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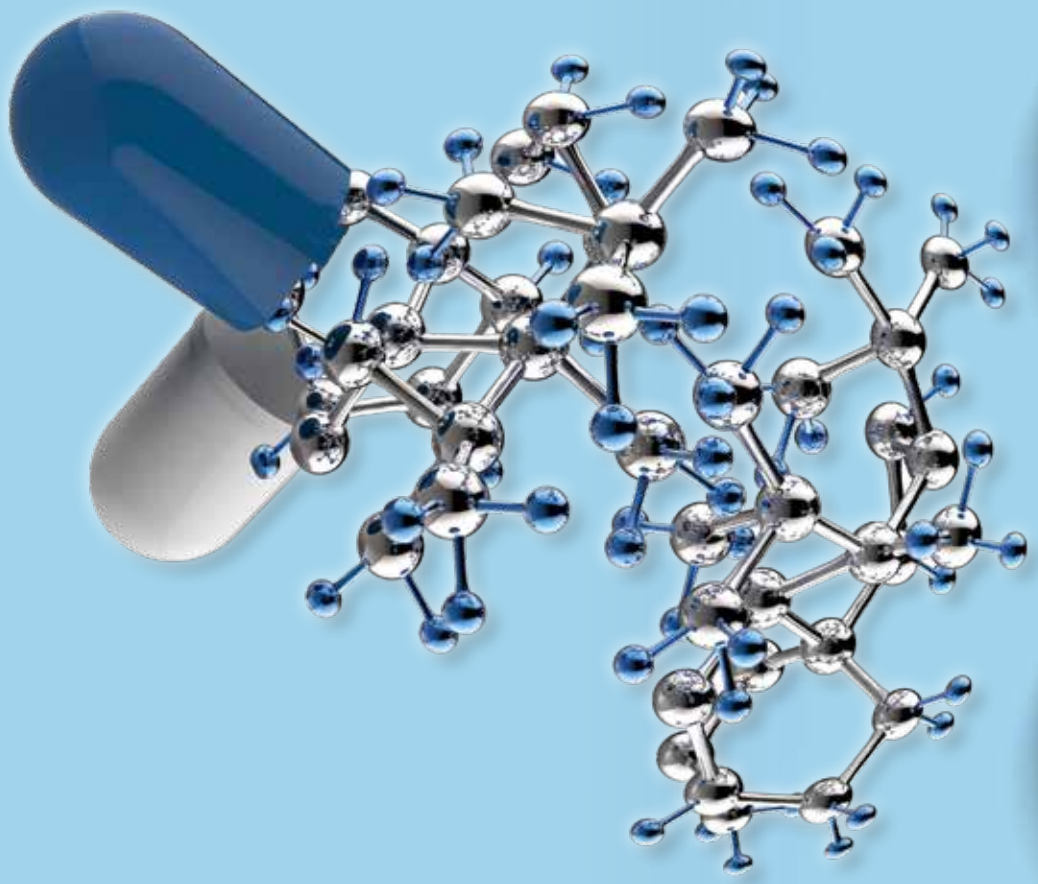

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, IV, 2023 | 2-27 ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

PHARMAKEFTIKI, 35, IV, 2023 | 2-27 REVIEW ARTICLE

Diagnosis and Treatment of Epilepsy using Nanotechnology, Artificial Intelligence and Internet of Things (IoT)#

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KEYWORDS:
Epilepsy; Nanotechnology; Artificial Intelligence; Diagnosis; Treatment

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ABSTRACT

Over 60 million people worldwide suffer from epilepsy. Despite the high prevalence of the disease, a clinical problem is emerging: The development of resistance to drug therapy. Drug delivery nanosystems, Artificial Intelligence (AI) and the Internet of Things (IoT) appear promising both at the diagnostic and therapeutic levels. This announcement is a review of clinical studies published in authoritative databases. These studies examine the application of drug delivery nanosystems in clinical therapy and diagnosis. At the same time, Artificial Intelligence (AI) and the Internet of Things (IoT) seem to give significant advantages to the treating physician. The application of Artificial Intelligence (AI), the use of Big Data through IoT-based networks, and the utilization of "smart" nanodevices can assist in clinical diagnosis and treatment. Also, drug delivery nanosystems improve biostability, reduce toxicity and control the release of the entrapped drug with better pharmacological effects. A typical example of diagnostic optimization is iron oxide nanoparticles as contrast agents in Magnetic Resonance Imaging (MRI). In addition, in the future, it is sought to design nanosensors that record an epileptic seizure, but have the ability to predict it by informing the patient from his mobile phone.

1. Introduction

Over 60 million people worldwide suffer from epilepsy. Despite the high prevalence of the disease, a clinical problem is emerging: The development of resistance to drug therapy. Thus, seizures become more frequent as the dose is reduced. Up to 40% of patients eventually develop resistance, increasing seizures, the risk of damage to an area of the brain, and mortality rates. Patients experience emotional and behavioral changes, seizures, convulsions, depression and, in some cases, loss of consciousness[1.] Pharmaceutical Nanotechnology has shown that it can contribute to diagnosis using nanoscale biosensors, which record the electrical excitability of the brain. The data can be used to develop algorithms that will be able to predict or characterize epileptic seizures[2]including added socio-economic burden. Unfortunately, only a few suppressive medicines are available, and a complete cure for the disease has not been found yet. Excluding the effectiveness of available therapies, the timely detection and monitoring of epilepsy are of utmost priority for early remediation and prevention. Inability to detect underlying epileptic signatures at early stage causes serious damage to the central nervous system (CNS. The treatment of epilepsy is often complicated by the inability of available antiepileptic drugs (AEDs) to cross the accessory blood-brain barrier (BBB), which could be overcome by appropriate drug delivery systems. The ideal system would provide localized and controlled release of AEDs at targeted sites in the brain to help reduce drug toxicity and enhance their efficacy. Several strategies for effective AED administration have been reported in the scientific literature. Nanotechnology-based systems appear to be a promising and innovative development as several drug delivery nanosystems have recently been reported as effective CNS delivery systems due to their increased biostability, ability to cross the blood-brain barrier (BBB) and their improved selectivity1. Nanotheranostics systems (nano therapeutics & diagnostics) combined with the Internet of Things (IoT) and artificial intelligence (AI) bring new possibilities for the immediate relief of epileptic seizures. Finally, the use of smartphones and portable devices (smart watches and fitness trackers) help to evaluate and monitor the neuronal impulses of the brain[3].

2. Definitions

Nanotechnology-based medicine (nanomedicine) refers to the characterization of surface properties and the design of nanocarriers for various medical strategies. Therapeutic agents are embedded or coated on nano-carriers, small colloidal or solid structural platforms ranging in size from 1 to 1000 nm. These NanoParticles (NPs) readily interact with the cellular environment at the molecular level to produce the desired physiological response. Nanotechnology-based AEDs have recently attracted attention due to their ability to penetrate the BBB, their improved selectivity and the potential for sustained drug release in the brain. Size, molecular weight (MB), copolymer ratio, corrosion mechanism and surface charge are important factors when considering the effectiveness of NPs. For example, the size of NPs is a very important determinant of effective BBB passage. NPs ranging from 35 to 64 nm have easy access to most neural tissues. The synthesis of NPs of specific size could be achieved through different preparation methods. As a result of the reduction in the sizes of NPs, the nanocarrier system exhibits a large surface area that can carry large doses of drugs, effectively reduce the peripheral drug toxicity and provide adequate drug delivery to their targets. The surface charge of NPs is also an important factor in determining their effectiveness in targeting the brain. It has been reported in the literature that neutral and mildly negatively charged NPs are more effective than positively charged NPs. On the other hand, positively charged NPs can make immediate changes in the BBB (albeit for a shorter duration) and are later cleared by the reticuloendothelial system (RES)[1.]

3. Pathophysiology and epidemiology of epilepsy

Epilepsy is a neurological disease characterized by

Table 1. Presentation of the advantages and disadvantages of using nanocarriers from studies performed for CNS diseases[1]. Table 1 is adapted from Reference 1.

Nano-carriers	Drugs used	Advantages	Disadvantages
Liposomes • PEG-Liposomes • Glycolipid conjugated • Immuno liposomes	GABA Phenytoin Thyrotropin	• Biocompatible • Size diversity • Molecular weight and hydrophilicity aids into effective encapsulation and entry to neural tissues skipping body defense machineries	• Susceptibility to RES clearing is more than the other NPs • Prone to phospholipid metabolic degradation leading short stay in the system • In earlier generations, shelf life stability was quite low
• Polymeric nanoparticles • PLGA • Poly(butylcyanoacrylate) • D,L-polyactide • Poly(ε-caprolactone) • Chitosan • Pullulan acetate-PEG • Poly(DL-lactide-co-glycolide) • Poly(glycolic acid)	β-Carotene Probenecid Thyrotropin Phenytoin Ethosuximide Clonazepam Valproate Loperamide Carbamazepine	• Biodegradable and biocompatible • Programmed drug release could be achieved by choosing apt polymer composition, ratio and molecular weight • Preparation easiness and greater stability	• Susceptibility to RES clearing and opsonization
Solid lipid nanoparticles • Chitosan	Diazepam Temozolomide Carbamazepine Riluzole Carvedilol	• Greater physical stability • Less toxicity • Greater surface area • Improve both drug loading and its efficacy • Multiple routes of administration	
Nano emulsion	Amiloride Olanzapine Carbamazepine Clonazepam Levetiracetam	• Stable preparations • Higher rate of absorption • Transmittable in multiple routes with less toxicity and irritation • BBB permeable	
Magnetic nanoparticles • Cobalt (Co) based • Nickel (Ni) based • Iron (Fe) based • Alginate-chitosan	Carbamazepine Alpha-methyl Tryptophan (diagnostic) Ethosuximide	• Precise modular control on transport and delivery to the targets • Minimum toxicity to other tissues	

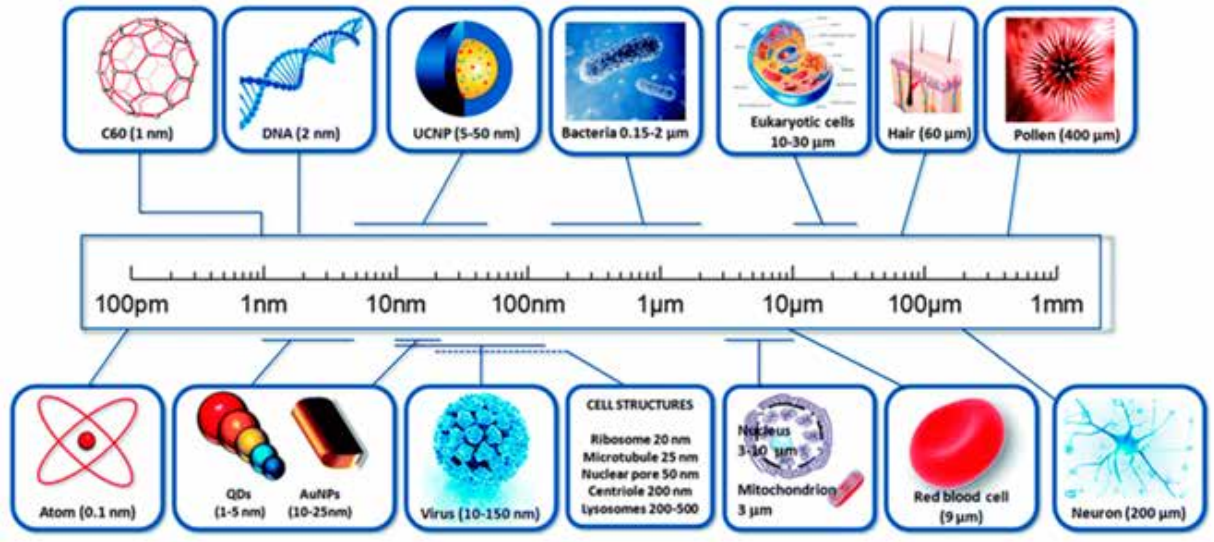

Figure 1. *Comparison of the size of nanosystems with biomolecules and cells. Adapted from ref. 4 (Creative Common CC BY license)[4].*

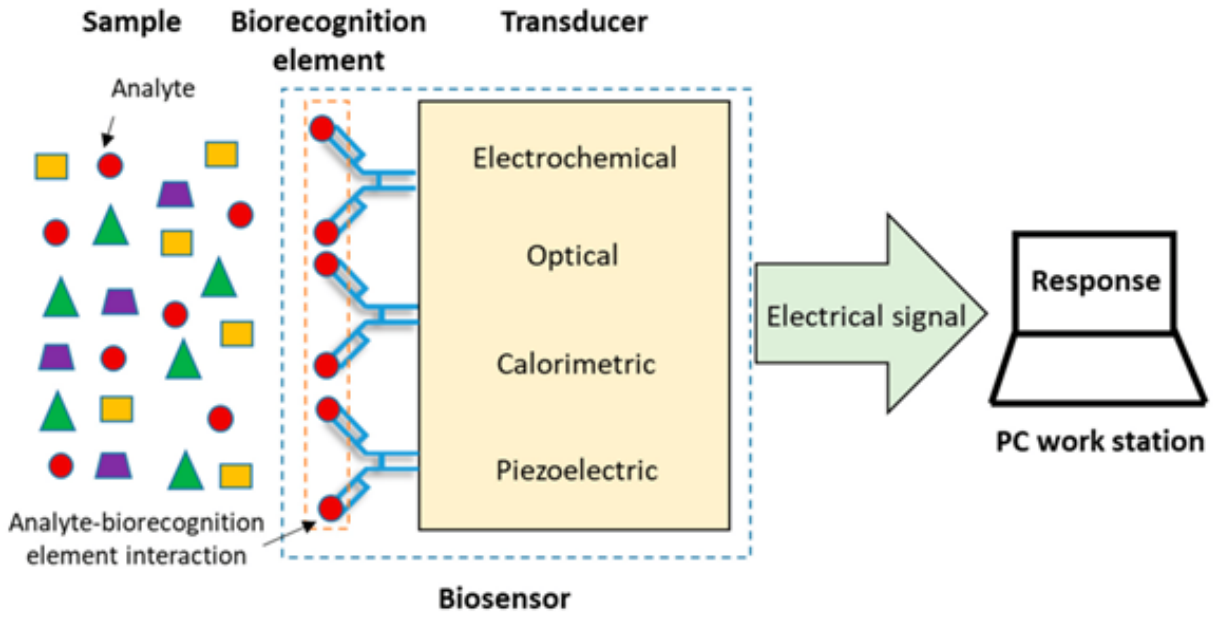

Figure 2. *Presentation of the parts of a biosensor. Adapted from from ref [12] (Creative Common CC BY license).*

abnormal electrical activity that causes seizures in various parts of the brain. This disease has neurological, cognitive, psychological and social effects and affects approximately 50 million people worldwide[5]. Estimates of the global prevalence and incidence of epilepsy vary from country to country. While it is more common in middle-income countries than in high-income countries, there is a significant increase in the number of patients in childhood compared to old age[6]. However, mortality is low. Unintention-

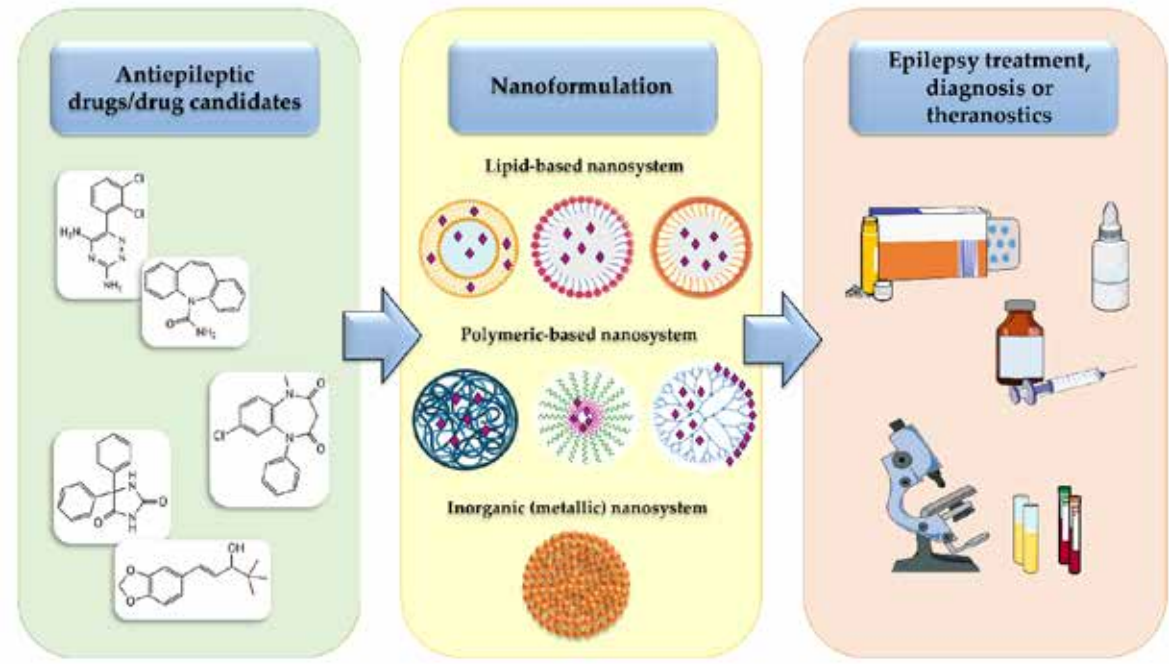

Figure 3. *Presentation of the steps for the formulation of nanomedicines in the treatment of epileptic brains. Adapted from ref. [13] (Creative Common CC BY license).*

al injuries and suicide were found to be among the deaths caused by epilepsy[7]. These results suggest that patients are psychologically affected by the lifelong continuation of the disease[8].

According to the World Health Organization (WHO), seizures are the result of excessive electrical discharges in a group of brain cells. These secretions can appear in many parts of the brain. The frequency of seizures caused by these secretions can range from 1 per year to several per day[8].

4. Diagnosis

Nanotechnology has been used as a diagnostic method in brain disorders. For example, iron oxide (magnetic) nanoparticles (MnPs) are applied as contrast agents in magnetic resonance imaging (MRI) of brain pathologies such as tumors, stroke, multiple sclerosis, acute diffuse encephalomyelitis, and trauma. Attached to non-radioactive drugs such as alpha methyl tryptophan, the magnetic nanoparticles are used for MRI imaging of normal brain functions and changes caused by epileptic activity. Conjugating nanoparticles to specific markers or antibodies can improve diagnostic specificity[9]. Technologies for monitoring neuronal activities are desirable for understanding the mysterious workings of the brain and the underlying mechanisms of neurological disorders such as epilepsy and Alzheimer's disease. In this regard, the potassium ion (K^+), as a key determinant of membrane potential, has been one of the main research targets, because the change in its concentration in the extracellular space directly affects the membrane potential of neurons and alters the intrinsic neuronal excitability and synaptic transmission. Therefore, much effort has been made to develop K+ sensors that have high sensitivity and selectivity. Until recently, K+-selective microelectrodes were considered the gold standard that allow us to measure extracellular K+ concentration ($[K^+]o$) with high temporal resolution[10].

In the effort to measure the change in potassium

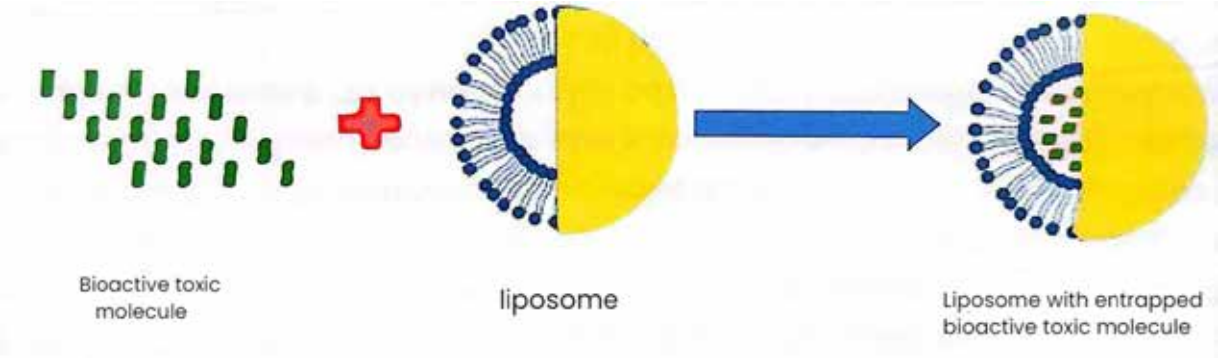

Figure 4. *Bioactive toxic molecule, liposome free of the bioactive molecule and liposome with the toxic bioactive molecule entrapped*[11]*.*

ion concentration in the extracellular space, a nanoscale biosensor was developed by a group led by Zhong Chen and Daishun Ling at Zhejiang University and Taeghwan Hyeon at IBS10. Biosensors are defined as devices used to detect of molecules and which have a biological component (enzyme, antibody, nucleic acid, whole cells or parts of tissues) combined with some physicochemical analytical part. In summary, biosensors give a measurable indication after reacting with the analyzed sample, without requiring the addition of reagents [11.]

A biosensor consists of three components:

1. A recognition biomolecule, responsible for the specificity of the sensor to the target molecule (e.g., receptor).

2. A detection element, which acts in some physicochemical way (optically, electrochemically, etc.).

3. A processor-signal converter or electronic processor, to present the results [11.]

The experiment's nanodevice consisted of an optical potassium indicator (a dye molecule that fluoresces in the presence of K^+) embedded in mesoporous silica nanoparticles shielded by an ultrathin layer of a potassium-permeable membrane. This membrane is very similar to the potassium channel in brain cells, and the pore size of the nanoparticles prevents other cations (including Na^+) from reaching the marker. This means that the device exclusively captured K^+ ions and could detect their presence at concentrations as low as 1.3 micromoles per liter. Thanks to this high sensitivity, the researchers were able to spatially map sub-milligram changes in extracellular K+ in three different regions of the mouse brain: the hippocampus, the amygdala, and the cortex10. After injecting the nanosensors into various locations in the brain of a test mouse, the team electrically simulated the mouse's hippocampus to induce a seizure and recorded the visual responses of the nanosensors. They then compared these measurements to those obtained from simultaneous measurements made using conventional electroencephalography (EEG). They found that in localized seizures, extracellular K+ concentration increases from the hippocampus to the amygdala and cortex over time, whereas in generalized seizures it increases almost simultaneously in all three brain regions[10].

5. Treatment

Nanotechnology is a rapidly developing field that has given new hope for the treatment of various disorders, including CNS diseases. The ability to cross the BBB and the specificity of targeting appear to be the main obstacles to the success of AEDs in pharmacotherapy. Applications of nano-based drug delivery systems in epilepsy are a promising solution that can overcome these limitations[1.]

It is important to note that the treatment of drug-resistant CNS disorders is one of the greatest

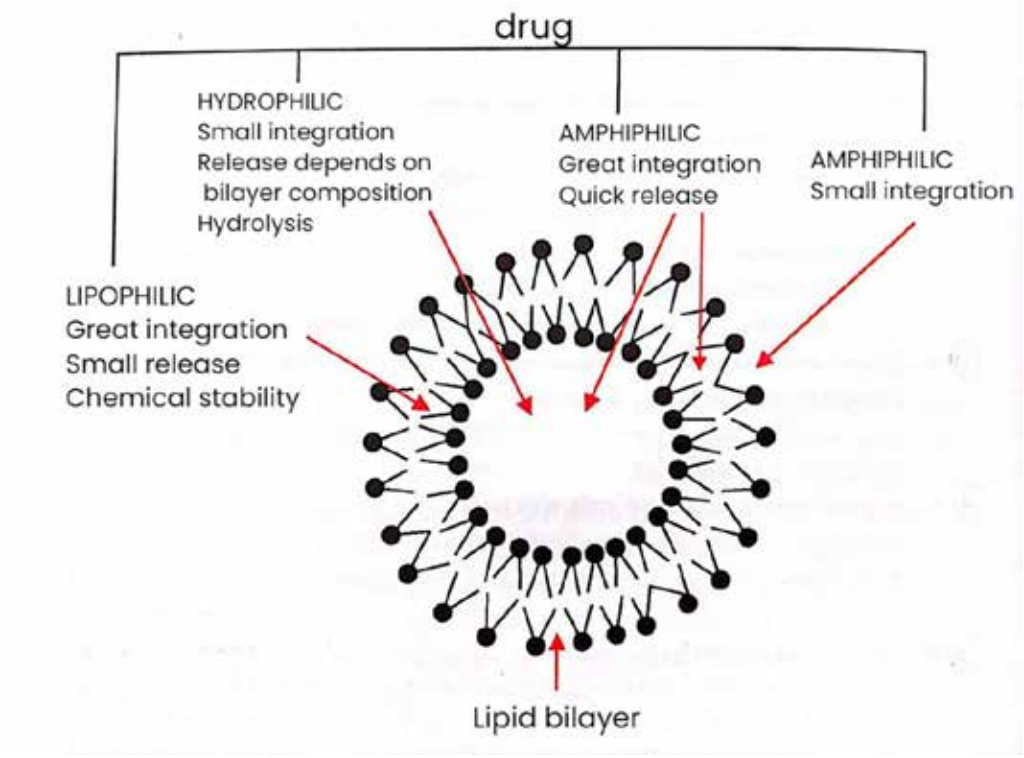

Figure 5. *Placement of the active molecules in the liposome according to their hydrophilicity*[11].

challenges in drug administration. However, there are currently no studies aimed at evaluating the effects of AEDs delivered by drug nanocarriers in experimental models or in patients with drug-resistant epilepsy[9].

Various nanocarriers can easily access targeted brain sites by manipulating the permeability of the BBB. Modification of NPs with surface ligands facilitates BBB crossing by nanocarriers. Due to the presence of transport molecules, such as growth factors, insulin and transferrin, at the BBB, NPs are desirable drug carriers in mapping strategies of epilepsy diagnosis and treatment. The blood-brain barrier acts as a neuroprotective barrier that prevents harmful substances from entering the brain while providing essential nutrients to the tissues. The BBB consists of a network of brain capillaries (microvessels) which are the smallest vessels of the vascular system, with a diameter of 3-7 μm. To ensure an efficient supply of nutrients and oxygen, approximately 100 billion of these microcapillaries are tightly packed and separated by only 40 μm. The transport of compounds into and out of the brain, leukocyte migration, and maintenance of brain microenvironment homeostasis are regulated by the microvascular endothelial cells of the BBB. Neighboring endothelial cells of brain capillaries contain tight junctions with multiple cell-cell protein interactions and some perforations and pinocytic vesicles[1].

6. Liposomes

Liposomes refer to unilamellar or multilamellar phospholipid vesicles that enclose a central aqueous compartment. Liposomes are the most studied AED delivery system due to their biocompatibility, biodegradability, and ability to encapsulate drugs with different lipophilicities and molecular weights. The ease of changing their dimensions, membrane fluidity and surface characteristics make them ideal

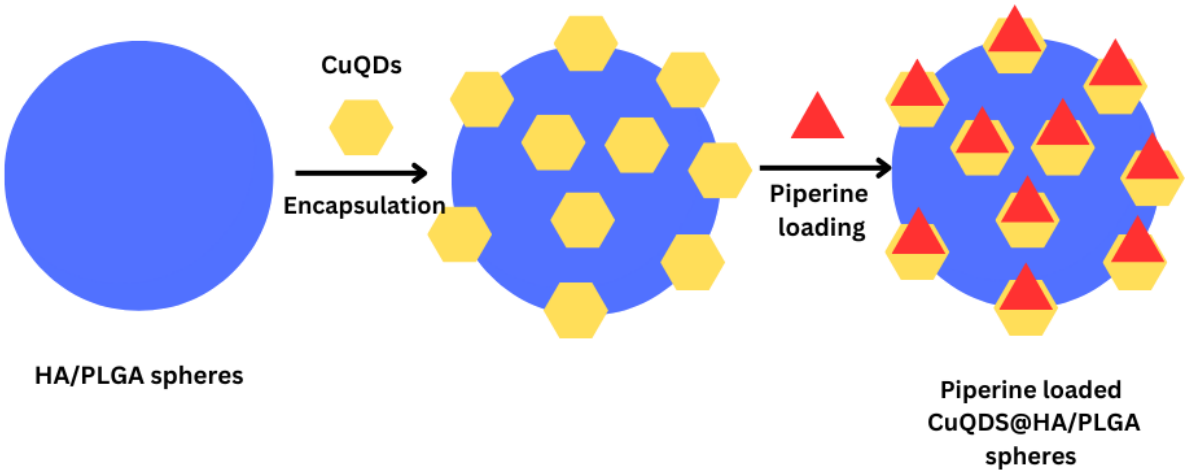

Figure 6. *Schematic presentation of nanocarrier formation and loading of Piperine drug molecules.*

nanocarriers[1].

There have been reports of enhanced bioavailability of drugs across cell membranes and minimization of enzymatic degradation using liposomal carriers. The half-life of liposomes can be improved through reduction of vesicle size, enhanced surface hydrophilicity, or the use of glycolipids and polyethylene glycol. The hydrophilic moieties form a periliposomal layer adjacent to the liposome surface and prevent opsonins from entering the liposome surface, which makes these nanocarriers invisible (Stealth) from direct clearance in the RES[1].

In order to implement stimulus (e.g. pH, temperature) drug release from liposomes, a pH- and temperature-sensitive liposome has been developed, which releases the drug content in response to an acidic environment and elevated temperature (41 °C-42°C) in a specific target tissue. In an early study, Loeb et al. (1982 and 1986) reported that liposome-encapsulated γ-aminobutyric acid (GABA) inhibited penicillin- and isoniazid-induced epileptogenicity in rodents. In another research program, a thyrotropin-encapsulated formulation produced extensive anticonvulsant activity and suppressed seizures in Amygdala-kindling rats (Kindling Amygdala is a type of Temporal Lobe Epilepsy (TLE)[14]. In another study, the same research group reported successful seizure prevention in response to certain threshold concentrations of liposomal GABA in Amygdala-kindling rats. Mori et al. (1995) used liposome-encapsulated phenytoin (PHT-L) to investigate status epilepticus in a rat model. They reported suppression of total seizures and sustained cortical activity in response to PHT-L.[15] Liposome compositions are used in the treatment of many malignant conditions in which secondary epilepsy is a characteristic feature. Brain tumors are often a major cause of seizures. Immunoliposomes are a successful strategy for optimal drug delivery to the brain. They are prepared by conjugating polyethylene glycol (PEG)-stabilized liposomes with monoclonal antibodies to the rat transferrin receptor. Immunoliposomes have been reported to deliver drugs at concentrations four times higher than PEG-liposomes due to targeted action. Some studies of PEGylated immunoliposomes with entrapped antineoplastic drugs have yielded encouraging results relative to the delivery of drugs without them entrapped in immunoliposomes due to targeted therapy. In one study, Yang et al. (2012) reported remission in mouse model glioma in response to guided chemotherapy using[16] PEGylated liposomal[17] doxorubicin combined with repetitive high intensity pulsed focused ultrasound. In another study, Anders et al. (2013) reported the improved of

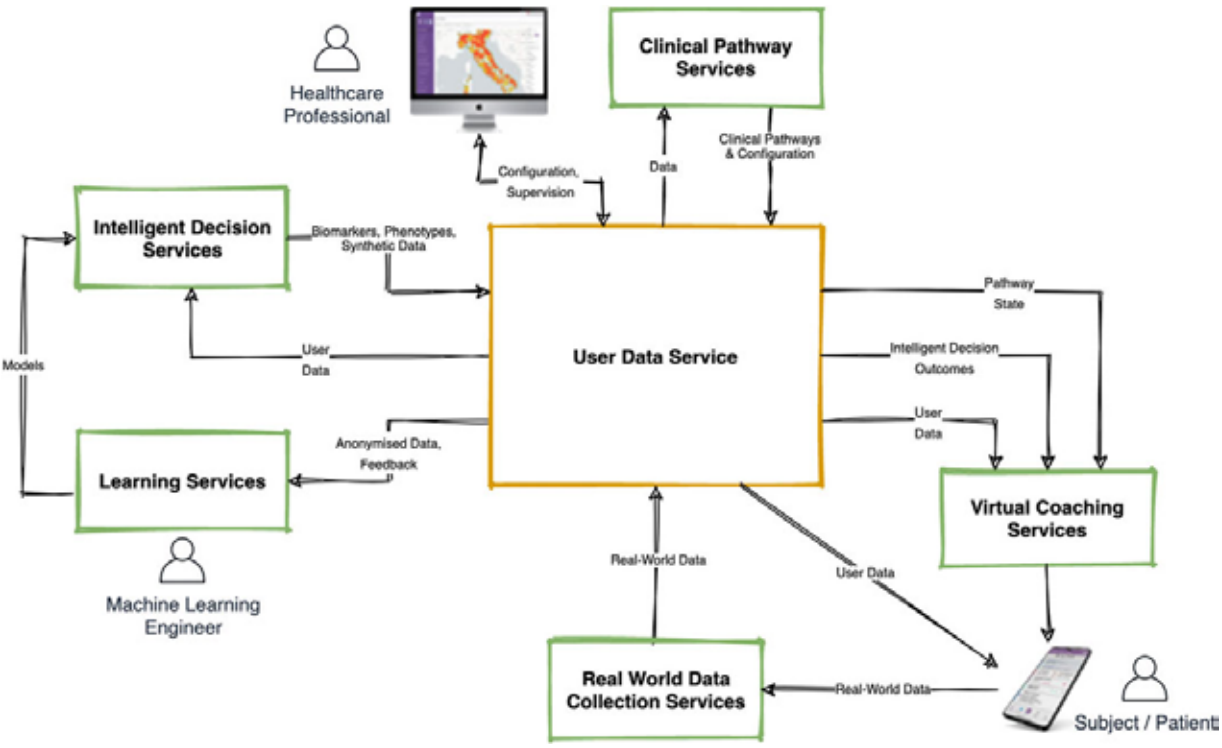

Figure 7. *Schematic representation of data transfer and storage in Healthentia's User Data Service (Cloud). Adapted from ref.[23] (Creative Common CC BY license).*

pharmacokinetics and efficacy[17].

Disadvantages of liposomal nanocarriers

Reported general limitations of liposomal formulations include rapid immune clearance if not surfaced with polyethylene glycol (PEG), from the bloodstream due to destruction by RES cells, low stability after prolonged storage, rapid metabolic degradation of of phospholipids and inability to provide sustained release of the entrapped drugs compared to other nanocarriers. Newer generations of liposomal AED formulations have greatly reduced some of these disadvantages, such as shelf life and stability, compared to previous generations of liposomal AED formulations. In addition, liposomal formulations of several pharmacologically active AEDs in preclinical development, including valproic acid, superoxide dismutase, GABA, and amiloride, are expected to provide new perspectives on drug delivery to the brain1. In addition to liposomes, there are also other nanocarriers, such as dendrimers, micelles, etc. each of which has its advantages and disadvantages in treating brain diseases[1.]

Nano-carriers have two main advantages. First, they have a higher surface-to-volume ratio, so they contribute to a significant reduction in drug concentration. As the dose is reduced, the side effects and toxicity of the drug will decrease. Second, drugs can target a specific tissue. Thus, the effect of the drug is further increased[8,10.]

The pharmacological approach to crossing the BBB is based on the modification, through medicinal chemistry, of a drug molecule to allow permeability of the BBB and render it impervious to drug efflux pumps, such as P-glycoprotein (PgP). One strategy is to develop highly lipophilic and small drugs, allowing them to successfully diffuse through brain endo-

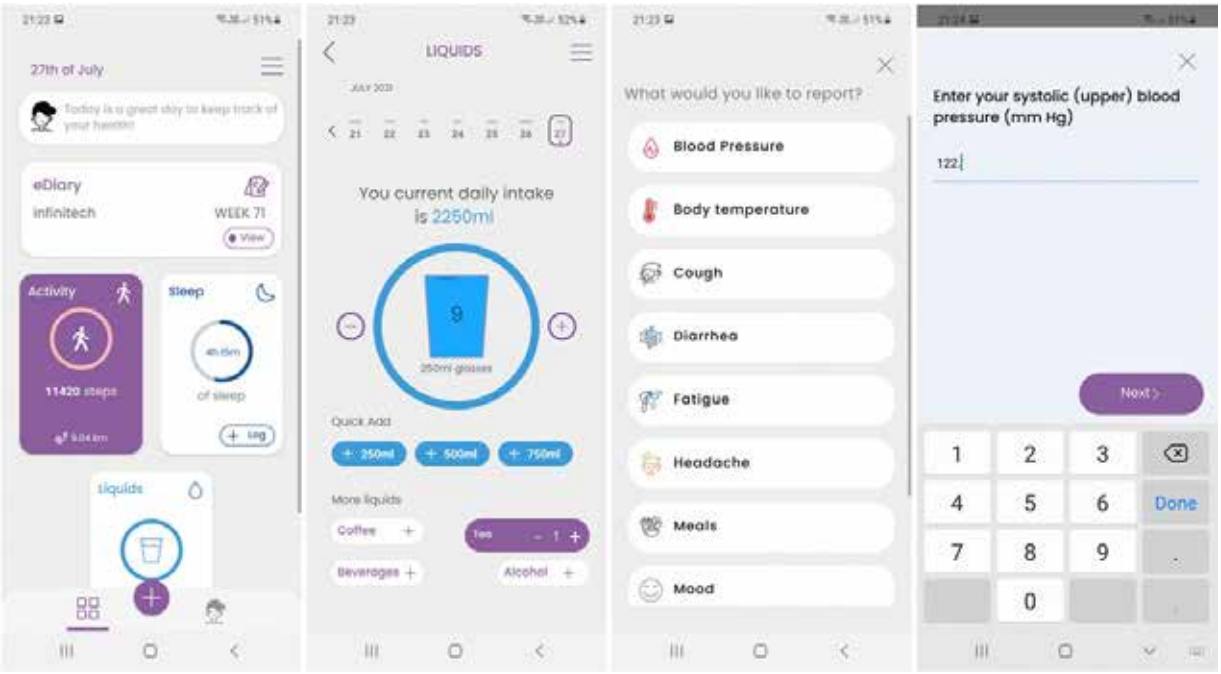

Figure 8. *Collection of Data from the application[19]. Adapted from ref.[23] (Creative Common CC BY license).*

thelial cells. Unfortunately, this strategy eliminates a huge number of potentially useful polar molecules that could be used to treat CNS disorders. An alternative strategy is to use small water-soluble drugs to facilitate crossing the BBB by the paracellular hydrophilic diffusion pathway, although the majority of these molecules are able to penetrate the mesoendothelial space of the cerebral vasculature up to the tight junctions and not beyond. In addition, it should be mentioned that modifications in the structure of the drug often lead to a loss of the biological activity of the drug[18].

In general, the physiological method shows more advantages, as it takes advantage of the existence of specific transcytotic molecule transport receptors expressed on the surface of the BBB in order for molecules to pass through it.

In recent years, liposomes have been investigated as carriers of drugs, of imaging contrast agents, and genes, particularly for the treatment and/or diagnosis of neurological diseases.

Therefore, it is important to gain a better understanding of the molecular mechanism of the disease and the development of improved diagnostic devices and therapies. Liposomes are promising carriers for CNS delivery[19].

The application of nanoelectronics can solve many problems. An example is the creation of a circuit that allows the normal flow of electricity but prevents the abnormal flow of electricity. This circuit must also be invisible to normal brain activity for the brain to function normally. Simply we need a damping circuit like a filter.

The device when attached to the ECM (Extracellular Matrixes) will eventually change the voltage at that point in the cell when an abnormality occurs[20].

In addition, a study focused on the synthesis of CuQDs@HA/PLGA microspheres for the treatment of epilepsy was carried out. Piperine drug molecules encapsulated with CuQDs@HA/PLGA nanovesicles significantly increased the anticonvulsant efficacy of PTZ-induced kindling in vivo animal models comparable to free piperine molecule treatment. The prepared CuQDs@HA/PLGA nanostructures proved to

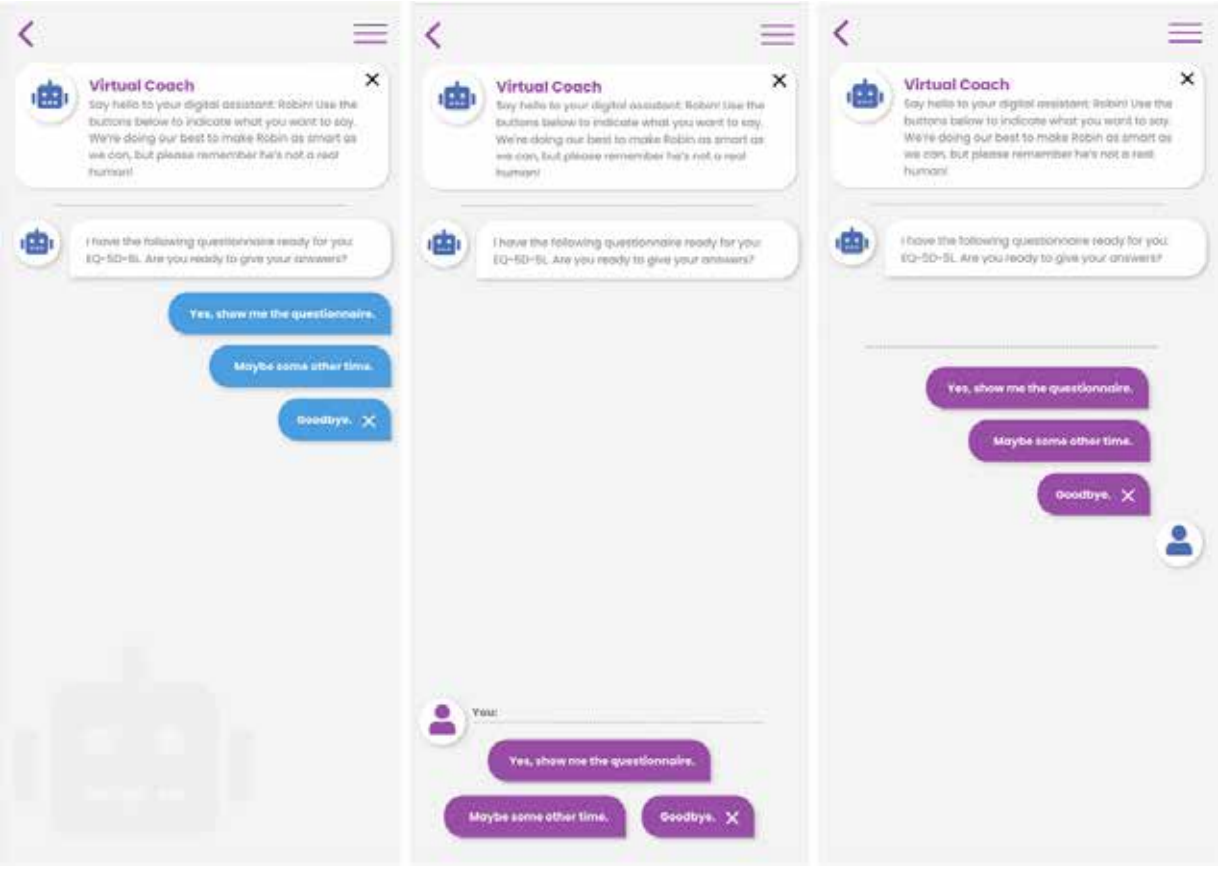

Figure 9. *Three different variations of dialog in chat box are shown[19]. Adapted from From ref [23] (Creative Common CC BY license).*

be a novel platform for antiepileptic drug delivery[21].

Finally, there is increasing interest in intranasal nanotechnology products in the management of epilepsy. The combination of nanotechnology with the nose-to-brain approach helps to increase the effectiveness of the treatment as the medicinal product reaches the target tissue more easily. It also minimizes side effects and patient non-compliance seen with other routes of administration[22].

7. New Technologies

7.1 The role of Nanotheranostics, Internet of Things and Artificial Intelligence in the Diagnosis and Treatment of Epilepsy

Nanotheranostics (nano therapeutics & diagnostics) systems are best proven in terms of targeted and controlled release, an integrated approach that takes into account the anatomy of each individual. Nanotheranostics help in profiling a disease and drugs, as well as understanding the relationship between host and disease. The management of epilepsy in a person is possible through "smart" nanotheranostic systems as they allow the detection and recognition of pathological signs. Nanotheranostics give the advantage of creating a database of information and with further clinical and economic developments may increase its widespread use in epilepsy. Nanotheranostics combined with the Internet of Things (IoT) and artificial intelligence (AI) bring new possibilities to epilepsy. In addition, the use of smartphones and wearable devices can (eg, smart watches and fitness trackers) assess and monitor neu-

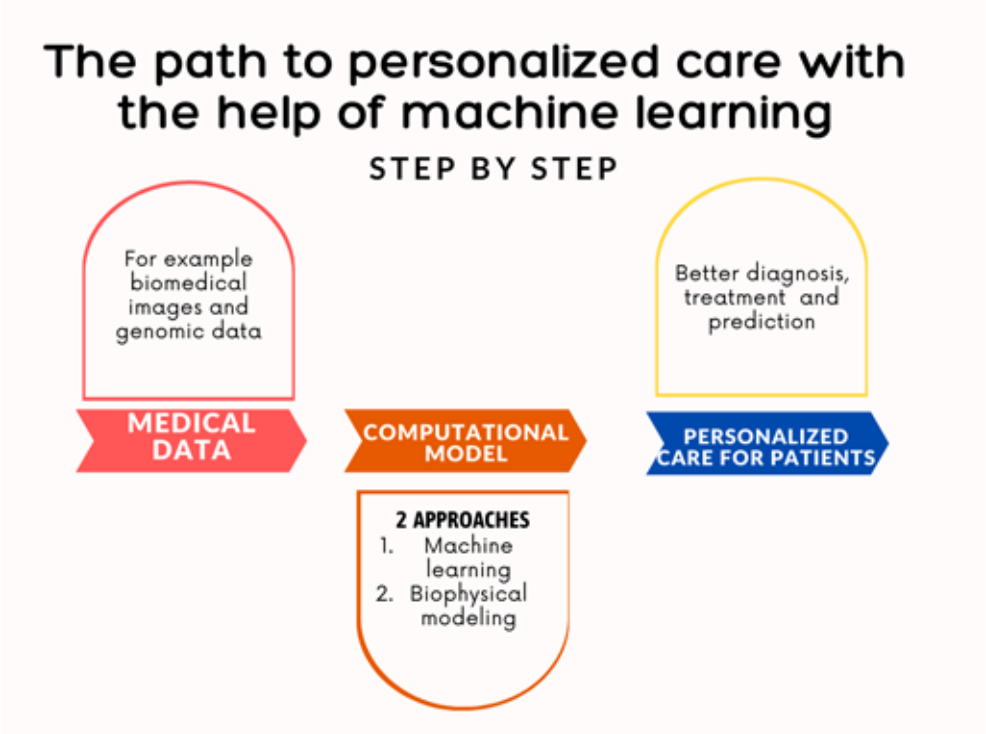

Figure 10. *Illustrating the path to personalized care with the help of machine learning.*

ronal firing or imbalance in the brain[3].

7.2 The application of Digital Therapeutics in Diagnosis and Therapy

More and more people worldwide need to deal with at least two chronic conditions for a significant part of their lives. Specifically, as the OECD (Organization for Economic Cooperation and Development) published in 2019, more than 30% of the population over 15 years old lives with at least 2 chronic diseases and this percentage is expected to increase in the coming years[23].

Thus, traditional health care provision is unable to meet the resulting individual and socioeconomic needs. Digital Therapeutics (DTx) is a form of digital health solutions that "provide evidence-based therapeutic interventions guided by high-quality software programs to prevent, manage or treat a medical disorder or disease". They are used independently or in combination with drugs, devices or other therapies to optimize patient care and health outcomes" (Digital Therapeutics Alliance, 2021). These, therefore, can relieve the health system from the ever-increasing pressure[23].

Healthentia is a CE (Conformité Européenne) Class I Medical Device currently used in more than 20 clinical studies. The Healthentia platform is an e-Clinical solution that captures clinical results from medical and Internet of Things (IoT) devices and offers intelligent services based on Artificial Intelligence (AI). In this way, virtual guidance is sought with no or very little human intervention. The healthcare professional is often available remotely and acts as a safety 'net' to oversee the automated system's decisions. E-coaching can take place in a variety of ways including: Short text messages e.g., website-like content e.g., video tutorials e.g. and realistic three-dimensional (3D) embedded interlocutors[23].

The healthcare professional can securely log into the Healthentia web portal and create interventions. At this point it invites the patient to participate and enables or disables modules such as patient fluid

intake. After the patient accepts the invitation and registers in the application, he fills in some personal information such as age, gender, weight and height. Also, it can connect the app to various apps (eg Fitbit or Garmin Activity Trackers) if they are enabled in the configuration stage to start collecting e.g. daily physical activity and sleep data. The patient receives various health questionnaires (which also include psychological questions) but can also enter data on his own initiative[23].

The real-world data collected is used in a number of ways. First, the patient can view their own information through the mobile app in order to increase awareness of their own behavior (an essential part of the virtual coaching part as described in Coaching: Virtual Coaching in Digital Therapeutics). Second, healthcare professionals (mainly in the case of clinical studies) can view patient data, at an individual level or through various dashboards that provide an overview of patient groups. Communication with the virtual coach is either initiated by the patient or the virtual coach can draw their attention when there is something important to discuss[23].

A new survey was conducted to evaluate the responses that artificial intelligence gives to patients. The researchers used 195 relevant questionnaires posted on Reddit r/AskDocs and answered by certified health professionals. They then posed the same questions to Open AI's GPT-4. A panel of experts who evaluated the results rated the AI responses as "good" and "very good". On the contrary, the answers of the doctors were characterized as "acceptable". It appeared, then, that the experts seemed to prefer the GPT-4 answers by 78.6% compared to the doctors' answers. The conclusion of the study published in JAMA Internal Medicine showed that AI messaging programs can be safely used by healthcare facilities to facilitate patients. It is pointed out, however, that the responses of the system must be under the supervision of doctors[24].

7.3 The application of Machine Learning to Epilepsy

For the seizure detection task, several studies have shown that models were trained through machine learning algorithms using specific features in the time and/or frequency domain based on scalp EEG. In another study, the researchers taught the model to distinguish normal from abnormal electrical activity automatically without requiring any manual process. They also found that seizure detection performance depends on the similarity of the seizure onset pattern between the training data and the test data, i.e., new data with a different onset pattern from that of the trained data could not be detected well. The models were also trained to detect an impending seizure several minutes before onset. In fact, they showed a sensitivity of 80-90%, but we must note that each study had a different prediction time (5'-60' before onset)[25].

In another clinical study the sensitivity of the models to detect seizures reached 65-100% in each patient but when data was obtained for that patient for more than a month. It is worth noting that these models are also particularly useful for the precise definition of the surgical target. Therefore, in addition to facilitating the diagnosis, they essentially help in the treatment as well[25].

Notable is the Multicentre Epilepsy Lesion Detection (MELD) project which used over 1,000 patient MRI scans from 22 global epilepsy centers to develop the algorithm, which provides reports of where abnormalities exist in cases of drug-resistant focal cortical dysplasia (FCD) - the main cause of epilepsy. The researchers then trained the algorithm on examples that had been labeled by expert radiologists as either healthy brains or having FCD – depending on their patterns and characteristics. The researchers found that overall the algorithm was able to detect FCD in 67% of cases (538 participants). Previously, radiologists in 178 of the participants were unable to find the abnormality on MRI but the MELD algorithm was able to identify FCD in 63% of these cases. This is especially important because if doctors can find the abnormality on a brain scan, then surgery to remove it can provide a cure[26].

8. Ethical Artificial Intelligence

Since 2014, private companies, research institutions, and public sector organizations have issued guidelines for ethical artificial intelligence (AI). However, despite the agreement that AI should be "ethical," there is debate over both what constitutes "ethical AI" and what ethical requirements, technical standards, and best practices are needed to implement it. A global convergence was thus found emerging around five ethical principles (transparency, fairness and justice, non-maleficence, responsibility and privacy)[27].

9. Discussion and Conclusions

Antiepileptic drugs are often used in the treatment of epilepsy, but the difficulty with these drugs is the emergence of resistance[8]. One hypothesis to explain pharmacoresistance in epilepsy is the overexpression of multidrug-resistant proteins, such as P-glycoprotein, in the endothelium of the blood-brain barrier[9]. Furthermore, due to AEF only drugs with a molecular mass < 400-500 daltons can cross the BBB. However, before drugs can reach the CNS, macrophages tend to phagocytose them within the RES (reticuloendothelial system) and astrocytes further limit drug accumulation in the brain. Tight barriers in the CNS prevent conventional drugs or chemotherapeutic agents from reaching targeted sites within the brain[1].

Medicines used to treat epilepsy must be administered effectively and safely so that the brain is protected. Therefore, new delivery systems should deliver drugs at concentrations determined to have high therapeutic efficacy in epilepsy without toxicity. Considering this information, there is a need to develop new treatment strategies. With the development of nanotechnology, it has been proven that nanoparticles as a drug delivery system are significantly effective in treating diseases[8].

Nanocarrier systems can fulfill many functions, such as being able to cross the blood-brain barrier (BBB) by passing a specific cell or signaling pathway, respond to endogenous stimulus, support nerve regeneration, and ensure cell survival. Nanotechnology holds the promise of controlling the concentration of drugs and delivering the drug to the target tissue across the BBB[8].

The combination of nanotechnology with Artificial Intelligence and the Internet of Things looks promising. The doctor will be able to monitor the patient's progress through a database as well as be informed of an upcoming seizure. All this combined with the high-quality data provided by Nanotechnology applications (e.g., nanobiosensors) open new avenues for faster and more accurate diagnosis as well as safer treatment.

Various nanoparticle (NP)-based drug delivery systems have been proposed and reported as great AED delivery systems. These when encapsulate antiepileptic drugs (AEDs) seem to be new weapons in the near future for the treatment of epilepsy1. Early and accurate diagnosis of the disease with the help of new technologies (Artificial Intelligence & IoT) as well as treatment using Nanotechnology can significantly improve the patient's quality of life. □

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, IV, 2023 | 18-33 ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

PHARMAKEFTIKI, 35, IV, 2023 | 18-33 REVIEW ARTICLE

Η Επίδραση των Οιστρογόνων και των Προγεστογόνων στην Εκδήλωση Μείζονων Ψυχικών Διαταραχών

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ABSTRACT

Τα οιστρογόνα φαίνεται να συμμετέχουν σε διάφορες λειτουργίες του οργανισμού, συμπεριλαμβανομένης και της λειτουργικότητας του εγκεφάλου, μέσω των οιστρογονικών υποδοχέων, επιδρώντας εκτός των άλλων και στην διαφοροποίηση ή και την εμφάνιση και προαγωγή, σοβαρών και μη, ψυχικών διαταραχών. Δείχνουν να εμπλέκονται στην κατάθλιψη, τη διπολική διαταραχή και τη σχιζοφρένεια, με τη διακύμανση των επιπέδων τους να είναι επιβαρυντικές ή επωφελείς. Σε αυτές τις ψυχικές διαταραχές τα προγεστερινοειδή διαφαίνεται να περιορίζουν την ευνοϊκή δράση των οιστρογόνων, τα οποία παρόλα αυτά είναι απαραίτητα σε ποικίλες περιπτώσεις, συμπεριλαμβανομένης της μείωσης των παρενεργειών των οιστρογόνων. Οι δράσεις αυτές των ορμονών και των συμπληρωμάτων τους φαίνεται να ασκούνται σε κυτταρικό επίπεδο, μέσω μεταγραφής και έκφρασης γονιδίων, αλλά και ρύθμισης της μιτοχονδριακής λειτουργίας, ενώ φαίνεται να παρεμβαίνουν σε όλα τα κύρια σηματοδοτικά μονοπάτια μέσω επιρροής της δράσης καίριων νευροδιαβιβαστών, διεγερτικών και κατασταλτικών. Επίσης, οι ορμόνες του φύλου φαίνεται να επιδρούν και στη ρύθμιση της διατροφικής συνήθειας, παράγοντας που δείχνει να μεταβάλλεται ιδιαίτερα κατά την εμφάνιση πολλών μείζονων ψυχικών διαταραχών, αλλά και λόγω της εκάστοτε αγωγής αυτών των παθήσεων. Έτσι θα ήταν σημαντική, η ανασκοπική μελέτη αυτών των μηχανισμών διασύνδεσης των ορμονών του φύλλου με τις ψυχικές διαταραχές καθώς και η πιθανή αντιμετώπιση τους μέσω ορμονικής θεραπείας, επικουρικά της υπάρχουσας αγωγής των ασθενών.

1. Εισαγωγή

Οι συναισθηματικές διαταραχές είναι από τις πιο συχνές ψυχικές διαταραχές, με τις γυναίκες να εμφανίζουν μεγαλύτερη ευπάθεια από τους άνδρες.[1] Ο σημαντικός ρόλος των γοναδικών ορμονών στις ψυχικές διαταραχές προτείνεται από τον αυξημένο επιπολασμό της κατάθλιψης κατά τη διάρκεια των αναπαραγωγικών ετών και την πιθανότητα εμφάνισης καταθλιπτικών επεισοδίων σε περιόδους μείωσης αυτών των ορμονών, ενώ η θεραπεία ορμονικής υποκατάστασης έχει δειχθεί ότι βελτιώνει ή και αποτρέπει τη μεταγεννητική και την μετεμμηνοπαυσιακή κατάθλιψη.[2] Οι συναφείς διαταραχές όπως το προεμμηνορροϊκό σύνδρομο, η μεταγεννητική κατάθλιψη και η προεμμηνοπαυσιακή κατάθλιψη σχετίζονται με χαμηλά επίπεδα οιστρογόνων στον ορό, ενώ η αμυγδαλή είναι μια βασική δομή που εμπλέκεται σε συναισθηματικές διαταραχές. Οι διακυμάνσεις των επιπέδων οιστρογόνων στις γυναίκες αναμένεται να έχουν σημαντικό αντίκτυπο στη λειτουργία της αμυγδαλής με βάση την έκφραση των υποδοχέων των οιστρογόνων (ER), και ιδίως του ERa.[3] Στην παρούσα ανασκόπηση επιχειρείται μία ανάλυση της εμπλοκής των οιστρογόνων στις ψυχιατρικές διαταραχές καθώς και η ανάλυση του μηχανισμού με τον οποίο συνδέονται υπό το πρίσμα της πιθανής εμπλοκής τους στη θεραπευτική προσέγγιση αυτών.

2. Συναισθηματικές διαταραχές

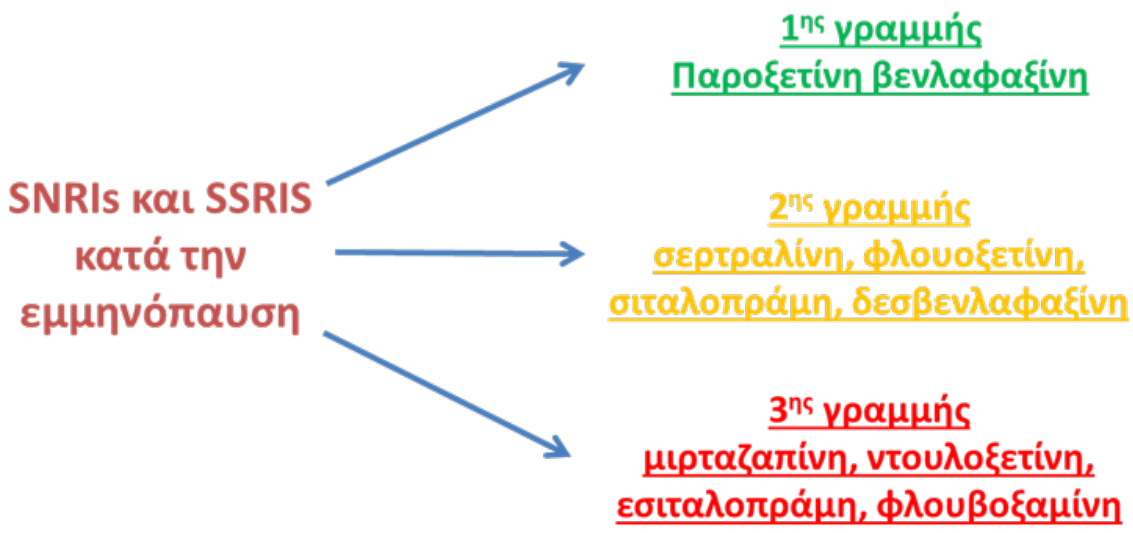
2.1. Κατάθλιψη

Υπάρχουν πολλοί τύποι κατάθλιψης που εμφανίζονται σε γυναίκες που παρουσιάζουν συναισθηματικές διαταραχές σε περιόδους ορμονικής διακύμανσης, που μπορούν να αντιμετωπιστούν αποτελεσματικά από τα οιστρογόνα. Αυτές είναι η μηνιαία κυκλική προεμμηνορροϊκή δυσφορική διαταραχή (PMDD), η μεταγεννητική κατάθλιψη που εμφανίζεται αρκετές εβδομάδες ή μήνες μετά τον τοκετό και επίσης η κατάθλιψη στους μήνες ορμονικών διακυμάνσεων που συμβαίνουν στη φάση μετάβασης προς την κλιμακτήριο.[4]

Θεμελιώδης για τη θεραπεία της κατάθλιψης με ορμονική θεραπεία είναι η σωστή διάγνωση του τύπου κατάθλιψης καθώς και αν πιθανά ανταποκρίνεται στα οιστρογόνα. Το πιο αξιοπρόσεκτο γεγονός είναι ότι η μέτρηση των επιπέδων ορμονών μπορεί να μην είναι χρήσιμη. Όλα τα παραδείγματα που δίνονται είναι σε προεμμηνοπαυσιακές γυναίκες που έχουν φυσιολογικά επίπεδα οιστραδιόλης, ορμόνης διέγερσης ωοθυλακίων και τεστοστερόνης, οι οποίες, αν και μπορεί να μην είναι βέλτιστες για την ασθενή, βρίσκονται συνήθως εντός του φυσιολογικού εύρους. Επομένως, το ιστορικό τους είναι ο πιο σημαντικός παράγοντας για τη διαμόρφωση της διάγνωσης και τη διαφοροδιάγνωση από άλλες αιτίες κατάθλιψης, ιδιαίτερα από τη διπολική διαταραχή, η οποία δεν αποτελεί μια ασυνήθιστα εσφαλμένη διάγνωση της κατάθλιψης σχετιζόμενη με την ορμονική ανισορροπία. Είναι γνωστό ότι η σοβαρότητα της διπολικής και μονοπολικής διαταραχής αλλάζει συχνά με τον εμμηνορροϊκό κύκλο, αλλά η συχνή διαγνωστική σύγχυση μεταξύ αυτών των ψυχιατρικών διαταραχών και της PMDD, η οποία απαιτεί πολύ διαφορετική θεραπεία, μερικές φορές δεν αναγνωρίζεται,[5] ενώ ιστορικό μεταγεννητικής κατάθλιψης σε μία ή περισσότερες από τις προηγούμενες εγκυμοσύνες είναι επίσης ισχυρός δείκτης κατάθλιψης, που δείχνει να έχει ορμονική βάση.[6]

Είναι πιθανό ότι η βασική αιτία της προεμμηνορροϊκής κατάθλιψης είναι η δυσανεξία στην ενδογενή προγεστερόνη μετά την ωορρηξία και φαίνεται ότι αυτοί οι ασθενείς είναι επίσης δυσανεκτικοί σε οποιοδήποτε προγεστογόνο, ενώ η έκταση της εξαρτάται από τη δόση και τη διάρκεια χορήγησης τους.[7] Επομένως, κάθε προγεστογόνο που ενδεχομένως να χρησιμοποιείται για ενδομήτρια προστασία σε αυτούς τους ασθενείς θα πρέπει ίσως να είναι εκείνο που παράγει τα λιγότερα συμπτώματα και να δίδεται στη χαμηλότερη αποτελεσματική δόση και για τον μικρότερο αριθμό ημερών. Για αυτόν τον λόγο, το δισκίο ελέγχου των γεννήσεων, αν και καταστέλλει την ωορρηξία, δεν είναι τόσο αποτελεσματικό λόγω του παρεχόμενου συστηματικά, σε ημερήσια βάση και συνεχώς προγεστογόνου για 21 ημέρες το μήνα.

Παρόλα αυτά, στις περισσότερες περιπτώσεις χορήγησης οιστρογόνου, οι ασθενείς θα χρειαστούν προγεστογόνο για την πρόληψη της υπερπλασίας



Εικόνα 1: *Κατηγοριοποίηση της χρήσης των SNRIs και SSRIs κατά την εμμηνόπαυση.*

του ενδομητρίου και της ακανόνιστης αιμορραγίας, αλλά, λόγω της δυσανεξίας στο προγεστογόνο σε αυτές τις γυναίκες, συνιστάται μικρότερη δόση και για μικρότερη διάρκεια. Το προγεστογόνο δροσπιρενόνη [(3-οξο-6α,7α,15α,16α-τετραϋδρο-7'Η,16'Η-δικυκλοπροπανο[6,7;15,16]-17α-πρεγν-4-εν-21,17-καρβολακτόνη] είναι ένα αντιανδρογονικό προγεστογόνο που έχει υποστηριχτεί ότι έχει αποτελεσματικότητα για τη θεραπεία της PMDD[8] και έχει οριστεί από ορισμένες μελέτες να είναι κατάλληλο ως θεραπεία πρώτης γραμμής.[9] Μια αποτελεσματική ορμονική θεραπεία για τη σοβαρή PMDD είναι η χρήση διαδερμικών οιστρογόνων για την καταστολή της ωορρηξίας.[10] Οι γυναίκες θα πρέπει να ειδοποιηθούν ότι μπορεί να αισθάνονται λιγότερο καλά τις πρώτες 2 εβδομάδες, μάλλον, με αλλαγές παρόμοιες όπως αυτές στη διάθεση που παρατηρούνται στις αρχές της εγκυμοσύνης και ότι η θεραπεία μπορεί να μην λειτουργεί για τον 1ο μήνα έως ότου η ωορρηξία έχει κατασταλεί.

Πρέπει να αναγνωριστεί ότι η μεταγεννητική κατάθλιψη μπορεί να είναι το σημείο καμπής στην ψυχική υγεία μιας γυναίκας που αργεί να συμβουλευτεί τον ιατρό της, και μπορεί να οδηγήσει σε ένα μακροχρόνιο ιστορικό κατάθλιψης, το οποίο ξεκινάει πολλά χρόνια, μετά από την εγκυμοσύνη, η οποία σχετίζεται με καλή διάθεση και πλήρη απουσία σημείων της κατάθλιψης, λόγω σταδιακά αυξημένης συγκέντρωσης οιστρογόνων. Επίσης, η κατάθλιψη κατά την εμμηνόπαυση οφείλεται σε ένα μείγμα πολλών παραγόντων, τα οποία μπορεί να εξαρτώνται από τα επίπεδα γυναικείων ορμονών στο σώμα. Το απλούστερο και πιο προβλέψιμο αποτέλεσμα της θεραπείας με οιστρογόνα είναι η ανακούφιση των αγγειοκινητικών συμπτωμάτων, όπως εξάψεις και νυχτερινές εφιδρώσεις, που προκαλούν αϋπνία, κόπωση, ανεπάρκεια και κατάθλιψη.[11] Παρόλα αυτά, τα αποτελέσματα της δράσης θεραπειών συστηματικά δρώσας ορμονικής υποκατάστασης δείχνουν να είναι αμφιλεγόμενα καθώς έχουν συσχετιστεί και με αύξηση του κινδύνου εμφάνισης κατάθλιψης, ιδιαίτερα σε γυναίκες έως 50 ετών, ενώ οι τοπικά χορηγούμενες θεραπείες έδειξαν μικρότερο κίνδυνο εμφάνισης κατάθλιψης.[12] Ωστόσο, προς το παρόν υπάρχει η τάση να χρησιμοποιούνται αντικαταθλιπτικά φάρμακα, με κυρίαρχα τα SSRIs (εκλεκτικοί αναστολείς της επαναπρόσληψης σεροτονίνης) και τα SNRIs (αναστολείς της επαναπρόσληψης σεροτονίνης και νορεπινεφρίνης), για αυτήν την ένδειξη, με την παροξετίνη και την βενλαφαξίνη να συνιστά-

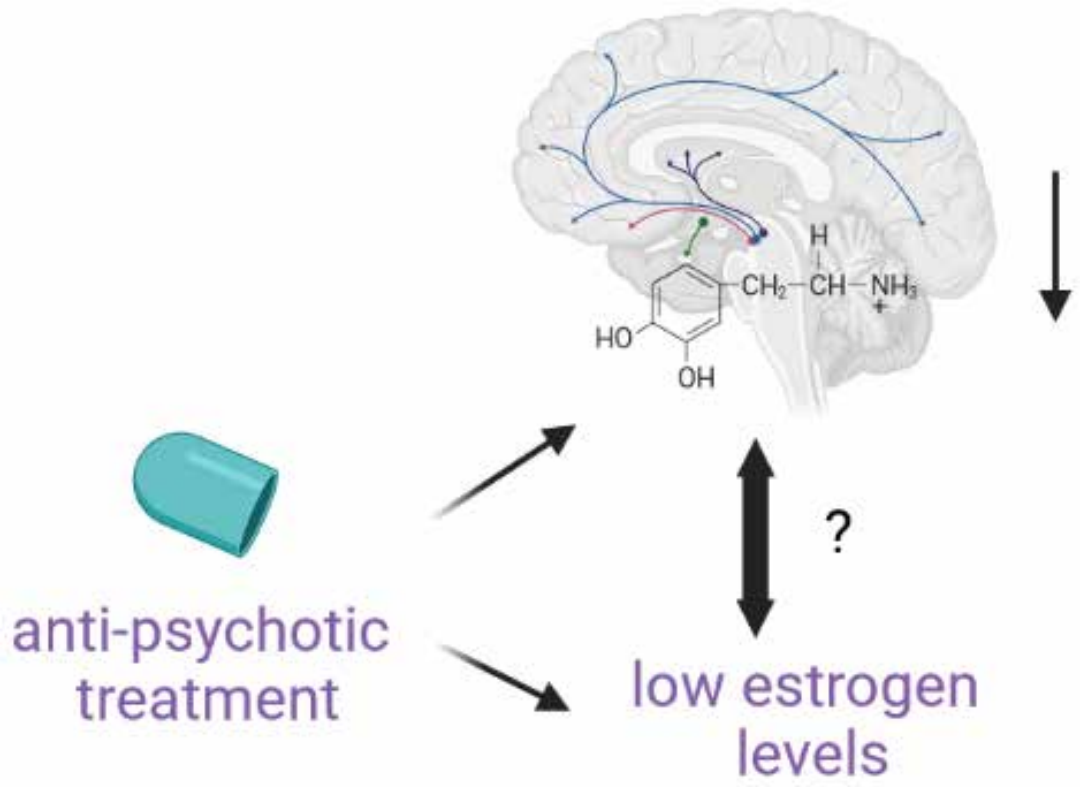
ται ως πρώτης γραμμής θεραπείες, και τα υπόλοιπα όπως σερτραλίνη, φλουοξετίνη, σιταλοπράμη και δεσβενλαφαξίνη ως δεύτερης, και τη μιρταζαπίνη, ντουλοξετίνη, εσιταλοπράμη και φλουβοξαμίνη να κατατάσσονται ως τελευταίες στη λίστα επιλογής (Εικόνα 1).[11,13]

Υπάρχει επίσης κατάθλιψη στη μεταβατική φάση, πριν από τη διακοπή της έμμηνου ρύσεως, που δεν σχετίζονται με τα αγγειοκινητικά ή ατροφικά συμπτώματα που ανταποκρίνονται σε οιστρογόνα.[14] Αυτός ο τύπος κατάθλιψης ξεκινά πολλά χρόνια πριν από τη διακοπή των περιόδων και ανταποκρίνεται στα οιστρογόνα πιο έντονα από την κατάθλιψη που εμφανίζεται στις ηλικιωμένες μετεμμηνοπαυσιακές γυναίκες,[15] ενώ έχει προταθεί ότι οι γυναίκες με κατάθλιψη που λαμβάνουν αντικαταθλιπτικά, κατά την κλιμακτήριο καλό θα ήταν επίσης να λαμβάνουν χαμηλές δόσεις οιστρογόνων για να βελτιώσουν την ανταπόκριση στη νόσο.[16]

2.2. Διπολική διαταραχή

Οι διπολικοί υποτύποι όπως η διπολική διαταραχή τύπου ΙΙ (BDII) και η ταχείας εναλλαγής είναι πιο συχνές στις γυναίκες από τους άνδρες και οι γυναίκες παρουσιάζουν συχνότερα από τους άνδρες συνδυαστικά καταθλιπτικά και μικτά επεισόδια.[17,18] Επιπλέον, αρκετές μελέτες έχουν δείξει ότι τα αναπαραγωγικά συμβάντα, ειδικά ο τοκετός, διαδραματίζουν κρίσιμο ρόλο στην πορεία της νόσου.[19] Σε μια μεγάλης έκτασης μακροχρόνια μελέτη, παρατηρήθηκε ότι οι γυναίκες με διπολική διαταραχή (BD) έχουν περισσότερες από 23 φορές περισσότερες πιθανότητες να εισαχθούν με επεισόδιο BD κατά τον πρώτο μήνα μετά τον τοκετό από ό,τι κατά τη διάρκεια της εγκυμοσύνης τους.[20] Τα οιστρογόνα φαίνεται να λειτουργούν ως αγωνιστές στο σεροτονινεργικό σύστημα, μειώνοντας τη δραστηριότητα της μονοαμινοξειδάσης και επηρεάζοντας τη μεταφορά της σεροτονίνης διανευρωνικά.[21] Και οι δύο διαδικασίες αυξάνουν τα επίπεδα σεροτονίνης στη σύναψη οδηγώντας σε πιθανή βελτίωση της διάθεσης.[22] Τα οιστρογόνα παίζουν επίσης ρόλο και σε άλλα συστήματα νευροδιαβιβαστών, καθώς αυξάνουν τη δραστηριότητα της νοραδρεναλίνης, δρουν εν μέρει ως χολινεργικοί αγωνιστές και μπορεί να μειώσουν την ευαισθησία του υποδοχέα της ντοπαμίνης D2, επιδρώντας θετικά σε σημαντικά μονοπάτια ρύθμισης των ψυχιατρικών διαταραχών.[21,23] Επίσης, έχει δειχθεί ότι σε γυναίκες με προδιάθεση για BD μετά τον τοκετό, υπήρχε αυξημένη ευαισθησία στους ντοπαμινεργικούς υποδοχείς στην ωχρινική φάση του εμμηνορροϊκού κύκλου, στις περιπτώσεις όπου οι γυναικείες ορμόνες του φύλου είναι σχετικά αυξημένες, ενώ αντίστοιχα φαίνεται τα ντοπαμινεργικά συστήματα σε γυναίκες με BD να έχουν αυξημένη ευαισθησία στις αλλαγές στις γυναικείες στεροειδείς ορμόνες.[24] Επιπλέον, ενδέχεται οι δράσεις των οιστρογόνων να εμπλέκουν και αλλαγές στο εντερικό μικροβίωμα, επηρεάζοντας με αυτό τον τρόπο και το σύστημα μικροβιώματος-εγκεφάλου επεμβαίνοντας σε διάφορες ψυχιατρικές διαταραχές.[25,26]

Η θεραπεία με οιστρογόνα αυξάνει την έκφραση της πρωτεϊνικής κινάσης C (PKC).[27] Τα βιοχημικά δεδομένα υποστηρίζουν την πιθανή εμπλοκή της PKC και των υποστρωμάτων της σε διπολικούς ασθενείς,[28] ενώ οι σταθεροποιητές της διάθεσης όπως το λίθιο και το βαλπροϊκό έχουν αποδειχθεί ότι είναι αναστολείς της PKC,[19] και η ταμοξιφαίνη [2-[4-[(Z)-1,2-διφαινυλοβουτ-1-ενυλο]φαινοξυ-]-N,N-διμεθυλοαιθαναμίνη], ένας εκλεκτικός τροποποιητής των οιστρογονικών υποδοχέων (SERM), που αλληλεπιδρά με ενδοκυτταρικούς υποδοχείς οιστρογόνων σε όργανα-στόχους ως αγωνιστής ή ανταγωνιστής οιστρογόνων, είναι και αναστολέας της PKC και θεωρήθηκε αποτελεσματικός στη θεραπεία της οξείας μανίας, καθώς η αναστολή της PKC δείχνει να είναι απαραίτητη στη θεραπεία της.[29] Μολαταύτα, δεν μπορεί να αποκλειστεί η δυνητική ανταγωνιστική επίδραση της ταμοξιφαίνης στους υποδοχείς οιστρογόνων, αν και θεραπεία με ταμοξιφαίνη δείχνει να προκαλεί σημαντικά υψηλότερα επίπεδα 17-β οιστραδιόλης [(8R,9S,13S,14S,17S)-13-μεθυλο-6,7,8,9,11,12,14,15,16,17-δεκαϋδροκυκλοπεντανο[a]φαινανθρενο-3,17-διολη] στον ορό σε προεμμηνοπαυσιακές γυναίκες με καρκίνο του μαστού σε σύγκριση με ασθενείς που δεν έλαβαν θεραπεία, αλλά επίσης αυξάνει και τη συχνότητα εμφάνισης κύστεων των ωοθηκών, ενώ οι ορμόνες διέγερσης των ωοθυλακίων (FSH) και η LH (ωχρινική ορμό-



Εικόνα 2: *Επίδραση των αντιψυχωσικών φαρμάκων στο σύστημα οιστρογόνων και ντομαπίνης και πιθανά στην αλληλεπίδραση τους.*

νη) παραμένουν αμετάβλητες, στοιχεία που μπορεί να υποδηλώνουν ότι η ταμοξιφαίνη δρα απευθείας στις ωοθήκες για να αυξήσει τη γένεση των στεροειδών.[30]

3. Σχιζοφρένεια

Τα επιδημιολογικά δεδομένα της σχιζοφρένειας αναφορικά με την ηλικία και το φύλο έδειξαν ότι υπάρχει διαφορά στην ηλικία κατά την έναρξη της σχιζοφρένειας μεταξύ των φύλων, κατά την οποία οι άνδρες φτάνουν σε μέγιστη εμφάνιση στις ηλικίες 18-24 ετών, ενώ για τις γυναίκες εμφανίζεται έως και 4 χρόνια αργότερα.[31] Επιπλέον, μόνο στις γυναίκες με σχιζοφρένεια εμφανίζεται μια δεύτερη αιχμή ηλικίας έναρξης στα 45-50 έτη.[32] Επίσης, δείχνει να υπάρχει αυξημένο ποσοστό επίπτωσης στους άνδρες (αναλογία 1,4 : 1), το οποίο έχει επαληθευτεί από 2 ανεξάρτητες μετα-αναλύσεις και παραμένει αυξημένο ακόμη και μετά τον έλεγχο για διάφορους παράγοντες διαφοροποίησης, όπως το εύρος ηλικίας, τα διαγνωστικά κριτήρια και τη μεροληψία.[33,34] Οι γυναίκες δείχνουν μια πιο ευνοϊκή αντιψυχωσική ανταπόκριση σε σχέση με τους άνδρες, έχουν λιγότερες περιπτώσεις νοσηλίας, προσαρμόζονται καλύτερα στην ασθένεια και παρουσιάζουν λιγότερη αναπηρία και μεγαλύτερη ικανότητα αυτοσυντήρησης.[35] Οι γυναίκες έχουν επίσης βελτιωμένη ποιότητα ζωής, ενώ είναι πιο πιθανό να είναι παντρεμένες, να παραμείνουν στην εργασία τους και να διατηρούν κοινωνικές επαφές με οικογένεια και φίλους.[36] Επιπλέον, οι άνδρες τείνουν να έχουν περισσότερες δομικές ανωμαλίες στον εγκέφαλο από τις γυναίκες, συμπεριλαμβανομένων των διευρυμένων κοιλιών και του μειωμένου όγκου του κροταφικού λοβού.[37,38]

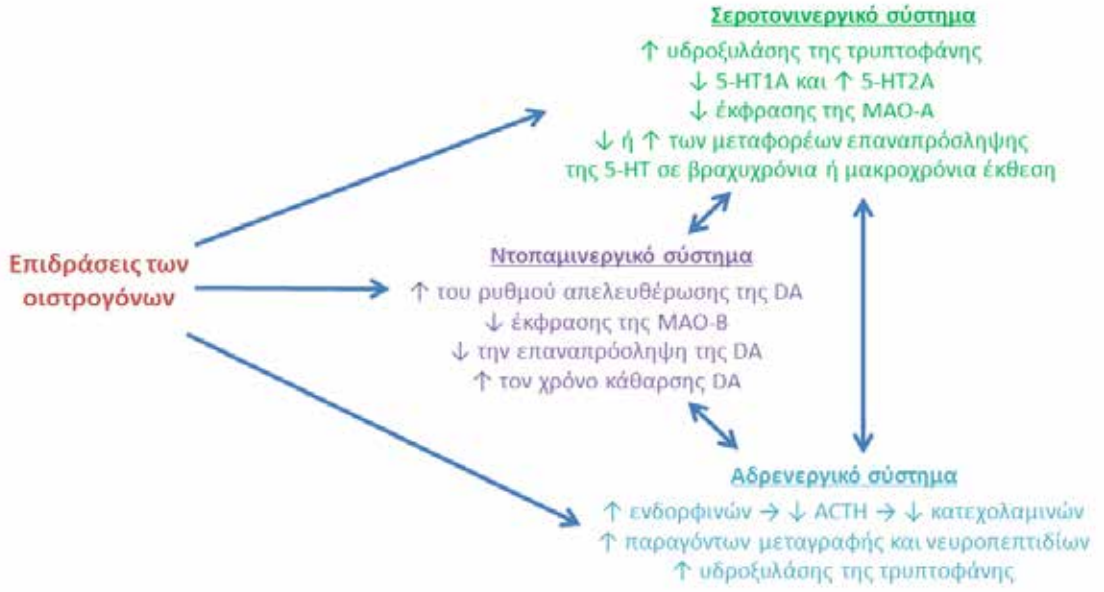
Τα ψυχωσικά συμπτώματα έχει δειχθεί να βελτιώνονται κατά τη διάρκεια της εγκυμοσύνης, και να επιδεινώνονται μετά τον τοκετό.[39] Αναφορές περιπτώσεων και κλινικές μελέτες έδειξαν ότι οι γυναίκες με σχιζοφρένεια δείχνουν αυξημένη σοβαρότητα των συμπτωμάτων, υψηλότερα ποσοστά υποτροπής και περισσότερες εισαγωγές στο νοσοκομείο σε περιόδους χαμηλών κυκλοφορούντων ορμονών φύλου, συμπεριλαμβανομένης της πρώιμης ωοθυλακικής φάσης, μετά τον τοκετό, αλλά και την εμμηνόπαυση. Αντίθετα, τα ποσοστά υποτροπής είναι λιγότερο συχνά και η σοβαρότητα των συμπτωμάτων μειώνεται σε περιόδους με υψηλή συστηματική κυκλοφορία των ορμονών του φύλου, συμπεριλαμβανομένης της εγκυμοσύνης και στα μέσα του ωχρινικού σταδίου του εμμηνορροϊκού κύκλου.[40,41] Σε μία αντίστοιχη μελέτη, η αξιολόγηση της διακύμανσης της σοβαρότητας των συμπτωμάτων σε γυναίκες, εξωτερικούς ασθενείς, με σχιζοφρένεια, κατά τη διάρκεια των δύο φάσεων του εμμηνορροϊκού κύκλου, παρατήρησε ότι η κατανομή των συμπτωμάτων ήταν σαφώς χαμηλή κατά τη διάρκεια του μέσου της ωχρινικής φάσης αλλά υψηλή κατά τη διάρκεια της έναρξης της παραγωγικής φάσης.[42]

Αντίστοιχα, σε γυναίκες με σχιζοφρένεια έχει παρατηρηθεί υποοιστρογοναιμία σε σχέση με τις φυσιολογικές, με παράλληλη τάση για εμφάνιση ανωμαλιών του κύκλου,[41] ενώ και μείωση των οιστρογόνων έχει βρεθεί μετά από εφαρμογή αντιψυχωσικής θεραπείας, ανεξαρτήτως του είδους της θεραπείας αυτής.[43] Αυτό το εύρημα δείχνει να είναι σημαντικό, καθώς ορισμένα αντιψυχωσικά μπορούν να προκαλέσουν υπερπρολακτιναιμία, η οποία οδηγεί σε μείωση των επιπέδων των οιστρογόνων.[44] Η υπερπρολακτιναιμία σχετίζεται κυρίως με αντιψυχωσικά, όπως η ρισπεριδόνη, η οποία εμποδίζει κυρίως τον υποδοχέα ντοπαμίνης D2, που ρυθμίζει την απελευθέρωση προλακτίνης από την υπόφυση. Το σημαντικό όμως να διευκρινιστεί είναι αν τα τροποποιημένα επίπεδα οιστρογόνων συμβαίνουν πριν ή μετά την έναρξη της νόσου. Προς αυτή την κατεύθυνση, η πρώιμη εφηβεία έχει συσχετιστεί με αργοπορημένη εμφάνιση της νόσου, πιθανά υποδεικνύοντας την σημασία των οιστρογόνων στην καθυστέρηση της εμφάνισης της.[41]

Σε προσπάθεια να διερευνηθεί το παραπάνω ζήτημα, σε ασθενείς με προεμμηνοπαυσιακή σχιζοφρένεια πρώτου επεισοδίου, σε σύγκριση με τους υγιείς μάρτυρες, οι ασθενείς με σχιζοφρένεια είχαν μεταγενέστερη εμμηνόρροια, ήπια αιμορραγία, τριχοφυΐα και μεγαλύτερη τάση για στειρότητα, ενώ φάνηκε και γοναδική δυσλειτουργία σε ένα δείγμα ασθενών.[45] Επίσης, ορισμένες διπλά τυφλές, ελεγχόμενες με εικονικό φάρμακο, τυχαιοποιημένες μελέτες δίνουν ενδείξεις ότι η θεραπεία με οιστρογόνα, που χορηγείται σε συνδυασμό με αντιψυχωσικά, είναι ευεργετική για τη σχιζοφρένεια, ιδιαίτερα στη μείωση των θετικών συμπτωμάτων, ενώ η per os χορήγηση 17β-οιστραδιόλης καθημερινά για οκτώ εβδομάδες, αλλά και η διαδερμική μέθοδος, βελτίωσε σημαντικά τα θετικά συμπτώματα σε προεμμηνοπαυσιακές γυναίκες με σχιζοφρένεια.[46,47] Παρόλα αυτά, υπήρξαν και αντίστοιχα παραδείγματα μελετών με καμία διαφοροποίηση στην εμφάνιση των συμπτωμάτων, κάτι το οποίο μπορεί να οφείλεται στη συνύπαρξη προγεστερόνης στο σκεύασμα, καθώς και στη φάση του ωοθηκικού κύκλου.[48]

Αντίστοιχα, σε μελέτη σε εμμηνοπαυσιακές σχιζοφρενείς γυναίκες βρέθηκε ότι απαιτούνταν χαμηλότερη δόση αντιψυχωσικών και εμφανίζονταν λιγότερο δυσμενή συμπτώματα σε περίπτωση θεραπείας ορμονικής υποκατάστασης, αποτέλεσμα που εμφανίστηκε μετά τη χρήση οιστραδιόλης, επί δύο εβδομάδες, ακόμα και σε άντρες (Lindamer 2001, Kulkarni 2011).[49,50] Επίσης, ο εκλεκτικός ρυθμιστής οιστρογονικών υποδοχέων ραλοξιφαίνη ([6-υδροξυ-2-(4-υδροξυφαινυλο)-1-βενζοθειεν-3-υλο][4-(2-πιπεριδιν-1-υλοαιθοξυ)φαινυλο]μεθανονη) έχει επίσης δοκιμαστεί σε γυναίκες με σχιζοφρένεια, με ευνοϊκά αποτελέσματα για τα θετικά, αρνητικά και γνωστικά συμπτώματα της νόσου.[42]

Ο ρόλος των οιστρογόνων στη γνωστική λειτουργία είναι ιδιαίτερης σημασίας για τη σχιζοφρένεια, καθώς τα γνωστικά ελλείμματα που σχετίζονται με την ασθένεια θεωρούνται τα πιο εξουθενωτικά συμπτώματα για τους ασθενείς και αυτά τα συμπτώματα αντιμετωπίζονται ελλιπώς χρησιμοποιώντας τα τρέχοντα αντιψυχωσικά, ενώ τα νευροπροστατευτικά αποτελέσματα είναι ένα άλλο βασικό συ-

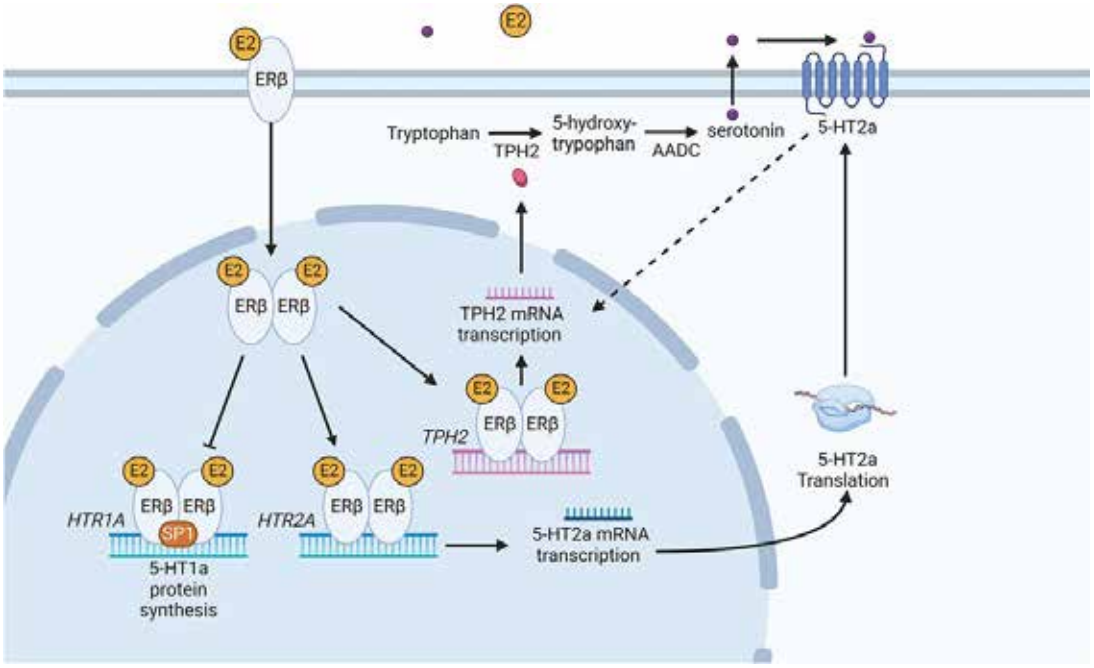


Εικόνα 3: *Επιδράσεις των οιστρογόνων στα συστήματα σεροτονίνης (5-HT), ντοπαμίνης (DA) και αδρεναλίνης-νοραδρεναλίνης (Α και ΝΑ).*

στατικό της δράσης των οιστρογόνων που σχετίζεται με τη σχιζοφρένεια.[51] Επιπλέον, έχει προταθεί ότι οι νευροπροστατευτικές δράσεις των οιστρογόνων μεσολαβούνται μέσω της διατήρησης της λειτουργίας των μιτοχονδρίων, και υπάρχουν αυξανόμενες ενδείξεις ότι η μιτοχονδριακή δυσλειτουργία παίζει εξίσου σημαντικό ρόλο στη σχιζοφρένεια.[52] Έχει διαπιστωθεί ότι υπάρχει μια θετική συσχέτιση με τα επίπεδα οιστραδιόλης στον ορό, συμπεριλαμβανομένων έξι γνωστικών τομέων, με τη λεκτική και χωρική μνήμη, να έχουν την πιο έντονη σχέση, ενώ βρέθηκε μειωμένη απόδοση στη λεκτική μνήμη και την εκτελεστική λειτουργία στην ομάδα με χαμηλή οιστραδιόλη.[53]

4. Επιδράσεις οιστρογόνων στα κύρια συστήματα νευροδιαβιβαστών

Τα ισχυρότερα στοιχεία για την επιρροή των οιστρογόνων επί των νευροδιαβιβαστών προέρχονται από μελέτες που εξετάζουν τα συστήματα ντοπαμίνης, σεροτονίνης και γλουταμινικού. Η διεγερτική επίδραση των οιστρογόνων στη δραστικότητα των ντοπαμινεργικών νευρώνων, ιδιαίτερα αυτών που βρίσκονται στο ραβδωτό σώμα και στον πυρήνα, είναι καλά διευκρινισμένη.[54] Μελέτες τρωκτικών έχουν δείξει ότι οι φάσεις της ντοπαμινεργικής μετάδοσης ποικίλλουν κατά τη διάρκεια του οιστρικού κύκλου, ενώ η απομάκρυνση της πρωταρχικής πηγής οιστραδιόλης μέσω ωοθηκεκτομής προκαλεί μόνιμη απώλεια πυκνότητας των νευρώνων της μέλανας ουσίας στα πρωτεύοντα.[55] Η θεραπεία με οιστραδιόλη μπορεί να ρυθμίσει τα επίπεδα των μεταφορέων, των υποδοχέων της ντοπαμίνης, τη σύνθεση, όπως και την απελευθέρωση και την ανανέωση της ντοπαμίνης, τόσο σε φλοιώδεις όσο και σε ραβδωτές περιοχές.[42] Η ωοθηκεκτομή σε αρουραίους έχει αποδειχθεί ότι μειώνει τα επίπεδα πρωτεΐνης του δραστικού μεταφορέα ντοπαμίνης (η οποία επανεισάγει τη ντοπαμίνη στον νευρώνα για ανακύκλωση ή αποικοδόμηση) και αυξάνει τα επίπεδα του υποδοχέα ντοπαμίνης. Η επακόλουθη θεραπεία σε επίμυες με ωοθηκεκτομή με 17β-οι-



Εικόνα 4: *Σχηματική απεικόνιση της οδού σύνθεσης σεροτονίνης μετά από αύξηση της οιστραδιόλης και της αναστολής και επαγωγής των υποδοχέων 5-HT1A και 5-HT2A αντίστοιχα. Η αύξηση της οιστροδιόλης (E2) οδηγεί στην ενεργοποίηση του υποδοχέα ERβ, προκαλώντας τον διμερισμό του. Αυτός αναστέλλει τη μεταγραφή του υποδοχέα HTR1A, ενώ προκαλεί και ενεργοποίηση της μεταγραφής του γονιδίου του υποδοχέα HTR2A, αυξάνοντας την πρωτεΐνη του υποδοχέα 5-HT2A στο κύτταρο, η οποία με τη σειρά της οδηγεί σε έμμεση αύξηση του mRNA και της πρωτεΐνης της υδροξυλάσης της τρυπτοφάνης 2 (TPH2) και στη συνέχεια τη μετατροπή της τρυπτοφάνης σε 5-υδροξυτρυπτοφάνη, και στη συνέχεια σε σεροτονίνη (5-υδροξυτρυπταμίνη) μέσω της αρωματικής αποκαρβοξυλάσης των L-αμινοξέων (AADC).*[59]

στραδιόλη ανέστρεψε την απώλεια του μεταφορέα ντοπαμίνης και επίσης μείωσε τα επίπεδα του υποδοχέα ντοπαμίνης D2 κάτω από εκείνα των ανέπαφων ομάδων ελέγχου.[56] Επίσης, η χρόνια θεραπεία με 17β-οιστραδιόλη σε πιθήκους που έχουν υποστεί ωοθηκεκτομή, αύξησε τα επίπεδα έκφρασης του μεταφορέα της ντοπαμίνης και την ενεργοποίηση της οδού σηματοδότησης Akt/GSK3, η οποία πιστεύεται ότι εξασθενεί στη σχιζοφρένεια.[57] Όσον αφορά στη θεραπεία με 17β-οιστραδιόλη, σε συνδυασμό με χρόνια χορήγηση αλοπεριδόλης, μείωσε την επαγόμενη από αμφεταμίνη κινητική υπερκινητικότητα σε θηλυκούς επίμυες, ενώ αυτή η επίδραση της οιστραδιόλης δεν παρατηρήθηκε όταν συνδυάστηκε με αλατούχο διάλυμα, υποδηλώνοντας ότι εμφανίζει αντιψυχωσικές ιδιότητες που ενισχύουν περαιτέρω τη λειτουργική αποτελεσματικότητα της αλοπεριδόλης.[58]

Έχουν αναφερθεί διαφορές μεταξύ των φύλων στη ρύθμιση της σηματοδότησης της σεροτονίνης, με τα επίπεδα mRNA του υποδοχέα σεροτονίνης 5-HT1A (Εικόνα 4)[59] να είναι χαμηλότερα στην αμυγδαλή και τον υποθάλαμο θηλυκών επιμύων σε σύγκριση με τους αρσενικούς, ενώ και η έκφραση του υποδοχέα 5-HT2A ήταν υψηλότερη στον ιππόκαμπο θηλυκών πειραματόζωων, και η θεραπεία τους με 17β-οιστραδιόλη έχει βρεθεί ότι βελτιώνει τη χωρική λειτουργική μνήμη και αυξάνει τα επίπεδα σεροτονίνης στον προμετωπιαίο φλοιό.[60] Επίσης, έχει δειχθεί ότι η θεραπεία υποκατάστασης οιστραδιό-

λης αύξησε τα επίπεδα του υποδοχέα σεροτονίνης 5-HT2A στον προμετωπιαίο φλοιό, ωστόσο, τα επίπεδα του 5-HT1A δεν μεταβλήθηκαν, υποδηλώνοντας την επιλεκτική δράση της οιστραδιόλης στους υποτύπους του υποδοχέα της σεροτονίνης.[42] Τα επίπεδα έκφρασης mRNA των 5-HT1A και των 5-HT2A υποδοχέων, καθώς επίσης και των ERa και ERβ, δεν έδειξαν σημαντικές διαφορές για τον 5-HT1A υποδοχέα. Αντιθέτως, φάνηκαν μειωμένα, επίπεδα έκφρασης mRNA του υποδοχέα 5-HT2A σε αρκετές από τις περιοχές του εγκεφάλου, σε περιπτώσεις κατάθλιψης, ενώ η οξεία χορήγηση 17β-οιστραδιόλης ομαλοποιούσε τα επίπεδα έκφρασης mRNA του 5-HT2A σε πολλές περιοχές του εγκεφάλου, εκτός από τον ιππόκαμπο.[3]

Η χρόνια θεραπεία με 17β-οιστραδιόλη έχει δειχθεί ότι ρυθμίζει την πυκνότητα των υποδοχέων γλουταμινικού, NMDA και AMPA, στον ιππόκαμπο αρουραίων, υποδηλώνοντας ποικίλες επιδράσεις των οιστρογόνων σε διαφορετικές περιοχές του εγκεφάλου στη σχιζοφρένεια.[61] Η ταμοξιφαίνη, έχει αποδειχθεί ότι αυξάνει την επαναπρόσληψη γλουταμινικού οξέος και επηρεάζει την ιονοτροπική και μεταβοτροπική δραστικότητα του υποδοχέα του γλουταμινικού στη σχιζοφρένεια, ρυθμίζοντας τη διαθεσιμότητα του στη συναπτική σχισμή.[62] Σε καλλιέργειες αστροκυττάρων, η ταμοξιφαίνη και η ραλοξιφαίνη έδειξαν να αυξάνουν τους μεταφορείς γλουταμινικού, EAAT1 και EAAT2, μέσω υποδοχέων ERa και ERβ, καθώς και μέσω του υποδοχέα του επιδερμικού αυξητικού παράγοντα και ρύθμισης της οδού NF-κB.[63] Ως εκ τούτου, μελέτες συμπεριφοράς σε ζώα υποδηλώνουν ότι οι επιδράσεις της οιστραδιόλης στη γλουταμινεργική σηματοδότηση μπορεί να εμπλέκονται σε συμπεριφορές σχετικές με τη σχιζοφρένεια, και ενώ υποδεικνύουν το ρόλο της οιστραδιόλης στη γλουταμινεργική δυσλειτουργία στη σχιζοφρένεια, παρόλα αυτά απαιτούνται περαιτέρω μελέτες για την υποστήριξη της πιθανά θεραπευτικής επίδρασης της οιστραδιόλης στη δυσλειτουργία αυτή.

Μια άλλη βιογενής αμίνη που έχει εμπλακεί ευρέως σε καταθλιπτικές, και όχι μόνο, διαταραχές είναι η νορεπινεφρίνη (NE). Οι διακριτοί υποπληθυσμοί των νευρώνων NE του εγκεφαλικού στελέχους εκφράζουν οιστρογονικούς υποδοχείς και η δραστηριότητα της NE αυξάνεται σαφώς ως απόκριση στα οιστρογόνα. Αυτό μπορεί να οφείλεται στο γεγονός ότι ορισμένα γονίδια που εκφράζονται από νευρώνες NE επηρεάζονται από οιστρογόνα, όπως παράγοντες μεταγραφής και νευροπεπτίδια.[64] Επίσης, έχει δειχθεί ότι η αυτοκτονία σχετίζεται σε μεγάλο βαθμό με συμπτώματα κατάθλιψης, ενώ διαπιστώθηκε σημαντική μείωση της έκφρασης mRNA του ERβ στα θύματα αυτοκτονίας σε σύγκριση με τα άτομα ελέγχου, ενώ αντίθετα, δεν υπήρχαν σημαντικές διαφορές για τα επίπεδα ERα.[65]

Όσον αφορά στη δράση των οιστρογονικών και προγεστογόνων στεροειδών επί του συστήματος GABA, η αλλοπρεγνανολόνη έχει βρεθεί ότι είναι η πιο ισχυρή από τους μεταβολίτες της προγεστερόνης, ακολουθούμενη από την πρεγνενολόνη.[66] Σε γυναίκες σε παραγωγική ηλικία, τα κυκλοφορούντα επίπεδα της αλλοπρεγνανολόνης και της πρεγνενολόνης, ακολουθούν εκείνα της προγεστερόνης, με υψηλότερες συγκεντρώσεις κατά τη διάρκεια της ωχρινικής φάσης από ό, τι στην ωοθυλακική φάση.[67] Ένα κρίσιμο σημείο στη συζήτηση σχετικά με τη σχέση μεταξύ της διάθεσης και των νευρωνικά δρώντων στεροειδών είναι το κατά πόσον τα αρνητικά συμπτώματα της διάθεσης αντικατοπτρίζουν πραγματικά μια επίδραση που προκαλείται από νευροδραστικά στεροειδή, όπως η αλλοπρεγνανολόνη, στο σύμπλεγμα υποδοχέων GABA-A. Οι βενζοδιαζεπίνες που χορηγούνται σε αγχολυτική δόση παρήγαγαν αρνητικά αποτελέσματα όπως και τα υψηλότερα επίπεδα προγεστερόνης/αλλοπρεγνανολόνης. Αυτά τα αποτελέσματα δείχνουν μια νευρική απόκριση που μπορεί να εμπλέκεται σε επαγόμενες από την προγεστερόνη ανεπιθύμητες ενέργειες στη διάθεση μέσω του υποδοχέα GABA-A, καθώς οι οξείες επιδράσεις της προγεστερόνης πιθανώς να μεσολαβούνται μέσω της κατασταλτικής δράσης της αλλοπρεγνανολόνης.[68] Ωστόσο, άλλη μελέτη δείχνει ότι ο κλασικός ενδοκρινικός πυρηνικός υποδοχέας προγεστερόνης δεν εμπλέκεται στην παθοφυσιολογία του προεμμηνορροϊκού συνδρόμου, όπως αποδεικνύεται από την αποτυχία του ανταγωνιστή του

υποδοχέα της προγεστερόνης (μιφεπριστόνη) να μειώσει τις φυσικές ή συμπεριφορικές εκδηλώσεις της προεμμηνορροϊκής δυσφορικής διαταραχής (PMDD).[69] Ωστόσο, έχει δειχθεί ότι η σοβαρότητα των προεμμηνορροϊκών συμπτωμάτων σε γυναίκες με PMDD σχετίζεται με την ευαισθησία τους στα στεροειδή και ότι αυτή η ευαισθησία ομαλοποιείται κατά τη διάρκεια της θεραπείας με εκλεκτικούς αναστολείς επαναπρόσληψης σεροτονίνης.[70] Αρνητικά συμπτώματα διάθεσης συναντώνται επίσης κατά τη διαδοχική προσθήκη προγεστογόνων ή προγεστερόνης στη θεραπεία με οιστρογόνα σε μετεμμηνοπαυσιακές γυναίκες. Σε ορισμένες γυναίκες, τα προγεσταγόνα και η προγεστερόνη φαίνεται να προκαλούν κυκλικά αρνητικά συμπτώματα διάθεσης, όπως κατάθλιψη, άγχος και ευερεθιστότητα. Επιπλέον, υψηλότερη δόση οιστρογόνων δείχνει να αυξάνει τα αρνητικά συμπτώματα της διάθεσης όταν χορηγείται με προγεσταγόνα, ενώ η δόση οιστρογόνου δεν δείχνει επηρεάζει την επιδείνωση της διάθεσης σε περίπτωση που χρησιμοποιείται μόνο του.[71]

5. Διατροφή και βουλιμία

Μελέτες δείχνουν να συσχετίζουν τις ορμόνες των ωοθηκών με κίνδυνο για διαταραγμένες διατροφικές συνήθειες.[72] Αυτές οι έρευνες επικεντρώθηκαν κυρίως στην υπερβολική κατανάλωση τροφής, μέσω μελετών σε πειραματόζωα, που περιγράφουν ότι τροποποιήσεις των ορμονών των ωοθηκών προκαλούν αλλαγές στην πρόσληψη τροφής.[73] Διαφαίνονται σημαντικές συσχετίσεις μεταξύ οιστρογόνων και πρόσληψης τροφής, έτσι ώστε η ωοθηκεκτομή θηλυκών ζώων να προκαλεί άμεσες και παρατεταμένες αυξήσεις στην πρόσληψη τροφής, ενώ η θεραπεία με οιστραδιόλη φαίνεται να αντιστρέφει αυτά τα αποτελέσματα. Η προγεστερόνη από την άλλη φαίνεται να ανταγωνίζεται τις επιδράσεις των οιστρογόνων, καθώς έχει λίγες άμεσες επιπτώσεις στην πρόσληψη τροφής σε ζώα με φυσιολογική ωοθηκική λειτουργία όταν χορηγείται μόνη της, αλλά ελαττώνει τις αλλαγές στην πρόσληψη που προκαλούνται από εξωγενή οιστραδιόλη σε ζώα με ωοθηκεκτομή[72] και προκαλεί ελαφρώς αυξημένη πρόσληψη τροφής σε άθικτους αρουραίους με φυσιολογική κυκλοφορία οιστραδιόλης. Αν και απαιτείται περισσότερη έρευνα για τον προσδιορισμό των ακριβών μηχανισμών των επιδράσεων της προγεστερόνης, τα ευρήματα μέχρι στιγμής υποδηλώνουν ότι οι δράσεις της προγεστερόνης εξαρτώνται από την παρουσία οιστρογόνων και ότι η πρωταρχική επιρροή της προγεστερόνης είναι να ανταγωνίζεται τις επιδράσεις είτε του ενδογενούς είτε του εξωγενούς οιστρογόνου στην πρόσληψη τροφής.

Μελέτες σε επίμυες διαπίστωσαν ότι η ωοθηκεκτομή αυξάνει την πρόσληψη τροφής και η χορήγηση θεραπείας με υψηλή δόση οιστραδιόλης και χαμηλής δόσης προγεστερόνης μειώνει την πρόσληψη τροφής με υψηλή περιεκτικότητα σε λιπαρά.[74] Στους ανθρώπους, έχουν εξεταστεί διαφορετικά ποσοστά πρόσληψης τροφής, υπερτροφίας και συναισθηματικών φάσεων κατά τη διάρκεια του έμμηνου κύκλου, που χαρακτηρίζονται από υψηλά επίπεδα οιστρογόνων, έναντι υψηλής προγεστερόνης. Τα αποτελέσματα υποστήριξαν την αυξημένη πρόσληψη τροφής, ακόμα και λόγω ψυχολογικών δράσεων, κατά τη διάρκεια της μέσης ωχρινικής φάσης σε σύγκριση με τη φάση της ωορρηξίας.[75] Τα δεδομένα υποδηλώνουν επίσης σημαντικές αλλαγές στον εγκέφαλο (π.χ. αυξημένος όγκος φαιάς ουσίας) στη διάρκεια του κύκλου της εμμήνου ρύσεως που σχετίζονται με αλλαγές στη συμπεριφορά (όπως πρόσληψη τροφής και αίσθημα ανταμοιβής) και πιστεύεται ότι οφείλονται εν μέρει και σε επιδράσεις των ορμονών επί του γονιδιώματος.[76] Ίσως οι συσχετίσεις μεταξύ των αλλαγών των ορμονών στον εμμηνορροϊκό κύκλο και των συναισθηματικών διαταραχών επί της διατροφής να αντανακλούν τις επαγόμενες από ορμόνες αλλαγές στη μεταγραφή γονιδίων σε γυναίκες που είναι γενετικά ευάλωτες σε βουλιμικό φαινότυπο. Αυτές οι γενετικές αλλαγές γίνονται εμφανείς μετά την ορμονική ενεργοποίηση, κατά την εφηβεία και την έναρξη των εμμηνορροϊκών κύκλων (περίοδος ανάπτυξης).

Επίσης, η σημαντική θετική σχέση μεταξύ της αρνητικής διάθεσης και της υπερβολικής συχνότη-

τας κατανάλωσης φαγητού, απεικονίζει τη σχέση μεταξύ των ορμονικών επιδράσεων με τους ψυχοκοινωνικούς παράγοντες και τη διατροφική συμπεριφορά. Οι ορμόνες των ωοθηκών μπορούν να επηρεάσουν τη βουλιμία μέσω διαφόρων μηχανισμών, συμπεριλαμβανομένων των συστημάτων χολεκυστοκίνης (CCK) και σεροτονίνης (5-HT).[77] Η μειωμένη λειτουργία των CCK και 5-HT έχει μελετηθεί ευρέως ως παράγοντας κινδύνου που διέπει τα βουλιμικά συμπτώματα.[77] Η επίδραση επί του κορεσμού της CCK ενισχύεται από αυξήσεις στην οιστραδιόλη, στη διάρκεια του κύκλου, και μειώνεται όταν τα επίπεδα της οιστραδιόλης είναι χαμηλά.[78]

6. Συμπερασματα

Συνοπτικά, οι νευροψυχιατρικές διαταραχές έχουν δείξει έντονες διαφορές μεταξύ των φύλων σε πολλές πτυχές των διαφόρων εμπλεκόμενων ασθενειών, συμπεριλαμβανομένης της πρώιμης ηλικίας έναρξης της νόσου, της πιο σοβαρής πορείας της ασθένειας, της κακής ανταπόκρισης στη θεραπεία και της δυσκολίας προσαρμοστικότητας σε ορισμένες ασθένειες στους άνδρες. Αυτή η ανασκόπηση υπογραμμίζει τη διαχρονική έρευνα που έχει περατωθεί για την κατανόηση των πιθανών προστατευτικών ή μη επιδράσεων της οιστραδιόλης και των προγεστογόνων σε σχέση με αυτές τις διαφορές στην εκδήλωση των κύριων ψυχιατρικών νόσων μεταξύ των δύο φύλων. Η έκταση αυτής της έρευνας κυμαίνεται από μελέτες που έχουν δείξει σαφώς τις περίπλοκες αλληλεπιδράσεις της οιστραδιόλης με τα κύρια συστήματα νευροδιαβιβαστών στον εγκέφαλο, και ειδικά εκείνα που εμπλέκονται στη σχιζοφρένεια και την κατάθλιψη, έως τα προκλινικά μοντέλα της νόσου που έχουν δείξει τη δυνατότητα της οιστραδιόλης είτε στην ενίσχυση της γνώσης και της μνήμης ή στα αναστρέψιμα ελλείμματα που αντανακλούν τα θετικά, αρνητικά και γνωστικά συμπτώματα. Κλινικές δοκιμές έδωσαν μια πολλά υποσχόμενη προοπτική για τη χρήση της οιστραδιόλης και την πιο πρόσφατη χρήση εκλεκτικών τροποποιητών των υποδοχέων των οιστρογόνων, ως συμπληρωματική θεραπεία για ασθενείς και των δύο φύλων, ενώ η χρήση προγεστενοειδών, σε ορισμένες περιπτώσεις, δείχνει να περιορίζει ή και να αναστρέφει πλήρως τις θετικές αυτές επιδράσεις. Βέβαια, παρόλα αυτά απαιτούνται επιπλέον μελέτες που να διερευνούν σε μεγαλύτερη έκταση και βάθος το μηχανισμό που διέπει την προστατευτική δράση της οιστραδιόλης στις ψυχιατρικές παθήσεις. Τέτοιες έρευνες είναι επίσης απαραίτητες σε όλες τις ψυχιατρικές διαταραχές όπου παρατηρούνται διαφορές μεταξύ των φύλων. □

The Effect of Estrogens and Progestogens on the Occurrence of Major Mental Disorders

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ABSTRACT

Estrogens seem to participate in various functions of the body, including the brain function, through estrogen receptors, affecting among others, the differentiation or the occurrence and promotion of serious or less serious mental disorders. Estrogens seem to be involved in depression, bipolar disorder and schizophrenia, with the fluctuation of their levels being aggravating or beneficial. In these mental disorders, progestogens appear to limit the beneficial effects of estrogens, which are nonetheless necessary in a variety of situations, including the reduction of the side effects of estrogens. These actions of hormones and their supplements seem to be exerted at the cellular level, through transcription and gene expression adjustment, but also regulation of mitochondrial function, while they seem to interfere with all the main signaling pathways, influencing the action of key excitatory and suppressive neurotransmitters. Furthermore, sex hormones seem to affect the regulation of eating habits, a factor that appears to change especially during many major mental disorders, but also due to the drug treatment of these diseases. It is therefore considered important the further study of these mechanisms, linking female hormones with mental disorders, as well as their possible treatment through hormonal therapy, possibly as adjunctive to the already prescribed for these diseases.

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, IV, 2023 | 34-46 ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

PHARMAKEFTIKI, 35, IV, 2023 | 34-46 REVIEW ARTICLE

Benefits and Complications of Using New Oral Anticoagulants (NOACS) in Elderly and Very Elderly Patients with Non-Valvular Atrial Fibrillation. A Comparative Study.

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ABSTRACT

Atrial fibrillation (AF) is the most common arrhythmia, with an increased frequency of occurrence in older age groups. AF can cause thromboembolic events with ischemic strokes being the most common. Therefore, the role of anticoagulant therapy is central. Until a few years ago, vitamin K antagonists (VKAs) represented the most used anticoagulant drugs for the prevention of thromboembolic complications of AF. However, VKAs had many problems in use, mainly due to their narrow therapeutic range, as well as multiple drug and food interactions. The increased requirements for frequent monitoring and dose adjustments led to decreased compliance and underuse. In recent years, new oral anticoagulant drugs (NOACs) have been developed, which have radically changed the management of patients with AF. NOACs include dabigatran, which is a direct thrombin inhibitor, and rivaroxaban, apixaban and edoxaban, which are direct inhibitors of factor Xa. Phase 3 studies, as well as subsequent analyses and real-world evaluations, have shown that NOACs are more effective in preventing stroke and systemic thrombosis in olders and are considered as the best choice for elderly (>65) and very elderly (>80) patients with AF, with clear clinical benefit against warfarin, while demonstrating at least an equivalent safety profile. There are of course several differences between them, with apixaban appearing as the drug with the best efficacy and safety profile in the elderly over 75 years of age. However, the choice of the most appropriate anticoagulant treatment should take into account the individual clinical profile of each patient, evaluating each time the benefits and risks of its use.

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice, affecting up to 33.5 million people worldwide. AF is not commonly found in people under the age of 65 years (<2%), but its frequency increases with age. The demographic aging of the population and the increase in the prevalence of other conditions that predispose to AF (obesity, hypertension, diabetes) have increased its impact, with about 9% of people aged 80 years and older developing the disease. AF can cause the formation of blood clots in the left atrium, which can then embolize into the systemic circulation and most commonly the cerebral arteries (ischemic stroke). These serious complications increase the rates of morbidity and mortality in elderly patients with AF[1-7].

The administration of anticoagulant therapy is a cornerstone and urgent need for the prevention and treatment of thromboembolic events in AF. However, it carries the risk of complications, with the most significant being bleeding, sometimes life-threatening, with the elderly and very elderly being more vulnerable[8-10]. For decades, the anticoagulant therapy of choice in AF for the prevention of thromboembolic events has been the vitamin K antagonists (VKAs), as well as suboptimal treatment with aspirin. However, the disadvantages they present have led to the development of new oral anticoagulants (NOACs). These drugs have been shown to be at least as effective as VKAs in preventing thromboembolism in non-valvular AF and maintain a favorable safety profile. NOACs include dabigatran, which is a direct thrombin inhibitor, and rivaroxaban, apixaban, edoxaban, which are direct factor Xa inhibitors. NOACs were designed to achieve anticoagulation therapy in a broad range of AF patients [1,2,11]. Despite the availability of these safer alternative drugs, the use of anticoagulant therapy remains suboptimal in elderly patients with AF, at risk for bleeding events[12].

The purpose of this review is to highlight the effectiveness and safety of NOACs compared to VKAs, as well as to present comparative studies aimed at selecting the most appropriate anticoagulant in the vulnerable group of elderly patients with non-valvular AF, taking into account the individual needs of each patient.

2. Method of Literature Search

The following keywords were used to search the MEDLINE database via the PubMed search engine for the writing of this review:

- NOACS AND atrial fibrillation
- NOACS AND olders
- Atrial fibrillation AND olders
- Risks of Noacs AND elderly
- Noacs AND (complicationsORbleeding)

Articles that concerned clinical trials, meta-analyses, reviews, and systematic reviews published in the last 5 years in the English language and referring to AF and the newer anticoagulant treatment, mainly in elderly patients, were selected.

3. Results

3.1 Stratification of Thromboembolic Risk in Patients with AF (Table 1)

European guidelines recommend the use of the CHA2DS2-VASc score for categorizing thromboembolic risk when making treatment decisions for patients with AF. Using this score helps identify low-risk patients who can be excluded from anticoagulant therapy, as well as those who have an indication for anticoagulant therapy. With a CHA2DS2-VASc score of 0 in men or 1 in women, the initiation of anticoagulant therapy is not recommended due to the absence of risk factors. With a score of 1 in men or 2 in women, the use of anticoagulant therapy should be considered, weighing individual bleeding risk against the risk of stroke. In men with a score of 2 or women with a score of 3, strong recommendation is given for anticoagulant therapy, as the benefits of its use significantly outweigh the risk [4,13,14].

3.2 Antiplatelet Therapy with Aspirin

In the past, prescribing aspirin to low-risk patients for stroke prevention was common practice, as it

Table 1: CHA2DS2-VASc Scale for Estimating Thromboembolic Risk
Scoring: Low risk (0), Moderate risk (1), High risk (2-9) [4,13,14]

Risk factor	CHA2DS2 -VASc
Syncope	1 point
Hypertension	1 point
Age	1 point (65–74 years old) 2 points (≥75 years old)
Diabetes	1 point
Previous stroke or systemic embolism	2 points
Vascular disease	1 point
Female gender	1 point (only as an additional risk)

reduced the risk of stroke to some extent and was considered safer for hemorrhagic events. However, in current European guidelines, it is clearly stated that "antiplatelet monotherapy is not recommended for stroke prevention in patients with AF, regardless of the risk of stroke." It has been proven that VKAs are superior to aspirin in stroke prevention and the rates of major bleeding are similar to aspirin, both in elderly and very elderly vulnerable patients [11,15].

3.3 Vitamin K Antagonists (VKAs) (Table 2)

Vitamin K antagonists (VKAs) (warfarin, acenocoumarol) have been the recommended oral anticoagulant protection against thromboembolic complications of AF for over 50 years. However, they have several disadvantages and limitations. They have a narrow therapeutic window, resulting in even minor deviations from therapeutic levels putting patients at

Table 2: Pharmacological characteristics of warfarin[6].

PROPERTIES	VITAMIN K ANTAGONISTS
TYPE	**Warfarin**
MECHANISM OF ACTION	It inhibits the synthesis of vitamin K-dependent clotting factors
TMAX (H)	90 minutes
HALF LIFE TIME	36-42 hours
MAXIMUM ANTICOAGULANT EFFECTIVENESS	5-7 days
EXCRETION	Liver
REDUCED DOSE	It should be avoided in liver failure
LABORATORY MONITORING	INR
ANTIDOTE	Vitamin K

Table 3: Pharmacological characteristics of NOACs [16,19,20].

PROPERTIES	DABIGATRAN	RIVAROXABAN	APIXABAN	EDOXABAN
MECHANISM OF ACTION	Direct inhibitor of thrombin	Factor Xa inhibitor	Factor Xa inhibitor	Factor Xa inhibitor
PRODRUG	Yes	No	No	No
HALF LIFE TIME (H)	12 - 14	6-13	12	12
TMAX (H)	1.5	2.5	3	1-5
BIOAVAILABILITY	7%	60%-80%	66%	60%
KIDNEY'S EXCRETION	80%	1/3 of the dose	Kidney clearance only 25%	Medium
LIVER METABOLISM	Low Does not get metabolized in CYP450	Medium (2/3 of the dose in the liver CYP (CYP3A4)	Medium CYP (CYP3A4)	Medium
INTERACTIONS WITH OTHER MEDICINES	P-gp inhibitors (ketoconazole, verapamil) P-gp inducers (rifampicin, carbamazepine)	Strong inducers and inhibitors of CYP3A4 Strong inhibitors of P-gp	Strong inducers and inhibitors of CYP3A4 and P-gp	Inducers and inhibitors of P-gp. Erythromycin, azithromycin, clarithromycin, ketoconazole, itraconazole
DOSE	150mg x 2 or 110mg x 2 and 75mg x 2 in case of kidney failure	20 mg daily 15 mg daily CrCl < 50 mL/min	5mg x 2 and 2,5mg x 2 if 2 of the following apply: age >80 years, weight< 60 kg, serum creatinine > 1,5 mg/dL	60 mg daily or 30 mg daily if CrCl< 50 mL/min or body weight <60kg
SPECIFIC REVERSAL AGENTS	Idarucizumab Aripazine	Andexanet alfa Aripazine	Andexanet alfa Aripazine	Andexanet alfa Aripazine

risk for serious complications (bleeding). Especially the elderly have an increased risk of bleeding (intracranial, gastrointestinal, etc.) due to age, comorbidities and polypharmacy. Therefore, regular monitoring of clotting with measurement of international normalized ratio (INR) and frequent dose adjustments are required to maintain a balance between effectiveness and safety in clinical practice. VKAs have slow onset of action and long half-life (dangerous when urgent surgery is needed). Genetic polymorphisms exhibited by each patient cause variable response. In addition, multiple interactions with other drugs and foods affect their activity and increase the demand for dose adjustment. All these limitations result in variable patient compliance and overall underutilization for the prevention of stroke [1,6,16].

3.4 Novel Oral Anticoagulants (NOACs) (Table 3)

NOACs (Novel Oral Anticoagulants) were designed to address the challenges of maintaining therapeutic anticoagulation in a broad range of patients with atrial fibrillation. NOACs are not antagonists of Vitamin K and include the drugs dabigatran, rivaroxaban, apixaban and edoxaban.

Most of clinical practice guidelines worldwide recommend NOACs over warfarin for the majority of non-valvular atrial fibrillation patients, as they have shown a favorable balance between efficacy and safety compared to VKAs, with significant reductions in stroke, intracranial hemorrhage and mortality. [1,2,15,17].

Advantages of NOACS in clinical practice are: [1,2,3,18]

- They are effective and safe for preventing thromboembolic events.
- Easy to use, with oral administration in a fixed dosing regimen once or twice a day.
- Have wide therapeutic windows.
- No need for laboratory monitoring of anticoagulant activity.
- Improved patient compliance, especially in the elderly.
- Rapid onset of action and short half-life after discontinuation.
- Half-life is well-defined (considering age-related increase due to renal function decrease).
- Predictable pharmacokinetics and pharmacodynamics with low inter-individual variability, even at an individual level.
- Fewer drug and food interactions.
- Cost-effectiveness for stroke prevention in non-valvular atrial fibrillation has been demonstrated in some studies.

Four large Phase III trials evaluated the non-inferiority of NOACs compared to VKAs in terms of further reducing the combined risk of stroke and systemic embolism by 19% and all-cause mortality[3].

DABIGATRAN

In the RE-LY trial, dabigatran was compared to warfarin. In the overall trial, dabigatran administered at a dose of 150mg twice daily, reduced the rate of stroke or systemic embolism by 34% with a similar rate of major bleeding. Dabigatran administered at a dose of 110mg twice daily, reduced the rate of major bleeding by 20% compared to warfarin and showed a similar rate of stroke or systemic embolism[11]. In the RE-LY trial, 7.258 (40%) of the participants were aged ≥75 years and the representation of this age group was well balanced in the three treatment categories (adjusted dose warfarin, dabigatran 110mg twice daily and dabigatran 150mg twice daily). Additionally, a subgroup analysis compared the safety and efficacy of dabigatran versus warfarin in four age groups (<75, 75 to <80, 80 to <85 and ≥85 years)[5]. The administration of dabigatran 110mg twice daily, reduced thromboembolic events by 12%, and the administration of dabigatran 150mg twice daily reduced the incidence of stroke or systemic embolism by 33%[11]. Age did not significantly affect these outcomes. However, this study showed a clear interaction between age and the risk of major bleeding in the three therapeutic categories[5]. Dabigatran 110mg twice daily was associated with major bleeding rates similar to warfarin (4.43% vs. 4.37%; p=0.89), while the dose of 150mg twice daily showed a higher risk of major bleeding (5.10% vs. 4.37% in the warfarin arm; p=0.07). This risk was related to extracranial major bleeding and was age dependent. Thus, the use of dabigatran 110mg twice daily was supported in patients aged ≥80 years, rather than dabigatran 150mg. The risk of intracranial bleeding was lower with both doses of dabigatran, regardless of age[12]. Both doses of dabigatran reduced mortality in patients aged <75 years, but were similar to warfarin in patients >85 years old[5].

RIVAROXABAN

The ROCKET-AF trial compared once-daily rivaroxaban 20mg with warfarin. In the overall trial, rivaroxaban was found to be equally effective as warfarin in preventing thromboembolic events, with a similar rate of major bleeding. In this study, 44% of patients were aged ≥75 years old. In this age group,

Table 4: Assessment of hemorrhagic risk according to the HAS-BLED scale: low risk (0), moderate risk (1-2), high risk (≥3). [13,22]

HAS-BLED	Points
Hypertension (syst>160 mmHg)	1
Kidney/liver failure (1 point each)	1 or 2
History of cerebrovascular accident (CVA) or stroke	1
Bleeding history	1
INR out of normal range	1
Age >65	1
Medicines (NSAIDs, antiplatelets) / alcohol (1 point each)	1 or 2

rivaroxaban was found to be non-inferior to warfarin in preventing thromboembolism, with a thromboembolic event rate of 2.3% (rivaroxaban) versus 2.9% (warfarin). As for bleeding events, the rates were similar, with a major bleeding rate of 4.9% in the rivaroxaban group versus 4.4% in the warfarin group. The difference in the above rate did not concern intracranial hemorrhage, which was lower in the rivaroxaban group, but rather extracranial hemorrhage, especially gastrointestinal bleeding, which was more frequent in elderly patients receiving rivaroxaban [5,11,12].

APIXABAN

The ARISTOTLE trial compared apixaban 5mg twice daily (or 2.5mg twice daily in patients with two or more of the following factors: age ≥ 80 years, body weight ≤ 60 kg, serum creatinine ≥ 1.5 mg/dL) with warfarin. In the overall trial, apixaban reduced the rate of thromboembolic events by 21% and the rate of major bleeding by 31%. In this study, 31% of patients were aged ≥ 75 years old. In this age group, apixaban reduced the incidence of stroke or systemic embolism by 29% and major bleeding by 36%. Both in the overall trial and in the subgroup of patients aged ≥ 75 years, the net clinical benefit favored apixaban, which reduced both thrombotic and major bleeding events [5,11,12].

EDOXABAN

The ENGAGE-AF trial compared edoxaban (at a full dose of 60mg or a reduced dose of 30mg once daily based on clinical criteria) with warfarin. In the overall trial, edoxaban reduced thromboembolic events by 13% and major bleeding by 20% compared to warfarin. In the study, 40% of patients were aged ≥ 75 years old. In this age group, the percentage of thromboembolic events was 1.9% in the edoxaban arm compared to 2.3% in the warfarin arm, and the percentage of major bleeding was 4% in the edoxaban arm compared to 4.8% in the warfarin arm. Therefore, edoxaban was at least as effective as warfarin in preventing stroke or systemic embolism regardless of age, while it had an additional benefit for patients aged ≥ 75 years due to the reduction in major bleeding [5,11].

3.5 Complications of NOACs in Elderly Patients

A bleeding event related to anticoagulant therapy is classified as major or non-major or minor, both of which are attributed to bleeding without a history of

prior injury or intervention[7]. A major bleeding event is characterized by extensive and/or symptomatic clinically significant bleeding, located in a critical organ/region that is potentially life-threatening, such as intracranial, intraspinal, pericardial, intraperitoneal/peritoneal, gastrointestinal, traumatic splenic rupture, and/or a decrease of at least 2 g/dl in hemoglobin or requires blood transfusion of at least 2 units[9], whereas, a minor (non-major) bleeding refers to a smaller extent of bleeding that is clinically apparent and requires management, such as hematuria, hemoptysis, epistaxis, intramuscular hematoma of the rectus abdominis muscle, etc.[7].

Specific factors have been implicated in the increased risk of bleeding in elderly patients with non-valvular atrial fibrillation, either related to the administered medication (type, dose) or non-pharmacological causes. Specifically, pre-existing comorbidities of the elderly (renal and hepatic dysfunction, diabetes, hypertension, COPD), geriatric syndromes (frailty, falls, cognitive impairment), hematologic disorders, systemic collagen diseases, thrombocytopenia, use of anti-inflammatory drugs (steroids or non-steroidal), antiplatelet agents (clopidogrel), history of bleeding (intracranial, gastrointestinal), smoking and alcohol use have been positively correlated[7].

The dual role of chronic kidney disease in the mechanism of hemorrhagic events in elderly patients with atrial fibrillation is noteworthy. On the one hand, the progressive reduction in glomerular filtration rate can affect the pharmacokinetics of NOACs due to their renal excretion, necessitating dose adjustments. On the other hand, renal dysfunction negatively affects platelet function (adhesion, aggregation), leading to disturbances in hemostasis that result in increased rates of intracranial and gastrointestinal bleeding[5,9,21].

3.6 Stratification for Hemorrhagic Risk (Table 4)

The assessment of hemorrhagic risk is performed using stratification models that include either clinical variables, such as the widely used HAS-BLED scale or biomarkers such as the ABC scale [age, biomarkers (GDF-15, cTn-hs, hemoglobin), history of bleeding]. Recently, the incorporation of new biomarkers related to cardiovascular function and the clotting process (fibrinogen plasma thrombus) is being investigated in order to improve the prognostic value of assessment models [7,13,22].

3.7 Management of Complications of NOACs in Elderly Patients (Figure 1)

The management of NOAC-associated bleeding poses a particular challenge for clinicians, as it is mainly characterized by internal bleeding with subtle clinical symptoms that can become life-threatening and the levels of anticoagulant therapy are not determined by routine coagulation laboratory tests. Specifically, the management of bleeding consists of measures for hemodynamic stabilization, blood product transfusion, and the use of specific or non-specific reversal agents. The approved specific reversal agents are idarucizumab, a monoclonal antibody that reverses dabigatran and Andexanet alfa, a recombinant coagulation factor Xa, with official approval for the reversal of rivaroxaban and apixaban. In addition, the effectiveness of new reversal agents, such as FXa(116L) and PER977 (arapazine/chiraparantag), as factor Xa reversals, is being investigated. Non-specific reversal agents that are administered adjunctively or in the absence of specific agents, include activated or non-activated prothrombin complex concentrate (4F-PCC or aPCC), antifibrinolytics such as tranexamic acid and desmopressin. Finally, it should be noted that reversal agents can sometimes have prothrombotic effects and should be strictly administered in life-threatening bleeding or pre-/perioperative in an urgent interventional procedure. It is important to closely follow the recommendations and guidelines of the treating physicians for the management of bleeding associated with NOACs [7,23,24,25].

3.8 Effectiveness of NOACS in Elderly Patients

The best available data support that NOACs have

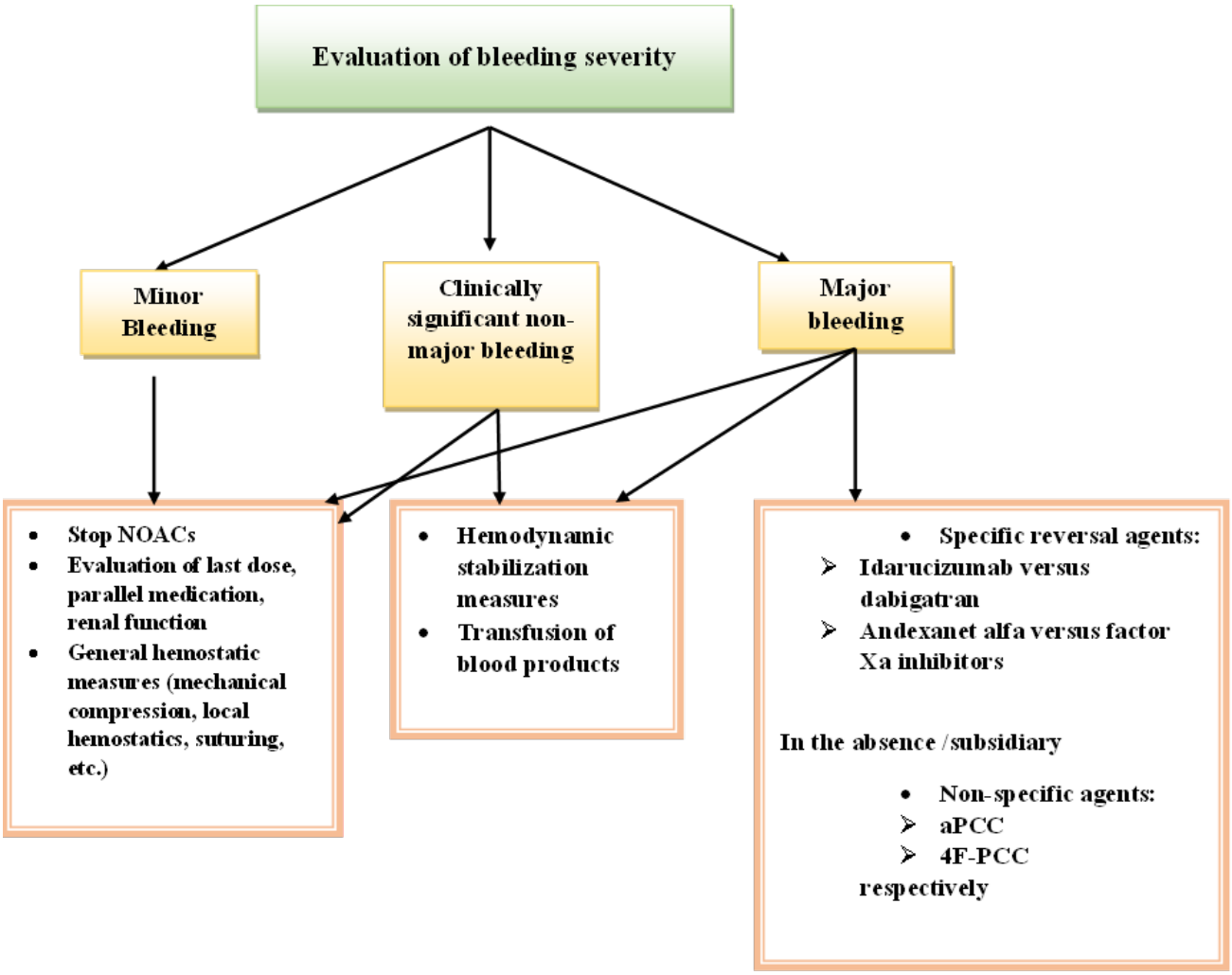

Figure 1: *Management of bleeding according to severity* [23,24,25.]

better effectiveness in the prevention of stroke and systemic thromboembolic events in elderly patients and are considered the preferred choice for elderly (over 65 years old) and very elderly (over 80 years old) patients with non-valvular atrial fibrillation, with a clear clinical benefit over warfarin [17,18].

The systematic review by Hulle et al. examined the impact of bleeding in 24,455 patients treated with NOACs compared to vitamin K antagonist and concluded that newer anticoagulants have a superior safety profile regarding all categories of bleeding events. A statistically significant difference was found in the rates of intracranial hemorrhage [7,26].

These results are also confirmed by the systematic review by Chai-Adisaksopha et al., which included randomized trials in a sample of 102,607 individuals with a median age of 70-73 years for individuals with atrial fibrillation. Specifically, a comparable safety profile was observed between NOACs and warfarin, with a significant 50% reduction in the frequency of intracranial hemorrhage in patients receiving NOACs compared to warfarin. However, the frequency of major gastrointestinal bleeding was increased with NOACs at a rate of 2.09% compared to 1.70%

with warfarin [7,9,27].

Regarding very elderly patients (over 80 years old), the meta-analysis by Bonanad et al., which included 16 studies and a sample of over 100,000 patients, revealed that newer anticoagulants were more effective and safer compared to warfarin for thromboembolic risk prevention. A statistically significant reduction of 43% in intracranial hemorrhage was observed, while the risk of major and gastrointestinal bleeding was at least equivalent, indicating that advanced age should not be a primary reason to withhold prescription of NOACs [28].

The latest review by Franco et al., examined 205 patients with an average age of 78 years old, who were under anticoagulant treatment, with respect to the occurrence of non-major bleeding. It was found that patients receiving NOACs had higher rates of gastrointestinal and genitourinary bleeding compared to those on warfarin[29].

A retrospective study by Khan et al., focused on the hemorrhagic complications of elderly patients undergoing treatment with NOACs. In total, it included 142 patients over the age of 75, of whom 36% had moderate/severe renal dysfunction. The results revealed that newer anticoagulants were a safe option for elderly individuals over the age of 75, with a hemorrhagic event rate of 1.37 per 100 person-years, while apixaban was found to be the safest among all [7,30].

The meta-analysis by Wolfe et al., examined over 100,000 patients in terms of the risk of intracranial hemorrhage comparing the four NOACs among themselves and with warfarin. Treatment with NOACs was found to be the safer option, while among the NOACs, dabigatran at a reduced dose (110 mg) was identified as superior in safety in the head-to-head comparative study, with a 56% reduction in risk compared to rivaroxaban, for this specific hemorrhagic complication[31].

In the retrospective study by Hou et al., the effect of rivaroxaban was evaluated in 299 patients over the age of 60, where adequate effectiveness against thromboembolic events was observed, while a moderate non-major bleeding occurred in 8.4% of patients, with a significant proportional increase in bleeding risk with age and dose[32].

In the NAXOS observational study by Ganse et al., involving a sample of 321,501 patients, it was observed that apixaban was superior in terms of safety, effectiveness, and mortality compared to vitamin K antagonists. Additionally, in the comparative study of NOACs, similar levels of effectiveness were attributed to all NOACs, with apixaban showing superior safety compared to rivaroxaban, while exhibiting at least a similar level of safety compared to dabigatran[33].

Lastly, according to a recent published literature review by Pazal et al., in which a comprehensive comparative study of NOACs was conducted in elderly patients with AF, apixaban was classified as the most suitable (effective/safe) for long-term treatment, ranking it in the highest category A of the FORTA scale, while other NOACs and warfarin were classified as beneficial alternatives in category FORTA B[34].

In conclusion, compared to older oral anticoagulants, NOACs are superior in preventing thromboembolic complications, while demonstrating at least an equivalent safety profile in elderly patients, even in those with moderate renal impairment[18].

Specifically:

- All NOACs are associated with similar or reduced risks of major bleeding compared to warfarin [2,35].
- Significant reduction in intracranial hemorrhage associated with the use of NOACs compared to warfarin[2,15,35].
- NOACs are associated with a higher rate of gastrointestinal bleeding compared to warfarin (the percentage varies depending on the selected NOAC and its dosage)[19,35].
- Especially for individuals aged 75 and older, the use of apixaban and edoxaban shown to be safer against gastrointestinal bleeding risk[7,9].
- There is no significant difference in the rates of fatal bleeding for any NOAC compared to warfarin[35].

Furthermore, the use of NOACs does not increase cardiovascular risk[35]. Finally, discontinuation of NOACs is easy and safe in case procedures such as sur-

gery, cardioversion, or catheter ablation are required in patients with AF[1].

4. Conclusion

The administration of anticoagulant therapy to elderly patients with non-valvular AF presents a challenge, due to the increased risk of both ischemic and hemorrhagic events. According to the recent scientific data, NOACs are an appropriate treatment option in this specific patient population, as they are superior in preventing thromboembolic complications compared to VKAs, while demonstrating at least an equivalent safety profile. However, there are several differences between them, with apixaban appearing as the drug with the best efficacy and safety profile in the elderly over 75 years of age.

Nevertheless, the clinical benefit that elderly patients with AF have from using NOACs is often undermined by clinicians due to the risk of hemorrhagic complications, mainly because of the absence of a laboratory method to determine anticoagulant activity, resulting in under-prescription. Additionally, despite the fact that the elderly are the majority users of anticoagulant therapy, they are underrepresented in clinical trials, particularly those over 80 years old, making it difficult to draw safe conclusions. Finally, the optimal recommended anticoagulation treatment in elderly patients is a combination of benefit/risk assessment through approved scoring scales (CHA2DS2 -VASc, HAS-BLED) as well as the patient's individual clinical profile (comorbidities, preferences). □

Οφέλη και Επιπλοκές από τη Χρήση Νεότερων από του Στόματος Αντιπηκτικών Φαρμάκων (NOACS) σε Ηλικιωμένους και πολύ Ηλικιωμένους Ασθενείς με Μη Βαλβιδική Κολπική Μαρμαρυγή. Συγκριτική Μελέτη.

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ΛΕΞΕΙΣ-ΚΛΕΙΔΙΑ: Κολπική μαρμαρυγή, NOACS, βαρφαρίνη, θρομβοεμβολή, ηλικιωμένοι

ΠΕΡΙΛΗΨΗ

Η κολπική μαρμαρυγή (ΚΜ) είναι η πιο συχνή αρρυθμία, με αυξημένη συχνότητα εμφάνισης σε μεγάλες ηλικιακές ομάδες. Η ΚΜ δύναται να προκαλέσει θρομβοεμβολικά συμβάντα με συνηθέστερα τα ισχαιμικά εγκεφαλικά επεισόδια. Ως εκ τούτου, ο ρόλος της αντιπηκτικής αγωγής είναι κεντρικός. Μέχρι πριν από λίγα χρόνια, οι ανταγωνιστές της βιταμίνης Κ (VKAs) αντιπροσώπευαν τα πιο συχνά χρησιμοποιούμενα αντιπηκτικά φάρμακα για την πρόληψη των θρομβοεμβολικών επιπλοκών της ΚΜ. Όμως, οι ανταγωνιστές της βιταμίνης Κ (VKAs) εμφάνιζαν πολλά προβλήματα στη χρήση, εξαιτίας κυρίως του στενού θεραπευτικού τους εύρους, αλλά και των πολλαπλών αλληλεπιδράσεων με

φάρμακα και τροφές. Οι αυξημένες απαιτήσεις για συχνή παρακολούθηση και προσαρμογές της δόσης οδηγούσε σε μειωμένη συμμόρφωση και υποχρησιμοποίηση. Τα τελευταία χρόνια αναπτύχθηκαν τα νέα από του στόματος αντιπηκτικά φάρμακα (NOACs), τα οποία άλλαξαν ριζικά τη διαχείριση των ασθενών με ΚΜ. Στα NOACs συμπεριλαμβάνονται η νταμπιγκατράνη που είναι άμεσος αναστολέας θρομβίνης και η ριβαροξαμπάνη, η απιξαμπάνη και η εντοξαμπάνη που είναι άμεσοι αναστολείς του παράγοντα Xa. Οι μελέτες φάσης 3, καθώς και μεταγενέστερες αναλύσεις και πραγματικές αξιολογήσεις έχουν αποδείξει ότι τα NOACs έχουν καλύτερη αποτελεσματικότητα στην πρόληψη του εγκεφαλικού και των συστηματικών θρομβοεμβολικών επεισοδίων σε ηλικιωμένους και θεωρούνται ως η καλύτερη επιλογή για ηλικιωμένους (άνω των 65) και πολύ ηλικιωμένους (άνω των 80) ασθενείς με ΚΜ, με καθαρό κλινικό όφελος έναντι της βαρφαρίνης, ενώ καταδεικνύουν ένα τουλάχιστον ισοδύναμο προφίλ ασφάλειας. Υπάρχουν βέβαια αρκετές διαφοροποιήσεις μεταξύ τους, με την απιξαμπάνη να εμφανίζεται ως το φάρμακο με το καλύτερο προφίλ αποτελεσματικότητας και ασφάλειας στους ηλικιωμένους άνω των 75 ετών. Ωστόσο, η επιλογή της καταλληλότερης αντιπηκτικής αγωγής θα πρέπει να λαμβάνει υπόψιν το ατομικό κλινικό προφίλ του εκάστοτε ασθενούς, αξιολογώντας κάθε φορά τα οφέλη και τους κινδύνους από τη χρήση της.

***ΣΥΓΓΡΑΦΕΑΣ ΓΙΑ ΕΠΙΚΟΙΝΩΝΙΑ:**
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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, IV, 2023 | 47-56 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 35, IV, 2023 | 47-56 RESEARCH ARTICLE

Chemometric Assisted UV-Vis Spectroscopic Study of Photostability of Some Beta-Blockers With Multivariate Curve Resolution-alternating Least Square (MCR-ALS) Method Using Soft and Hard Modelling Approach.

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ABSTRACT

The present work focuses on the use of simple UV-Vis spectrophotometric methods along with chemometric tools to understand the photodegradation and degradation kinetics of five Beta blockers namely atenolol, bisoprolol, metoprolol, propranolol, and sotalol. All the molecules were exposed to sunlight for 24 hours and UV spectra were recorded between 200 -400 nm for a specific interval of time. Amber colour and transparent glass material were used to study the penetration effect of sunlight. The recorded UV absorbance spectra were subjected to Multivariate curve resolution alternating least square method using soft and hard modelling constraints to get an understanding of the number of pure components and their concentration in the reaction mixture. Atenolol, Bisoprolol, Metoprolol, and Sotalol were found to be stable even after exposure to direct sunlight, but Propranolol shows photodegradation through a two-step mechanism Photodegradation kinetics was studied for Propranolol using kinetic constrain under study conditions. ALS optimization shows percentage lack of fit value was found to be 0.0073 with 99.98% variance explained. The standard deviation of residual against experimental data was found to be 0.0129 which suggests the statistical significance of the developed model. The rate constants of the first (k1) and second (k2) steps were calculated as 0.00315 h^{-1} and 0.0149 h−1 respectively.

1. Introduction

Photostability of drug molecules is a very important aspect when it comes to the formulation of the drug molecule. Photostability of the drug molecule is related mainly to its chemical structure and the presence of some characteristic functional groups. Photochemical reactions can lead to the decomposition of the drug molecule which may result in the formation of some degradation products that may be harmful to the human being. The study of photodegradation reaction is very essential at the time of formulation development and selection of the packaging material for the formulation. These photochemical reactions may change the physical or chemical nature of the molecule and the formulation. Drug molecules can undergo decomposition by absorbing photons that are emitted by sunlight may be in the form of UV -VIS light or Infrared radiations.

Most of the time chromatographic methods like high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), and high performance thin

layer chromatography (HPTLC) is used for identification of the drug degradation. Multivariate curve resolution alternating least square MCR-ALS is a chemometric approach that can be used to demonstrate the stability of the drug molecule using spectrophotometric data. It helps to understand multiple sources of variability in spectroscopic measurements and can be used to investigate complex chemical reactions by assessing the number of constituents involved in the reaction process, along with their concentration profile. Kinetic constrain when added as a hard modelling tool predict the kinetics of the photodegradation reaction with determination of rate constants (k).[1-4]

The beta blockers are primarily used in the therapy of cardiovascular diseases. Beta blockers are the primary choice of drugs for cardiovascular diseases.[5] The first beta-blocking drug used in cardiovascular therapy was Propranolol,[9]. It functions as a nonselective beta blocker with membrane stabilizing properties. Bisoprolol, Atenolol, Metoprolol and Sotalol is [6-9] are the four members of that fall under the category of beta1-adrenergic receptor blockers. They have a similar chemical structure and are capable of selectively blocking beta2-adrenergic receptors found in the lungs and vascular smooth muscle. All currently available beta blockers share an amino-alkanol side chain and an aromatic group, which is phenyl in the case of all drugs except Propranolol which possess naphthyl ring as a structural feature.[10]

Vamsi et al. in their review on photocatalysis of beta blockers enlisted various studies carried out on different beta blockers alone or in comparison with use of different catalytic reagents[10]. The current study is mainly focused on the use of simple UV Spectroscopy along with MCR-ALS as a chemometric approach to study the photostability of five beta blockers. To the best of our knowledge chemometrics assisted photo stability study of different beta blockers was not reported in the literature.

Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) is a chemometric method which aims towards resolution of number of components in an unknown sample. The MCR algorithm performs an approximation of variation in the X axis related data with bilinear two-factor matrices having reduced size along with pure response profile. ALS performs optimization of spectral and concentration profiles of species present in reaction mixture simultaneously with a iterative fashion. This method can be applied to environmental samples, chemical mixtures, chromatographic and spectroscopic data.[11].

According to published research, combining soft and Hard modelling MCR-ALS approaches can reveal information about the reaction kinetics and also reduces the rotational ambiguity effect of soft modelling.

The first step of MCR-ALS process is to build up the data matrix, D. In the rows of this data matrix represents different samples or sampling points whereas columns represents absorbance spectra , bilinear relation between the experimental data, the concentrations and the pure spectra of the components is assumed, In matrix form, this bilinear model is expressed by equation (1).

$$D = CST + E \quad (1)$$

where D(I,J) is the matrix of experimental data, of

dimensions I samples (spectra) by J wavelengths; C(I,K) is the matrix of concentration profiles of the different K analytes presents in the samples; ST(K,J) is the spectra matrix, whose K rows contain the pure spectra associate with the K species present in the samples; E(I,J) is the matrix associated to the experimental error This method decomposes the data matrix to product of two matrices: C and ST where C and ST and are concentration and spectral profile matrices, respectively having actual chemical significance.[12-18]

2. Material and Method

Material

Atenolol, Bisoprolol, Metoprolol, Sotalol and Propranolol were procured as gift samples from reputed pharmaceutical manufacturing companies from Maharashtra state. Glass distilled water was used for preparation of all solutions.

A double beam UV-Vis Spectrophotometer (Jasco; model V-650) having a 10-millimeter quartz cell was used to record the UV-Vis spectra. Data was recorded for an interval of 1 nm wavelength. MCR-ALS was performed by utilizing the MATLAB environment (MathWorks, Natick, MA; version 10 a). using the MCR-ALS function of MATLAB which was developed and designed by Tauler along with de Juan. The toolbox can be found by using the link http://www.ub.es/gesq/mcr/mcr.htm on the MCR website.

Method

Standard stock solutions of all drug molecules were divided into four different volumetric flasks with two transparent and two amber colour volumetric flask. One pair of flasks (one amber and one transparent were placed in direct sunlight and one pair inside the room at 25^0C to study effect of sunlight on UV spectra of each drug molecule namely propranolol, atenolol, metoprolol, bisoprolol and sotalol. Samples were pipetted out from each flask after an interval of 30 mins and the solution was subjected to recording of UV spectra between 200-400nm range as all the drug molecules show good absorption between this ranges which makes it easy to record the spectra of all the drug molecules. Readings were recorded for 24 hours over a period of 4 days. For development of model data in the range of 220 nm to 300 nm was selected.[19]

The data obtained from the UV spectra was used to study the susceptibility of an analyte towards photodegradation. MCR-ALS toolbox was used which employs an algorithm to solve the basic bilinear MCR model. Initially rough estimate of number of components can be obtained with different methods like principal component analysis (PCA)[20-23]. Assessment of singular data can provide a preliminary information about the number of chemical components present in the sample. First, singular value decomposition (SVD) was applied to the data to estimate the number of components. SVD is based on a algorithm where in matrix D is broken down in to the product of three; an orthogonal matrix U, a diagonal matrix S, and the transpose of an orthogonal matrix V[24] as following:

A = USVT

This step is followed by Evolving factor analysis (EFA) which was performed in forward and backward direction. Final EFA results gave the confirmation about the number of components contributing to the sample.

EFA is a local rank analysis method that detects the emergence and decay of the components in the data set and provides concentration profiles assuming a sequential order of emergence-decay for all components in the system. EFA provides a set of initial estimates and relevant additional information, such as the windows of existence of the components in the system. Forward EFA starts with the calculation of the eigenvalues (EVs) of the first row and is continued far all the rows in the data matrix along with generation of loading and score matrix. Thus PCA is performed on complete data set. The point where EV raise above the noise level indicate appearance of new substance which can increase rank by one. Similarly the number of EVs which are above the noise level shows the number of compounds evolved.

In analogy to forward EFA, backward EFA starts with measurements from the last row and EVs for

whole data matrix is calculated. In this case An increase of an EV above the noise level indicates disappearance of the compound from the system. Thus, an EFA graph provides information on both the appearance and the disappearance of the analytes.[25]

Soft modelling gives the values of concentration profile of all species present in the sample and it was used to calculate the rate of reaction based on initial and final concentration of each species. Hard modelling constrain of kinetics was applied to study the exact value of rate constant. The combined soft and Hard modelling gives the estimates of number of species along with their spectral profile and rate constants for each reaction step.

Hard-modelling approaches of fitting multivariate absorption data are based on mathematical relationships, which describe the measurements quantitatively. In chemical kinetics, the analysis is based on the kinetic model or reaction mechanism, which quantitatively describes the reactions and all concentrations in the solution under investigation. In combined soft and hard modelling approach kinetic profiles in concentration submatrix is used as input for a non-linear multivariate kinetic fit. Fitting is directly done on the concentration profiles and there are no spectral contributions to be considered. The resulting fitted kinetic profiles update the soft-modelled ones, and the rate constants values can be obtained for each step involved in the reaction.[16]

3. Results and Discussion

Figures 1-5 show changes in the spectra of the beta blockers studied according to the different exposure conditions figure at left represent sample treated inside room (room temperature) whereas figure to the right represents sample exposed to direct sunlight outside the room. If we look at the absorbance spectra, most of the beta blocker molecules studied, did not show any significant change in the shape of spectra, (Fig 1 to 4). Only a small decrease in absorbance was observed suggesting slight degradation of the drug molecule without formation of any new chemical species or degradation product except in the case of propranolol.

However, UV absorbance spectra of propranolol solution which was exposed to direct sunlight in the transparent flask, showed clear changes in the shape of the spectra absorbance of solution. At 248 nm absorbance goes on decreasing, while at the same time absorbance increases at 280 nm. An isosbestic point was observed at 276 nm (Figure 5,6). This observation indicates that there is formation of new product or degradation product. In order to understand the number of species formed during degradation of Propranolol solution, a chemometric approach through multivariate curve resolution- Alternating Least square was used.

First step of SVD shows presence of three chemical entities in the reaction mixture which can be confirmed based on eigenvalues. This result was confirmed by evolving factor analysis (EFA)s with three principal components to be considered for the pure variable detection. Initial estimates step also shows three separate spectra's with different concentration profiles. This data was further pushed for the forward and backward evolving factor analysis (EFA) (Figure 7)

The graph in the forward and backward EFA demonstrated that three lines intersect with each other. The result was confirmed by the EFA plot, as a large gap is shown in the forward and backward EFA plot between the first three eigenvalues and the remaining, (Figure 7). This is one more proof that three chemical species may be present during the photodegradation reaction of the drug under investigation. This experimental study suggests that the photodegradation of propranolol may be preceded in two steps. Figure 8 shows EFA based concentration profiles of chemical species present in the sample. The plot of concentration profile suggests two step mechanism of photocatalysis of propranolol like A→B→C in transparent flask up on exposure to direct sunlight, where A is pure propranolol and B and C are the intermediate and final products, respectively.

In the final step of optimization spectral and concentration profiles are optimized simultaneously in iterative way. Figure 9 represent the concentration profile of the species present in the sample during course of reaction against time in minutes. The re-

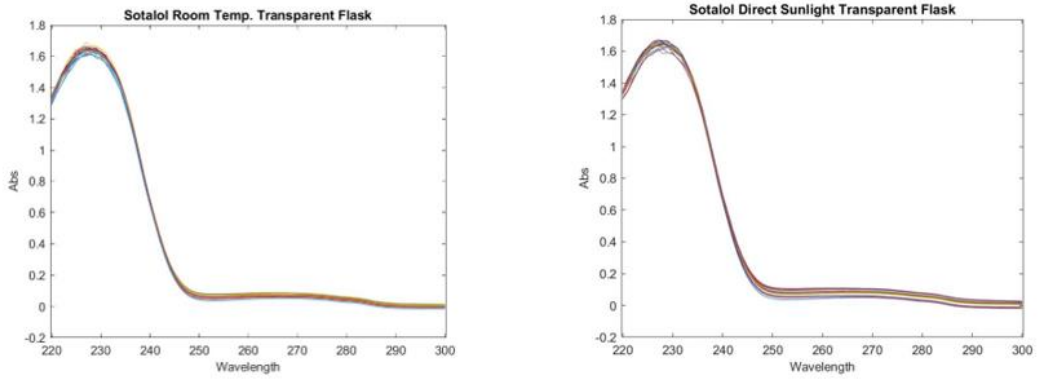

Figure 1: *Absorbance Spectras of Sotalol within room and direct exposure to sunlight outside*

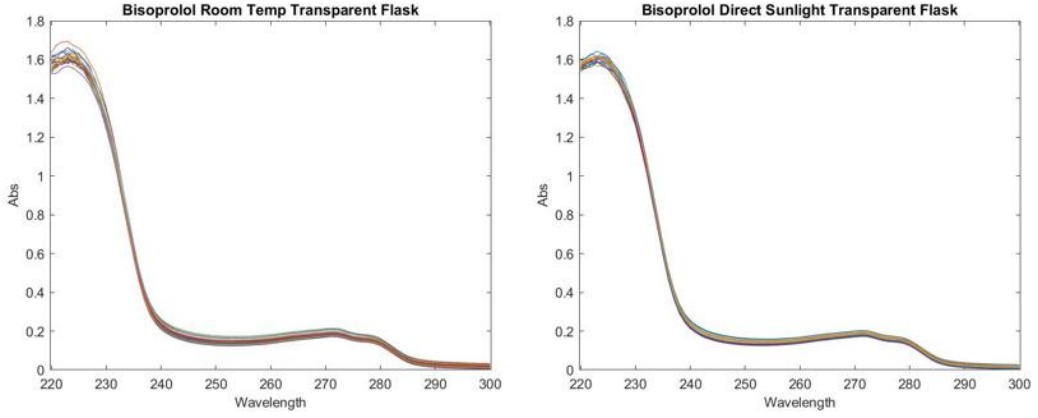

Figure 2: *Absorbance Spectra of Bisoprolol within room and direct exposure to sunlight outside*

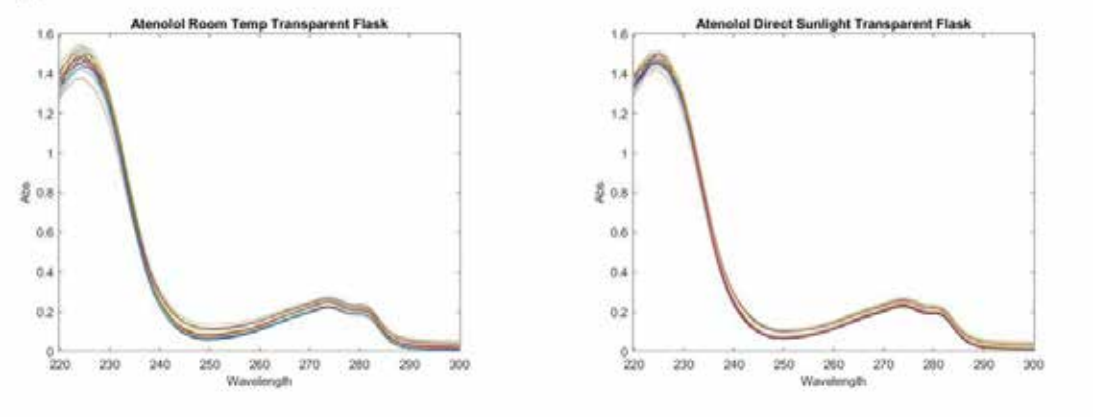

Figure 3: *Absorbance Spectra of Atenolol within room and direct exposure to sunlight outside*

sults of optimization are statistically very significant and are shown in Table 1.

Results of the MCR-ALS shows that the photodegradation of propranolol follows an consecutive two step reaction with the value of (k1) 0.00315 h^{-1} and (k2) 0.0149 h−1 as the rate constants obtained after use of combined soft and hard modelling approach for first and second step of reaction respectively.

It was observed that only propranolol shows major photodegradation along with formation of new species or degradation product in the reaction. In view of this we have studied the chemical structures of all five beta blockers (Figure 10)

As observed in figure 10, the chemical structures of atenolol, bisoprolol, metoprolol, and sotalol contain benzene as their backbone structure and only propranolol consists of naphthalene as an aromatic ring in its structural formula. It can be said that propranolol reacts more rapidly than beta blockers like atenolol, bisoprolol, metoprolol and sotalol. Salvatore et al.[26] have proposed that naphthalene skeleton in propranolol structure is responsible for initiation of photolysis reaction. Uwai et al [27] showed that when the propranolol was used in a solid state, the hydrolysis of the acetal did not appear to proceed. Thus, the C –O bond of the naphthyl ether was cleaved by

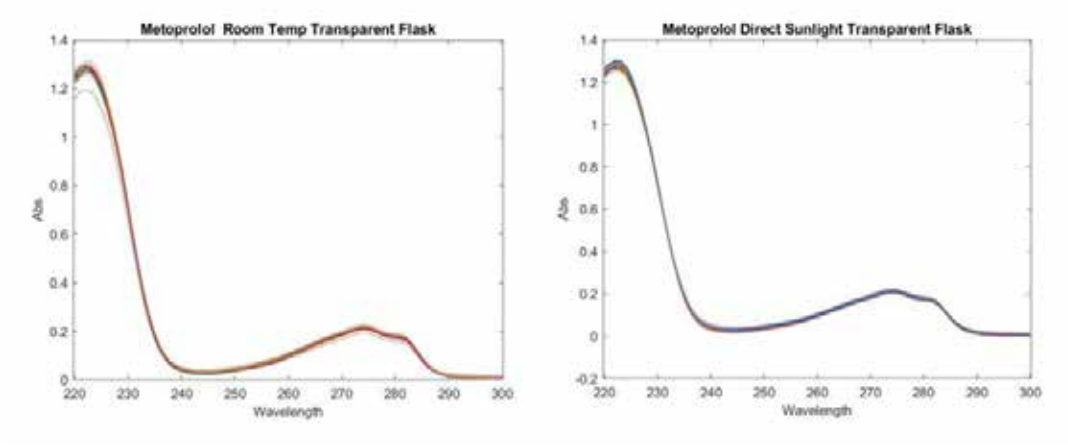

Figure 4: *Absorbance Spectra of within room and direct exposure to sunlight outside*

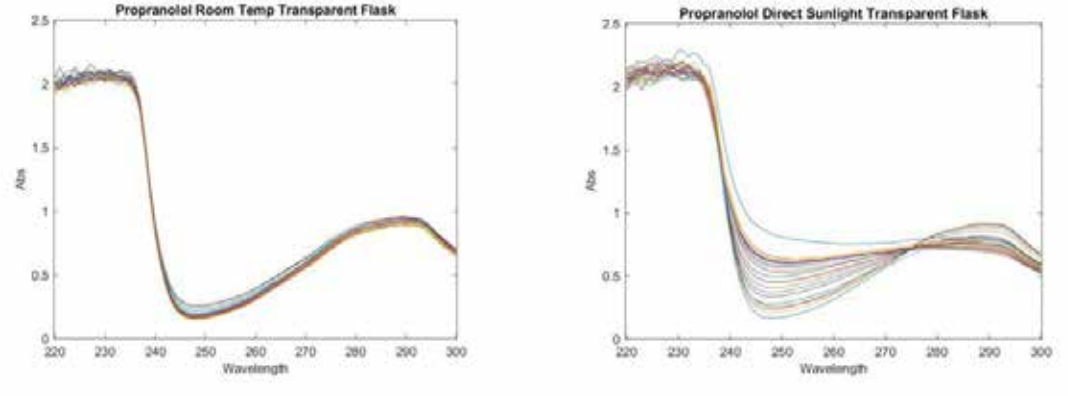

Figure 5: *UV -Absorbance Spectra of Propranolol within room and direct exposure to sunlight outside*

Sr. No.	Factor	Results
1	Standard Deviation of Residuals Vs Expt. Data	0.0129
2	Lack of Fit (LOF in %)	0.0073
3	Percent of Variance Explained	99.98

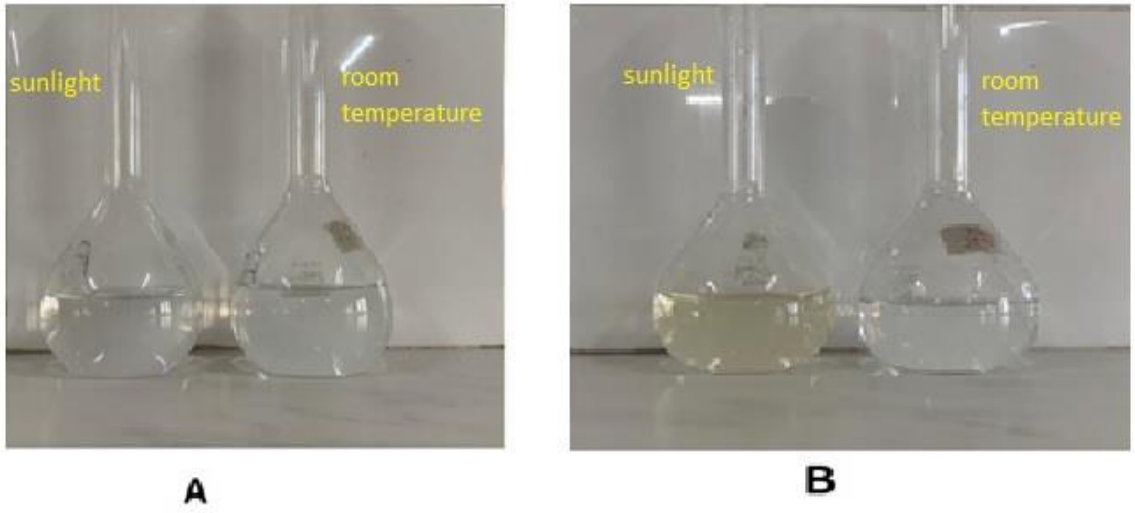

Figure 6: *Physical changes in the Propranolol solution A. Initial Sample solutions B. Sample solution on exposure to direct sunlight.*

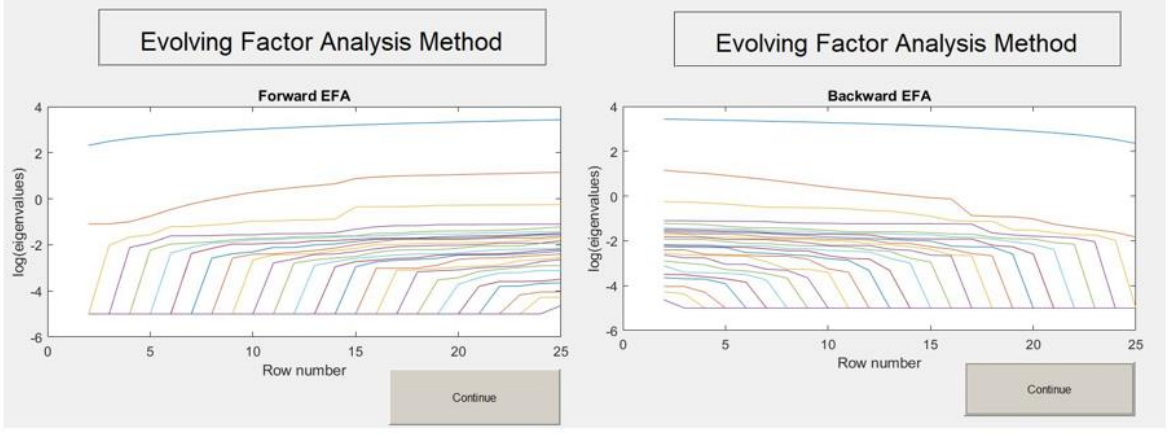

Figure 7: *Forward Evolving Factor Analysis and Backward Evolving Factor Analysis*

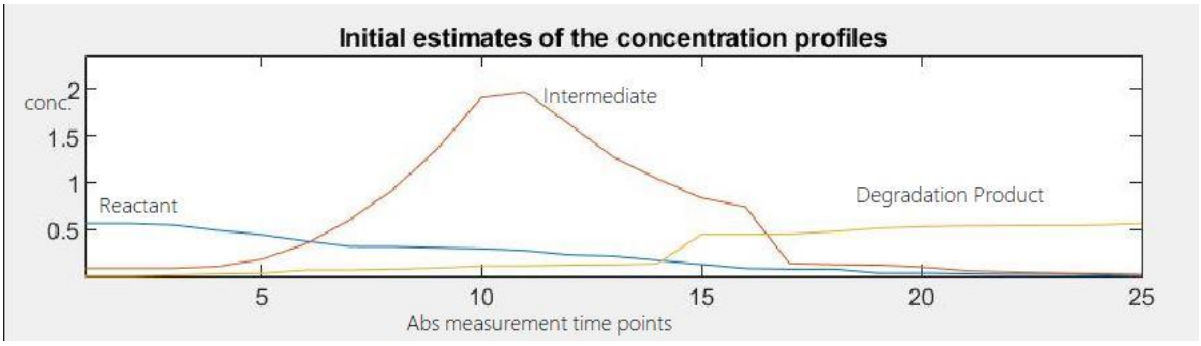

Figure 8: *Concentration profile obtained by EFA*

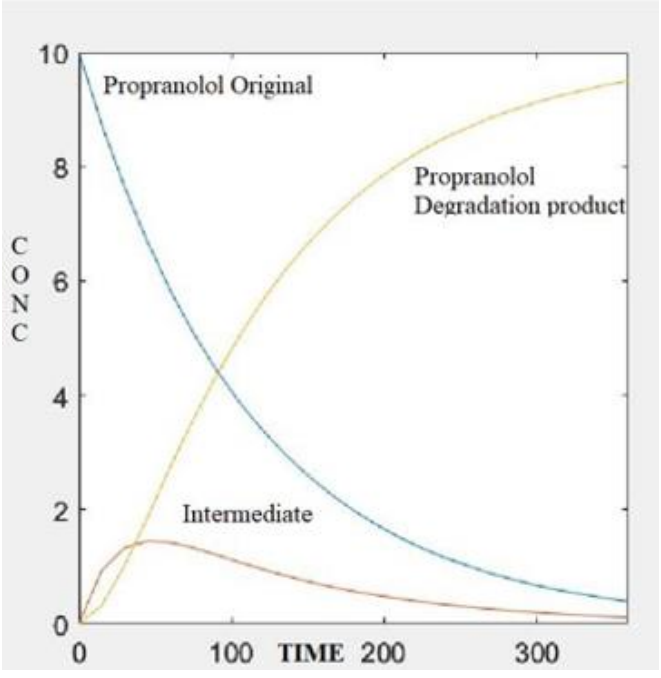

Figure 9: *Results of ALS optimization*

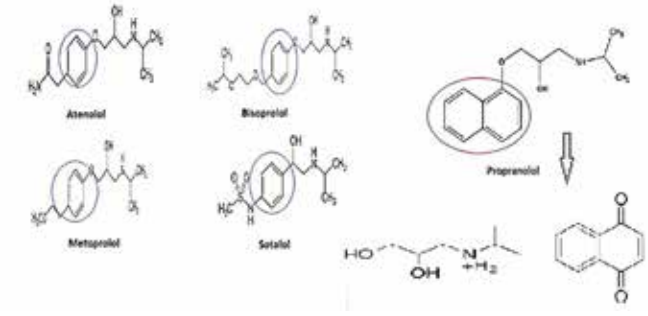

Figure 10: *Chemical Structures of studied beta-blockers and possible breakdown of Propranolol*

a thermally activated crossing of the bonding generating 1-naphthol and the remaining side chain as shown in figure 10. [27,28]

4. Conclusion

Five members of beta blockers were evaluated for their susceptibility to the solar photodegradation in transparent and amber colour containers along with variations in temperature. Clear changes in the absorption spectral profile of propranolol was observed, other beta blocker molecules showed very negligible decrease in the concentration on direct exposure to sunlight in both type of containers. Combined Soft and Hard modelling MCR-ALS resolve pure spectra and kinetics of photocatalysis of propranolol with the rate constants was obtained, it was shown that propranolol undergoes photodegradation via a two-step mechanism with formation of an intermediate species. Concentration profile obtained through MCR-ALS analysis shows that initial concentration of propranolol decreases along with formation of reaction intermediate, and a new final product with increasing concentration can be seen.

This behaviour of the propranolol was due to presence of a naphthalene moiety in the chemical structure as compared to other members which contain benzene moiety in structure.

We can conclude that simple UV spectrophotometric method with the aid of chemometrics can be used as an alternate qualitative tool for HPLC to study stability of drug molecules under various stress conditions. □

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, IV, 2023 | 57-63 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 35, IV, 2023 | 57-63 RESEARCH ARTICLE

Characterization of the renal safety profiles of coumacines

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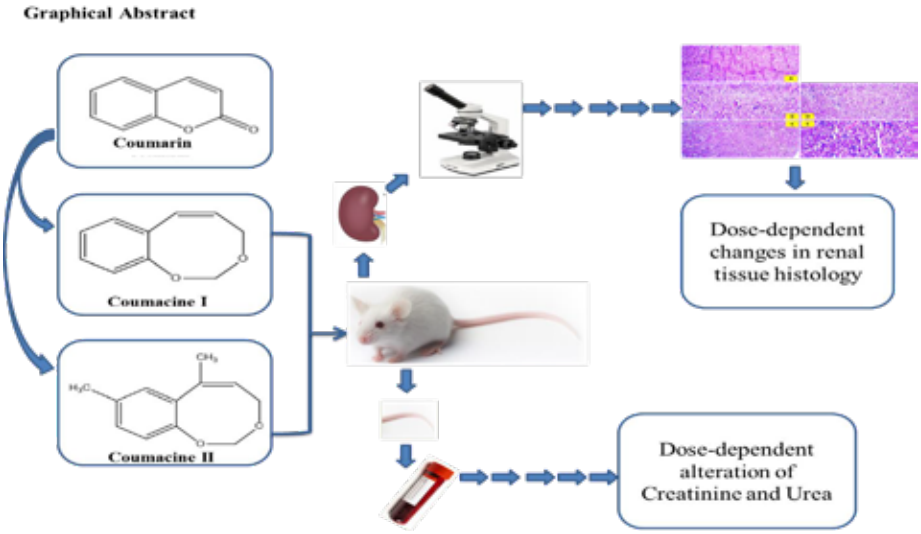
ABSTRACT

The bottleneck step following the synthesis and characterization of the drug in the pharmaceutical setting is their adverse effects in the light of useful therapeutic doses. The kidney as the main clearance organ is the target for metereos of chemicals, xenobiotics, and drug metabolites. The present study aimed at characterizing the renal safety profile of newly synthesized coumarins-coumacine I and II. To do so, a mouse model was used with a total of 30 mice subclassified into 5 groups; Group 1 (Control group): given placebo vehicle IP for five consecutive days, Group 2: given Coumacine I at a dose of 250mg/kg IP, Group 3: given Coumacine I at dose 500mg/kg IP, Group 4: given Coumacine II at dose 250mg/kg IP, and Group 5: given Coumacine II at dose 500mg/kg IP, for each treated group coumacines given for five consecutive days. Blood samples were withdrawn at the end of the study from sacrificed animals and kidneys harvested for histological study. The results confirmed that serum creatinine and urea rose significantly ($p<0.05$) in the high-dose group compared to the control or low-dose group. Histological study revealed that mild degenerative changes are associated with a low dose of coumacine compared to moderate or severe degeneration associated with a high dose of either coumacines. This pilot study provides promising future direction for the discovery of new medication with anticoagulant therapy with improved pharmacokinetics or additional pharmacodynamic properties.



1. **Introduction**

Coumarins are a significant and broad class of oxygen heterocycles that are frequently discovered as secondary metabolites produced by plants[1]. The coumarins can be generally classified into 7 groups; simple coumarins, furanocoumarins, pyranocoumarins, dicoumarins, dihydrofuranocoumarins, isocoumarins, and phenylcoumarins[2]. Coumarines are a widely expanded group of drugs in the last decade several members were introduced in the pipeline of therapeutic applications in clinical settings[3]. The biochemical and pharmacological aspects with wide therapeutic efficacy of coumarin make this group of clinical importance including their application in high protein oedema[4], chronic infections[5], cancer treatment[6], antioxidants[7], anti-inflammatory[8], blood coagulation and anticoagulant[9].

Formerly, coumarin has been used as rodenticide due to its vitamin K inhibition properties resulting in internal bleeding and death[10]. The nucleus structure of coumarin enriched the group for the synthesis of new compounds and thereby increased new derivatives in a timely manner[11].

The area of research concerning drug discovery and development was increasingly challenging due to the potential involvement of various parameters in the path of synthesis and experimentation[12], especially in terms of the adverse effect profile of the newly synthesized drugs. Adverse effects on vital organs are the first on the pipeline to be considered with highlighted focuses on the kidney as a major clearance organ[13]. Heterocyclic compounds are increasingly reported as a rich chemical resource for new drug developments[14]. New heterocyclic compounds, under name of coumacines, were synthesized and subsequently characterized for their physicochemical properties by Mustafa Y.F. in 2018 (Figure 1)[15]. The therapeutic potential regarding their antioxidants, anticancer, and other effects have been subsequently characterized in a series of studies[13-15]. Moreover, their adverse effects profile and dosage toxicity were reported using laboratory animals[16].

Coumarines as well-known anticoagulant drugs have potentially been reported to preserve renal functions providing protection against renal damaging compounds[17]. Being excreted though kidney, coumarins need dose adjustment in patients with renal dysfunction[18]. The present study aimed to characterize the safety profile of renal toxicity in experimental animals using the newly derived coumacine compounds, namely coumacine I (CMI), and coumacine II(CMII) as a target for future product release into phase II trial.

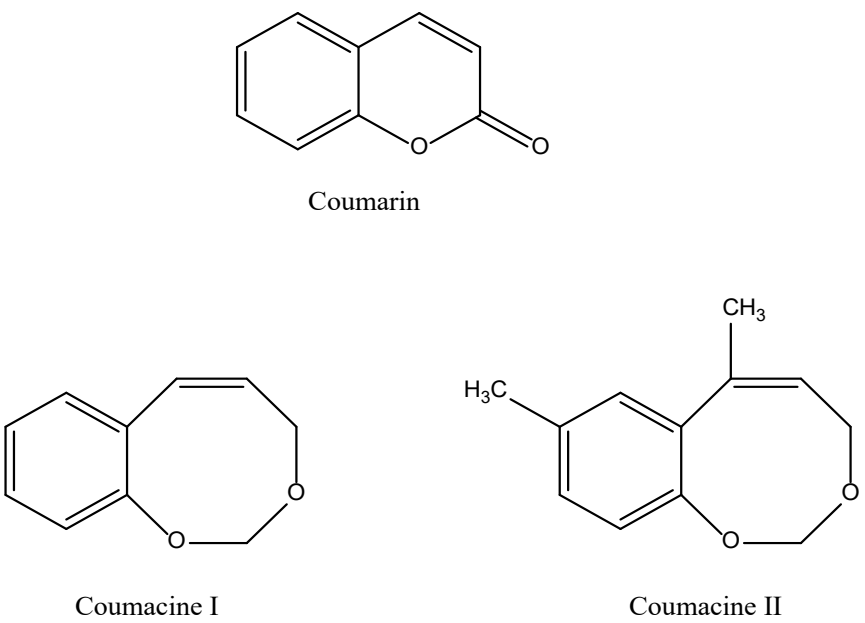


Figure 1. *Chemical backbones of coumarin chemical nucleus, coumacine I (CMI), and coumacine II (CMII)[15].*

2. Materials, and Methods

Chemicals: Coumacine I and II were originally synthesized by Mustafa YF[15] in the College of Pharmacy/university of Mosul. Characterised for physicochemical properties and potentially a candidate for subsequent biological activity. In the present study, Coumacine I and II have been dissolved instantly in a 10% solution of hydroxypropyl beta-cyclodextrin (HPBCD) to enhance their solubility.

Animal treatment: Replicates of 6 mice per group were used in the present study (30 mice in total, 8 weeks age, 2-3 mice per cage, temperature 23-25 ˚C and humidity of 50-55%). Standard food pellets *ad libitum* and free access to water. The 30 mice were divided as follows:

Group 1 (Control group): given 10% HPBCD solution IP for five consecutive days

Group 2: given Coumacine I at a dose 250mg/kg IP for five consecutive days

Group 3: given Coumacine I at a dose 500mg/kg IP for five consecutive days

Group 4: given Coumacine II at a dose 250mg/kg IP for five consecutive days

Group 5: given Coumacine II at a dose 500mg/kg IP for five consecutive days

Blood samples were withdrawn from all mice on day 6, serum was isolated, and samples were frozen for further analysis.

Renal function tests measurements: As per manufacturer instructions creatinine (kit supplied by Biolabo Cat No. 90107, France) was measured by Enzymatic Method. The principle of this assay is based on the lysis of endogenous creatinine in samples through two consecutive steps, which culminate in the formation of hydrogen peroxide. The amount of hydrogen peroxide produced is reciprocally related to the concentration of creatinine in the samples. The colourless hydrogen peroxide was converted to a chromogenic compound via n-ethyl-n-sulphopropyl-m-toludine and 4-amino antipyrin to the coloured compound to be detected at an optical density of 545 nm[19].

As per manufacturer instructions blood urea (kit supplied by Biolabo Cat No. 90107, France) was measured by Enzymatic Method. The principle of as-

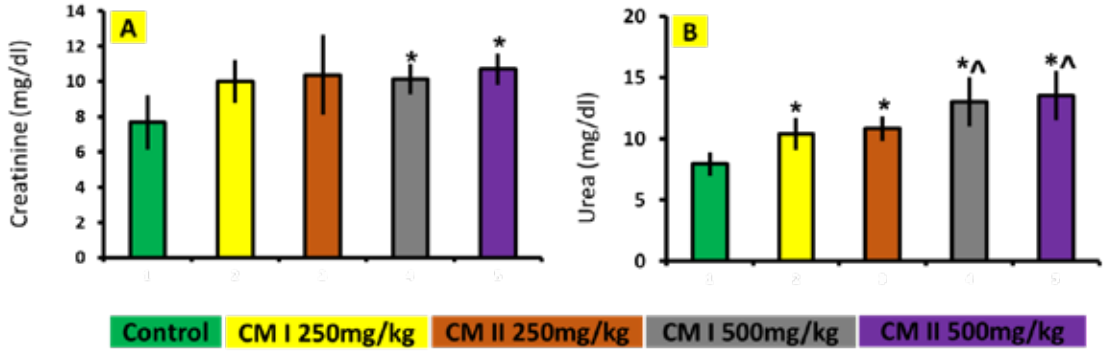


Figure 2. *Renal function tests of coumacine I and II at different doses (A) creatinine, (B) Urea. Data expressed as mean±SD. *^P< 0.05, *significant as compared to control group, ^significant as compared to coumacine I and coumacine II group at low dose.*

say based on lysis of endogenous creatine through two consecutive steps ending with the formation of hydrogen peroxide reciprocally related to creatine in the samples. The colourless hydrogen peroxide were converted to a chromogenic compound via n-ethyl-n-sulphopropyl-m-toludine and 4-aminoantipyrin to coloured compound to be detected at an optical density of 545nm[20].

Histological study: Kidney harvested from sacrificed mice immediately, were quickly washed with normal saline and fixed overnight in formalin. The next day, samples were embedded in paraffin blocks. These tissue blocks were sectioned (5 µm) stained with eosin-hematoxyline, and examined under a light microscope[21].

Statistics: Analysis of parametric data was conducted using IBM SPSS statistics 25 software. To compare groups, one-way ANOVA with Bonferroni tests as a post hoc test was performed to determine variations among groups. Data were expressed as mean values ±SD. Using a power of 80% or greater to determine sample sizes and an alpha error level (P) of ≤ 0.05 in all experiments is necessary for statistical significance.

3.Results

Analysis of the results concerning the renal function tests has revealed that the level of serum creatinine rose significantly ($p<0.05$) in high-dose of CMI (10.1±0.9 mg/dl) and CM II (10.7±0.9 mg/dl) compared to the control group (7.7±1.5 mg/dl). However, serum creatinine levels in low dose of CMI (10±1.2 mg/dl) and CMII (10.4±2.3 mg/dl) have shown non-significant differences when compared to the control group (7.7±1.5 mg/dl) (Figure 2A).

Analysis of the results concerning the level of serum urea revealed significant increase ($p<0.05$) in high-dose of CM I (13±2 mg/dl) and CMII (13.5±2 mg/dl) compared to the control group (8±1 mg/dl) or low-dose CMI (10.4±1.3) and CMII (10.8±1), (Figure 2B).

The histological study of the exposed tissue to coumacines was associated with oedematous changes, tissue degeneration, vacuole formation, and angioedema. However, these changes were represented as severe damage with high doses of coumacine I and II compared to low-dose coumacines or normal histology of the control group (Figure 3)

4. Discussion

The present study confirmed that the newly derived compounds from coumarins namely coumacine I and II are relatively safe particularly at low doses, inducing minimal renal toxicity at 250mg/kg whether CMI or CMII. This assumption was confirmed by measured

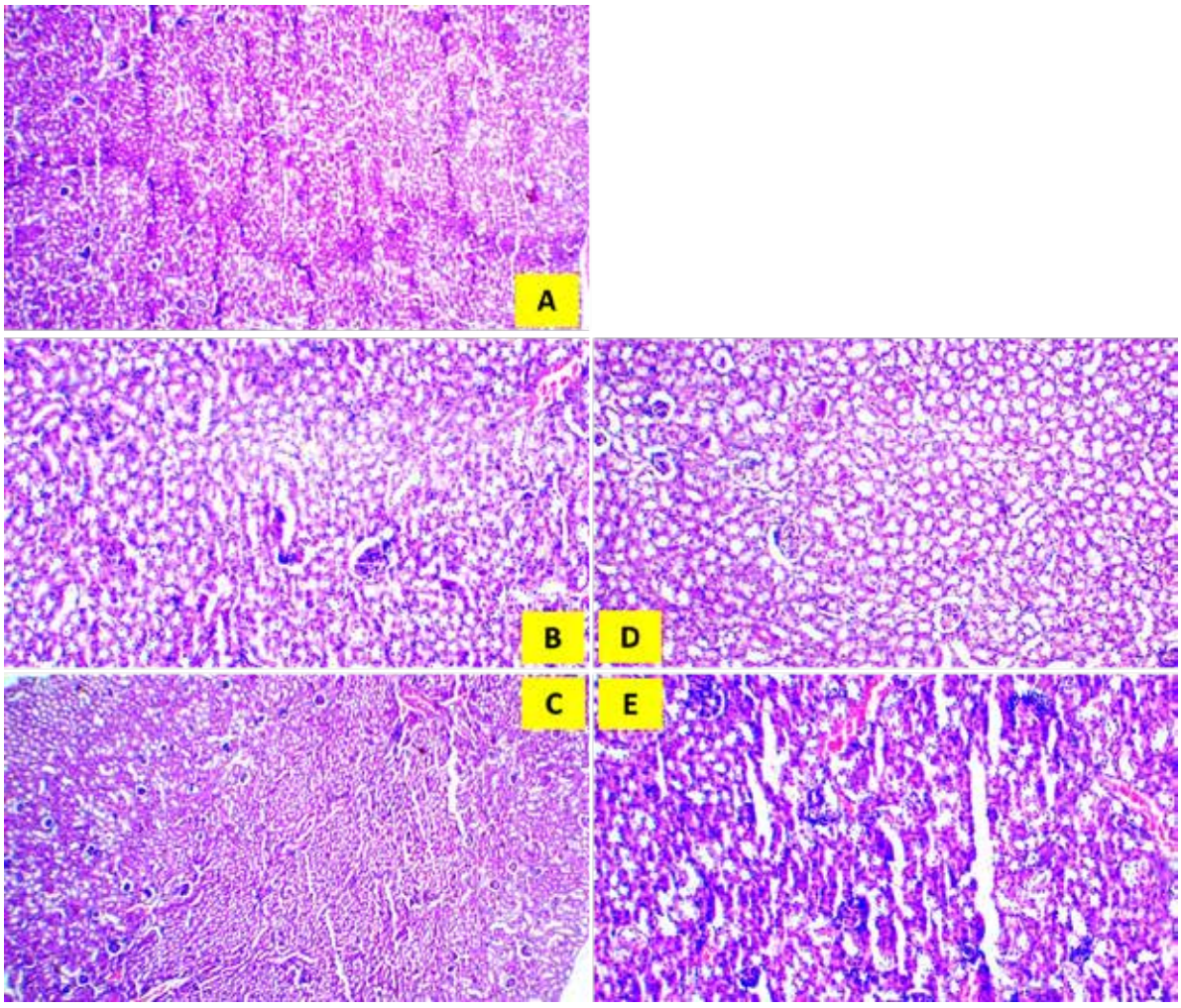


Figure 3. *Shows renal tissue morphology A) control group showed intact renal collecting tubules, glomeruli, and renal tubules B) renal tissue exposed to a subchronic dose of coumacine I at 250 mg/kg which shows no clear difference from the control C) coumacine II at 250 mg/kg which shows oedema and signs of degeneration D) coumacine I at 500 mg/kg renal tissue shows mild changes and degenerations E) coumacine II 500 mg/kg show dramatic signs of degeneration, necrosis, addition to in nephrons units.*

renal function tests and histological kidney characterization. The outcome revealed that low doses induced minimal elevation in urea and creatinine alongside minimal histological changes compared to high-dose of either coumacines. Most of the available literature was directed towards a well-established and clinically used coumarin (warfarin). Therefore, the present study could be considered a pilot study.

The standards of coumarin toxicity have recently been reported as a part of structural variations since fluorocoumarins (e.g. psoralen and angelicin) have been reported as a renal damaging entity. Several studies have shown that psoralen and angelicin can cause severe kidney damage in animals and humans. Fluorocoumarins are a type of coumarin that contain a fluorine atom. This structural variation makes

them more toxic than other coumarins. Psoralen and angelicin are two of the most commonly studied fluorocoumarins. They are often used in the treatment of skin disorders such as psoriasis and vitiligo. However, they have been found to cause kidney damage in some patients. The mechanism by which psoralen and angelicin cause kidney damage is not yet fully understood. However, it is believed that they may interfere with the normal functioning of the kidneys. They may also cause inflammation and oxidative stress within the kidneys, leading to damage and dysfunction[22-24.] Renal vascular congestion, inflammatory cell infiltration, and renal capillary dilatation has been reported with a combination of cisplatin with psoralen or angelicin[25.] Conversely, daphentin (a herbal-derived coumarin analogue) blocked cisplatin-induced renal toxicity, as confirmed by measured renal function tests, reduced pro-inflammatory parameters, shifted the balance toward antioxidant activity, and blocked apoptosis[26.]

On the other hand, simple coumarin might induce no harmful effects, and perhaps some of them might paradoxically induce beneficial effects, such as esculetin has shown that it improved the prognosis of patients with diabetic nephropathy mainly inducing antioxidant enzymes imparting tissue protection[27], however, imperatorin as a simple coumarin has reported renal toxicity in mice models, thereby the coumarin toxicity is structural rather than group-based[28-30].

5. Conclusion

Low doses of newly discovered coumacines have greatly shown safe or mild effects on the kidney compared to high doses providing a template for the discovery of new derivatives with improved pharmacokinetic and pharmacodynamics profiles. □

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, IV, 2023 | 64-70 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 35, IV, 2023 | 64-70 RESEARCH ARTICLE

The Efficacy of Spiramycin-based Triple Therapy for First-Line Helicobacter Pylori Eradication

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ABSTRACT

Background/ Objectives: To evaluate the efficacy and safety of spiramycin-based triple therapy for first-line Helicobacter pylori eradication.

Materials and Methods: One hundred and twenty-two dyspeptic patients infected with H. pylori, who had not received previous eradication treatment were randomly divided into two groups. The study group of 70 patients received pantoprazole 40 mg twice a day, spiramycin 1,5 M.U.I., and metronidazole 250 mg (film-coated tablet), three times a day for 10 days. Meanwhile, the control group consisting of 52 patients received standard triple therapy with pantoprazole, clarithromycin, and amoxicillin for 14 days. One month after the completion of therapy, H. pylori status was assessed. If the test for H. pylori was negative, it was considered that eradication had been successfully performed.

Results: In the study group, H. pylori was eradicated in 52 patients (74.3%), whereas in the control group, it was eradicated in 45 of them (86.58%). Although eradication was higher in the second group, the difference between the two groups was not statistically significant (p = 0.097). Regarding the side effects of the ordered therapies, 12 (54.5%) patients were sick in the first group, while 10 (45.5%) in the second group. Common adverse effects were nausea, abdominal pain, and diarrhea. Again, there was no statistical difference between these two groups (p = 0.266).

Conclusion: In our study, it was not proven that spiramycin is more effective than clarithromycin in the eradication rate of H. pylori. No significant statistical difference was found between the study group and the control group. Also, in terms of side effects, there is no difference between the two groups.

1.Introduction

Despite the presence of a large number of antibiotics, the degree of eradication of H. pylori is not satisfactory. Resistance of H. pylori to antibiotics has reached high levels globally, causing the eradication rates of H. pylori to decline. This prompts us to always look for new protocols that will eradicate H. pylori in desirable values.

More than half of the world's population is infected with H. pylori, which always causes chronic gastritis, and can progress to more serious complications such as peptic ulcer, gastric adenocarcinoma, and MALT gastric lymphoma. The majority of the patients, except the structural and functional changes as a result of the active chronic inflammation of the gastric mucosa, have no other clinical symptoms. [1]

Kyoto's Consensus Conference 2015, based on the objective pathologic criteria, defined H. pylori gastritis as an infectious disease, regardless of the presence or the absence of the symptoms or clinical complications. In the new International Classification of Disease, 11th Revision, H. pylori gastritis is included as a separate nosological entity. Based on this, all patients infected with H. pylori should be treated, regardless of the presence or the absence of the symptoms. [2]

Studies done with serology or endoscopy showed a high prevalence of Helicobacter pylori in the Albanian population (54%- 92.1%). [3, 4, 5, 6, 7]

Over the last four decades, the prevalence of antibiotic resistance has gradually increased. This increasing resistance of H. pylori to previously effective antibiotics has become a major concern and requires a careful selection of therapies and re-evaluation of therapeutic strategies.[8]

Bacterial resistance is one of the biggest threats to global health. Both the WHO and the European Union Council advise the careful use of antibiotics to avoid the development of bacterial resistance. [9]

In 1952, spiramycin was discovered as a product of Streptomyces *ambofaciens*. Since 1955 it has been used as a preparation for oral administration. The antibacterial action spectrum of spiramycin is quite broad and typical of macrolides. It compasses most of the pathogens involved in respiratory tract infections, including Gram-negative and Gram-positive cocci, Parvobacteriaceae, Legionella spp., Chlamydia spp., Ureaplasma urealyticum, M. pneumoniae, and Listeria monocytogenes, but not Enterobacteriaceae. Spiramycin is active against many bacteria that have acquired resistance to erythromycin and other macrolides. [10, 11, 12]

Since its discovery in 1952, spiramycin has been used in the treatment of various infections in the human body. It has been widely used, especially in the treatment of Toxoplasma gondii in pregnant women.[12]

During the literature review, we saw that the studies related to the role of spiramycin in the eradication of H. pylori are few. Although few, in some studies with adults[13, 14] and children[15,16] spiramycin has been shown to be effective in eradicating H. pylori infection with high eradication rates of 91-95%. Based on these studies, spiramycin is not inferior compared to amoxicillin and oxytetracycline in the eradication of H.pylori.

The first study that was done regarding the in vivo effect of spiramycin in the eradication of H. pylori was done by Berstad et al. (1995). The percentage of eradication of these patients and healing of peptic ulcers was 91.3%. Side effects that limited daily activities were smaller than those that occurred during the administration of oxytetracycline.[16]

The second study published regarding the effect of spiramycin in the eradication of H. pylori is the study by Olafsson et al. (1999). One hundred and eighty-three patients were treated for ten days in 4 different groups. Intention to treat eradication rates were 93.5% for the first group, 91.3% for the second group, 93.6% for the third group and 88.6% for the fourth group. 33% of patients had side effects, and women had more complaints (p=0.0002). So, the eradication rate for the two groups with spiramycin was 91.3% and 88.6%.[17]

There are two studies that should be mentioned that were done regarding the effect of spiramycin in the eradication of H. pylori in children.

The first study aimed to evaluate the effect of spiramycin compared to amoxicillin in the treatment of

H. pylori with triple therapy with metronidazole and lansoprazole. In the first group out of 14 patients, 12 were eradicated (85.7%), while in the second group 8 out of 11 were eradicated (72.7%), there was no difference between these two groups, p>0.5. Limited side effects were observed in one patient with spiramycin (abdominal pain) and in two cases with lansoprazole (mouth dryness). Therapeutic compliance was excellent. [18]

The second study was also conducted by the same authors and aimed to determine metronidazole resistance in bacterial eradication and the improvement of histological gastritis determined by endoscopic and histological examinations. Eradication in the first AML (Amoxicillin, Metronidazole, Lansoprazole) group was 83.3%, it was not significantly different from the second SML (Spiramycin, Metronidazole, Lansoprazole) group 63.6%, p=0.3).21

As we have seen, the success of H. pylori eradication with different combinations of spiramycin in the literature ranges from 63% to 93.6%. Our eradication rate is similar to those results.

The aim of antimicrobial therapy is to eradicate reliably H. pylori infection in the majority (eg, ≥90%) of patients.

Treatment of H. pylori infection remains challenging. Antibiotic resistance is one of the factors that affect the success of H. pylori eradication. Clarithromycin is one of the key factors in the eradication of H. pylori. Recent studies show primary resistance to clarithromycin in Europe at 21.4% (Italy 36.9%, Croatia 34.6%, Greece 30%, and Bulgaria 26.9%). Here we should also mention the resistance to clarithromycin and metronidazole at the same time, which in this study was 9.7%. Resistance to the other three antibiotics was exceptional; 0.2% for amoxicillin, 0.9% for rifampicin, and 0% for tetracycline.[8]

It is not known about the resistance of H. pylori to clarithromycin and other antibiotics in Kosovo. But if we take into consideration the high values of resistance to clarithromycin in Southern European countries that surround Kosovo, we can assume that resistance to clarithromycin in Kosovo is very high, anyhow over 15%. Another thing that should be mentioned is that resistance to clarithromycin is associated with the use of macrolides in the community. For this reason, extreme caution is preferred when prescribing eradication therapy.[8]

Claritomycin is the most expensive drug used in the eradication of H. pylori. Spiramycin is an older drug than clarithromycin, which was discovered in 1984. [17]

Since it is much more affordable than clarithromycin, we used spiramycin which is in the form of a film-coated tablet Spiramycine / metronidazole 1.5 M.U.I. /250 mg GERDA, a new product on the Kosovar market. To our knowledge, this is the only study conducted with this product.

The aim of our study was to evaluate the efficacy of spiramycin and to monitor the occurrence of side effects while receiving H.pylori eradication treatment based on spiramycin.

2.Materials and Methods

This prospective controlled randomized open-label clinical trial is designed to evaluate the efficacy of spiramycin-based triple therapy for first-line H. pylori eradication. This study was approved by the Ethics Committee of the Kosovo Doctors Chamber (07.07.2022, nr. 108/2022) and conducted from July 2022 to January 2023. The sample consists of 122 patients with dyspeptic complaints diagnosed with H. pylori infection in the Gastroenterology Clinic of the University Clinical Center of Kosovo and two private clinics. Diagnosis of H. pylori was based on the presence of Helicobacter pylori stool antigen, positive urea breath test, and positive urease test.

Exclusion criteria: Patients who were previously treated with H. pylori eradication therapy, those who have used PPIs and antibiotics within the last 4 weeks, those with penicillin and PPI allergies, patients with previous gastric surgery, the coexistence of malignancy, renal failure, and pregnant women.

The diagnosis of the H. pylori infection is made if one of these examinations results is positive. For those who underwent endoscopy, two biopsies were taken for the urease test.

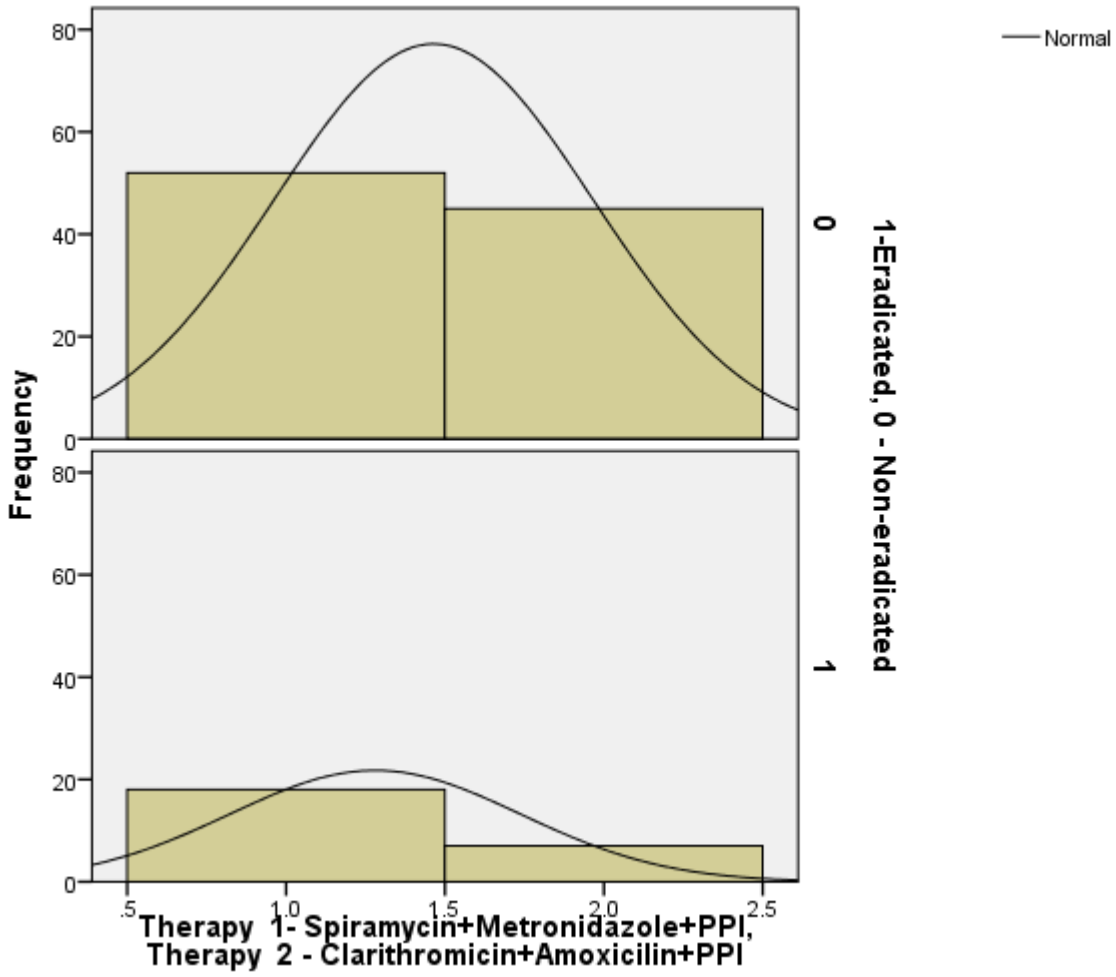

Figure 1 Eradication rate between groups (p= 0.099)

Therapy regimen

The study group, including 70 patients, received pantoprazole 40 mg twice a day, half an hour before meals, for 10 days; spiramycin 1,5 M.U.I. and metronidazole 250 mg in the form of film-coated tablets Spiramycine/Metronidazole GERDA 1.5 M.U.I./250 mg three times daily with food for 10 days. The control group, including 52 patients, received pantoprazole 40 mg twice a day, half an hour before meals, clarithromycin 500 mg twice a day after meals, and amoxicillin 1000 mg twice a day after meals, for 14 days.

In advance, the possible side effects were explained to the patients and their consent to participate in the study was obtained.

After the end of the therapy, the patients did not take any proton pump inhibitor for two weeks and bismuth or any other antibiotic for four weeks. Four to six weeks after the end of the eradication therapy, the success of the therapy has been checked with H. pylori stool antigen, urea breath test, and urease test. In those who underwent endoscopy, two biopsies were taken for a urease test. The negative result of one of these tests was defined as successful eradication.

Statistical Analysis Data was evaluated by the Statistical Package for the Social Sciences (SPSS) 22 computer program. Statistical analyses were carried

Table 1. Demographic characteristics, side effects, and eradication rates of our patients

	Study group	Control group	p
Patients (nr, %)	70 (57.4%)	52 (42.6%)	0.497
Mean age ±SD	41.17±SD 12.565		
Sex (M/F)	M-38(54.3%) F- 32 (45.7%)	27 (51.9%) 25 (48.1%)	0.855
Side effects	12 (54.5%)	10 (45.5%)	0.266
Eradication rate	52 (74.3 %)	45 (85.6%)	0.097

out by T-tests and chi-square tests. P values less than 0.005 were significant.

3.Results

This study included 122 patients with H. pylori infection, 46.72% of them females, with a mean age of 41.17±SD12.565 (18-60). The patients were split into two groups. There were no differences between these groups in terms of gender (p=0.855). The eradication rate in the study group was 74.3%, whereas the control group had an eradication rate of 85.6%. No statistical differences were observed between the two groups, (p=0.099). (Figure 1)

Regarding side effects, in the study group, 12 patients had mild complaints which were nausea, abdominal pain and diarrhea. The most common complaint in the study group was nausea. In the control group, there were 10 patients with mild complaints, with nausea being the most common. Even though the study group had a larger number of patients with complaints there was no statistical difference between the two groups(p=0.266). There were no significant side effects that would force patients to stop the therapy. All patients completed their therapies, (Table 1).

4. Discussion

Our study did not prove that a combination of spiramycin/metronidazole is more effective than clarithromycin-based triple therapy, in the eradication rate of H. pylori. No significant statistical difference was found between the study group and the control group. Also, in terms of side effects, there is no difference between the two groups. In this study, which is the first in our country regarding the efficiency of spiramycin in the eradication of H. pylori, we found a value of 74.3 % in the success rate of the eradication of H. pylori. Based on Graham's scale, it seems that we are dealing with the eradication rate of the category of C or fair (85-89%).[18] At the same time, we saw that the percentage of side effects was low. We did not have any patients who discontinued the therapy due to side effects. The eradication rate of H. pylori with standard triple therapy in Kosovo ranges between 61,3 % and 71%.[19,20]

The study limitations

- H. pylori cultures, antibiotic susceptibility, and drug resistance were not performed,
- Only the presence or absence of side effects was considered, without measuring their severity,
- The small number of patients in the study groups.

5. Conclusion

According to our results, the combination spiramycin/metronidazole is not more effective than clarithromycin-based triple therapy in the eradication rate of H. pylori., since there was not significant statistical difference between the study group and the control group. Also, in terms of side effects, no difference between the two groups was observed. However, the spiramycin/metronidazole combination has an important advantage due to its lower cost. □

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, IV, 2023 | 71-81 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 35, IV, 2023 | 71-81 RESEARCH ARTICLE

The study of prostate-protective effect of dry extract from reishi mushrooms on the model of testosterone-induced prostatic hyperplasia

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ABSTRACT

The aim of this work was to study the prostate-protective properties of dry extract from reishi mushrooms in testosterone-induced benign prostatic hyperplasia in rats.

Materials and methods. Simulated benign prostatic hyperplasia was reproduced by subcutaneous injection of testosterone propionate solution at a dose of 3 mg/kg to male rats for 21 days. The dry extract of reishi mushrooms was administered intragastrically to experimental animals daily at a dose of 100 mg/kg body weight simultaneously with subcutaneous injection of testosterone propionate for 3 weeks. The comparison drug «Prostatophyt" in a dilution of 1:10 at a dose of 1 ml/100 g of the animal's body weight was administered intragastrically according to the same scheme as the reishi extract. Prostate homogenate and rat blood serum were tested. The prostate-protective effect of the reishi mushroom extract was evaluated by the activity of acid and alkaline phosphatase, the content of TBA-active products and reduced glutathione. The phosphatase index was calculated, which is the ratio of

acid phosphatase to alkaline phosphatase (AP/ALP).

Results. Subcutaneous administration of testosterone propionate to male rats for 21 days probably leads to disruption of phosphatases activity, increase in TBA-active products and decrease in the content of reduced glutathione in blood serum and prostate homogenate of affected animals.

It was established that the dry extract from reishi mushrooms in the model of testosterone-induced benign prostatic hyperplasia in white rats inhibits the processes of lipid peroxidation and normalizes the antioxidant/prooxidant balance.

Conclusion. Dry extract from reishi mushrooms has a prostate-protective effect in conditions of testosterone-induced prostate adenoma due to its antioxidant properties, which are caused by the presence of biologically active substances in mushrooms, in particular polysaccharides, flavonoids, vitamin C and steroid compounds.

1. Introduction

Currently, the treatment of benign prostatic hyperplasia (BPH) remains an urgent problem. According to the latest data, 15-25% of men aged 50-65 suffer from this disease, which, causing lower urinary tract dysfunction (lower urinary tract symptoms), significantly reduces the quality of life[1, 2].

According to the recommendations of the European Association of Urologists in 2021, 6 groups of drugs can be used for the treatment of prostate adenoma: α1-adrenoblockers, 5α-reductase inhibitors, muscarinic receptor antagonists, phosphodiesterase type 5 inhibitors, vasopressin analogue and herbal preparations, including the extract of saw palmetto (Serenoa repens) were recognized as the most effective and well-studied[3, 4]. All the groups of drugs listed above have proven clinical effectiveness, are used to treat overactive bladder, lower urinary tract symptoms in BPH, but not all patients are helped by standard therapy. There are data confirming the prostate-protective effect of burdock root and leaf extracts[5, 6]. There is a development of side effects of medicines, especially of synthetic origin. Most herbal preparations are multicomponent, which increases the likelihood of an unpredictable allergic reaction[7, 8]. Therefore, the search for new effective and safe drugs for the treatment of BPH and chronic prostatitis is still ongoing.

The study of reishi mushrooms dry extract (RMDE), which, due to its rich chemical composition, can have a complex pharmacological effect[9, 10, 11] is promising in this direction. Since it is a drug of natural origin, it can be used for a long time without significant side effects, which is relevant in the therapy of prostate adenoma and CP.

The aim of our work was to experimentally study the prostate-protective properties of dry extract from reishi mushrooms in testosterone-induced benign prostatic hyperplasia in rats.

2. Material and methods

2.1. Material

The material of the experimental work was a dry extract from reishi mushrooms. The research was performed on white male rats weighing 200-250 g, which were kept on a standard diet of the vivarium of of I. Horbachevsky Ternopil National Medical University. All experiments were performed in accordance with good laboratory practice (GLP) and bioethics according to the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes"[12]. The conducted re-

search was approved by the Ethics Committee of I. Horbachevsky Ternopil National Medical University (excerpt from the protocol №72 from 06.01.2023).

2.2. Experimental induction of benign prostatic hyperplasia

Modeling of BPH in white male rats was carried out by injecting a solution of testosterone propionate at a dose of 3 mg/kg subcutaneously for 21 days. Reishi mushroom extract was administered intragastrically to the experimental animals daily at a dose of 100 mg/kg of the animal's body weight simultaneously with subcutaneous administration of testosterone propionate for 21 days. The dose of 100 mg/kg of animal body weight for RMDE was chosen based on our previous studies, which found it to be conditionally therapeutic for experimental carcinogenesis in rats[9, 13]. As a comparison drug for the prostate-protective action of RMDE, "Prostatophyt" (manufacturer - "Eim Scientific and Production Pharmaceutical Company" LLC, Kharkiv, Ukraine) was used in a 1:10 dilution at a dose of 1 ml/100 g of animal body weight, which was administered intragastrically according to the same scheme as the studied extract.

2.3. Experimental design

The design of the experimental work included seven groups of animals, 8 rats in each: Group 1– animals that were given the appropriate amount of purified water daily (for 3 weeks), control (C); Group 2 and 3 - animals that were subcutaneously injected with a solution of testosterone propionate, control pathology (CP), 15th and 22nd days of the experiment; Group 4 and 5 – rats, which were treated with a dry extract of reishi mushrooms simultaneously with subcutaneous injection of testosterone propionate, 15th and 22nd days of the experiment; Group 6 and 7 – animals that were treated with the reference drug "Prostatophyt" simultaneously with subcutaneous injection of testosterone propionate, 15th and 22nd days of the experiment.

On the 15th and 22nd day from the beginning of the study, animals were removed from the experiment by euthanasia using sodium thiopental. Prostate glands (PG) and seminal vesicles (SV) were isolated, and relative mass was determined. The homogenate of PG and blood serum of rats were also subjected to research, the relative weight of the prostate gland, seminal vesicles was isolated and determined.

2.4. Methods

The prostate-protective effect of RMDE was assessed by the activity of acid (AP)[14] and alkaline (ALP) phosphatase[15], the content of TBA-active products (TBA-AP)[16] and reduced glutathione (GSH) [16] in blood serum and homogenate of animal prostates. The phosphatase index was calculated, as the ratio of acid phosphatase to alkaline phosphatase (AP/ALP) and indirectly characterizes the degree of androgen saturation of the body[6].

2.5. Histopathological studies

The collection of material for histological and histochemical studies was carried out according to the generally accepted methodology[17]. Pieces of the rat's prostate gland were fixed in a 10 % formalin solution, while the duration of exposure did not exceed 1-2 days. Next, the pieces were dehydrated in alcohols of increasing concentration in an AT-4 machine for histological processing of tissues, and embedded in paraffin blocks. Sections with a thickness of 5-7 μm obtained on a sled microtome MC-2 were stained with hematoxylin and eosin. Slides were viewed under a light microscope at 100x and 200x magnification.

2.6. Statistical analysis

Statistical analysis of the data was performed using STATISTICA 13 (TIBCO Software Inc., 2018). Parametric and nonparametric methods of evaluation of the obtained data were used for statistical processing of the results. For all indices, the arithmetic mean of the sample (M) and the error of the arithmetic mean (m) were calculated. The reliability of the difference between the values between the

Table 1: Effect of reishi mushrooms extract on the relative weight of the reproductive system organs under conditions of simulated BPH (M±m; n=56)

Index/Group of animals	Relative weight of organs, g/100 g of body weight			
	Prostate gland		Seminal vesicles	
	15th day	22th day	15th day	22th day
C	0.31±0.02	0.31±0.02	0.48±0.03	0.48±0.03
CP	0.47±0.03*	0.55±0.06*	0,87±0,03*	1,08±0,05*
CP+RMDE	0.35±0.02**	0.37±0.02**	0,63±0,05**	0,57±0,04**
CP+Prostatophyt	0.34±0.02**	0.35±0.03**	0,55±0,02**	0,54±0,04**

Note. Here and in the following tables: ** – probable changes between the indicator of control and testosterone-affected animals; ** – probable changes between the indicator of testosterone-affected and treated animals; p<0,05.*

independent quantitative values was determined by the Mann-Whitney test. The difference between the values was considered probable at $p < 0.05$[18, 19].

3. Results

3.1. Effect of RMDE on the relative weight of the reproductive system organs, on serum biochemical indicators and biochemical indicators in rat prostate homogenate under the conditions of simulated BPH

Subcutaneous administration of testosterone propionate at a dose of 3 mg/kg for 21 days caused statistically significant changes in the relative mass of androgen-dependent organs compared to rats in the control group: an increase in PG – by 1.5 and 1.8 times and an increase in seminal vesicles – by 1.8 and 2.2 times on the 15th and 22nd days of the study, which indicates the development of BPH (Table 1).

The use of RMDE and the comparison drug for 21 days to correct the PG damage caused a statistically significant decrease in the relative weight of PG and SV in both terms of the study compared to CP group. RMDE and prostatophyte showed similar effectiveness, under their influence the relative mass of androgen-dependent organs approached the indicators of the control group.

The next stage of our research was to study the activities of acid and alkaline phosphatase in the blood serum and prostate homogenate of animals with simulated hyperplasia of the PG and after the use of RMDE and prostatophyte.

Acid phosphatase (AP) is a lysosomal enzyme found in almost all tissues of the body. Its highest concentration is in the prostate gland (prostatic fraction), then in the liver, spleen, erythrocytes (externally lysosomal localization), platelets, and bone marrow. That is why the blood serum analysis for the determination of acid phosphatase is used to detect prostate carcinoma in men. A high activity of AP in macrophages and osteoclasts is also noted. As for the activity of the enzyme in the prostate gland, it is not detected until puberty[3].

Alkaline phosphatase (ALP) is an enzyme that affects lipid metabolism and the process of calcium deposition in bone tissue. Under the influence of the enzyme, reactions related to the release and free movement of phosphorus in the blood are carried out, ALP catalyzes the separation of phosphoric acid from its organic compounds. LF is extremely common in the body. It is found in the intestinal mucosa, osteoblasts, walls of bile ducts of the liver, placenta, lactating mammary gland, prostate gland[6].

The development of inflammatory processes in the prostate under the influence of testosterone is evidenced by an increase in the activity of acid and alka-

Table 2: Effect of reishi mushrooms extract on serum biochemical indicators of rats under conditions of testosterone-induced hyperplasia of the PG, day 15 (M±m; n=56)

Index/Group of animals	C	CP	CP+RMDE	CP+Prostatophyt
AP, nmol/l*hour	6.70±0.38	12.84±0.63*	7.50±0.28**	7.18±0.36**
ALP, nmol/l*hour	10.45±0.36	18.05±0.55*	12.03±0.56**	11.21±0.39**
AP/ALP	0.64±0.02	0.72±0.05*	0.64±0.05	0.64±0.03
TBA-AP, μmol/l	2.06±0.11	3.37±0.16*	2.78±0.12**	2.55±0.14**
GSH, mmol/l	1.75±0.06	0.96±0.05*	1.54±0.03**	1.61±0.03**

Table 3: Effect of reishi mushrooms extract on serum biochemical indicators of rats under conditions of testosterone-induced hyperplasia of the PG, day 22 (M±m; n=56)

Index/Group of animals	C	CP	CP+RMDE	CP+Prostatophyt
AP, nmol/l*hour	6.70±0.38	19.07±0.66*	8.69±0.51**	7.70±0.32**
ALP, nmol/l*hour	10.45±0.36	25.27±1.10*	12.88±0.77**	11.45±0.35**
AP/ALP	0.64±0.02	0.76±0.03*	0.68±0.01**	0.67±0.02**
TBA-AP, μmol/l	2.06±0.11	4.55±0.24*	2.84±0.15**	2.30±0.10**
GSH, mmol/l	1.75±0.06	0.73±0.04*	1.39±0.09**	1.53±0.07**

line phosphatases in the blood serum of animals with simulated hyperplasia of the prostate gland. Thus, AP activity increased by 1.9 and 2.8 times, and ALP increased by 1.7 and 2.4 times relative to the control on the 15th and 22nd days of the experiment (Tables 2, 3).

In the CP group, the AP/ALP ratio in the blood serum increased by 1.2 times by the end of the experiment, which indicates an increase in the permeability of the membranes of the acini, which are the final branches of the ducts. Acini are surrounded by secretory cells in the prostate gland and stimulate the flow of prostate-specific enzyme into the blood [6].

Administration of the dry reishi mushroom extract to animals in parallel with testosterone propionate contributed to the normalization of phosphatases activity already on the 15th day of the experiment. By the end of the study, the activity of AP in the blood serum of rats under the influence of RMDE decreased by 2.2 times, the activity of ALP - by 2.0 times compared to the group of control pathology. The use of the reference drug also reduced the activity of AP and ALP by 2.5 times and 2.2 times, respectively, in affected animals on the 22nd day of the experiment. The use of RMDE and prostatophyte led to a probable decrease in the ratio of phosphatases (AP/ALP) on the 22nd day of the study compared to the control pathology group (Table 3).

When studying the activity of AP in the homogenate of the prostate of animals, it was established that under the influence of testosterone there was a probable decrease in the activity of the enzyme by 1.5 and 2.2 times compared to the control group on the 15th and 22nd days of the experiment. This indicates a violation of the functional state of the PG. ALP activity in the prostate homogenate of white rats increased by 1.6 and 1.9 times on the 15th and 22nd days of the study compared to the control (Tables 4, 5).

Table 4: Effect of reishi mushrooms extract on biochemical indicators in rat prostate homogenate under conditions of testosterone-induced prostate hyperplasia, day 15 (M±m; n=56)

Index/Group of animals	C	CP	CP+RMDE	CP+Prostatophyt
AP, nmol/kg*hour	20.20±0.34	13.83±0.46*	17.67±0.31**	18.69±0.35**
ALP, nmol/kg*hour	23.59±0.89	36.75±1.26*	26.25±1.16**	25.38±0.86**
AP/ALP	0.87±0.04	0.38±0.02*	0.68±0.04**	0.74±0.04**
TBA-AP, μmol/kg	13.44±0.82	22.41±1.12*	16.25±0.92**	15.26±0.60**
GSH, mmol/kg	1.26±0.06	0.77±0.03*	1.09±0.03**	1.16±0.03**

Table 5: Effect of reishi mushrooms extract on biochemical indicators in rat prostate homogenate under conditions of testosterone-induced prostate hyperplasia, day 22 (M±m; n=56)

Index/Group of animals	C	CP	CP+RMDE	CP+Prostatophyt
AP, nmol/kg*hour	20.20±0.34	9.10±0.37*	17.17±0.59**	18.98±0.27**
ALP, nmol/kg*hour	23.59±0.89	44.75±2.23*	28.97±1.01**	25.27±1.11**
AP/ALP	0.87±0.04	0.21±0.01*	0.60±0.03**	0.76±0.03**
TBA-AP, μmol/kg	13.44±0.82	27.96±0.97*	16.42±0.78**	14.58±0.62**
GSH, mmol/kg	1.26±0.06	0.55±0.05*	1.03±0.09**	1.17±0.05**

In the prostate homogenate of the affected animals, the AP/ALP ratio was also determined, which decreased by 4.1 times compared to the control by the end of the experiment.

After the use of RMDE, AP activity in rat prostate homogenate increased by 1.9 times, ALP activity decreased by 1.5 times on the 22nd day of the study compared to the control pathology group. Under the influence of the prostatophyte, the activity of AP in the examined tissue probably increased by 2.1 times by the end of the experiment, the activity of ALP decreased by 1.8 times compared to CP. Under the influence of RMDE and the reference drug, the AP/ALP ratio in the animal prostate homogenate increased by 2.9 and 3.7 times, respectively, relative to the affected rats on the 22nd day of the study. This testifies to the ability of the studied extract and prostatophyte to stabilize acinus membranes.

The development of hyperplasia of the prostate gland under the influence of testosterone was accompanied by an increase in the processes of free radical oxidation. Thus, in the blood serum of the affected animals, a probable increase in the content of TBA-AP was observed by 1.6 and 2.2 times on the 15th and 22nd days of the experiment, which, in turn, caused corresponding changes in the antioxidant system of rats - a decrease in the level of reduced glutathione (GSH) by 1.8 and 2.4 times (Tables 2, 3). The administration of RMDE and the reference drug in parallel with testosterone contributed to a probable decrease in the content of TBA-AP, and most significantly on the 22nd day of the study in the blood serum of rats by 1.6 and 2.0 times and an increase in the level of GSH by 1.9 and 2.1 times, respectively, relative to the group of affected animals.

The content of TBA-AP in the prostate homogenate

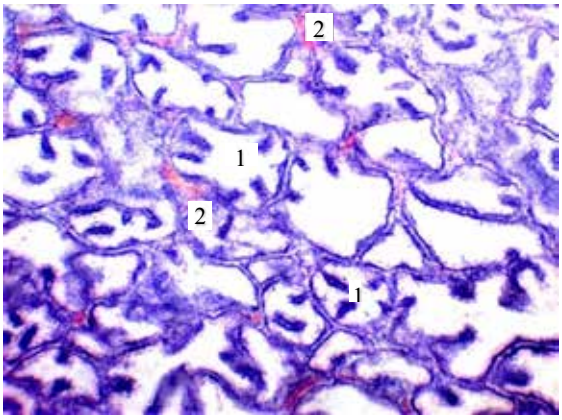

Photo 1: *Prostate gland of a control group rat. The glands are lined with prismatic epithelium and form numerous folds (1). Vessels full of blood (2). Staining with hematoxylin and eosin. ×100.*

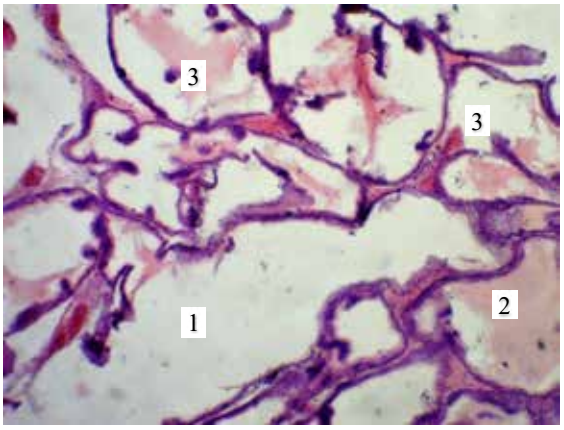

Photo 2: *Prostate gland of a rat with simulated BPH. Cystic transformation of the glands (1), flattening of the epithelial lining (2) and uneven distribution of the secretion (3). Staining with hematoxylin and eosin. ×200.*

of the group of animals with control pathology increased by 1.7 and 2.1 times, the content of GSH decreased by 1.6 and 2.3 times compared to the control on the 15th and 22nd days after the start of testosterone administration (tables 4, 5). The use of RMDE and prostatophyte probably reduced the content of TBA-AP in the prostate homogenate throughout the study, and most of all on the 22nd day of the experiment - by 1.7 and 1.9 times compared to CP. The content of GSH in the homogenate of the prostate of animals probably increased under the influence of the studied extract by 1.4 and 1.9 times, and under the influence of the comparison drug - by 1.5 and 2.1 times on the 15th and 22nd days of the experiment compared to the affected animals (Tables 4, 5).

3.2. Histopathology study

Histologically, the parenchymal component of the prostate gland of rats is represented by numerous alveolar-tubular glands. The basement membrane is lined with a layer of secretory cells - high prismatic epithelium, which together form folds protruding into the lumen of the glands. Peeled epitheliocytes are often found in the lumen of the glands, which is considered a normal process of cell removal. There are also characteristic morphological signs of high functional activity of the glands - the lumens are filled with a secret of various tinctorial properties. In addition to secretory cells, there are small basal cells with signs of mitotic activity.

The stroma of the gland is represented by connective tissue cords that depart from the capsule of the organ and smooth myocytes with a large number of blood microvessels. Venules, as a rule, are full-blooded (Photo 1).

Histological analysis of prostate tissues of affected animals revealed significantly different structural changes compared to the control group. First of all, they concerned the glandular component. The lumens of the glands are significantly expanded and deformed. The epithelium located on the basement membrane is represented by cubic or flattened cells. Nuclei reversion from the basal part of the cells to the apical part is often observed. A decrease in the number of basal cells is characteristic. At the same time, binucleated epitheliocytes appear. The number of folds, as well as the height of the preserved

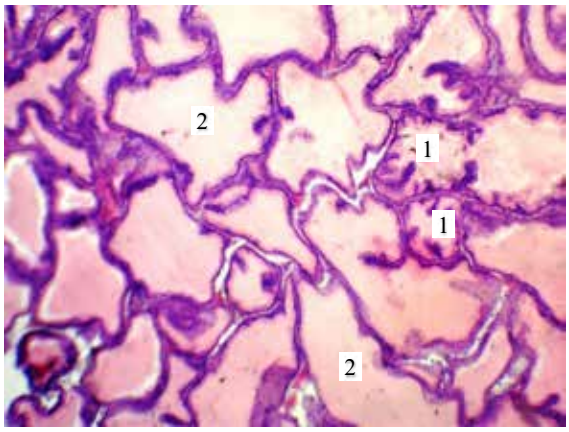

Photo 3: *Prostate gland of the rat after correction of BPH by prostatophyte. Some of the glands are identical to control glands (1), others - with signs of cystic transformation (2). The discharge is distributed evenly. Staining with hematoxylin and eosin. ×100.*

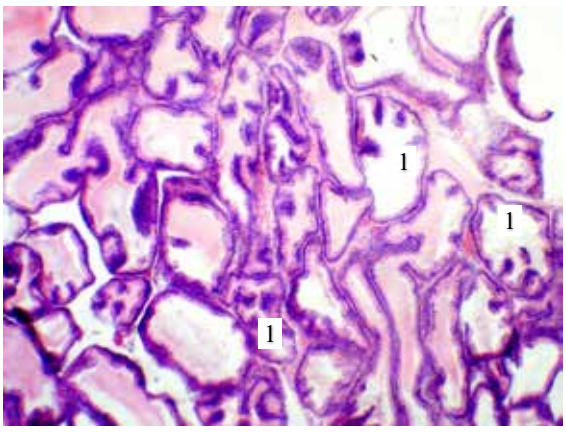

Photo 4: *Rat prostate after correction of BPH with reishi mushroom extract. Glands of tubular-alveolar structure with papillary folds inside (1). Staining with hematoxylin and eosin. ×100*.

ones, has been drastically reduced. The secretion is unevenly distributed, there are many lumens of the glands that do not contain secretion (Photo 2).

During the histological examination of the tissues of the prostate gland of animals that received the comparison drug, we observed a sufficiently clear positive dynamics. The parenchymal component in many areas was represented by alveolar-tubular glands similar to the control group. The secretory cells that cover the basement membrane have a high prismatic shape. Together with the underlying thin stroma, they form papilla-like growths in the lumen of the glands, the discharge of which is distributed evenly. Proliferative activity of basal cells was occasionally noted. The stroma is dominated by venous full blood of the microcirculatory channel. At the same time, often, next to the described changes, cells of various sizes typical for the prostate gland of affected animals were adjacent - deformed, cystic enlarged glands with flattened or cubical epithelium, which formed single short folds. The discharge is mostly evenly distributed (Photo 3).

Histological analysis of the tissues of the prostate gland of experimental animals, which were injected with RMDE, showed significant positive changes in the structure of the organ. The glandular component in most of the studied cases was similar to the control manifestations and was represented by typical glands of alveolar-tubular structure. A high prismatic, functionally active epithelium is located on the basal membrane, and folds and papilla-like outgrowths protrude into the lumen. The gaps are filled with a secret. However, a similar picture was not regularly observed everywhere. Changes typical for BPH with glandular ectasia, atrophied and partially exfoliated epithelium, and atrophied folds were also observed (Photo 4).

The intermediate tissue of the gland is almost evenly distributed between the glands and is represented by collagen fibers and smooth myocytes. The microcirculatory channel was characterized by full blood of its venous link.

4. Discussion

According to scientific research, up to 35% of patients with chronic prostatitis and up to 25% of patients with BPH receive drugs of natural origin. Thus, in many randomized placebo-controlled studies, the

high effectiveness of medicinal products containing biologically active substances of natural origin (extracts of sabal palm fruits, African plum bark, nettle roots, slangy grass, etc.) has been proven. These agents are able to reduce inflammatory processes and slow down the proliferation of PG tissues, normalize the level of androgens, exhibit angioprotective, antioxidant, antihypoxic effects, increase potency and libido[1, 3, 20].

Evaluation of the development of the simulated pathology of PG and the effectiveness of the reishi mushroom extract was carried out by biochemical parameters of blood serum and PG homogenate, changes in the mass of PG and SV. To confirm the results of the experiment, a histological examination of the prostate gland of rats with testosterone-induced BPH and after the use of RMDE and the reference drug was performed.

Administration of testosterone propionate to male rats for 3 weeks led to the development of the animals prostate gland pathology, which was indicated by a significant increase in the relative mass of PG and SV compared to the control.

Under the influence of RMDE, the normalization of the mass of the PG and SV was noted almost to the values of the control group, which indicates a decrease in proliferative processes, and therefore the restoration of the structural and functional state of the PG and androgen sensitivity in the organ, disturbed by testosterone-induced BPH in rats.

The course of the simulated pathology was characterized by a probable, relative to the values of the control group, an increase in the level of ALP and AP in blood serum. According to the literature, an increase in the activity of these enzymes, especially acid phosphatase, which is a prostate-specific enzyme, indicates an increase in proliferative processes and the development of hyperplasia of the organ[6]. In the prostate homogenate of animals of the CP group, a significant decrease in AP activity and an increase in ALP activity were observed relative to the control.

The introduction of testosterone into male rats leads to the activation of the processes of peroxidation, primarily lipoperoxidation. Lipid peroxidation (LPO) is a physiological process that ensures the normal functioning of cells. However, its deviation from the norm causes damage and death of cells[4, 9]. TBA-AP are the primary products of LPO, which in further transformations cause membrane destabilization, destruction and cell death[6]. Testosterone propionate caused a probable increase in the content of TBA-AP in blood serum and prostate homogenate of animals.

The main role in the neutralization of hydroperoxides, formed during the activation of free radical oxidation processes, is played by the system glutathione peroxidase - glutathione reductase - reduced glutathione. Restored glutathione participates in the protection of cells from oxidative stress and supports the functioning of the body's detoxification system[6]. Exposure of albino rats to testosterone resulted in a significant decrease in GSH in both tissues tested.

The results of our experimental work correlate with the data of other researchers, who highlight the important role of the antioxidant/prooxidant balance in the pathogenesis of chronic prostatitis[4, 6]. Activation of lipid peroxidation against the background of reduced antioxidant protection causes a violation of the permeability of biological membranes, leads to an increase in destructive and dystrophic processes in the prostate gland and causes cell death..

RMDE effectively affected the indicators of the oxidative status of rats under conditions of testosterone-induced BPH, minimizing the disruption of AP and ALP activities, reducing the content of TBA-active products, and increasing the content of reduced glutathione in blood serum and prostate homogenate of affected animals almost to the control level. This confirms the presence of antioxidant properties in reishi mushrooms. Prostatophyte also had a positive effect on the investigated biochemical indicators.

5. **Conclusions**

It was experimentally proven that the subcutaneous administration of testosterone propionate to white male rats at a dose of 3 mg/kg body weight of the animals for 21 days led to a violation of the

functional state of the prostate gland, which was characterized by an increase in the mass of prostate and seminal vesicles of the affected animals. Activation of LPO processes and imbalance in the antioxidant protection system were observed. The results of the conducted studies showed the pronounced effectiveness of the dry extract from reishi mushrooms, the introduction of which to male rats almost completely restored the balance in the peroxidation system/antioxidant system. In terms of effectiveness, the studied extract was close to the comparison drug «Prostatophyte».

The prostate-protective effect of reishi mushrooms extract is probably due to its antioxidant properties, which are associated with the presence of polysaccharides, flavonoids and steroid compounds in the chemical composition of the mushrooms.

Microscopic analysis of rat prostate tissues showed positive dynamics of the structural changes under the condition of exposure to reishi mushroom extract and prostatophyte. □

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Conflicts of interest: *The authors report no financial or any other conflicts of interest in this work.*

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ΕΚΔΗΛΩΣΕΙΣ - MEETINGS

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International Symposium on Chemical Biology (EFMC-ISCB)
https://www.efmc-iscb.org/

• DECEMBER 8, 2023 BRUSSELS, BELGIUM
MedChem 2023
https://www.medchem.be/

• JANUARY 28-FEBRUARY 1, 2024 ST. ANTON, AUSTRIA
4th Alpine Winter Conference on Medicinal and Synthetic Chemistry
https://www.alpinewinterconference.org/

•APRIL 8-11, 2024 UTRECHT, THE NETHERLANDS
EFMC-ACSMEDI Medicinal Chemistry Frontiers

• APRIL 8-11, 2024 UTRECHT, THE NETHERLANDS
EFMC | ACSMEDI Medicinal Chemistry Frontiers 2024
Joint Symposium on Medicinal Chemistry
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• 23-25 MAY 2024 DEBRECEN, HUNGARY
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Tetrahedron Symposium i
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•23-26 JUNE 2024 ATHENS, GREECE
9th European Congress of Pharmacology (EPHAR2024)
Megaron Athens International Conference Center
https://ephar2024.org/

•SEPTEMBER 1-5, 2024 ROME ITALY
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