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We would like to inform our readers that this issue is being published on the second anniversary of death of the member and honourable president of editorial of the Bosnian Journal of Basic Medical Sciences, Academician Seid Huković, who contributed to the foundation of the solid basis of communication between scientists from all over the world, especially young ones, in the war period when the bleakness of everyday life destroyed all on its way.

For that reason, editorial board made decision to issue a reprint about Academician Seid Huković, primarily published on November 2001, together with the review written by Academician Muhamed Filipović.

His colleagues, pupils, friends, and acquaintances will never forget a man who connected many generations of scientists, and whose name is deeply engraved in the history of development of medical sciences in Bosnia and Herzegovina.

During his lifetime, Academician Seid Huković linked the most significant names of the world scientific masterminds in the field of fundamental medical sciences, with whom he jointly contributed to the creation of a world treasury of discoveries that served the humankind.

While remembering him on his death anniversary, in the name of all of us, our big Thank You goes out to him.

Sarajevo, July, 2003

Editorial Board
ACADEMICIAN - SEID HUKOVIC
(1925-2001)

We were surprised with the news that a great scientist, pedagogue and humanist passed away on 5 July 2001.

In poor health, academician Seid Hukovic had daily and without break, actively performed very delicate scientific, cultural, humanitarian and educational tasks in the BiH Academy of Arts and Sciences (ANU BiH), at the University, in branch associations, citizens' associations, editorial offices of scientific and expert magazines. He was present in all segments of social developments.

And as the American and German pharmacologist and Nobel Laureate Otto Loewi (1873-1961) was a rare person who, in his career, brought together several generations of scientists whose names are deeply engrained in the history of medical science, the working years of academician Seid Hukovic links the most significant names of scientific master minds in the field of fundamental medical sciences and with whom he jointly contributed to the creation of a world treasury of discoveries that serve the humankind.

The life path of Seid Hukovic leads through two catastrophes: first, through the period of brutal developments of the World War II (1941-1945) and second, between 1992-1995 when, before the eyes of the world community, a drama took place, and unlike the classic war one, it imperiled all human, spiritual and civilized matters in our country. This period in time was particularly difficult for an elderly person, who, in this time of social turbulence, was responsible for preservation of dignity and integrity of academicians and the BiH Academy of Sciences and Arts as it's the then president. In poor health, often left to one's own resources and without support, every day he passed through parts of the city where people were being injured or killed and visited with the fellow citizens, colleagues, scientists, students, post-graduate students, doctoral candidates, those specializing in particular fields, academicians, humanitarian workers and was getting to all those places where anti-war, humanitarian, cultural, civilized and all other support of intellectual elite was expected.
Academician Seid Hukovic was born in Sarajevo on 1 May 1925. He completed elementary school and grammar school in Sarajevo and Medical Faculty in Zagreb with honors. He started working at the clinic in Sarajevo (1951) and then at the Institute for Pharmacology and Toxicology of the Medical Faculty in Sarajevo (1953), where, following his retirement in 1991, he was awarded the title Professor Emeritus.

Shortly after he started working at the Medical Faculty, he had established close cooperation with leading scientists in the country and abroad. He had been well informed of current researches in all fields of medical science. Together with many local and foreign scientists, he had worked on research projects, had had joint presentations at scientific symposiums, congresses and he published scientific papers in famous worlds magazines.

By analyzing several hundred references of Seid Hukovic, one can follow the development periods of medical science, fields that have been subject of scientists' greatest attention, new techniques as well as follow the development of basic medical sciences here, slow at first but later developing local basic medical sciences.

From 1953 to 1970, fields of morpho-physiological sciences were almost integrated. Teaching physiology at Medical Faculties included both biochemistry and patho-physiology. Number of engaged teachers was poor and the new scientific generation has only started to form. Therefore the cooperation of all pre-clinic teachers was a necessity and the commitment depended upon the current issues that were most frequently jointly researched and published. That is clearly visible in the published works of teachers-scientists of the Medical Faculty in Sarajevo who more or less all, even in spite of certain individual traits, worked as one research team.

In this period of time (1953-1960), first scientific publications of Seid Hukovic appear. He publishes them independently or in cooperation with other teachers and associates of the Medical Faculty in Sarajevo (Pavao Stern, V. Fukarek, P. Kosak, A. Rimski, Z. Madjarek, A. Misirlija, Z. Besarevic, R. Milin, M. Ciglar) and scientists from other research laboratories of the world (S. Cassentini, A. De Foli, L. Martini, Tani F., Sulla H. Burn). Scientific problems published in these 43 works belong to the field of pharmacology, physiology and patho-physiology of VNS (?), cardiovascular system and digestive tract, muscle physiology and endocrine system and immunology. They were published both in local and international magazines (Medical archive, Sarajevo; Ztschr. Kreislaufforsch.; Gastroenterologia.; Arch. Exp. Path. Pharmacoil.; Makedon. Med. Pregled, Naturwissenschaften; Neuropsihijatrija; Alergia und Asthma, Arch. Intern. Pharmacodyn.; Bolletino della Societa Italiana di bilogia sperimentale; Wiener klin. Wochenschr.; Naturwisenschaften; Acta Physiol. Pharmacoil. Neederlandica and others).

He defended his doctorate thesis (1958) at the Medical Faculty in Sarajevo and published the research results in "Dissertation Bull. Scientifique". The dissertation contains results on effects of serotonin and its enzymes involved in its metabolism in different model illnesses.
According to the number of published works, mostly in world scientific literature, it is evident that Seid Hukovic with his manager Pavao Stern represents a significant scientific potential of the Medical Faculty and the Sarajevo University. At that time, their names are generously quoted by the world scientific prominent scholars, doing research in the field of basic medical sciences. That is how the works of P. Stern and those he published with S. Hukovic were at the top, by its importance and topicality.

Then follows a period of research and publication of findings that cause a great interest in scientific circles.

Since the very beginning, Seid Hukovic attempts to introduce a number of objective criteria for assessment of experimental results. He researches a possibility of qualitative and quantitative identification of effects of endogenous and exogenous biological active substances on suitable biological objects. He engaged his wife Bubic Iduza, a known professor of anatomy at the Medical Faculty in Sarajevo, in this project. Her anatomist meticulousness was of particular use in the process of preparing isolated organs on which it is possible to test endogenous and exogenous biologically active substances and get reproducible, credible and implicit results of effects on certain organs, organic systems and the organism as a whole.

Work on isolated organs was known for several decades. Rudolf Morgan (1904) studied mechanical activities of smooth musculature on the segment of small intestine.

In time, through perfection of the isolated organs method, numerous researchers were able to register the moves of small intestine’s segment for several hours with the control and maintenance of constant Ph, temperature, isotony and iso-iony. Today, modern techniques offer the possibility of automatic registration, visualization and recording even those changes our senses do not detect.

The monograph of M.J. Rand and Michelson "The guts of the matter: Contribution of studies on smooth muscle to discoveries in pharmacology" contains the most credible evidence of the contribution of Seid Hukovic to the discovery of suitable isolated mono-tissue and poly-tissue test models. We will only quote parts of the second chapter titled “The vasa deferentia”.

Judging by the few subtitles in the same chapter ("2.2. Evidence for noradrenergic transmission in the vas deferens", 2.3. Doubts about noradrenergic transmission in the vas deferens: Effects of alpha adrenoreceptors antagonists"; 2.4. The two phases of the response of the vas deferens to nerve stimulation"), one can anticipate what the discovery of preparation of isolated vas deferens represented to the pharmacological and physiological research. One can also judge its scale and popularity through a number of published works in the last three decades. This preparation was extensively used in experiments for identification and characterization of biologically active substances that produce effects through adrenergic receptors.
Contemporaries of Seid Hukovic were obsessed with the receptor theory, introduced in the middle of spring last year and significance of which in medical science and biology in general is compared to that of atom theory in physics.

The basic classification of receptors starts in the works of Ahlquist (1948), through research of intrinsic activity and affinity as basis for molecular and biological quantification and identification of receptor subtypes.

Expansion of research of adrenergic biologically active substances, including techniques of isolated organs, particularly the isolated vas deferens, resulted in accumulation of evidence on existence of a vast number of receptors. Modern physiological and pharmacological techniques provided an answer to fundamental scientific questions on their functional importance, research possibilities of effects of exogenous, endogenous and synthetic biological active substances on certain organs, organ systems and organism as a whole and ultimately possible therapy effects.

Somewhat later, a method of synthetic radio-ligand, particularly the method of their connection, was used. Some of them can selectively activate or block receptors, resulting in the discovery of different receptor groups and characterization of numerous subtypes.

Until recently, unthinkable findings were available through molecular and biological research. Established was the exact structure of receptor genes and their ultimate products - receptor proteins. Today, that offers a possibility to research function and regulation of receptor processes. Up to five years ago, cloned were 150 genes of receptors connected to G-proteins and their number increases daily.

Today, the question of WHERE FROM and WHAT FOR is such a large number of receptor subtypes?

Scientifically funded, exact answer is only to come. However, based on previous cognition, it can be presumed that control mechanisms in adjustment of function's biochemistry are in question. Thus, effect research of interactions of different biologically active substances, whether applied or endogenously created, with adrenergic receptors, is particularly interesting to physicians who do not only deal with treatments but also research consequences of autonomous nervous system response to stress. By disturbing normal regulatory mechanisms, stress causes the state of disharmony in the physical and emotional life, which in the case of long duration, reflects itself in changes on highly specialized structures, that is - specific mechanisms of integration and correlation that guard bodily homeostasis.

Identified receptors subtypes are mutually different also through primary structure and functional differences relate to one or more of the following:
- differences in affinity toward natural hormones or neurotransmitter,
- differences in affinity for agonist or/and antagonists
- difference in anatomic location
- differences in mechanism of signal transduction and
- differences in regulation of receptor density (developmental, physiological or pathological).

Out of all approaches to identification of receptors, the most historically acceptable one was the functional approach that studies the effects of biologically active substances on isolated organs with or without the connecting nerves and on different organ systems.

Even next to all the advantages of described modern research methods used by the receptor theory, especially the molecular and biological one, the model systems in general, particularly those fourteen introduced by Seid Hukovic in scientific methodology represent the beginning of research in selection and pharmacological identification and characterization for modern pharmacologists and researchers working in pharmaceutical industry.

Seid Hukovic studies the effects of microtoxins and toxins of external and internal environment polluters on isolated organs. This is about the results of multidisciplinary research done within the implementation of American and other project tasks.

The efforts of Seid Hukovic to introduce the model of illness through provocation of artificial hypertension into the experimental pharmacology should be particularly mentioned. He studied the effects of bacterial toxins, nerve poisons, botulinus toxins, chemical and animal products.

His engagement in and his doubtless contribution to the reform of medical education is significant also. He was at the forefront in that field, at the level of community of Medical Faculties in former Yugoslavia.

As the education system here and in the world is full of outdated methodologies, Seid Hukovic cooperated with international education institutes that studied the existing systems and created modern education systems at universities. In that sense, he was at the forefront of those who sought replacement of the old with the new models in the system of undergraduate and permanent post-graduate education. His contribution in the field of post-graduate education consisted of imperative and constant innovation of existing program contents, adding on the existing ones and defining new ones. As the changes in medicine take place in great rapidity, every day modern technology essentially changes diagnostics, practice and methodology. Everything that happens in the field of medicine is of essential importance to the life and health of the population. That is why he insisted on such a reform of education of all the different medical staff that will through the system of automatism enable a dynamic educational process.
through daily mandatory following of all innovations that appear in the world and at home. This system commits the employees to education throughout their whole work careers. As in fundamental, experimental scientific research work, Seid Hukovic insists on assessment of education system through constant checks, rejection of everything that does not give better results, which does not provide better quality with greater rationality. He worked in a studious manner, without stopping, up until the moment when he left us for ever. Testament of that are numerous textbooks, scripts, monographs that students, physicians, stomatologists and pharmacists extensively use. However, the most valuable thing is what the students, those specializing, assistant and doctoral candidates heard from their modest teacher in direct contact. That contact with students was an integral part of practical training in regular post-graduate studies. However, Seid Hukovic had daily appointments with all those who needed advice, assistance or solution to a problem in direct conversation. He never denied the young people the possibility to address him, seek assistance, advice, recommendation and everything else they would ask him for. There were no limits to his love for his colleagues. He shared his enormous experience in a distinctive manner. An interlocutor, while talking to his professor, had a feeling that he is appreciated, respected, that his opinion is very important, that he has all the qualities of a future scientist and that he will certainly succeed with appropriate effort. By preserving the dignity of every person, he managed to plant in the seed of curiosity and animate a large number of young people for scientific and research work.

There is an impressive number of specialists, masters of science and doctors he was mentor to. Seid Hukovic's students take prominent positions in the country and abroad.

For his significant contribution to science, as a prominent world pharmacologist, he was member of all national and the greatest number of world associations of pharmacologists and physiologists as well as editorials of the most important world magazines. We will mention only some associations he was member of:

- Deutsche Pharmacologische Geselsschaft,
- British Pharmacological Society
- British Physiological Society
- European Society for Biochemical Pharmacology
- Royal Society of Medicine
- BiH Society of Pharmacologists, President
- Society of Physiologists and Pharmacologists of Yugoslavia (he was Vice-President)
- Member of BiH Society of Physicians etc.
- Member and Honorable President of the BiH Association of Basic Medical Scientists

He was editor-in-chief or member of editorial of numerous scientific and scholarly magazines at home and in the world. We are mentioning only a few he was member of for the longest time, and in some he was member till the end of his life:
Seid Hukovic had been invited to all world congresses, symposiums and other forms of communications with current world scientists. Most frequently, he was either the organizer or co-organizer.

He had established close cooperation with the largest local and international pharmaceutical producers in the expert field. As head of the administration office of the Institute for pharmacology and toxicology, he provided services that were legally defined. The Institute was a educational basis for specialized training in clinical pharmacology, drugs control and other pharmaceutical disciplines.

He was awarded with Certificate of Merit, Plaque and Diploma of Cambridge University, Men of Achievement Diploma, Medal of the Helsinki University, Plaque of the City of Sarajevo, Medical Faculty and University of Sarajevo, Prize of 27 July, three high state awards:

- Medal of Labor with gold wreath
- Medal of Merits for people with silver lines and
- Medal of Labor with red flag.

He was elected correspondent member of ANU BiH in 1968 and he became regular member in 1972. At the time, he was ANU BiH's youngest member. His name can be found in a large number of world encyclopedias. In the Academy of Sciences and Arts, he held the post of Secretary of medical department and President in the last decade. Academician Muhamed Filipovic, Vice-President of ANU BiH, writes about Seid Hukovic as member of ANU, in this issue.

This issue of JABMS is dedicated to the life and work of deceased academician Seid Hukovic. With two reviews, one written by ANU BiH Vice-President academician Muhamed Filipovic and other by the magazine's editorial, we are publishing a reprint of the following scholarly publications:

ASSOCIATION OF BASIC MEDICAL SCIENCES
OF BOSNIA AND HERZEGOVINA
Esteemed and Dear Seid!

Fifteen days ago we were making arrangements how to mark your scientific jubilee and we determined the particulars we will, God willing, put into action, even in your absence. There has long been a scientific ambiance you continuously enhanced in the Institute you have headed for many years. Many young people have completed their masters and doctorate thesis with your help. Today, they are the pillars of BiH medicine.

At this moment, I would like to say farewell to you, a great man and a giant in medical science. Therefore, I will present what are your medical discoveries of worldly value to this saddened gathering.

I told you how the other day we received a letter from our Italian colleagues, asking us to send them the materials from the First Symposium on P substance, held more than thirty years ago. Its importance in the algesic processes is revealing itself to the world these years only.

Or your unique contribution to physiological and pharmacological research in vitro when you added nerve responsible for innervation to a mass of isolated tissue and thus made the organ operate naturally, physiologically. Likewise, pharmacology gained a completely new experimental momentum, because we were able to, conditionally said, follow almost normal functions of organs and tissues and replace them with medications. Until then, for almost two hundred years, pharmacologists and physiologists had experimented with tissue mass without appertaining natural innervation.

Or your leadership in experiments where twenty years ago we demonstrated that clear specific characteristics do not exist in receptors. Leading world pharmacologists have included that in their books. A lot is being done today on undefined primordial formations of organism that can transform themselves into something specific.

Your work in the field of toxicology has brought this Institute a reputation of prominent toxicological center, where examined were the poison of lathamodeus, botulinus toxin and many nerve poisons. You have also founded the Academy of Science’s Board for research of chemical weapons during the aggression on our country and enabled us to know the size of our imperiled state.

With you, we had taken a great step ahead, past the basic pharmacology and had advanced our medical research with clinical and pharmacological methods. In Bosnia and Herzegovina, there are more specialists – clinical pharmacologists, than in many other countries with similar demographic characteristics. Clinical pharmacy, and pharmacy in general, developed along with this. You were not just a medical scholar, you were a leading person of all health-related and pedagogical sciences.

In order for all this to happen, it was necessary to have a person who mastered his calling to philosophical proportions. Only the scientist of your cerebral ability could have theoretically organized a medical and every other idea to its carrying out and proven value. In the world, others verified the values of your discoveries in their doctorates and demonstrated your outlined directions. That is what we - your successors - as well as our young associates do, publishing dozens of articles in world magazines and at congresses’ sessions. Scientific unrest with which you have contaminated almost forty of your generations and hundreds of
post-graduate students of all medical schools cannot be calmed. Scientific methodology has arrived in all parts of our beautiful homeland and the world.

The scientific reputation you acquired in the world enabled you to be a cosmopolite or, as you often pointed out, that a real person carries primeval tolerance and understanding of people. That is why in many social events, you have been an active participant and often a regulator of key developments. Bosnia and Herzegovina has few persons of your greatness and it will only now begin to discover what you meant and were worth to it.

To your wife Hilduza, your son Nedim and your relatives, we express our condolences with a message that they can, at any moment, rely on us, your colleagues and your Institute, as their trusted friends.

May you, the great one, rest in peace.

Muhamed Filipović
Human cloning is considered in theological and philosophical circles largely from the ethical standpoint. The arguments against human cloning in this type of contemporary theological and philosophical apologetics generally focus on three criteria which, it is claimed, the advocates of human cloning fail to take into consideration. These are:

a) moderation
b) limits
c) the entity as a whole

Permit me to set out in brief the main points of reference on which the arguments are based, in the same order.

**Human cloning and the criterion of moderation**

When debating how modern civilization differs from the civilizations of ancient Babylon, Egypt, Persia, India, China, Greece, and the Maya and Aztec civilizations, or with those of mediaeval Christendom and Islam, for example, there are many points at which one may give accurate and truthful answers. But certainly, to arrive at the appropriate answers, one must agree beforehand on the criteria by which the comparison between modern and ancient or mediaeval civilizations is to be made.

If we start, say, from the **criterion of moderation**, which the religions of all these ancient civilizations taught, it is clear that in ancient times the maxim of moderation in all things protected not only nature from the onslaughts of humankind, but also human nature from human assault.

The philosophers and men of religion who founded the Axial Age - Socrates, Buddha, Confucious and others - incorporated into their teachings and preachings certain interdictions beginning with "Do not", "Thou shalt not". This "Do not" advises us to be circumspect in regard to our actions: for humankind experiences incomparably greater misfortunes as the result of human action than from inaction. More human tears have been shed as a result of the malign use of human knowledge than from the inaction of ignorance.

There can be no criterion of moderation without interdiction. It is perfectly understandable, therefore, that the human cultures and religions of ancient times were based above all on commandments forbidding humankind to act in certain ways. The Bible and the Qur'an have their Ten Commandments, most of them interdictions. People knew, of course, that this "Thou shalt not" did not belittle the human being, but rather affirmed human dignity, uniqueness and moral rectitude on this earth, among the mineral, plant and animal worlds, and even among the spirit civilizations that the religions called angels, jinns, and satans.

The heavens forbade the mineral, plant and animal worlds nothing, nor, eo ipso, were they commanded as humankind is commanded. These worlds, or what we call nature, live out the balance that is bestowed on them - indeed, they are balance itself.

The way God maintains that balance in nature often seems harsh or cruel to us. Animals eat one another, plants come to life and burgeon in spring but with the relentless onset of autumn, nature dies down again. Population explosions among locusts bring corresponding increases in the flocks of birds that feed on them. We see in all this how the world of nature is pleasing to God, however obscure or puzzling the way He has ordained it may seem to our minds, however little sense we may be able to make of earthquakes, floods, volcanic eruptions, or destructive tornados. There is no human court that can put God or nature on trial for the ravages wrought by natural disasters. However horrifyingly powerful the technology we now possess, our most effective response to an ordinary earthquake is still our humble prayers to God.

And yet, these events in the world of nature, impenetrable to our minds, are but the incessant manifestation of natural equilibrium. And it is only ourselves who are able to inflict deliberate disorder on the mineral, plant and animal worlds.

As a result, humankind must be commanded to observe balance and moderation, for we are not merely beings of necessity and nature, but beings of freedom and culture.

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1 They say that Buddha taught inaction and abstention from speech: he sat silent beneath the Bo-dhi tree, and acted but little in Kapilavastu for forty years, teaching his disciples to behave in the same way.
In short, the heavens have sought to bridle us, human beings, with endless constraints. We are not merely beings of nature, not merely part of the natural animal environment; we are world beings. And this means that we are beings with immense potential, both good and evil as well as ethically neutral. It is no doubt because of this human potential, because of these multiple relationships between humankind and the world, that we have been hedged about with so many religious interdictions. They stand before us, before our views, our actions and our thoughts, as a warning or caution.

Modern theology asserts that the religions of the ancient civilizations affirm men and women as universal beings, but that the universe of religious commandments says human universality is contradictory and dangerous. The infinite starry heavens above us call to us, inviting us to invoke the scientific, irresponsible technological mind against human nature. Human cloning is going too far, and present-day theological writings see it as a kind of rebellion against the Divine order in which His creatures are born.

If we recite a Biblical or Qur'anic commandment - Thou shalt respect thy father and thy mother, say, or a prohibition, such as Thou shalt not commit adultery - it awakens in us a sense of limits, of the fact that a parent is an inviolable boundary to the child and the child to the parent, the mother - in her motherhood - to her son, and that she watches over that boundary purely as a mother.

True, in exercising our relative freedom, we may not only transgress those religious prohibitions and go beyond the bounds, but also break the laws of the state. Human cloning, and the cloning of other living beings, is a direct betrayal of the criterion of moderation, consistent with which humankind must live on this earth.

The criterion of the limit

Here we come to the criterion of limits, the bounds that the moral law within us warns us of, whenever we permit it to do so. The great religions - Christianity, Islam, Judaism, Buddhism and others - all count on that moral threshold within us, and the main calling of those ancient teachings, as before and since what cultural anthropolog- 

2 This modern civilization of ours, rightly called technological, has trampled many criteria underfoot, including that of moderation. I invite everyone to reflect on the fact that as we are discussion our topic, at least five hundred million private vehicles in motion the world over, at least two thousand aircraft are in the air, and that never before has so much fuel been consumed on earth at the hand of man. The spirit of the times in which we are living is that our planet is infinite and that all its resources are inexhaustible.

3 The Qur'an refers to the people of Lot, who went beyond the bounds of married life.

4 In his studies of so-called primitive societies and communities untouched by the civilizations of either Christendom or Islam, Claude Levi-Strauss noted that they have perfect customs and systems of taboos that forbid murder, theft, incest and the like. All the fundamental family regulations very similar to those of the great religions are already to be found in these primitive societies. A mother is a mother, and is a limit; a daughter is a daughter, and she too is a limit. Levi-Strauss convincingly demonstrates that primordial humans always have a primordial belief in God. (See Claude Levi-Strauss, Anthropologie structurale dux, Libraire Plon, Paris, 1973).
Human cloning, as theological and many philosophical writings on cloning note, is a violation of the limits set by God. Theologians and philosophers who are opposed to cloning ask simple questions such as: Who is the mother of the clone? Who is its father? Is a cloned person deprived of the mystery of natural creation and spontaneity? Do we have the right, by cloning someone, to deprive him or her of the distinctiveness that is the result of creation?

In short, do we have the right to copy someone's face, that miraculous bodily island through which our soul manifests itself, and with which that soul regards all the wonders of this world. That face we each have is so diverse, so utterly ours. It is the seal of God, the Divine warrant of our authenticity, a guarantee that we are not a copy or a counterfeit; it is the warrant that He has created us as a unique entity, thereby dedicating to us the Divine Universality, the Universality of His Mercy which He bestowed upon us at the moment of our creation.

But why do we need to be reminded of these limits?

Probably because we are faced with many broad paths, far broader than any other creature. While religion claimed that these paths may be safely trodden only by those whose provisions for the journey include an inviolable respect for the limits, the technological age has made us chafe against those limits. The technological spirit celebrates Prometheus and his theft of fire from God. At the height of the technological age, as the twentieth century is known, when more than fifty million people were killed in the two world wars alone, Karl Jaspers, prompted by a sense of responsibility in his philosophical thought, developed the doctrine of the human being in extreme situations - situations at the boundary. Birth is a boundary, sex - being born as male or female - is a boundary, being born rather than cloned is a boundary, language is a boundary, disease is a boundary, corporeality is a boundary, spirituality is a boundary, death is a boundary. It is a boundary, an extreme situation, that we have our own, not someone else's face and person, and that we share this with no one else.

Karl Jaspers' message in his doctrine of extreme situations in human existence is clear: there is and can be no technology that is able in a moral fashion to transcend or abolish these extreme situations, these boundaries of humankind.

The criterion of the entity as a whole

The acquisition of an awareness of and respect for the limits enables us to recognize the criterion of moderation, which is extremely important. The criteria of moderation and of the limits are closely related to that of the separate entity. The human individual, a bird, a blade of grass, an earthworm: all these are separate entities, individual examples of the whole. But every being experiences its separate entity within another, wider whole. The entity in which a human being is human is not an autonomous entity. We are still somehow connected with the multiplicity of things known and unknown, joined by countless umbilical cords that can never be severed, linking us to visible and invisible entities. We breathe the air, are able to walk thanks to the weight of the entire world, draw our nourishment from the animal and plant world as a whole, and so on. It is as though our separate human entities, like our human destiny, were articulated into the entities and destinies that surround us like a myriad concentric circles. This is how things are when we consider them in their outward aspects.

But an entity also has its inner aspects. Theologians and philosophers who oppose cloning claim that the Divine act of creation takes place through the creation of an entity, not by copying it or creating a part-entity. A grain of
wheat is an entity, an ant is an entity, a bird is an entity, a human being is an entity. Creation is always the creation of an entity. It is impossible to give birth just to a heart, or a pair of lungs, or other organs required for transplant.

True, there are attempts to ascribe human intentions to cloning, with assertions that by cloning or copying we shall obtain what we need - a heart, kidneys, a knee-joint or whatever. And to obtain the part, we need to clone the whole, since the only way to get the part is to take it from the whole that made it possible.

Theologians are unanimous in their view that if cloning succeeds, it will raise a whole range of ethical, legal and moral issues. It is a deprivation of mother, father, kin-dred; a deprivation of what we call the soul, the self, the individual view of the infinity of the heavens. Whatever the outcome of cloning may be as an entity, the ban on killing it to obtain the organs that some say it will offer will still stand.

Cloning is yet another attempt to use technology to escape death or, if that is impossible, to defer it. Cloning is an attempt by technology to steal from God the mystery of creation, so that we might laugh at eternity without experiencing death.

The religions claim that there is eternity, but that the only way to it is through death.

I recall a short verse from the Qur'anic Sura an-Najm (the Star: v. 24): "Does man imagine that it is his due to have all that he might wish for?"
Abstract

Drugs, natural medicinal plant, animals and mineral materials, have a large and various application in official pharmacy and medicine. Carriers of multilateral pharmacological effects that those drugs shown, are chemically define as active components that are present in them. Methods of qualitative and quantitative analysis are used for the chemical investigation of components that drugs contain. Method of thin layer chromatography has been shown as very reliable.

According to the chemical investigation of single drugs, it is possible to define a group of compound or single compound comparing them with standards. Relating to the usage of method of thin layer chromatography, it has been carried out investigation on presence of coumarins and flavonoids in domestic plant material that have wide everyday usage. Coumarins and flavonoids from the point of view of chemical belonging are phenol derivatives with important pharmacological effects.

Applying method of thin layer chromatography, it is detected presence of coumarins and flavonoids substances in plant material that has been tested. *Anethi graveolens fructus et folium* (fruit and leaf of dill), *Anethum graveolens* L., Apiaceae, *Avenae sativae fructus* (fruit of oats), *Avena sativa* L., Poaceae and *Asperulae odoratae herba* (sweet woodruff), *Asperula odorata* L., Rubiaceae. Chromatograms are developed in systems cyclohexane-ethylacetat (13:7) and toluene-ether (1:1) saturated with 10% acetic acid, and visualisation by observing on UV lamp (254 and 366 nm), spraying with reagents KOH (10% ethanol solution) and diphenylboryloxyethylamine (1% methanol solution).

Key words: TLC, coumarins, flavonoids, dill, oats, sweet woodruff

Introduction

Chromatography belongs to the group of analytical separation methods that are applied for identification, purification and determination of contents of substances or preparations. The separation of different components using chromatography is based on dynamic distribution of dissolve substance between two fazes that are unmixed and one of them must be mobile in relation to one other. The immobile faze slow down movement of the melted components, enabling single substances to be separated under certain conditions. Using chromatographic methods makes possible to separate substances, which have small difference from the point of view of the chemical structure, because they have similar physical and chemical properties, that chromatography put in advantage respect to other methods (1, 2).

Analysis of natural raw material, drugs and isolated substances, according to principles of pharmacognosy is carried out mostly by using methods of thin layer chromatography and column chromatography (3, 4). They were applied for qualitative and quantitative analysis of drugs, natural products and preparation with natural components.

A series of advantages, respect to the other chromatographic methods, gives the priority in usage of thin layer chromatography in pharmacognostic and phytochemical analysis of drugs. It distinguished, in addition to relatively fast application, well separation that can be archived using small quantities of samples, and with thin layer of adsorbents, and the results obtained are reproducible under standardised conditions.

In that sense it was carry out analysis of chemical composition of plant material using method of thin layer chromatography and that was shown in this work. It was carry out the analysis of following drugs *Anethi graveolens fructus et folium* (fruit and leaf of dill), *Anethum graveolens* L., Apiaceae, *Avenae sativae fructus* (fruit of oats), *Avena sativa* L., Poaceae and *Asperulae odoratae herba* (sweet woodruff), *Asperula odorata* L., Rubiaceae.

Dill is herbaceous annual plant that was grown as spice plant in the garden, but also in plantation. It is of use its mature fruit, leaf, but also the upper overgrown part of the plant in bloom as herbs. The fruits contain ethereous oil with carvone, limonene, than fatty oil, coumarin derivatives, and proteins. Leaf or herbs contain essential oil, vitamin C, pro-vitamin A, flavonoids. It is in use as carminative, stomachic, diuretic, bland sedative, lactagogue. Use of fruits extract as antispasmodic in light
forms of chronic coronary insufficiency, preventively against asthma and contraction of abdominal organs, is very important. As specie is in a large use (5, 6).

Oath is cultivated cereal that grows everywhere, even in a poor field. It is in use-husked fruit obtained from cultivated plant. It contain silicon acid, flavonoids, avenacin, avenacoside, coumarins, vitamin C and B-complex, amide, proteins, lecithin, fatty oils, mineral material, especially iron, iodine, wax and sugars. It is in use as food, dietetic and medicine. It use as sedative and diuretic in homeopathic medicine. It is very important as stimulant of immunity (6, 7).

Sweet woodruff is herbaceous plant wide spread in forest area especially in beech forests. It is useful over ground part of the plant during flowering period. Drug contains coumarins, iridoid glycosides, tannins, bitter substances and essential oil. Preparations with sweet woodruff are used as anti-inflammatory, lymphokinetic, antispasmodic and as diuretic, bland sedative and aromatic. The drug is not explored and used sufficiently (8, 9).

Investigation of the above mentioned drugs were carried out with thin layer chromatography method according to the principles of this method. Adsorbent was silica gel 60 F254, on which were applied methanolic extracts of drugs and respectively coumarins and flavonoids standards. Chromatograms were developed in two systems that literatures cite for analysis of coumarins and flavonoids phenolic derivatives.

In this way we would like to ascertain possibly optimisation in separation of these components that limit use of drugs that contain them (10, 11). Separated coumarins and flavonoids spots show blue-white and yellow fluorescence under UV-lamp on 254 and 366 nm that became more intense after spraying with reagents ethanolic potassium hydroxide and diphenylboryioxyethylamin. This is very evident after spraying with second reagent under UV-366 nm. The results we obtained were shown on photos and tables that contain Rf-values of separated spots.

Standard substances fraxin, fraxetin, aesculin, aesculetin were supplemented with coumarin substances obtained synthetically.

Their aim of usage is comparison between coumarin nucleus behaviours, such is contained in natural material and which is result of metabolic process of natural living and plant environment, and those, which are, produced synthetically (12).

Material and methods

In this work the analysis have been carried out on presence of coumarins and flavonoids in selected plant material by using method of thin layer chromatography. This method was used for qualitative chemical analysis. Plant species we utilized for analysis are: dill, *Anetum graveolens* L., Apiaceae, oats, *Avena sativa* L., Poaceae and sweet woodruff *Asperula odorata* L., Rubiaceae. All of three plant species give drugs that are: dill, *Anethi graveolens* fructus, fruit (Figure 1), *Anethi graveolens* folium, leaf (Figure 2), oats, *Avenae sativae* fructus, husked fruits (Figure 3) and sweet woodruff, *Asperulae herba*, over ground part (Figure 4). Fruit and leaf of dill had been collected in surroundings of Sarajevo and dried as drug. Leaf was dried binding over ground parts, herb, keeping it on dray and ventilated place. The fruit was collected in mature state, and was dried in thin layer on drayed and ventilated place. Along with draying of leaf of dill, it is necessary to conserve its green colour, while with its fruit the contact with humidity should be avoided. Mature fruits of oats were collected on ownership in surrounding of Sarajevo. Sweet woodruff was acquired as already prepared drug.

Method of thin layer chromatography was carried out under following conditions.

Extracts of plant material, of investigated drugs, were prepared in the following way: powdered drug (1g) is extracted with methanol (10ml) for 30 minutes under reflux on the water bath; filtrate is evaporated to about 1 ml, and 10 µl is used for TLC investigation. In this way,
extracts are prepared and bring on chromatogram by following sequence:

1. *Anethi graveolens fructus*,
2. *Anethi graveolens folium*,
3. *Avenae sativae fructus*,
4. *Asperulae herba*.

Methanolic solution (0.1%) of coumarins and flavonoids substances is used for standard, more precisely coumarin derivatives fraxin, fraxetin, aesculin, aesculetin and three coumarins as synthetic substances. Fraxin and aesculin are hetero-side compounds, fraxetin and aesculetin are their corresponding aglicos, which are liberated by hydrolysis of hetero-sides, during which glycolic part is separated. Synthetic coumarins are utilized as standards because of theirs coumarin structure which can give information in correlation with natural coumarin substances from their analytics as well affects point of view.

For standards substances of flavonoid structure are used methanolic solution (0.1%) of quercetin ("Kemika", Zagreb) and rutin ("Merck", Darmstadt). Pre-coated TLC plates with adsorbent silica gel 60 F254, layer thickness 0.25, "Riedel-de-Haën" Seelze, Germany, dimension 20x20 cm are used. Extracts were brought on thin layer with capillary, dotted with Nanomat IV "Camag", Muttenz, Switzerland.

Chromatograms are developed in systems of solvents, which are specific for coumarins and flavonoids substances, cites in literature like that one (3).

Presence of those derivatives is investigated by developing in systems

a) Cyclohexane-ethylacetate (13:7); developing values d2 is 14.5 cm (travelling of mobile phase was 60 min).
b) Toluene-ether (1:1), saturated with 10% acetic acid; developing values d2 is 14.5 cm (travelling of mobile phase was 55 min).

Detection of developed spots is carrying out according to the principles of visualisation of chromatograms. First, chromatograms are observed under UV-lamp on 2 standard wavelengths, 254 nm and 366 nm. Fluorescence of separated spots in comparing to fluorescence of standards could be considered reliable information in identification of analysed coumarin and flavonoid derivatives. This substance gives intensive fluorescent spots of blue-white and yellow colour as standards and those corresponding spots got from drug’s extracts. Chromatograms are sprayed with reagents for identification, attaining reinforcement of intensity of fluorescence, thanks to them. Reagents are following:

a) KOH-ethanolic (10%), reagents for identification of presence of coumarins substances
b) Diphenylboryioxyethylamin (Merck, Darmstadt)-methanolic (1%), reagents for identification of flavonoids substances.

The chromatograms we obtained are presented in original photos made after developing and observing under UV-lamp before and after spaying with abovementioned regents (Figure 5,5a,5b,6,6a,6b,7,7a,7b,8,8a,8b).

Results and discussion

In this work chromatographic analysis have been carried out on presence of coumarins and flavonoids substances in selected plant material. Method of thin layer chromatography, which is quoted in pharmacopoeia’s regulations for analysis of drugs but also other substances, it is utilised for qualitative chemical analysis of drugs (13, 14). The plant material we analysed, leaf and fruit of dill, fruit of oats and sweet woodruff, are drugs in everyday usage and that is why chemical study represents an important information. Taking food into man’s organism is not the only importance of this studies but also the possibility of preparation of different pharmaceutical medicaments intended to the specific pharmacologic indication. From viewpoint of pharmacological affect, coumarins and flavonoids represent very important groups of pharmacologically active chemical compounds present in plant material, but also the substances that are obtained semi-synthetically or synthetically trying to meliorate pharmacological efficiency.

By chromatographic analysis of drugs, Anethi graveolens fructus, Anethi graveolens folium, Avenae sativae fructus and Asperulae herba, with corresponding standards, the chromatograms we obtained gives next results:

![Figure 5](image_url)

**Figure 5.** System, cyclohexane: ethylacetat (13:7); Visualization UV-254 nm

![Figure 5a](image_url)

**Figure 5a.** System, cyclohexane: ethylacetat (13:7); Visualization UV-366 nm

![Figure 5b](image_url)

**Figure 5b.** System, cyclohexane: ethylacetat (13:7); Visualization UV-366 nm, sprayed with KOH/EtOH (10%)

The presence of coumarin substances in analysed plant material under determinate conditions, after visualisation with UV-lamp and spraying chromatogram with ethanolic solution of potassium. Standard of hetero-side fraxin and its aglicon fraxetin, then hetero-side aesculin and its aglicon aesculetin belongs to the group of coumarin sub
stances with basic coumarin nucleus. The same structure had the synthetic coumarin substances, which we used in experiment.

All samples we examined, on start line show the spots which correspond to standards of fraxin (A) and aesculin (C), blue fluorescence (UV-254 nm), intensively blue-white (UV-366 nm), that reinforce after chemical treatment with ethanolic KOH, with Rf-0.01, values which are in correlation with literature data about this coumarin aglicons (3). Standard of fraxetin (B) has Rf-0.05, and the spot is blue-brown (UV-254), more exactly blue-dark
Standard of aesculetin (D) has Rf-0.08, in zone of fluorescence 254 nm show blue spots, or blue-white fluorescents on 366 nm. From synthetic coumarin compounds, S3 show blue-brown fluorescence (UV-254 nm), Rf-0.05, as aesculetin. In system toluene-ether, saturated with acetic acid, synthesised compound remain on start line together with standards of fraxin and aesculin. As we mentioned before all comparative coumarin standards has the basic coumarin structure. For the separation of coumarin substances, the system we applied on had shown to be good, apposite to system toluene-ether, saturated with acetic acid, a little bit better then system cyclohexane-ethylacetat (Figure 5, 5a, 5b, 6, 6a, 6b, Table 1).

Methanolic extracts Anethi graveolens fructus, and Anethi graveolens folium in system cyclohexane-ethylacetat gives the spots of brown fluorescence (UV-254 nm) on start line, as standard quercetin, Rf-0.01. Dark-brown zone of fluorescence on Rf-0.15 show extract of Asperulae herba together with rutin standard (UV-254 nm). From extract of Avenae sativae fructus remain on start line spot of light-brown fluorescence (UV-254 nm), Rf-0.09, which is identical with behaviour of rutin standard, Rf-0.11.

In zone UV-366 nm, fluorescence of spots is blue (Rf-0.01), and after spraying with reagents diphenylboryoxyethylamin, the spots show intensive yellow fluorescence (Rf-0.01), and Rf-0.34 that is given from methanolic extract of Asperulae herba.

In system toluene-ether, saturated with acetic acid, from extract of Avenae sativae fructus the separated component in zone UV-366 nm show yellow fluorescence (Rf-0.16) the same as rutin (Rf-0.15), with close Rf values (Figure 7, 7a, 7b, 8, 8a, 8b, Table 2).

The results we obtained from chromatographic analysis of examined drugs, show that they contain coumarin and flavonoid substances, as part of there own metabolism, which is in correspondence with literature quotation From the type of coumarins and flavonoids point of view, especially their percentage contents, investigations continue, by using other necessary methods of qualitative and especially quantitative analysis.

The results obtained, also, show the justify utilize of this drugs as medicament natural raw material, and especially present contribution on better and bigger employment of sweet woodruff in pharmacy and medicine.
### Table 1. Rf-values of separated components (analyses of coumarins)

<table>
<thead>
<tr>
<th>Sample</th>
<th>System I*</th>
<th>System II**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Anethi graveolens fructus</em></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.45</td>
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<tr>
<td></td>
<td>0.84</td>
<td>0.80</td>
</tr>
<tr>
<td>2. <em>Anethi graveolens folium</em></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.13</td>
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<tr>
<td></td>
<td>0.30</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>3. <em>Aveanae sativae fructus</em></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>4. <em>Asperulae odoratae herba</em></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>0.98</td>
<td>0.96</td>
</tr>
<tr>
<td>A Fraxin</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>B Fraxetin</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>C Eseculin</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>D Esculetin</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>S1 Synthetic coumarin</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>S2 Synthetic coumarin</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>S3 Synthetic coumarin</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* System I - cyclohexane: ethylacetat (13:7)
** System II - toluene: ether (1:1), saturated with 10% acetic acid

### Table 2. Rf-values of separated components (analyses of flavonoids)

<table>
<thead>
<tr>
<th>Sample</th>
<th>System I*</th>
<th>System II**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Anethi graveolens fructus</em></td>
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<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.2</td>
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<td></td>
<td>0.32</td>
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<td>0.37</td>
<td>0.38</td>
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<tr>
<td>2. <em>Anethi graveolens folium</em></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>3. <em>Aveanae sativae fructus</em></td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>4. <em>Asperulae odoratae herba</em></td>
<td>0.34</td>
<td>0.01</td>
</tr>
<tr>
<td>E Quercetin</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>F Rutin</td>
<td>0.11</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* System I - cyclohexane: ethylacetat (13:7)
** System II - toluene: ether (1:1), saturated with 10% acetic acid
References


13. European Pharmacopeia, 3rd editon; Council of Europe, Strazburg, 1997

Abstract

Panic disorder (PD) is an acute psychobiologic reaction manifested by intense anxiety and panic attacks, that occur unpredictably with subjective sense of intense apprehension or terror, accompanied by temporary loss of the ability to plan, think, or reason and the intense desire to escape or flee the situation. Panic attacks may last from a few seconds to an hour or longer. Symptoms typically include, among others, palpitations, tachycardia, hypertension, chest pain, dyspnoea, and fear of losing control or going crazy and vague feeling of imminent doom or death. Since pharmacotherapy of PD includes the administration of selective serotonin reuptake inhibitors and tricyclic antidepressants, the objective of this study was to perform a pilot double blind clinical trial designed to compare the effects of two studied drugs in the treatment of PD.

A total number of 40 patients with a history of panic disorder were randomly assigned into two groups of 20 patients each. Hamilton anxiety rating scale and Standard Psychiatric Interview were methods for PD assessment. One group was treated with clomipramine hydrochloride (ANAFRANIL®) 75 mg/day and the other with fluoxetine (OXETIN®) 60 mg/day. Both drugs were administrated by mouth (PO) two times-a-day in equally divided doses for 6 weeks.

Both studied agents produced similar antipanic effectiveness. Favourable response was achieved in 95% of patients treated with fluoxetine and 90% of patients treated with clomipramine. The onset of antipanic effects was quicker in all clomipramine treated patients, while fluoxetine produced more-favourable response in male patients. The duration of treatment with both antidepressants studied should be at least 10 weeks, instead of 6 weeks.

Key words: panic disorder, clomipramine hydrochloride, fluoxetine hydrochloride, antipanic effectiveness.

Introduction

Panic disorder is a psychiatric disorder characterized by recurrent and unpredictable panic attacks involving a feeling of terrifying fear and extreme discomfort with an impending sense of doom (BENNETT et al., 1998). It may be accompanied by temporary loss of the ability to plan, think, or reason and the intense desire to escape or flee the situation. This disorder affects approximately 2% to 4% of the population (BALLenger et al., 1998). It usually begins in the late adolescence or early adulthood and affects women two to three times more often than men.

Panic attacks are the main feature of panic disorder, but other problems may also include anticipatory anxiety, panic-related phobias, poor overall well-being, and disability (BALLenger et al., 1998). Whilst panic disorder is uncommon, affecting less than 1% of the population in a six month period, panic attack ase common, affecting more than 1/3 of the population in a single year. Panic attacks usually last from a few seconds to an hour or longer, vary in frequency from several times a day to once a month and they are accompanied by the strong body reactions or profound physiological effects.

Symptoms of a panic attack typically include chest pain or discomfort, choking, dizziness, unsteady feelings or faintness, fear of dying, fear of becoming insane or of losing control, feelings of unreality, strangeness or detachment from the environment, flushes or chills, nausea or abdominal distress, numbness or tingling sensations, palpitations or accelerated heart rate, shortness of breath or smothering sensation, sweating, and trembling or shaking.

Most persons suffering from panic attacks recover without treatment, and a few develop panic disorder. For these persons, especially without treatment, panic disorder follows a chronic waxing and waning course. Pharmacotherapy and behaviour therapy usually help to control symptoms of panic attacks. There is strong evidence suggesting that selective serotonin reuptake inhibitors and tricyclic antidepressants are effective therapies (BALLenger et al, 1998). These drugs can prevent or greatly reduce the number and intensity of panic attacks and are usually recommended as first-line agents for the treatment of panic disorder (BENNETT et al, 1998).

Objective

Since pharmacotherapy of panic disorder includes the administration of selective serotonin reuptake inhibitors and tricyclic antidepressants as first-line agents in the
treatment of this disorder, the objective of this study was to perform a pilot double-blind clinical trial designed to compare the effects of the representatives of those two groups of drugs in the treatment of panic disorder.

**Patient and trial characteristics**

**Type of trial**
A pilot randomized double-blind trial.

**Patient selection**
A total number of 40 patients (23 males and 17 females) with a history of panic disorder, between 35-45 years of age and of similar educational background.

**Panic disorder assessment**
- Hamilton anxiety rating scale (HAMA) (HAMILTON, 1959)
- Standard Psychiatric Interview (SPI)

**Inclusion criteria**
- Only HAMA diagnostic criteria proven panic disorder
- Only SPI diagnostic criteria proven panic disorder

**Exclusion criteria**
- History of anxiety-depressive disorder
- History of generalized anxiety
- History of abdominal upset or gastrointestinal complaints (hiatus hernia)

**Drug administration**
Patients with proven panic disorder were randomly assigned into two groups. Each group of 20 patients was treated with one of two investigated drugs, which were administered by mouth two times-a-day in equally divided doses for 6 weeks:

- One group (12 males and 8 females) was treated with **clomipramine hydrochloride** (ANAFRANIL®) 75 mg/day b.i.d. Initial dose of 25 mg/day was increased gradually to a maximum of 75 mg/day during the first 2 weeks (depending on patient tolerance).
- The other group (11 males and 9 females) was treated with **fluoxetine hydrochloride** (OXETIN®) 60 mg/day b.i.d. Initial dose of 20 mg/day was increased gradually to a maximum of 60 mg/day during the first 2 weeks (depending on patient tolerance).

**Results**

*Favourable response* (reduced frequency and severity of panic attacks, prolongation of the period of clinical remission from panic attacks, decrease in anxiety and phobic avoidance and improved quality of life) was achieved in 19 (95%) patients treated with **fluoxetine hydrochloride** and 18 (90%) patients treated with **clomipramine hydrochloride**.

**Clomipramine hydrochloride** produced quicker effects (onset after 2 weeks) and complete recovery in almost all treated patients with less (20%) adverse reactions (tremor, nausea) in 4 patients, sex regardless (2 females and 2 males).

**Fluoxetine hydrochloride** showed a greater but slower efficacy (onset after 3 weeks) in male patients with more (35%) adverse reactions in seven female patients (drowsiness, nausea, vomiting, sweating, hyperhydrosis, insomnia).

The effectiveness of clomipramine hydrochloride and fluoxetine hydrochloride treatment with regard to sex is shown in Graphs 1 and 2, respectively.

**Discussion**

Panic induction has three postulated neurochemical pathways: benzodiazepine receptor binding, noradrenergic function, and serotonergic function. Evidence suggests that it is the serotonergic component that modulates the proposed noradrenergic and benzodiazepine receptor binding mechanisms (De VANE, 1997). The relationship between serotonin and anxiety is very complex (NUTT, 1998). There are two opposing theories: one involves an excess of serotonin and the other a serotonin deficit. Multiple regions of the brain appear to be involved along with the serotonin receptors (NUTT, 1998).

Patients suffering from panic disorder should be told that their disorder results from both biologic and psychological dysfunction and that pharmacotherapy and behaviour therapy usually help control symptoms. Since pharmacotherapy of panic disorder may include the administration of selective serotonin reuptake inhibitors and tricyclic antidepressants, as effective first-line initial agents for the treatment of this disorder, the objective of this study was to perform a *pilot double-blind clinical trial* designed to compare the effects of the representatives of those two groups of drugs in the treatment of panic disorder. **Clomipramine hydrochloride** (ANAFRANIL®) and **fluoxetine hydrochloride** (OXETIN®) were representatives of tricyclic antidepressants and **selective serotonin reuptake inhibitors**, respectively.

The exact mechanism of action of clomipramine is not known. The drug is classified as a tertiary amine tricyclic antidepressant with very potent serotonin uptake blocking activity and moderate blocking activity for noradrenaline (BERTILSSON et al., 1974; ASBERG et al., 1977). On the other hand, its active metabolite, desmethylclomipramine, is a potent noradrenaline uptake inhibitor...
and may retain some serotonin uptake inhibition (BENFIELD et al., 1980). Fluoxetine is a "second-generation" antidepressant agent, which is a specific inhibitor of serotonin reuptake (STARK et al., 1985). Treatment initiation of these agents is recommended at low doses with slow increases (BALLINGER et al, 1998). Outcome of drug therapy has not been associated with baseline frequency of panic attacks (DAVIDSON, 1998). However, level of phobic avoidance at baseline has been an important predictor (DAVIDSON, 1998).

It has been shown that both agents used produced similar antipanic effectiveness, but more- favourable response was achieved with fluoxetine hydrochloride (95%) than with clomipramine hydrochloride (90%). Clomipramine hydrochloride (ANAFRANIL®) produced quicker effects (onset after 2 weeks) and complete recovery in almost all treated patients, sex regardless. Fluoxetine hydrochloride (OXETIN®) showed a greater but slower efficacy (onset after 3 weeks) in males, than in females. These results are in agreement with other data sources. Clomipramine has been studied with favourable results (den BOER, 1998) and it has been found to be effective in the treatment of panic attacks during four clinical trials using this drug. It has been reported (CAILLARD et al., 1999) that low-dose clomipramine (60 mg/kg) was as

Graph 1. The effectiveness of clomipramine hydrochloride (ANAFRANIL®) (75 mg/day p.o.) treatment by sex

Graph 2. The effectiveness of fluoxetine hydrochloride (OXETIN®) (60 mg/day p.o.) treatment by sex
effective as high-dose clomipramine (150 mg/kg) in the treatment of panic attacks (the number of DSM-III-R symptoms of panic attacks was decreased) in a multi-centre clinical trial which lasted 8 weeks. BROOCKS et al. (1998) have reported that in a randomized, placebo-controlled, 10-week study, clomipramine (increasing doses over three weeks up to 112.5 mg/day) was found to be more effective for the treatment of panic disorder, and that significant (p<0.001) improvement was seen after only 4 weeks. It has been shown (PAPP et al., 1997) that clomipramine (initial dose of 10 mg/day was increased slowly to the mean daily dosage of 96.9 mg after 13 weeks of treatment) produced marked or moderate improvements in 84% of patients with panic disorder. PERNÁ et al. (1997) have conducted double-blind, randomized, placebo-controlled clinical study using clomipramine (10 mg for 3 days and 20 mg for 4 days) and have published that this drug was effective in decreasing panic attacks of patients with panic disorder. Fluoxetine has been found to be effective for treating panic disorder and to be useful, when administered in weekly doses, for preventing recurrence of panic disorder during two clinical trials using this drug. It has been reported (MICHELSON et al, 1998) that fluoxetine (10 or 20 mg/day) was effective (significant reduction in the Clinical Global Impression improvement scores and significant reduction in total panic attack frequency) and tolerated well in patients with confirmed panic disorder in a 10-week, double-blind, randomized, placebo-controlled clinical trial. EMMANUEL et al. (1999) have published that fluoxetine (10-60 mg/week) prevented recurrence of panic attacks in patients with panic disorder for periods of 1 to 26 months.

Patients with panic disorder are particularly sensitive to the adverse effects of medicines. These patients often misinterpret them as anxiety symptoms and thus start the vicious cycle of escalating anxiety that leads to further panic attacks (BALDWIN and BIRTWISTLE, 1998). Clomipramine hydrochloride (ANAFRANIL®) produced less (20%) adverse reactions (tremor, nausea) in four patients, sex regardless (2 females and 2 males). Fluoxetine hydrochloride (OXETIN®) showed more (35%) adverse reactions in seven female patients (drowsiness, nausea, vomiting, sweating, hyperhydration, insomnia).

**Conclusions**

A pilot clinical trial, designed to compare the effects of the representatives of tricyclic antidepressants and selective serotonin reuptake inhibitors in the treatment of panic disorder, was performed.

Two agents used were: clomipramine hydrochloride (ANAFRANIL®) as a representative of tricyclic antidepressants and fluoxetine hydrochloride (OXETIN®) as a representative of selective serotonin reuptake inhibitors.

Both agents used produced similar antipanic effectiveness, but more-favourable response was achieved with fluoxetine hydrochloride (95%) than with clomipramine hydrochloride (90%).

The onset of antipanic effects was quicker (two weeks) in clomipramine hydrochloride treated patients, than in fluoxetine hydrochloride treated patients (three weeks).

The effectiveness of clomipramine hydrochloride and fluoxetine hydrochloride treatment with regard to sex was assessed.

Fluoxetine hydrochloride produced favourable response sex regardless (10 males and 9 females), while clomipramine hydrochloride produced more favourable response in male patients (12 males and 6 females).

Adverse effects were less pronounced in all clomipramine hydrochloride treated patients (20%) sex regardless and more noticeable in female patients (35%) treated with fluoxetine hydrochloride.

The duration of treatment with representatives of both groups studied should be at least 10 weeks instead of 6 weeks.
References


**Abstract**

An increased understanding of the phenomenon of polymorphism should enable pharmaceutical scientists to gain control over the crystallization process in order to selectively obtain the desired polymorph or suppress the growth of an undesired one. Phase changes during processing and scale-up are a problem, which may be avoided by carefully designed initial small-scale studies. The availability of detailed structural data, combined with strategic design of substrates and additives, has led to significant advances in the control over the polymorphs obtained in a particular crystallization. With all the information available from these initial studies, it should be possible to design and to select processing conditions which would give a desired polymorph and maintain the desired form throughout the various stages of drug processing and manufacture.

**Key words**: polymorphs, identification, prediction

**Introduction**

Physical characterization of the active pharmaceutical ingredients is crucial for the successful development of the final drug product (1,2). It has long been known that pharmaceutical solids can exist in more than one solid form (crystal, amorphous). The different solid forms of a drug can display significantly different physical and chemical properties, including color, morphology, stability, dissolution, and bioavailability. Typically, the most thermodynamic stable form is chosen for development into the final dosage product, but more recently, metastable forms have been utilised due to enhanced dissolution or bioavailability profiles.

The common crystalline forms found for a given drug substance are polymorphs and solvates. Crystalline polymorphs have the same chemical composition but different internal crystal structures and, therefore, possess different physico-chemical properties. The different crystal structures in polymorphs arise when the drug substance crystallizes in different crystal packing arrangements and/or different conformations. The occurrence of polymorphism is quite common among organic molecules, and a large number of polymorphic drug compounds have been noted (2).

Solvates, also called pseudopolymorphs, are crystalline solid substances containing solvent molecules within the crystal structure. If the incorporated solvent is water, a solvate is termed a hydrate. Because different crystalline polymorphs and solvates differ in crystal packing, and/or molecular conformation as well as in lattice energy and entropy, there are usually significant differences in their physical properties, such as density, hardness, compressibility, refractive index, melting point, enthalpy of fusion, vapour pressure, solubility, dissolution rate, other thermodynamic and kinetic properties and even colour (3).

Differences in physical properties of various solid forms have an important effect on the processing of drug substances into drug products, while differences in solubility may have implications on the absorption of the active drug from its dosage form, by affecting the dissolution rate and possibly the mass transport of the molecules (4).

These concerns have led to an increased regulatory interest in understanding the solid-state properties and behaviour of drug substances. For example, for approval of a new drug Food and Drug Administration (FDA) states that "appropriate" analytical procedures need to be used to detect polymorphs, hydrates and amorphous forms of the drug substance. It is also very important to control the crystal form of the drug substance during the various stages of product development (1), because any phase change due to polymorph interconversions, desolvation of solvates, formation of hydrates and change in the degree of crystallinity can alter the bioavailability of the drug. When going through a phase transition, a solid drug may undergo a change in its thermodynamic properties, with consequent changes in its dissolution and transport characteristics (5).

Processes such as lyophilization and spray drying may lead to the formation of the amorphous form of drug, which tends to be less stable and more hygroscopic than the crystalline product. Also, processing stresses, such as drying, grinding, milling, wet granulation, and compaction accelerate the phase transitions in pharmaceutical solids. Keeping these factors in mind, it is desirable to choose the most stable polymorphic form of the drug in the beginning and to control the crystal form and the distributions in size and shape of the drug crystals during the

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**Recent advances in the identification and prediction of polymorphs**

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entire process of development. The presence of a metastable form during processing or in the final dosage form often leads to instability of drug release as a result of phase transformation (6).

Crystallization plays a critical role in controlling the crystalline form and the distribution in size and shape of the drug (7). A crystalline phase is created as a consequence of molecular aggregation processes in solution that lead to the formation of nuclei, which achieve a certain size during the nucleation phase to enable growth into macroscopic crystals to take place during the growth phase. The factors affecting the rate and mechanisms by which crystals are formed are: solubility, supersaturation, rate at which supersaturation and desupersaturation occur, temperature, and the reactivity of surfaces towards nucleation.

The various forces are responsible for holding the organic crystalline solids together, such as nonbonded interactions and hydrogen bonding (8).

Various analytical methods are being currently used to characterize the crystalline form of the drug during the various steps of processing and development. With advances in analytical methods, the current focus of research in the solid-state area is to understand polymorphism and pseudopolymorphism at the molecular level. Knowledge of the crystal packing arrangements and the various intermolecular forces involved in the different packing arrangements will help in the prediction and preparation of the most stable polymorphs of a given compound.

Understanding of the physicochemical properties of polymorphs and solvates (hydrates) is of primary importance to the selection of a suitable crystalline form and development of a successful pharmaceutical product.

**Types of polymorphism**

Polymorphs are classified, based on differences in the thermodynamic properties, as enantiotropes or monotropes, depending upon whether one form can transform reversibly to another or not. In an enantiotropic system, a reversible transition between polymorphs is possible at a definite transition temperature below the melting point. In a monotropic system, no reversible transition is observed between the polymorphs below the melting point. Burger and Ramburger (9,10) have developed four rules to determine qualitatively the enantiotropic or monotropic nature of the relationship between polymorphs.

These rules are:
- the heat of transition rule,
- heat of fusion rule,
- infrared rule and
- density rule.

If it is established that the polymorphs of a particular drug are enantiotropic or monotropic, then the next goal is to define the thermodynamically stable (or metastable) domain of each crystalline phase of a substance as a function of temperature.

In recent years, the main focus of research has been the characterization of polymorphs arising from structural differences in the crystal lattice. It has been established for some time that organic molecules are capable of forming different crystal lattices through two different mechanisms. One of the mechanisms is termed packing polymorphism, and represents instances where conformationally relatively rigid molecules can be assembled into different three-dimensional structures through the invocation of different intermolecular mechanisms. The other mechanism is termed conformational polymorphism and arises when a nonconformationally rigid molecule can be folded into different arrangements, which subsequently can be packed into alternative crystal structures. The distinction between packing polymorphism and conformational polymorphism is somewhat artificial because different packing arrangements impose different conformations on the molecules, however slight, and different conformations will inevitably pack differently (11).

**Phase transformations in the solid state**

Studies of phase transformations in the solid state are important, because the sudden appearance or disappearance of a crystalline form can threaten process development, and can lead to serious pharmaceutical consequences if the transformation occurs in the dosage forms. Hence, an understanding of the kinetics and mechanism of phase transformations is of practical importance. The rearrangement of molecules into a new structure during phase transformation may or may not involve a solvent or vapor phase. To explain the mechanism of solid-solid physical transition, four steps have been proposed:

(a) molecular loosening in the initial phase;
(b) formation of an intermediate solid solution;
(c) nucleation of the new solid phase and
(d) growth of the new phase (11).

In an interesting study, Skwierczynski (12) has proposed a two-environment model to describe the decomposition
reaction kinetics of a crystalline solid, aspartame. The decomposition reaction of aspartame is a simple unimolecular thermally-induced aminolysis and the reaction proceeds under anhydrous conditions, i.e., water is not a reactant (13). This model links the chemistry of the solid-state reaction with the molecular mobility of the reactant as the reaction proceeds. The advantage of this model is that it can be used to determine the shelf life of a product from kinetic data gathered at elevated temperatures. Apart from solid-solid physical transformations, solution-mediated physical transformations among polymorphs are also known to occur in processes, such as wet granulation and during dissolution testing.

The main challenge in managing the phenomenon of multiple solid forms of a drug is the inability to predict the number of forms that can be expected in a given case. This prediction would involve quantification of the myriad intermolecular forces within any proposed crystal structure as well as the ability to postulate the likely packing modes for a given molecule in all its configurations.

Accurate theoretical prediction of polymorphs from studies of molecular dynamics and crystal structure generation would be of outstanding importance in drug research (14).

More research is now being directed towards developing computational tools to understand the nature of polymorphism and to predict polymorphic forms at an early stage in the drug development process. The recent developments in computational chemistry allow the prediction of possible polymorphic forms based only on the molecular structure of the drug. The Polymorph Predictor, from Molecular Simulations, is currently the only commercial software package that can predict the possible polymorphs of an organic compound from its molecular structure (15).

The package developed by Karfunkel and co-workers (16) uses a Monte Carlo simulated annealing approach to generate thousands of possible crystal packing alternatives for a given molecule. Each of the unique crystal structures is then subjected to a lattice energy minimization to obtain the relative stability ranking of the various packing possibilities and the resulting lowest-energy structures are the potential polymorphs. This method has been successfully employed to generate known polymorphs of primidone and progesterone, starting from the molecular structures alone (17). The theoretical predictions of lattice energies, entropies, morphologies and polymorphs should stimulate experimental activities and vice versa. The current crystal-modeling efforts have the potential of producing more quantitative tools for bridging structures and properties, which could help in creating solid forms with desired properties (18).

There are many limitations in using computational methods for predicting polymorphs theoretically. The first limitation is that the ab initio screening is useful only for nonionic rigid molecules. For more complex systems, the method is very useful for generating plausible crystal structures, but it is not accurate enough to determine which of these possible structures can actually be crystallized (19).

In addition, the limitations in computer power can restrict the use of this method for predicting polymorphs of complex molecules. An issue of concern is that the existing methods only predict the lattice energies, which relate to internal energies or enthalpies of the crystals. However, the relative thermodynamic stability of polymorphs is determined by the Gibbs free energy, which is a linear function of both enthalpy and entropy. Predictions of the relative stability of polymorphs will be more accurate when the entropies, as well as lattice energies, are considered. Application of molecular dynamics may enable the entropies to be calculated. Hence, no general method is currently available for the prediction or interpretation of the properties of complicated polymorphic or pseudopolymorphic systems.

**Conclusions**

It is very important to choose the most suitable form of the crystalline drug in the initial stages of drug development. Systematic isolation and early characterization of the largest number of possible forms of a drug reduces the chances of surprises at the late production stage due to identification of a new crystalline form or phase change. With the development of more sophisticated computational tools, the main focus of many investigators is to be able to predict all the possible forms of a drug from its molecular structure. Understanding the origins of the multiple solid forms of a drug molecule, either due to differences in packing arrangement or conformation of the molecules, becomes the first step in prediction.

When crystal structures can be calculated with certainty, it will be possible to predict the various polymorphs of a compound and this information could be used to guide experimental studies. This goal may be difficult to achieve owing to the complex molecular structures of...
new organic molecules and the presence of several molecules in each asymmetric unit, but the future development of improved force fields and increased computational speeds, may make it achievable.

It is important to make every effort to prepare and to identify the most stable polymorph in order to guide the selection of the optimal form for development. The emergence of sensitive methods and the use of combination techniques, facilitate the identification and the more accurate characterization of the various polymorphs of a drug molecule.

An increased understanding of the phenomenon of polymorphism should enable pharmaceutical scientists to gain control over the crystallization process in order to selectively obtain the desired polymorph or suppress the growth of an undesired one. Phase changes during processing and scale-up are a problem, which may be avoided by carefully designed initial small-scale studies. The availability of detailed structural data, combined with strategic design of substrates and additives, has led to significant advances in the control over the polymorphs obtained in a particular crystallization. With all the information available from these initial studies, it should be possible to design and to select processing conditions which would give a desired polymorph and maintain the desired form throughout the various stages of drug processing and manufacture.
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Polytrauma with significant lesion of peripheral nerves is a specific war injury. It is also one of the most delicate problems in rehabilitation treatment because it requires a close cooperation with surgeon and timely surgical interventions. Based on our experience, the best results in the treatment of injured persons with lesion of peripheral nerves have been accomplished after the surgical treatment. Results in the neurolysis were better than those accomplished in neurorrhaphy.

Total of 436 patients with lesion of peripheral nerves were recorded and 56 patients with plexus lesion. Out of this number, 78 patients (about 15%) had surgical treatment (41 neurorrhaphy and 37 neurolysis). Due to lack of adequate ENMG diagnostics, the objective valorisation of treatment outcome was not possible.

Key words: war injures, peripheral nerves lesion, rehabilitation.

Introduction

Characteristics of war injuries are polytrauma with severe damage of tissue and peripheral nerves lesion. Consequences of the injuries accompanied with nervous system damage are delicate rehabilitation problem. Like in all other injuries, success of the therapy depends on timely and adequate surgical treatment of patients and proper rehabilitation concept for every single case. It also depends on application of all available classic and modern treatment devices, on long-term work with patients and optimal active patient participation.

Material and methods

Data about treatment results of all patients with extremity peripheral nerves lesion, in ambulance "PRAXIS" during the period from 1993 to 1996, were analysed. Physical treatment results were valorised by estimation of a success. Success of treatment is expressed as results of clinical condition after treatment, objectively valorised by using following scheme:

- Grade "0" - zero: unchangeable condition (without treatment results),
- Grade "2" - two: minimal changes,
- Grade "3" - three: satisfied functional changes with consequence (sensory or motor),
- Grade "4" - four: good changes and satisfied function restitution with minimal consequence,
- Grade "5" - five: good restitution without consequence of injuries or diseases.

Using retrospective analysis we registered and sorted all patients with war peripheral nerve lesion that were treated surgically or conservative, with physical medicine procedures and rehabilitation methods in Centre for Physical Medicine and Rehabilitation "PRAXIS". Using a clinical and neurology findings, physiology measurement as manual muscle test, muscle tonus and contraction tests and sensibility, we valorised a success of the treatment for every single patient. Data are expressed according to mentioned scheme using scale from 5 to 0. Gained results are statistically calculated and presented in tables and graphics.

Results and discussion

In Centre for Physical Medicine and Rehabilitation "PRAXIS", there were 454 or 28% patients with peripheral nerve system injuries in the 4 years period (Table 1)

According to location of lesion:
- n. ishiadicus 98 (21.5% of all isolated nerve lesion)
- n. ulnaris 74 (16.3%)

It is treated 59 surgically treated patients (12.9%)
- neurorrhaphy procedure done in 30 patients
- neurolysis procedure done in 29 patients (Table 2)

Plexus injuries had 55 patients (12%)
- plexus brachialis 37 patients (8%)
- L/S plexus 18 patients (4%)

It is registered 230 (50.6%) patients with peripheral nerve injures of arms and 224 patients (49.4%) with injures of legs.
Table 1 presents figures about the peripheral nerve injury structures. All of 454 of injured patients with nerve lesion have been surgically treated after the report. Evaluation of the success of carried medical rehabilitation in patients with peripheral nerve lesions is presented in Table 2 and Graph 2. Only 5% of patients have no consequences after the rehabilitation. It is significant that almost half of the patients (43%) have a minimal consequence. About one fifth (19%) have a minimal consequence after medical rehabilitation.

Table 2. Evaluation of the success of treatment of patients with peripheral nerve lesion treated in ambulance “PRAXIS” in the period 1993 - 1996

| 5 (complete restitution) | 23 | 5.06% |
| 4 (with minimal consequences) | 197 | 43.39% |
| 3 (satisfied functional restitution) | 148 | 32.59% |
| 2 and 1 (with minimal changes) | 70 | 15.42% |
| 0 (without changes) | 16 | 3.52% |

Discussion
Based on experience of "PRAXIS" Centre for Physical Medicine and Rehabilitation, peripheral nerve lesions are one of the most delicate problems in medical rehabilitation. There are many reasons for that:

- massive peripheral nerve lesions in war victims reach an epidemic level
- long duration of the treatment, persistence and patience of therapist and patients
- inadequate diagnostic procedure (ENMG was not in work in Sarajevo during the war)
- multiple injuries with combined muscle, bone and vascular structure lesions.

Results of conservative treatment of peripheral nerve injuries in the war show that application of standard physical procedure with electro-acupuncture stimulation (own experience) is successful in high percentage. If physical treatment starts earlier and carries out long enough, success is indicatively higher. Everyday patient-physician contact during the treatment is very important because of psychological moment and continuous monitoring of objective conditions. Contact is necessary for timely neurosurgery and plastic surgery cooperative intervention, which should increase, in high percentage (average 81%), functional status of patients. Out of 454 treated patients with peripheral nerves lesions in "PRAXIS", according to clinical parameters, the best results were obtained at isolated n. ulnaris lesion (72%), but according to functional status the best results were at n. ulnaris.

Table 1. Structure of no surgically and surgically treated patients with peripheral nerves lesion treated in the period 1993-1996 in "PRAXIS"

| N. ulnaris | 74 | 32.20% |
| N. medianus | 47 | 20.40% |
| N. radialis | 39 | 17.00% |
| N. axillaris | 6 | 2.60% |
| Senso-cutaneous branches | 3 | 1.30% |
| Combined multinevre injuries | 24 | 10.40% |
| Plexus brachialis injuries | 37 | 16.00% |
| Total of nerves injuries of arms | 230 | 100.00% |
| N. ischiadicus | 98 | 43.75% |
| N. femoralis | 22 | 9.82% |
| N. tibialis | 13 | 5.81% |
| N. peroneus | 53 | 23.66% |
| Senso-cutaneous branches | 13 | 5.81% |
| Combined multinevre injuries | 7 | 3.12% |
| Plexus L/S injuries | 18 | 8.03% |
| TOTAL | 224 | 100.00% |
| Total of nerve injuries of legs | 454 | 31 | 28 | 59 (12.9%) |
medianus lesion (94%). The poorest results were obtained in n. ischiadicus complete lesion (52%) and isolated n. peroneus lesion (65.7%).

War injuries were diverse, mostly combined and multiply locomotor system injuries with peripheral nerves lesions (10.7%).

Some of injuries treated in "PRAXIS" (total 454 cases) were plexus injuries (cervicalis and L/S - 55 injures) and n. ischiadicus injuries (98 injures).

From other isolated nerve lesions, the most registered were injuries of n. ulnaris (74 cases or 17%) and injures of n. peronealis (53 cases or 12%).

After rehabilitation treatment there was only 5% of patients without consequences. It is indicative that almost half of the patients (43%) had a minimal consequence. About one fifth (19%) had a minimal consequence after medical rehabilitation.

**Graph 1.** Peripheral nerves injuries structure of patients treated in ambulance "PRAXIS" in the period 1993 - 1996

**Graph 2.** Success of the treatment of patients with nerve lesion of arms treated in ambulance "PRAXIS" in the period 1993-1996
Conclusion

Treatment of war peripheral nerve lesion is important from the point of view of carrying out a medical rehabilitation measurement (10.7% of all heavy war injuries). In spite of difficult working conditions in war period, it was possible to achieve good results by using a complex approach to every single case, with persistence and patience work and patient motivation to be engaged in own rehabilitation. Treatment should be long, started as soon as possible after the injury, with application of all available methods, especially electro stimulation, individually programmed kinesio-therapy, massage and thermo therapeutic procedures. Application of electro-acupuncture stimulation on acupuncture spots was useful for fast and successful rehabilitation in most of the patients. There was no ENMG diagnostic procedure to help in differentiation of the number of sub clinical lesions, following a success of the treatment and final condition evaluation.

References


Cardiac troponin I: the gold standard in acute myocardial infarction diagnosis

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Abstract

Cardiovascular diseases are leading cause of morbidity in the world. Measurement of the level of biochemical markers in the serum is one of World Health Organisation (WHO) criteria in diagnosing acute myocardial infarction (AMI). Non-specific clinical state of patients and insufficiently sensitive electrocardiographic (ECG) diagnostics, at patient's hospital admission time, point out the importance of biochemical markers in acute myocardial infarction diagnosis. Technology development and new diagnostic methods lead to the invention of highly sensitive and specific marker as myocardial damage evidence. Cardiac Troponin I (cTnI) is specific marker for myocardial damage. Its elevation in the serum within myocardial ischemia symptomatology is important in diagnosis of myocardial infarction.

Keywords: acute myocardial infarction; cardiac troponin I.


Introduction

Biochemical markers of myocardial damage are essential for diagnosis, risk stratification and selection of the ACS patients' treatment. Testing of the patient for AMI diagnosis has been rapidly changed in comparison to the traditional enzymatic assay and mass measurement of the specific and sensitive protein markers. The measurement of troponin I or troponin T in the blood has been accepted by the Joint Committee of the ESC/ACC as a standard biomarker for the diagnosis of acute myocardial infarction. Due to that, clinicians can initiate proper treatment as quickly as possible after the correct MI diagnosis.

Acute coronary syndrome - pathogenesis

Myocardial infarct is a terminal event of syndrome called acute coronary syndrome. Long duration of coronary ischemia is a cause of the myocardial infarction with following tissue necrosis. ACS starts with asymptomatic coronary artery diseases, progresses to stable and non-stable angina, ECG non-Q-wave MI, transmural infarction, cardiac arrhythmia and death. All forms of the syndrome (Figure 1), including non-stable angina, ECG non-Q-wave MI, and Q-wave MI, share a common pathogenic substrate: atherosclerotic lesion of coronary arteries. When atherosclerotic plaque ruptures or erodes, pathophysiological processes are triggered resulting in thrombus formation. Inflammatory and thrombotic mechanism are involved in pathogenesis of the syndrome. Inflammatory reactions result in plaque rupture and give possibilities of the onset of coagulation process that finishes with thrombus formation. When thrombus formation results in reduction or cessation of the blood flow within the affected coronary vessel, the resulting imbalance between oxygen require and supply produces the clinical manifestation of ischemia.

Figure 1. Spectrum of acute coronary syndromes

Acute myocardial infarction diagnosis

According to WHO recommendations, a traditional approach to patients with suspected acute myocardial infarction is based on three criteria:
clinical symptoms that points on ischemia (chest discomfort that lasts about 30 minutes)
- electrocardiographic changes (depression or elevation of ST segment or T wave inversion)
- biochemical markers detection

According to WHO recommendations, myocardial infarction is defined as a presence of two out of three mentioned criteria.

The leading cardinal symptom of patients with acute coronary syndrome is chest pain that demands further clinical evaluation. Pain is a result of the imbalance between myocardial oxygen supply and need.

Electrocardiography is a method that enables the further risk stratification of patients with chest pain. If thrombus formation completely occludes coronary artery, ECG pattern involves development of the typical Q-wave. In case of transitory or non-complete occlusion, ECG changes have less prognostic importance. Unstable angina is diagnosed by the conventional enzyme markers being in referential values or minimally elevated. Biochemical markers in AMI diagnosis are very important in cases of non-specific clinical symptomatology and untypical electrocardiographic changes.

A need for the redefinition of MI comes from the presence of advanced technology and highly sensitive cardiac markers which could detect small infarcts of less than 1.0 g of necrosis that do not cause diagnostic ECG changes or functional abnormalities.

About 30% of patients with previously diagnosed non-stable angina pectoris have elevated cTnI level in the blood. In such patients there is no evidence of elevated CK or CK-MB isoenzyme activity.

Recommendations of the Joint European Society of Cardiology (ESC) and American College of Cardiology (ACC) Committee in consensus document advice to re-examine the definition and diagnosis of MI. MI is a minimal myocardial necrosis followed by the elevation of cardiac troponin I in the blood.

New definition of myocardial necrosis is "maximal concentration of troponin T or troponin I that exceeds the 99% of the value for the referent control group, on at least one occasion during the first 24 hours following the evidence of clinical event."

Importance of the new definition is that the previously diagnosed severe stable and non-stable angina is now re-diagnosed as myocardial infarction. Suggested ESC/ACC criteria differ from WHO criteria in the presence of two parameters in MI diagnosis: elevated cardiac markers with another changes, whether it is ECG changes or typical ischemic chest pain (Figure 2.)

Biochemical markers in diagnosis of acute myocardial infarction

Biochemical markers usage in the acute myocardial infarction diagnosis began after the year 1954 when La Due and his associates reported their investigation results about aspartate aminotransferase elevation in MI patients.

In last twenty years, the gold standard in laboratory diagnosis of MI has been measurement of CK-MB activity. Increase or decrease in its activity has been used in diagnosis and monitoring of the treating course of patients with MI. This isoenzyme is not completely specific for the myocardial muscle. Its share in total myocardial muscle mass is just 3% from the total creatine kinase quantity.

CK-MB isoenzyme increases in myocardium as a response to ischemia. It has been considered for a long time that CK-MB is not detectable in skeletal muscles containing the majority of the CK-MM isoenzyme. Now we know that under the control of regulation genes, CK-MB content in skeletal muscle increases after injuries or during the regeneration process. Due to this fact, there is...
possibility of the masking of CK-MB release from the myocardium in the case of simultaneously damaged skeletal and heart muscles. Another problem is a small sensitivity in the first couple of hours following the infarction onset. Reviewing these acknowledgements raises a need of finding a marker with high sensitivity and specificity for the heart muscle.

**Troponin complex**

While searching for the sensitive and specific laboratory test in urgent MI diagnosis a troponin complex has been found. Troponin complex consists of three subunits: troponin I (TnI), troponin C (TnC), and troponin T (TnT). This complex (Figure 3).

**Figure 3** Troponin complex components

![Figure 3](www.abbottdiagnostics.com/medical_conditions/heart_disease)

Different function, molecular weight and the origin of protein subunits are associated with the distinct aminoacid sequences encoded by separate genes. Troponin I is an inhibitory unit that regulates contraction of skeletal and heart muscle. It inhibits Mg\(^{2+}\) activated actinomyosin ATP-ase activity. Its molecular weight is 23 000 Dalton.

Troponin I has three isoforms: two for skeletal muscles (slow and fast ones) and one for the heart muscle. Aminoacid sequence of the cardiac isoform makes troponin specific for the heart muscle. The N-terminus of the cTnI has 31 additional amino acid residues that are not present in skeletal troponin isoforms, allowing the development of specific antibodies as a condition for the development of a new diagnostic test. The majority of cTnI is bound to contractile apparatus (97%) and about 3% of it is a free cytosolic component. After cell necrosis, cTnI releases into the circulation with other troponin complex subunits.

Concentration of cTnI elevates almost at the same time as CK-MB concentration after the onset of symptoms, but its increase is higher and persists 5-7 days. This could be explained by the higher content of cTnI in myocardium (4-6 mg/g wet weight of tissue) in comparison to the CK-MB content. The initial cTnI rise is seen during the first couple of hours, with the peak level achieved between 12 and 24 hours after the estimated time of myocardial necrosis.

Half-life of cTnI in the serum is about 90 minutes. The possible time of the cardiac troponin I detection is related to the extent of myocardial damage. Long duration of the elevated values points out the prolonged protein release from the infarcted area. Decreased concentration of cTnI points out the reparation of myocardial damage. Cardiac specificity is proved in several clinical conditions without the detection of cTnI elevation in the blood:

- muscular dystrophy patients
- marathon and exercise sport activities
- muscle injuries
- renal diseases

Suitability of cTnI to “The Gold standard” for the Diagnosis Acute Myocardial Infarction:

- cTnI is only found in cardiac muscle during adult life and embriogenesis
- cTnI is not synthesised in response to skeletal muscle injury
- The antibodies used in the assay for cTnI do not cross-react with the skeletal muscle troponin I
- cTnI is not elevated in patients suffering from the variety of clinical conditions
- Elevation of cTnI closely correlates with the evidence of myocardial injury demonstrated by echocardiography
- There is 13-fold greater concentration of the cTnI in the heart in comparison to the CK-MB concentration
- Elevation of the cTnI is at least as sensitive in the clinical detection of cardiac injury as CK-MB elevation
- Risk of the 24-hour and 30-day death or MI with detected positive CK-MB results is lower than with the detected positive troponin I results.

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Introduction of the new analytical approaches to the doping control on the XIV Winter Olympic Games

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Key words: doping control, anabolic steroids, exogenous testosterone, gas chromatography-mass spectrometry, XIV Winter Olympic Games

Summary

In this paper we present introduction and development of some new analytical methods for identification of anabolic steroids, their metabolites and certain hormones, especially determination of exogenous testosterone by means of gas chromatography-mass spectrometry. Identification of central nervous stimulants and corticosteroids has been performed by high performance liquid chromatography.

In desire to achieve better results, to increase strength and endurance, to sharpen reflexes and to reduce stress and anxiety athletes as well as other people use different pharmacological substances, hormones or even illicit drugs. Use of these substances without medical supervision can lead to adverse effects to one's health or even cause a death. At the same time, use of such substances means a kind of cheat that could not be accepted. This is why International Olympic Committee started at 1968 with official doping control that is permanently carried out and continuously increasing number of banned substances. Doping control demands for discover and development of new sensitive and specific methods for detection of banned substances and their metabolites in urine and blood.

Doping control consists of two parts: sample collection and analytical treatment of sample. Collecting procedure varies depending on types of sports; it is different during Olympic games and different on other competitions. Sample collection procedure consists of system for athlete's selection, their approach to the field station for sampling, sample transport to the laboratory and documentation that trace chain-of-custody for each sample. Sampling procedure is described in details since there are the most possibilities for sample manipulation and generally this part of doping control has the most of athletes complain. Laboratory for doping control has its own characteristics. It is similar to the laboratory for clinical biochemistry qualified for pharmacokinetic studies of drugs metabolism, identification of metabolites in body fluids, especially endogenous and exogenous hormones using specific sophisticated analytical equipment. Specific demands for such laboratory are capability for identification of large number of drug classes and large number of chemically similar substances in each class. Ultimate precision is absolutely necessary since consequences of positive result reach individual competitor, his sport federation and country as well. Laboratories are rigorously reviewed and evaluated through the International Olympic Committee (IOC) reaccreditations and proficiency programme. Drugs and metabolites are identified using the most sensitive and specific methods and every result is accompanied by complete documentation obtained during doping control.

When the first large scale test were officially instituted at the X Olympic Winter Games in Grenobl and the Games of the XIX Olympiad in Mexico City in 1968 equality of the treatment among athletes was easily established. As there had previously been no rules, the International Federations easily accepted those prepared by the IOC containing the procedures and the list of banned substances. From that time up to now following prohibited classes substances and prohibits methods are subject for doping control: stimulants, narcotics, anabolic agents including anabolic androgenic steroids, diuretics, peptide hormones, mimetic and analogues, chorionic gonadotropine (hCG), pituitari and synthetic gonadotropines (LH), corticoestroides (ACTH), growth hormone (hGH), insulin-like growth factor (IGF-1), erythropoietin (EPO) and insulin. Prohibited methods include following procedures: blood doping, administering of artificial oxygen carriers or plasma expanders, pharmacological, chemical and physical manipulation. In certain circumstances, following substances are prohibited: alcohol, cannabinoids, local anaesthetics, glucocorticosteroids and -blockers. Problems associated with abuse of anabolic steroids and testosterone started during Olympic games in Moscow in 1980. At that time identification of these substances was carried out by means of radio immunoassay (RIA). RIA as the analytical method lacks enough specificity for these classes of substances because it is not possible to distinguish between or substituted cyclopananoperhydrophenanthrenes and doping control demands for unequivocal identification of substance and its metabolite. So, Medical Commission of IOC decided that for next Olympic games in Sarajevo and Los Angeles new methods for anabolic steroid analysis should be develop-
oped and introduced in routine. Quantification of exogenous testosterone and caffeine in urine should be done as well, together with identification of corticosteroids. This conclusion of IOC introduced quantitative analysis in doping control for the first time. That caused another problem for laboratory staff. Joint project of Sarajevo and Los Angeles laboratories was to simultaneously investigate determination of testosterone an anabolic steroids using RIA and hyphenated system gas chromatography-mass spectrometry (GC-MS). These investigations demanded for modern equipment and Organizing Committee of XIV Winter Olympic Games purchased adequate high-resolution gas chromatography-mass spectrometry system.

Quantification of exogenous testosterone was a specific problem. Presence of exogenous testosterone significantly influences normal hormonal profile of androgenic hormones and their metabolites. Detection of these changes was solved by tracing ratio of testosterone and its metabolites androsterone and etiocholanolone, as well as ratio of testosterone and other androgenic hormones: epitestosterone, dehydroepiandrosterone, androsten-dione, 5β-androstanedione, 11β-hydroxyandrostenedione and 11β-hydroxyetiocholanolone (1-8). Significant indicator of the presence of exogenous testosterone is ratio of testosterone and epitestosterone in urine. If this ratio exceeds 6 that means obvious presence of exogenous testosterone. In this case, quantification was performed by Single Ion Recording (SIR) technique that enables simultaneous tracing of ions in correlation with their chromatographic retention times, while other ions are not registered. Single Ion Recording technique gains sensitivity by approximately 3 orders of magnitude and at the same time avoids registration of impurities from biological matrix.

In table 1 monitored androgenic hormones are listed with molecular ions of their trimethylsilyl derivatives and retention times relative to deuterated testosterone. Figure 1 shows fragmentograms of androgenic hormones in urine sample oh healthy male.

During Olympic games in Sarajevo and Los Angeles simultaneous analysis of steroids by RIA and GC-MS were performed. After completed investigation, Medical Commission of IOC accepted GC-MS as the official method for identification of anabolic steroids and quantification of exogenous testosterone (9-17), while RIA was accepted as a screening technique. High performance liquid chromatography (HPLC) was introduced for quantification of caffeine in urine (18). The definition of positive results is a concentration in urine greater than 12 µg/mL. HPLC was also used for analysis of pemoline (19,24,26) and other stimulants (9, 20, 23, 25).

Identification of β-blockers and their metabolites was also subject of our collaboration with Institute for Pharmacology, UCLA, Los Angeles (27-30). Systemic use of glucocorticosteroides is prohibits when administered orally, rectally or by intravenous or intramuscular injection. Some contributions in this field we gave with investigations presented in different journals (31-33).

Particular problem for laboratory for doping control is accreditation by IOC. Accreditation is performed by analysis of ten samples in exactly 48 hours, under supervision of IOC expert. Every result has to be absolutely correct followed by complete documentation including methodology and results interpretation. Analysis in a doping control can be interfered by some masking agents that could be intentionally added to the samples. The best-known agents of such kind are bromantane, probenecide and diuretics. There are some

<table>
<thead>
<tr>
<th>Steroid hormones</th>
<th>Monitored molecular ion (M⁺)</th>
<th>Relative retention time*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androsterone</td>
<td>434</td>
<td>0.819</td>
</tr>
<tr>
<td>Etiocholanolone</td>
<td>434</td>
<td>0.836</td>
</tr>
<tr>
<td>Dehydroepiandrosterone (DHEA)</td>
<td>432</td>
<td>0.912</td>
</tr>
<tr>
<td>5β-androstandione</td>
<td>432</td>
<td>0.939</td>
</tr>
<tr>
<td>Epitestosterone</td>
<td>432</td>
<td>0.948</td>
</tr>
<tr>
<td>Testosterone</td>
<td>432</td>
<td>1.002</td>
</tr>
<tr>
<td>D₇-Testosterone</td>
<td>434</td>
<td>1.000</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>430</td>
<td>0.987</td>
</tr>
<tr>
<td>5β-hydroxy-androsterone</td>
<td>522</td>
<td>1.023</td>
</tr>
<tr>
<td>5β-hydroxy-etiocholanolone</td>
<td>522</td>
<td>1.039</td>
</tr>
</tbody>
</table>

*Relative retention time expressed in relation to D₇-Testosterone
other possibilities for manipulation with sample, like dilution by simultaneous use of diuretics, which put them on the list of banned substances as well. Use of substances that change urine pH value making it acidic or alkaline might slow down excretion of banned substances and their metabolites.

Great problem in analysis is presence of the substances with similar chemical structure. These substances undergo similar metabolic changes and some of them give the same metabolites. This is the case with methyl testosterone and methandriol. Metabolic pathway of these substances is shown in figure 2.

Reliable identification of these substances is possible only by identification of their metabolites and elucidation of their pharmacokinetics. Presence of substances like methyl testosterone and methandriol might lead to false results and they are often used by IOC for doping laboratory testing during competitions.

Laboratory for doping control must have adequate equipment, highly trained multidisciplinary staff, pure test sub-
stances, specific software, bank of spectra and different analytical data including fragmentation pathways, molecular masses and so on. Specific for doping control laboratory is collection of so-called "physiological urines" that are collected in different time course and that serve for metabolite tracking.

A team of 20 sub specialists carried out accreditation of Sarajevo laboratory: chemists, pharmacists, biologists, physicians and computer experts. Intensive preparations were carried out for a one year and during Olympic games 60 persons worked in laboratory (12 doctors of science, 3 masters of science, chemists, pharmacists, physicians, technicians).

During preparation of laboratory we made intensive collaboration with prof. dr A. Becket and prof. dr D. Cowen, Chelsea College of Science, London; prof. dr Manfred A. Donicke, Bundes Institut für Sportwisenschaft - Institut für Biochemie, Köln; prof. dr V. Semjonov, head of Doping control laboratory in Moscow and prof. dr Catlin, Institute for Pharmacology, UCLA.

Collaborators from our laboratory were on education in Institute Jožef Stefan, Ljubljana (at prof. dr J. Marsel and prof. dr B. Kralj); in Manchester, VG Analytical Education Center (now Micromass) and in Köln attending "Workshop in Dope Analysis of Anabolic Steroids". We are taking the advantage of this opportunity to thank them for their collaboration and help.

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Computed tomography review of the osseous structures of the orbital apex

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Abstract

In this paper, we described osseous anatomy of the orbital apex using CT in axial and coronal projections. The main osseous landmarks facilitate the evaluation of orbital apex in radiology, especially on the axial and coronal CT scans. These landmarks include so called optic strut, small segment of the greater wing of the sphenoid bone and upper part of the pterygopalatine fossa. We also concentrate attention upon visualisation and review of the optic canal, superior and inferior orbital fissure, pterygopalatine fossa and foramen rotundum.

Key words: orbital apex, CT, optic canal, superior orbital fissure, inferior orbital fissure, pterygopalatine fossa, foramen rotundum.

Introduction

The top of the orbital cavity or orbital apex is defined as the area between the posterior ethmoidal foramen on one side, and optic canal and superior orbital fissure, on other side. The roof of the orbital apex consists of lower side of the lesser wing of the sphenoid bone; its medial wall creates the lateral wall of the etmoidal sinus, medial wall creates the greater wing of the sphenoid bone, and the basis is the orbital process of the palatine bone. The orbital apex through the optic canal and superior orbital fissure is establishing the communication with the sellar region, and together with it, it represents the clinical-anatomical and radiological entity known as the cranio-orbital junction. This area is affected by different pathological processes which include fracture of the sphenoid bone with consecutive lesion of intracanalicular or intracranial part of optic nerve, infectious processes (orbital pseudotumour and Tolosa-Hunt syndrome), vascular lesions (e.g. aneurysm, carotid-cavernous shunts, thrombosis of cavernous sinus etc.), and tumours with the most frequent appearing of pituitary adenoma, meningioma and craniopharyngioma (1).

From the above mentioned we can conclude that the osseous anatomy of the orbital apex is very complicated and that it understands the knowing of the optic canal, superior and inferior orbital fissures, pterygopalatine fossa and foramen rotundum (2,3,4,5). Knowing these structures, its relations and variations, as well as the position and the orientation is the key point in evaluation (diagnostics and therapy) of the fractures, tumours and the inflammatory processes in the area of orbital apex, as well as the neighbouring topographical areas, firstly the middle cranial fossa, pterygopalatine and infratemporal fossa (1,2,3,4,5,6,7).

Adequate presentation and description of osseous anatomy of the orbital apex by the method of computed tomography (CT) demands knowing of it's appear in three dimensions (Figure 1 and Figure 2)

Figure 1 Osseous structures of the cranio-orbital junction. Viewed from the above:

1. fossa pterygopalatina, 2. foramen rotundum,
3. canalis pterygoideus, 4. canalis palatinus major,
5. foramen sphenopalatinum, 6. fissura orbitalis inferior,
7. sulcus infraorbitalis,
8. paries inferior orbitae,
9. cellulae ethmoidales,
10. foramen ovale,
11. foramen spinosum.

Testut / Latarjet - Traite D’Anatomie Humaine, Tome 1, page 281.
Osseous structures of the cranio-orbital Osseous structures of the orbital apex
1. canalis opticus,
2. optic strut,
3. fissura orbitalis superior,
4. fissura orbitalis inferior

By this we can have the complete insight in "normal" appearance of the osseous structures of the orbital apex, and analyse the changes which are seen with different pathological conditions by which it may be primarily or secondarily affected.

Aim of work

The objective of this paper is defining and the presentation of main osseous orientation points (landmarks) by the method of computed tomography, which will help the evaluation of the structure of orbital apex in normal and pathological circumstances.

Material and methods

As the material for the preparation of these papers, we used 50 craniums from the osteological collection of the Institute for Anatomy of the School of Medicine in Sarajevo. With the osteological material, by anthropometrical analyses we defined main osseous orientation points of the orbital apex and the ratios of osseous structures in so-called cranio-orbital junction and the junction of the orbital cavity with the extracranial topographic areas. CT findings in axial and coronal projections are made by the apparatus Somattom ART with the width of layers of 2.3 and 5 mm. The osseous orientation points, defined at the bone material are shown at CT records.

Results

The optic canal (canalis opticus) forms the angle of 40° -50° with the sagittal plane of the head. It is limited medially by the body of the sphenoid bone, towards the superior root of the lesser wing of the sphenoid bone, inferolaterally by so called optic strut (which represents the inferior root of the lesser wing), and totally laterally by the anterior clinoid process (Figure 3).

Inferolaterally from the optic canal, separated from it with above mentioned optic strut, there is the superior orbital fissure (fissura orbitalis superior). It is located between greater and lesser wing of the sphenoid bone (Figure 4).

The communication of middle cranial and pterygopalatine fossa is established through the foramen rotundum (foramen rotundum), located in the upper part of the sphenoid bone's greater wing. In question is in fact parasagittally positioned small thin canal, directed from the back area and laterally towards front and medi ally - anteromedially direction (Figure 5).

The communication of middle cranial and pterygopalatine fossa is established through the foramen rotundum.

The pterygopalatine fossa (fossa pterygopalatina) and the inferior orbital fissure (fissura orbitalis inferior) have the common relations (Figure 6).
Figure 4  Axial CT scan through the upper part of the orbital cavity (red arrow - foramen rotundum, yellow arrow - fissura orbitalis superior)

Figure 5  Axial CT scan through the lower part of the orbital cavity (black arrow - fissura orbitalis inferior, yellow arrow - foramen sphenopalatinum, red arrows - canalis rotundus)

Figure 6  Coronal CT scan through the anterior part of the pterygopalatine fossa (white arrow - fissura orbitalis inferior, red arrow - foramen sphenopalatinum, green arrow - fissura pterygomaxillaris)
The inferior orbital fissure with the sagittal plane of head creates the angle of around 45°. It is located between the lateral and the inferior wall of the orbital cavity and it is best noticed if the cranium is observed from aside and slightly from back. The sphenoid bone creates the lateral margin of the inferior orbital fissure, and superior and posterior wall of the pterygopalatine fossa. The maxillary bone creates the medial margin of the inferior orbital fissure and together with orbital process of the palatine bone creates the anterior wall of the pterygopalatine fossa. The anterior margin of the inferior orbital fissure is created by the zygomatic bone. The anterior part of the inferior orbital fissure is widen, while its posterior part (best shown in coronal CT) seems to be the thin, obliquely positioned gap, surrounded laterally by the sphenoid bone, and medially by the maxillary bone. Inferolaterally, through the inferior orbital fissure there is the communication between the orbital cavity and the infratemporalis fossa (fossa infratemporalis).

**Discussion and conclusion**

Important osseous orientation landmarks are making easier the evaluation of the orbital apex with axial and coronal CT scans (1,2,3,4). These orientation points include, firstly, so called optic strut (which separates the optic canal and the superior orbital fissure) small segment of the greater wing of the sphenoid bone (which separates the foramen rotundum and the superior orbital fissure), as well as upper part of the pterygopalatine fossa, which is screened at axial CT scans. Knowing of the osseous orientation points is of crucial importance in diagnostics of many pathological processes by which the orbital apex is primarily or secondarily affected, but also in the diagnostics of processes in the closest intracranial and extracranial surroundings (1,2,3,6,8,9,10).

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Iontophoresis: fundamentals, developments and application

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Abstract

The skin is an excellent barrier to the transport of charged compounds and large molecules. Many substances of present and potential therapeutic utility carry charge at physiological pH, have high molecular weights and/or are hydrophilic and, consequently, do not transport well across the skin. Pathways for the transport of small ions do appear to exist through the skin and flow along these pathways can be substantially enhanced by iontophoresis.

Key words: iontophoresis, transport, patches, application

Introduction

Drug delivery technology allows the right dose of an active pharmaceutical ingredient to be delivered at the right time, and most importantly, to the right site. It is playing an increasingly important role in lowering the cost of health care. New formulations are no longer simply line extensions intended to prolong a drug’s lifecycle, but strategic weapons that have real economic value in disease management. For major drug companies, the addition of an improved drug delivery formulation, either clinically or through improved patient compliance, can often mean the difference between being on or off a formulaury.

Transdermal drug delivery systems (TDDs) facilitate the passage of therapeutic quantities of drug substances through the skin and into the general circulation for their systemic effects. Evidence of percutaneous drug absorption may be found through measurable blood levels of the drug, detectable excretion of the drug and/or its metabolites in the urine, and through the clinical response of the patient to the administered drug therapy. With transdermal drug delivery, the blood concentration needed to achieve therapeutic efficacy may be determined by comparative analysis of patient response to drug blood levels. For transdermal drug delivery, it is considered ideal if the drug penetrates through the skin to the underlying blood supply without drug build up in the dermal layers.

Multiple methods exist for transdermal drug delivery. There is great interest between pharmaceutical scientists to develop physical methods and chemical permeation enhancers that can increase the percutaneous absorption of therapeutic agents.

Definition

Iontophoresis is a process or a technique which involves the transport of ionic (charged) molecules into a tissue by the passage of a direct electric current through an electrolyte solution containing the ionic molecules to be delivered, using an appropriate electrode polarity. It involves the transfer of ions into the body by an electromotive force. Ions with positive charge are driven into the skin at the anode and those with negative charge at the cathode.

This technique is used to enhance the transdermal transport of drugs by applying a small current through a reservoir that contains ionized species of drug. Positive or negative electrodes are placed between the drug reservoir and the skin. Positive ions are introduced in the skin from positive electrode, and negative ions from a negative electrode.

Principles of ionic transport in an electric field

The transport of ions under the influence of a uniform electric field was first studied by Planck. The Nernst-Planck equation, a fundamental equation widely used to describe the membrane transport of ions (1,2) is written as:

$$J_i = -D_i \frac{dC_i}{dx} + \frac{D_i z_i e_i E C_i}{kT}$$

where $J_i$ is the flux of ions across the membrane, $D_i$ is the diffusion coefficient of the ion $i$ (in the x direction), $C_i$ is the concentration of ions with valence $z_i$ and electron charge $e_i$, $E$ the electric field. The term $kT$ is the thermal energy of the system where $k$ is the Boltzmann constant and $T$ is the absolute temperature.

A better appreciation of the meaning of this equation may be achieved by considering the case of a nonelectrolyte in which case the charge, $z_e$, equals zero. In this case, Nernst-Planck equation becomes:
which is Fick’s first law of diffusion. On the other hand, for an ion with a uniform concentration throughout the system (dC/dx=0), the Nernst-Planck equation becomes:

\[ J_i = -D_i \frac{dC_i}{dx} \]

The Nernst-Planck equation may be thus interpreted as implying that when a concentration gradient and an electric field both exist, the ionic flux is a linear sum of the fluxes that would arise from each effect alone. Verification of the validity of the Nernst-Planck equation can be achieved by using thermodynamic expressions for chemical potential, since the driving force on the \( i \)th species is the negative gradient of its chemical potential.

### Electrical properties of the skin

Stratum corneum is composed of layers of horny cells which are a good insulator, and forms the principal barrier of the body to electrical conductivity. The relative conductivity of various tissues is known to be about equal to their water content, and the water content in the stratum corneum is about 20%, much lower than the normal physiological level of 70%. As the stratum corneum forms the high electric layer, it is a very important element for skin impedance. To understand the concept of impedance, the term called capacitance must be introduced: a pair of plates are arranged at parallel position and separated by a very small distance and connected to a battery which allows electrons to distribute over the lower plate. The electrons on the lower plate then induce a positive charge on the upper plate, so that more electrons can now flow into the lower plate from the battery. This arrangement of parallel plates acts as a capacitor (or condenser) and this property is called capacitance. Thus, the capacitance of a capacitor is its ability to store an electric charge, flowing into it in the form of current. Biological tissues such as skin tissue also have a capacitance because of their ability to store electrons, and are thus electrically capacitors.

### Iontophoretic patches

An iontophoretic patch (Figure 1) consists of three main components (3):

1. An aqueous drug reservoir that is usually biocompatible gel or absorbent-pad material. Positively charged ions are placed at the positive pole, while negatively charged ions are placed at the negative pole.

2. A return reservoir that completes the circuit. Typically, this circuit is a saline formulation.

3. An electronic controller that is programmable to give a complicate dosing feature.

**Figure 1. Simplified representation of the components of an iontophoretic patch.**

Transdermal drug delivery platform using an adhesive patch containing medication and a small electronic dose controller. The patch consists of two pre-loaded reservoirs, a drug reservoir that contains the drug to be delivered and a return reservoir with saline to complete the circuit. The controller contains a battery and a pre-programmed microcomputer to control the electrical charge. The two components connect through an interface (Figure 2).

**Figure 2. Iontophoretic system-way of drug delivery**

Factors affecting iontophoretic transport

Many factors have been shown to affect the results of iontophoresis. These include the physiochemical properties of the compound (molecular size, charge, concentra-
tion), drug formulation (type of vehicle, buffer, pH, viscosity, presence of other ions), equipment used (available current range, constant vs. pulsed current, type of electrode), biological variations (skin site, regional blood flow, age, sex), skin temperature and duration of iontophoresis.

A) Influence of pH

The pH is of importance for the iontophoretic delivery of drugs. The optimum is a compound that exists predominantly in an ionised form. When the pH decreases, the concentration of hydrogen ions increases, and a vascular reaction (vasodilatation) is initiated because of C-fibre activation. Thus, it is important to keep the pH as close as possible to 7, at least when working with vasodilators. At pH 5.5 and below, there is an increasing risk for vascular reactions due to the high concentration of hydrogen ions rather than the compound used. Since hydronium ions are small, they penetrate the skin more easily than larger drug ions.

B) Current strength

There is a linear relationship between the observed flux of a number of compounds and the applied current. With the electrode area of 1 cm², the current is limited to 1 mA due to patient comfort considerations. This current should not be applied for more than three minutes because of local skin irritation and burns. With increasing current, the risk of non-specific vascular reactions (vasodilatations) increases. At a current of 0.4 to 0.5 mA/cm², such a vascular reaction is initiated after a few seconds of iontophoresis with deionised or tap water.

C) Ionic competition

In a solution of sodium chloride, there is an equal quantity of negative (Cl⁻) and positive (Na⁺) ions. Migration of a sodium ion requires that an ion of the opposite charge is in close vicinity. The latter ion of opposite charge is referred to as a counter-ion. An ion of equal charge but of a different type is referred to as a co-ion.

When using iontophoresis, it is important to know that pH adjustment is performed by adding buffering agents. The use of buffering agents adds co-ions which are usually smaller and more mobile than the ion to be delivered. This results in a reduction of the number of drug ions to be delivered through the tissue barrier by the applied current. This means that when a positively charged drug is diluted in saline, the sodium ions will compete with the amount of drug ions to be delivered. Ideally, the use of a buffer system should be avoided in iontophoresis, but if this is not possible, alternative buffers consisting of ions with low mobility or conductivity are preferred.

D) Drug concentration

Depending on the drug used, the steady-state flux (ion movement) has been shown to increase with increasing concentration of the solute in the donor compartment, i.e. in the delivery electrode. A limiting factor to be considered is the strength of the current used. At higher drug concentrations, the transport may become independent of concentration, probably because of the saturation of the boundary layer relative to the donor bulk solution (4).

E) Molecular size

It has been shown that the permeability coefficients in positively charged, negatively charged and uncharged solutes across excised human skin are a function of molecular size. When the molecular size increases, the permeability coefficient decreases. However, there are certain solutes with a relatively high molecular size (e.g. insulin, vasopressin and several growth hormones), which have also been shown to penetrate the skin barrier into the systemic circulation.

F) Convective or electro-osmotic transport

When performing iontophoresis with a specific current, the flow of ions across the membrane induces a flow of solvent called electro-osmosis. Compared to the ion transport, the electro-osmotic contribution is small. The penetration of uncharged substances (e.g. bovine serum albumin) has been shown to be facilitated by the volume flow effect induced by an applied potential difference across the membrane. Iontophoresis has also been observed to enhance the penetration of a number of dipolar ions (zwitterionic substances, such as phenylalanine) . Most of these substances have been shown to be delivered in significantly higher amounts by anodic delivery than by cathodic delivery. In general, iontophoresis is more effective for charged compounds, especially monovalent ions.

G) Current - continuous vs. pulsed mode

Application of a continuous current over a long period of time can modulate iontophoretic delivery. Continuous DC current may result in skin polarisation, which can reduce the efficiency of iontophoretic delivery in proportion to the length of current application. This polarisation can be overcome by using pulsed DC, a direct current that is delivered periodically. During the "off time", the skin becomes depolarised and returns to its initial unpolarised status. The enhanced skin depolarisation using pulsed DC can, however, decrease the efficiency of pulsed transport if the frequency is too high (5).
H) Physiological factors

Iontophoresis reduces intra- and inter-subject variability in the delivery rate. This is an inherent disadvantage with the passive absorption technique. Experiments *in vivo* and *in vitro* give support for clinical findings that there are small differences in the flux rate following transdermal iontophoresis between males and females, as well as between hairy and hairless skin. The status of the vascular bed is also important; for instance, a pre-constricted vascular bed decreases the drug flux through the skin while a dilated vascular bed increases the yield of the drug through the skin.

Optimising iontophoretic transport

1. Iontophoretic transport can be regulated by varying the applied current density and area of application. A current density that is too high may be unpleasant for the patient. If possible, avoid using current settings that result in more than 500 mA/cm². At high current densities, there is a significant risk for unspecific electrically mediated vasodilatation that is not drug related.

2. The pH of the formulation should be optimised to ensure maximum ionisation of the compound. To prevent pH drifts during the iontophoresis, the choice of electrodes is of importance. With a correct electrode material, decreased solubility and precipitation of the compound are avoided.

3. Before iontophoresis is carried out, carefully clean the skin area to be used with deionised water or preferably 70 per cent alcohol. Cleaning will decrease the current needed and minimise the risk for local spots of high current density, which could result in C-fibre activation, vasodilatation and local micro-burns.

Advantages and disadvantages of iontophoresis

Advantages of iontophoresis

There are several advantages to iontophoretic drug delivery methods:

- The risk of infection is reduced because it is non-invasive there is no mechanical penetration or disruption of the skin to cause pain.

- Drug solutions are delivered directly to the treatment site without the disadvantages of injections or orally administered drugs, avoiding first pass metabolism.

- It eliminates multiple dosing per day.

- It provides a relatively pain-free option for patients who are reluctant or unable to receive injections. For example, with appropriate controls, iontophoresis can be an extremely attractive method for delivering drugs such as analgesics for the treatment of acute pain - the drug can be rapidly delivered for absorption and can, with appropriate controlling technology, be self-administered on demand by the patient

- It minimizes the potential for further tissue trauma that can occur with increased pressure from a fluid bolus injection.

Disadvantages of iontophoresis

Major side-effects are very rare when using iontophoresis as a diagnostic tool. However, minor reactions such as itching, erythema and general irritation of the iontophoretic skin surface are common. There is an increased risk of minor reactions if the exposure time and/or current are increased, and with some drugs like histamine, capsaicin and acetylcholine. Some drugs induce long-lasting skin pigmentation after iontophoretic application, where the intensity of skin discoloration is proportional to the exposure time.

The current density across the pores in the skin may be higher than the current per unit area applied, depending on the density of pores in a given area. These spots of high current density increase the possibility of current-induced skin damage.

It was shown (6) that the skin resistance was always less than the initial value when a current of 0.16 mA was applied for 10 minutes. This may result in a permanent skin damage. This phenomenon may explain the sudden vascular response with iontophoresis of deionised water, which seems not to be related to the dose. Under the microscope, small spots of skin damage within the pore area could be recognised. The vasodilatation initiated in this way may be caused by activation of nociceptive fibres terminating in the epidermis, which initiate an axonreflex mediated vascular response.

Although widely available, iontophoresis, to date, has been minimally employed. Technical issues have had to be overcome, in order to take full advantage of the approach. For example, the skin is relatively impermeable, and drug ions do not cross easily into the underlying tissue. Consequently, only smaller molecular weight drugs (generally under 10,000 Daltons) that are water-soluble are good candidates for delivery. Additionally, some patients, with certain formulations, experience redness, burning, and/or itching at the administration site. Perhaps a key disadvantage to the technology has been the lack of adequate delivery control and a low-cost,
long-lasting power source - optimizing the device relies on electrical current to deliver the drugs over a prescribed period of time in a carefully controlled manner

Contraindications for iontophoresis

Contraindications for iontophoresis are important in patients with higher susceptibility to applied currents. Such patients include those carrying electrically-sensitive implanted devices such as cardiac pacemakers, those who are hypersensitive to the drug to be applied, or those with broken or damaged skin surfaces.

Application of iontophoresis

Iontophoresis can be considered as an interesting alternative to parenteral route, particularly in the case of peptide drugs (e.g. insulin, calcitonin), macromolecular substances in ionized state at physiological pH values, that are poor absorbed and extensively degraded by proteolytic enzymes in the gastro-intestinal tract, showing extremely low bioavailability when administered orally.

Iontophoresis has been extensively investigated for local absorption of topically applied drugs (diagnosis of cystic fibrosis with pilocarpine, local anaesthetic with lidocaine, in the therapy of osteoarthritis, rheumatism, tendonitis-glucocorticoids, non-steroid analgetics, in the therapy of surface tissue diseases Herpes Simplex infections and psoriasis-acyclovir and khellin).

Conclusions

Iontophoresis offers the benefits of being painless and non-invasive. In addition, there is no danger of infection or damage due to needle insertion or to impact from a bolus of fluid. The local concentration of the drug is high, while the systemic concentration is minimal. Only minute amounts of the drug reach the systemic circulation, greatly reducing side effects. Drug dosage is accurately controlled by controlling the quantity of electrical current used to transfer the drug. Exposure to mild electrical current provides added therapeutic effects. Contraindications with this modality pertain to sensitivity to the drug rather than to the modality itself. The manufacturer suggests avoiding electrode placement so that the current pathway crosses the heart or the brain. Also the area of the eye should be avoided. Abraded skin or new scar tissue should be avoided as these areas are sensitive to electrical current, making the treatment uncomfortable. The equipment available today is efficient and miniaturized. The possibility of shock or burns, a problem with iontophoresis in the past, are now eliminated by advanced electrode design and modulated current. Iontophoresis has the potential to provide substantial benefits when this mode of therapy is applied in the appropriate manner (7). There is little doubt that many substances can be introduced into the body by this method. Iontophoresis offers a means of introducing medications through the surface of the skin in a safe, easy and painless manner. As with many new modes of therapy, however, there is need for more studies that document the use and effects of iontophoresis in various clinical situations.

References


Abstract

Coumarin and its derivatives are reactive compounds, suitable for many syntheses. They are used as anticoagulants, antibacterial, animistic compounds. The interest in coumarins has increased because it was found that they reduce the HIV virus activity.

The synthesis of 4-arylaminocoumarin derivatives from 4-hydroxycoumarin, has been carried out, and their antimycotic effects were tested. In the QSAR (quantitative structure-activity relationship) QSPR (quantitative structure-property-activity relationship) study we have used physicochemical properties and topological indices (Balaban index J(G), Wiener index W(G), information-theoretical index I(G), and valence connectivity index (G), to predict bioactivity of the newly synthesized coumarin compounds. By using methods of molecular modelling, the relationships between structure, properties and activity of coumarin compounds have been investigated.

The best QSPR models were obtained using valence connectivity index or combination indices. According Rekker's method the best correlation of calculated values log P, has been obtained with the model based on the inhibition zone (I) 4-arylaminocoumarin derivatives expressed in mm.

The results obtained in this study enable further synthesis of new coumarin derivatives and predict their biological activity and properties.

Keywords: QSPR, QSAR, derivates of 4-arylaminocoumarin

Introduction

Studies of natural and synthetic coumarins and its derivatives have been present for a number of years. Coumarins and their derivatives are characterised with very good chemical reactivity and different bioactivity. A great number of synthesized derivatives are biologically active, and many of them are applied in therapy as anticoagulant, antibacterial and antifungal agents (1). Recently the interest in coumarins has increased significantly because it was found that they reduce the HIV virus activity. (2, 3) A molecule of 4-hydroxycoumarin is very reactive and suitable for many syntheses. Due to the exceptional reactivity, we synthesized new 4-arylaminocoumarin derivatives from 4-hydroxicoumarin. Derivatives of 4-arylaminocoumarin from 4-hydroxycoumarins were synthesized and their microbiological activity were examined. Newly prepared coumarin derivatives have various constituents, and according to that, they can exhibit potential microbiological activity; therefore, the microbiological activity of these derivatives in case of various species of bacteria and fungi was tested. The results obtained indicate that newly synthesized compounds have much better animistic properties than antibacterial (4).

QSAR analysis is a useful tool for examining the relationship between the biological activities, the physicochemical properties and the molecular structures of a series of compounds. The fundamental axiom of QSAR and QSPR modelling is that the structures of molecules are reflected in their biological activities and physicochemical properties (5). Most molecular activities and properties can be represented by a single number, but molecular structure cannot similarly be represented. The representation of molecular structures by numbers is a way to encode the structural information in QSAR and QSPR studies. The modelling process reduced to a correlation between two set of numbers, one sets of numbers representing the molecular bioactivity or property and the other set representing the molecular structure. This correlation is meaningful only if it is carried out for a larger set of molecules.

In this work, several quantitative QSPR models and QSAR models with topological indices will be used to study of some physicochemical properties and bioactivity of newly synthesized coumarins have been investigated. Topological indices have been mainly used for purpose of correlating the properties of molecules with their topological structure, since most of biological processes are difficult to quantify accurately. Lipophilicity has been investigated by determining the log P (partition coefficient). Lipophilicity is a property of a molecule, which depends on structure and can be changed by the modifications in the molecular structure. (6)

Material and methods

Synthesis of derivatives of 4-arylaminocoumarin and their microbiological investigation

The synthesis of 4-arylaminocoumarins derivatives and their spectral characteristics, elementary analysis and results of microbiological activity were described in our previous investigation (4). The microbiological activity
of compounds was tested by the diffusion method on species Candida albicans 5934 (4) and are shown as inhibition zones expressed in mm. The results of synthesis, structure of 4-arylaminocoumarin derivatives and their microbiological activity are shown in Table 1.

According to the method of encoding the structural information, the QSAR and QSPR models may be classified into three major groups (7):

1. empirical models
2. quantum - chemical models
3. non-empirical models.

In our research, we applied non-empirical QSAR-QSPR models, topological indices and log P. In QSAR modelling of lipophilicity of our compounds, the following topological indices were used: the Wiener index. The valence-connectivity indices, the Balaban index and information-theoretical index. In order to describing of molecular properties our compounds we are used values of log P. These values for the studied compounds were calculated according to the method of Rekker (8).

**Topological indices**

**The valence connectivity index, \( \chi_v(G) \)**

The molecular connectivity index, \( \chi_v(G) \) of molecular graph G is defined as (9):

\[
\chi_v(G) = \sum_{i,j} d(i) d(j)^{-0.5}
\]

where the sum is taken over all edges of G. In equation d (i) and d (j) are the valences of vertices i and j making up edge i-j.

For heterosystems, the connectivity index is given in terms of valence delta values \( \delta(i) \) and \( \delta(j) \) of atoms i and j. In that case it is denoted by \( \chi^* \).

This type of connectivity index is called the valence connectivity index (10) and is defined as

\[
\chi^* = \sum_{i,j} [\delta(i) \delta(j)]^{-0.5}
\]

where the sum is taken over all bonds i-j of the molecule. In this case edge of G has a weight of \( \delta(i) \delta(j) \).

**The Balaban Index, J(G)**

Balaban index J (G) represents the extended connectivity. This index, denoted by J (G), is defined as (11):

\[
J(G) = \frac{M}{\mu+1} \sum_{\text{edges}} (d(i)d(j))^{0.5}
\]

Where is:

M - the number of edges in G;
\( \mu \) - cyclomatic number of G;
\( d(i) \) - distance sum (i = 1, 2, 3, ..., N-Number of vertices in G).

The distance sum for a vertex i represents the sum of all entries in the corresponding row (or column) of the D distance matrix:

\[
d_i = \sum_{j=1}^{N} (D)_{ij}
\]

The cyclomatic number \( \mu \) of a polycyclic graph G is equal to the minimum number of edges necessary to be erased from G in order to transform it onto the related acyclic subgraph:

\[
\mu = M - N + 1
\]

Barysz at al. have modified the elements of the distance matrix for heterosystems (12):

1) diagonal elements

\[
(D)_{ij} = 1 - \frac{Z_c}{Z_i}
\]

\( Z_c = 6 \) and \( Z_i \) is the atomic number of the given element;

2) off-diagonal elements

\[
(D)_{ij} = \sum_{r} k_r
\]

where \( k_r \), the bond parameter is:

\[
k_r = \frac{1}{b_r} \frac{(Z_c^2)}{(Z_iZ_j)}
\]

\( b_r \) is the bond weight that has values 1 for single bond, 2 for double bond. For different types of heterobonds, the values are given in the literature (13).

**The Information-Theoretic Index, I(G)**

This index was calculated by the modification of Shannon’s relation (14):

\[
I(G) = - \sum_{i=1}^{n} \frac{2N_i}{N(N-1)} \log \frac{2N_i}{N(N-1)}
\]

n - the number of different sets of elements

\( N_i \) - the number of elements in the i-th set of elements and the sum is over all sets of elements.

**The Wiener Index, W(G)**

The Wiener Index, W(G) of a structure G can be obtained from the distance matrix D of the corresponding hydrogen-depleted chemical graph G as the half-summation of the elements of D(15):
\( W(G) = \frac{1}{2} \sum_{ij} (D)_{ij} \)

\((D)_{ij}\) - off-elements of \(D(G)\) which stand for the shortest distance in terms of the number of bonds between atoms \(i\) and \(j\) in \(G\).

The calculation of partition coefficient logP (octanol/water)

Values the log \(P(o/w)\) for the series coumarines are calculated according to the method of Nys and Rekker (16,17):

\[ P(o/w) = \sum_{i=1}^{n} a_i f_i \]

\(f\) - the hydrophobic fragmental constant, the lipophilicity contribution of a constituent part of a structure to the total lipophilicity.

\(a\) - numerical factor which indicating the incidence of fragment \((f_n)\) in the structure.

TAM program for the calculation of topological indices in QSPR and QSAR studies was used.

Results

The results of synthesis, structure of 4-arylaminocoumarin derivatives and their microbiological activity and selected physicochemical properties: molecular mass \((M.m.)\), melting point \((M.p.)\) partition coefficient \((\log P)\) calculated according to Rekker’s method, van der Waals-volume \((Vw)\) and inhibition zones of arilaminocoumarin derivatives expressed in mm, and topologic indices: Wiener index \(W(G)\), information-theoretical index \(I(G)\), Balaban index \(J(G)\) and valence connectivity index \(\chi_v(G)\) are shown in Table 1.

Discussion

In this work, several quantitative QSPR and QSAR models for predicting properties and activity of 4-arylamino- coumarin derivatives are considered. As one might expect, different properties were best modelled with different regression and different indices. We have compared each physicochemical property with each topological index. To test the quality and accuracy of derived models, the following statistical parameters were used: \(n\) is the number of data points, \(r\) the correlation coefficient, \(s\) the standard deviation. Molecular mass shows best correlation with valence connectivity index \(\chi_v(G)\) (Figure 1). It is obvious that by increase of the molecule by substituents CH3, OCH3 and OC2H5 the correlation is decreased.

\[ \chi_v(G)=50.1754 - 0.3544 \cdot M.m + 0.0007 \cdot M.m^2 \]

\(n=10\)

\(r=0.9039\)

\(s=0.14\)

Van der Walls volume shows best correlation with valence connectivity index \(\chi_v(G)\)

\[ \chi_v(G)=3.2435 + 1.3225 \cdot V(W) \]

\(n=10\)

\(r=0.9667\)

\(s=0.08\)

Table 1. The structure of prepared 4-arylamino-coumarin, the selected physiochemical properties: molecular mass \((M.m.)\), melting point \((M.p.)\) partition coefficient \((\log P)\) calculated according to Rekker’s method, van der Waals-volume \((Vw)\) and inhibition zones of arilaminocoumarin derivatives expressed in mm, and topologic indices: Wiener index \(W(G)\), information-theoretical index \(I(G)\), Balaban index \(J(G)\) and valence connectivity index \(\chi_v(G)\).

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<th>(I(G))</th>
<th>(J(G))</th>
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</table>
The valence connectivity index $\chi_v(G)$ shows best correlation with Wiener index $W(G)$ (Figure 3). Connectivity index characterizes the structure best in these investigations. The sizes of the molecule and ramification are characterised by the valence connectivity index, the compounds are much related and characteristic groups methyl, methoxy, contributed the increase valence connectivity index as well as the Wiener index.

**Figure 3** The parabolic QSPR model of valence connectivity index $\chi_v(G)$ of 4-arylaminocoumarin derivatives based on the Wiener index ($W(G)$).

\[
W(G) = 12771.32 \chi_v(G) - 40988.60 - 966.31 \chi_v(G)^2
\]

\[n=10\]
\[r=0.9482\]
\[s=51.16\]

Table 1 shows the values of log $P$ which for the investigated compounds are all negative, indicating a decrease of lipophilicity. The QSAR model based on the partition coefficient has shown the best correlation with the inhibition zone (I) 4-arylaminocoumarin derivatives expressed in mm (Figure 4).

**Figure 4** The parabolic QSPR model of inhibition zone I (mm) of 4-arylaminocoumarin derivatives based on the partition coefficient log $P$

\[
I = 19.8574 + 9.1083 \log P + 1.8379 \log P^2
\]

\[n=10\]
\[r=0.9121\]
\[s=2.28\]

The partition coefficient was found most suitable in QSPR study and correlation with tested activity to Candida albicans 5934 is shown as the inhibition zone (I) of certain 4-arylaminocoumarin derivatives expressed in mm. The best activity to Candida albicans was shown by the compounds 4 and 9. Better activity to Candida albicans was shown by the compound with substituent on R1 position. The results obtained indicate that zone inhibition increases with lipophilicity.

**Conclusion**

We have investigated linear and several nonlinear relationships between the topological indices as discussed in previous section, and selected properties of 4-arylaminocoumarin derivatives.

In our investigations, the best QSPR models were obtained using valence connectivity index or combination indices. The best QSAR model was based on the partition coefficient and it has shown the best correlation with the inhibition zone (I) 4-arylaminocoumarin derivatives expressed in mm. All newly synthesized compounds have negative values of log $P$, and indicate decrease of lipophilicity. CH$_3$ group and Cl increased lipophilicity and antimycotic activity, especially when the substituent was on R1 position. Other examined indices and regression analysis have not a good correlation and are irrelevant for the purpose of this study. The results obtained in this study will enable targeted synthesis of new coumarin derivatives of defined physicochemical properties and desired biological activity.
References


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Third page - footnotes to the title, if any. List of any non-standard abbreviations.

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Number the remaining pages consecutively and type the author’s(s) last name(s) at the top of each page. Write in the first person (except summary) and the active voice whenever possible.

Keep the INTRODUCTION brief, stating clearly the purpose of the article and its relation to other papers on the same subject. Do not give an extensive review of literature.

Provide enough information in the MATERIAL AND METHODS section to enable other investigators to repeat the experiments.

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In the DISCUSSION interpret the results, state their meaning and draw conclusions. Do not simply repeat the results.

Start each section on a separate sheet.
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Tables and illustration: Tables and illustrations (both numbered in Arabic numerals) should be prepared on separate sheets. Tables require a heading, and figures a legend. Only good drawings and original photographs can be accepted: negatives or photocopies cannot be used. On the back of each illustration, indicate its number, the author’s name, and “top”.

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