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EDITORIAL

Nerve Agents – a Clear and Present Danger to Mankind

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ABSTRACT

This editorial is written on the occasion of the 25th anniversary of the infamous sarin and VX terrorist attacks in Japan, in order to increase the awareness of the potential terrorist use of nerve agents and to urge the preparedness to cope with its consequences. Nerve agents are extremely toxic organophosphorus acetylcholinesterase inhibitors, divided in three known groups: G-, V- and A-agents. G-agents tabun, sarin and soman were synthesised in Nazi Germany (1938-1944), V-agents including VX by the British in the 1950s and A-agents or Novichok agents between 1971 and 1993 in the Soviet Union. The use or alleged use of tabun and sarin was mentioned in connection with the Iraq-Iran war (1980-1988) and the Syrian conflict in 2013 and 2017. Sarin and VX were used for terrorist purposes by the Japanese religious sect AUM Shinrikyo in 1994 and 1995. The assassination of Kim Jong Nam with VX took place in Kuala Lumpur, Malesia in 2017, while the 2018 Salisbury and Amesbury poisonings in the UK were ascribed to the so-called Novichok agent A234. Milder cases of poisoning with nerve agents is accompanied by predominantly muscarinic symptomatology and more massive intoxications with mainly nicotinic and central symptoms. Treatment consists of use of atropine, oximes and anticonvulsants.

Key words: nerve agent, tabun, sarin, soman, VX, Novichok, atropine, oxime, anticonvulsant.

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INTRODUCTION

The reason why I decided to exercise my editorial prerogatives and write this editorial, was commemoration of quarter of a century after the first terrorist attack with some nerve agent took place – it happened in Matsumoto, Japan, on 27 June 1994 and sarin was then released in the residential part of the city, with 600 people injured and 7 dead. This grim "jubilee" falls also close to the 25th anniversary of the December 1994/January 1995 Osaka and Tokyo VX attacks, with three people poisoned, one of whom died, and heralds quarter of a century after the infamous 20 March 1995 Tokyo subway sarin attack that injured some 5,000 men and women and claimed lives of 12 people. Use or alleged use of sarin, VX and a Novichok agent wrote a new page in the history of terrorism and constitute a clear warning that the healthcare systems should be alert and prepared for their use. This is very important, since in many cases precious time was lost because physicians could not recognise the symptomatology of intoxication with an anticholinesterase compound.

BRIEF HISTORY OF NERVE AGENTS

Ever since they were conceived as a new and the deadliest class of chemical warfare agents in late 1930s in Gerhard Schrader's laboratory of IG Farbenindustrie in Nazi Germany (hence the NATO code G-agents, for German), nerve agents have threatened to be the most nefarious means of mass destruction. After the synthesis

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of tabun in 1936, sarin (1938) and soman (1944) followed, coded by North Atlantic Treaty Organisation (NATO) GA, GB and GD, respectively, but none of them was used during the World War II. The same goes for the second subclass of nerve agents (V-agents; V was for "venomous") synthesised by the British in the 1950s. The best known among them is VX that became part of the US nerve agent armamentarium. Leading military powers stockpiled in the 1950s and 1960s a substantial arsenal of various nerve agents. Later they were banned by the Chemical Weapons Convention (CWC) of 1993 that came into effect in 1997.

The first documented use of nerve agents was in the Iraq-Iran war (1980-1988), when tabun and sarin use by the Iraqis was proven, along with blistering agent sulphur mustard1. Use of sarin was reported in the Syrian conflict in 2013 and 2017.[2] Although every use of nerve agents represents act of violation of CWC and automatically constitutes a criminal act against humanity, what gave a new dimension to the problem was their abuse by the non-governmental entities for the purpose of terrorism. The Japanese religious cult AUM Shinrikyo used sarin on two purposes in Matsumoto in June 1994[3] and in Tokyo subway in March 19954 for massive intoxications and VX in December 1994 and January 1995 for assassination of individuals – ex-AUM members and anti-AUM activists, with one fatality, out of three victims.[5]

Similar pattern - percutaneous poisoning with VX was used at the Kula Lumpur International Airport, Malaysia, to assassinate Kim Jong Nam, a North Korean president's half-brother in February 2017.[6] A member of a special, most toxic and least publicly known subclass of Russian nerve agents called A-agents or the Novichok ("newcomer" in Russian) agents, was allegedly used in the Salisbury poisonings in the UK on 4 March 2018, when the Russian double agent Sergey Skripal and his daughter Yulia Skripal were poisoned, along with a police officer who investigated the case. Novichok agent A234 was found at the Skripal residence in a perfume bottle.[7,8] All three of them recovered after a lengthy hospitalisation. On 30 June 2018, two people were poisoned with identical clinical presentation after spraying themselves from a container found discarded in a park in Amesbury, some eight miles from Salisbury; one of them died.[9]

Actually, the pattern of terrorist use of sarin and VX or Novichok agent – for massive inhalation poisonings and individual percutaneous assassinations, respectively reflects differences in their physico-chemical properties. Although all nerve agents are frequently called "nerve gases" they are not gases at all. At room temperature, they are either easily evaporating liquids (G-agents) or thick, oily liquids that almost do not evaporate at room temperature at all (V-agents). In fact, sarin has around 2,100-fold higher volatility than VX.[10]

MECHANISM OF TOXICITY OF NERVE AGENTS, SIGNS AND SYMPTOMS

All these subclasses of nerve agents share the same mechanism – irreversible inhibition of acetylcholinesterase (AChE), with consequential accumulation of high concentrations of acetylcholine and hyperstimulation of postsynaptic cholinoceptors – muscarinic and nicotinic ones. Milder forms of poisoning are accompanied by miosis, rhinorrhoea, tightness in chest and hypersalivation, while more massive intoxications result in mixture of muscarinic, nicotinic and central symptoms, including tonic-clonic convulsions, failure of the respiratory and vasoactive centre and death.

Classically, symptoms of poisonings with nerve agents are divided into three categories: muscarinic, nicotinic and central. (1) Muscarinic signs and symptoms include miosis and compromised accommodation of the eye (hence dim vision), increased secretion of exocrine glands (hypersalivation, bronchorrhoea), increased smooth muscle tone (bronchoconstriction, stimulated peristaltic bowel movements) and hypotension and bradycardia. (2) Stimulation of nicotinic receptors results in skeletal muscle fasciculation and, due to stimulation of nicotinic receptors in sympathetic ganglia, in mydriasis, hypertension and tachycardia. (3) Central effects encompass headache, vertigo, vomiting, convulsions and cessation of the central respiratory drive.[11] It is believed that even the central effects of nerve agents are consequence of the stimulation of muscarinic and nicotinic receptors in the brain, followed by the secondary stimulation of glutamatergic and opioidergic structures.[12] Poisoning with high doses of nerve agents brings

sometimes the dominance of nicotinic signs and symptoms over the expected muscarinic ones, which masks the clinical presentation, postpones the establishment of the correct diagnosis and hence timely administration of atropine and other antidotes.[13] Such cases were indeed described in sarin-intoxicated patients in the Tokyo subway attack.[4]

THERAPY OF NERVE AGENT POISONING

Standard therapy is triple: administration of sufficiently high doses of atropine, AChE reactivators - oximes, such as pralidoxime salts (2-PAM or P2S), obidoxime (LüH-6) and asoxime (HI-6) and anticonvulsants, mainly diazepam or midazolam.14 Although atropine itself and especially when overdosed, exerts some adverse effects (tachycardia, xerostomia, impaired cognitive functions), it is essential to repeat the single shots of 2 mg each until the proper atropinisation is reached. It is a doctrinary position that mild atropine overdose is less dangerous than untreated anticholinestarese poisoning.[11]

Use of oximes as unique causal antidotes is absolutely preferable, if there are some oximes at hand, but even if there is none, sufficiently high doses of atropine are essential for survival. Anticonvulsants are used as gamma-aminobutyric acid (GABA)-receptor agonists (eg benzodiazepines diazepam or midazolam and barbiturates, such as thiopental sodium) and it is important that they are administered timely, before the convulsions enter deeper into the so-called glutamatergic phase, where only antagonists of glutamate receptors (eg ketamine, memantine or procyclidine) can be effectively utilised.[15]

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CONFLICT OF INTEREST

None.

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ORIGINAL ARICLE

The Effects of Certain Gasotransmitters Inhibition on Homocysteine Acutely Induced Changes on Rat Cardiac Acetylcholinesterase Activity

Marko Djuric[1], Slavica Mutavdzin[2], Dragana Loncar-Stojiljkovic[3], Sanja Kostic[4], Mirjana B. Colovic[5], Danijela Krstic[6], Vladimir Zivkovic[7], Vladimir Jakovljevic[7], and Dragan M. Djuric[2]

ABSTRACT

Background/Aim: Hyperhomocysteinaemia is linked to higher level of acetylcholinesterase (AChE) in brain, but there is insufficient information on influence of homocysteine (Hcy) and gasotransmitters on cardiac AChE. Thus, the aim of this study was to evaluate the influence of certain gasotransmitter inhibitors in Hcy-induced changes on rat cardiac AChE activity.

Methods: Research was performed on 72 male Wistar albino rats distributed into 6 groups: 1) Control group – saline (1 ml 0.9 % NaCl ip); 2) DL-Hcy (8 mmol/kg ip DL homocysteine (DL-Hcy); 3) L-NAME (10 mg/kg ip N$^{\omega}$-Nitro-L-arginine methyl ester (L-NAME), inhibitor of NO production); 4) DL-PAG (50 mg/kg ip DL-propargylglycine (DL-PAG), inhibitor of H2S production); 5) DL-Hcy+L-NAME (8 mmol/kg ip DL-Hcy + 10 mg/kg ip L-NAME); and 6) DL-Hcy+DL-PAG (8 mmol/kg ip DL-Hcy + 50 mg/kg ip DL-PAG). All tested substances were administered in a single dose, intraperitoneally, 60 minutes before animals' sacrifice. AChE activity was measured in the rats' cardiac tissue homogenate.

Results: Administration of Hcy and L-NAME induced significant decrease in AChE activity compared with control condition. Administration of DL-PAG, DL-Hcy+L-NAME and DL-Hcy+DL-PAG did not change AChE activity compared with the control group.

Conclusion: The effects of acute Hcy administration on the cardiac AChE activity are partially mediated via interaction with tested gasotransmitters.

Key words: acetylcholinesterase, heart, homocysteine, inhibition.

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INTRODUCTION

Hyperhomocysteinaemia is an independent predictor of different diseases such as, cardiovascular and cerebrovascular diseases, neurodegeneration and cancer.[1] Increased blood homocysteine (Hcy) level independently predicted all-cause and cardiovascular mortality in the general and especially in the older population,[2] and it has been recognised as a risk factor for cardiovascular diseases.[3,4] Different studies[5-9] have demonstrated that increased Hcy concentration was correlated with poor prognosis in patients with acute coronary syndrome, but other studies results were contradictory.[8,10-13] Previous study revealed that increased Hcy may be associated with elevated oxidative stress and inhibition of the butyrylcholinesterase (BuChE) activity.[14]

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This may result, probably, in cardiovascular diseases and consequently in an increase of the mortality risk.[14] It has been reported that after both, acute and chronic Hcy administration in rats, serum BuChE activity was significantly decreased. Antioxidants, such as vitamins E and C, avoided the decrease of this enzyme activity caused by acute Hcy administration, implying that free radicals are responsible for reducing BuChE activity under conditions of acute hyperhomocysteinaemia.[14] Another study showed that the increased concentration of Hcy in serum decreases the activity of acetylcholinesterase (AChE).[15] Many tissues, and especially the nerve tissue, are rich in AChE and BuChE.[16] BuChE is the most abundant cholinesterase in serum[17], while AChE is primarily present in membranes of erythrocytes.[18] AChE is an enzyme belonging to the group of serine hydrolase with the primary function of hydrolysing neurotransmitter acetylcholine.[19] It is mainly located at cholinergic brain synapses and neuromuscular junctions. Although less than there, AChE activity could be also very important for functioning of cholinergic system within the heart.[20] Even if it hydrolyses acetylcholine, it is demonstrated that AChE is also present in hematopoietic tissue and cancer cells that are not innervated by cholinergic system.[21]

Cetrain signaling gaseous molecules or gasotransmitters, such as nitric oxide (NO) and hydrogen sulfide (H2S) participate in effects of Hcy-thiolactone on the coronary circulation and myocardial function.[22,23] Gasotransmitters have many important roles. They participate in the regulation of inflammation, modulation of mitochondria respiration and activation of antioxidant enzymes and consequently have essential role in oxidative stress regulation, so their cardiac effects are expected and reasonable.[24] It is demonstrated that S-nitroso-Hcy inhibits hydrogen peroxide production with participation of NO.[25] H2S lowers plasma Hcy level and it scavenge reactive oxygen species and functions as an antioxidant.[26,27]

However, connection between Hcy and certain gasotransmiter effects is still not fully understood. Taken into consideration assumptions referring to existence of AChE within the heart[20], as well as insufficient data about complex interaction between gasotransimtters and Hcy in the cardiac muscle, researches in order to evaluate the role of gasotransmitters (NO and H2S) on Hcy-induced effects on AChE in rat heart are necessary. Therefore, the aim of this study was to assess the effects of acute administration of DL-Hcy, as well as administration of DL-Hcy together with specific inhibitors of different gasotransmitters, such as Nω-nitro-L-arginine methyl ester (L-NAME) and DL-propargyl Glycine (DL-PAG) on AChE activity in rat heart tissue.

METHODS

Physiological Assay and Experimental Protocol

Experiment was performed on male Wistar albino rats (n = 72, 12 in each experimental group, 10 weeks old, body weight 250 ± 30 g). Experimental animals were housed in pairs with standard food and water available ad libitum. The ambient conditions were strictly controlled (air temperature of 22±1°C, relative humidity of 50%, and a cycle of light: dark 12:12 hours, starting light period at 8 AM). In all experimental groups, tested substances were administered in a single dose, intraperitoneally (*ip*), 60 minutes before sacrificing of animals. The experimental animals were distributed randomly in one of six groups: 1) Control group – saline (1 ml 0.9% NaCl *ip*, pH 7.4); 2) DL-Hcy group (8 mmol/kg *ip* DL homocysteine); 3) L-NAME group (10 mg/kg *ip* L-NAME as inhibitor of NO production via inhibition of nitric oxide synthase); 4) DL-PAG group (50 mg/kg *ip* DL-PAG as inhibitor of H2S production via inhibition of cystathionine gamma lyase); 5) DL-Hcy+L-NAME group (8 mmol/kg *ip* DL-Hcy + 10 mg/kg *ip* L-NAME); 6) DL-Hcy+DL-PAG group (8 mmol/kg *ip* DL-Hcy + 50 mg/kg *ip* DL-PAG).

All experimental procedures were done in agreement with prescribed legislation (EU Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 86/609/EES) and the principles of ethics.

Tissue preparation

Sixty minutes after *ip* administration of tested substances, the rats were euthanised by decapitation. Whole rats' hearts were isolated and the blood was stored in test tubes coated in heparin. The hearts were rinsed in cold phosphate buffer pH 8.0, and homogenised in the same buffer.

The final tissue concentration was 20 mg tissue per 1 ml buffer.

Biochemical analyses

For the biochemical analyses blood was collected through a glass funnel and placed in appropriate vacutainers coated in heparin. After the collection, the samples remained at room temperature for 15 minutes and then they were centrifuged (15 min x 3000 rpm) and in the obtained plasma Hcy concentration was analyzed. Following the sacrificing of rats, AChE activity was determined in samples of cardiac tissue homogenate.

Determination of plasma Hcy

For this process the samples were analyzed using the electrochemiluminescence method (ECL-electrochemiluminescence immunoassay system, ADVIA Centaur XP System, Siemens Healthcare GmbH, Erlangen, Germany). The reference value for Hcy was < 15 µmol/l.

Determination of AChE activity

The specific activity of AChE in samples of cardiac tissue homogenate was determined *in vitro* by method of Ellman. The method is based on reaction of a colouring reagent (5, 5-dithio-bis-2-nitrobenzoic acid, DTNB) with the hydrolysis product of thioholine substrate, acetylcholine iodide (AChI), thioholine, to give the compound 5-thio-2-nitro-benzoate-yellow color, whose intensity is proportional to the specific activity of AChE.[28]

Chemicals used

All chemicals were of p.a. grade quality and were purchased from Sigma Aldrich (Germany).

Statistical analyses

One-way analysis of variance (ANOVA), followed by Tukey's Post Hoc Test was used for testing statistical significance after testing normality of parameters distribution. Statistical calculation was done using SPSS computer program (SPSS Inc. Chicago, SAD). Values were presented as mean ± SEM. P < 0.05 was considered statistically significant.

RESULTS

Determination of Hcy

In the Control group plasma Hcy was 10.4 ± 0.6 µmol/l, while in all other plasma samples levels of measured Hcy were higher than 65 µmol/l. These results demonstrate moderate hyperhomocysteinemia (30-100 µmol/l).

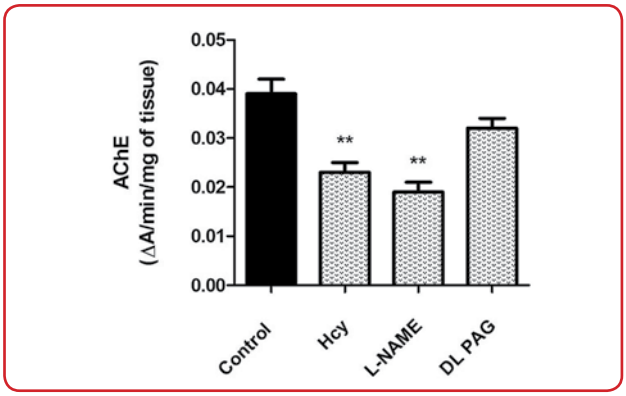

Figure 1 a

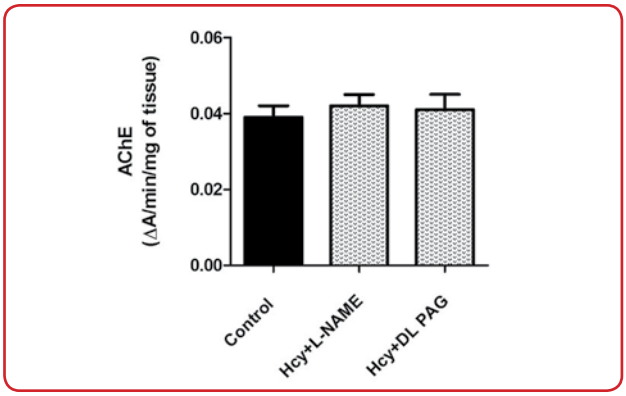

Figure 1 b

Cardiac tissue homogenate AChE activity

Administration of Hcy (0.023±0.002 ΔA/min/mg of tissue, Figure 1. a) and L-NAME (0.019±0.002 ΔA/min/mg of tissue, Figure 1. a) induced significant decrease in AChE activity compared with the control group (0.039±0.003 ΔA/min/mg of tissue). Administration of DL-PAG (0.041±0.004 ΔA/min/mg of tissue, Figure 1. a), DL-Hcy+L-NAME (0.042±0.003 ΔA/min/mg of tissue, Figure 1. b) and DL-Hcy+DL-PAG (0.041±0.004 ΔA/min/mg of tissue, Figure 1. b) did not induce significant changes in AChE activity compared with control condition.

DISCUSSION

Few studies have proven certain cardioprotective effects of ACh, but only in conditions of hypoxaemia, ischaemia and inflammation.[29,30] All these conditions are highly correlated with oxidative stress and ROS production. Mentioned studies have shown that these effects are

achieved by cytokine inhibition[30], but also by activating muscarinic receptors and NO production.[29] This could explain the significant reduction of AChE activity in cardiac tissue, observed in this study, in acutely induced hyperhomocysteinaemia as a compensatory mechanism, leading to increase of ACh. Even more prominent decrease of AChE activity occured during application of L-NAME, which is also known as a non-selective muscarinic antagonist.[29] Contrary to this point of view, it could be assumed that one of potential mechanisms through which Hcy manifests its pro-arrhythmogenic potential[31] might be decrease of AChE activity, having in mind that AChE is mainly located in region of SA and AV nodles.[32] Additional investigations are needed to determine whether this is a compensatory mechanism for direct effect of Hcy.

Findings of this study are corroborated by other studies that also showed AChE reduction.[15] Stefanello and coworkers, investigated the effects of Hcy (500 μM) on other cholinesterase involved in ACh degradation, ie BuChE, and found that Hcy strongly inhibited activity of this esterase in rats, as well.[15] Also, few years later, the same authors examined and compared acute and chronic effects of Hcy on BuChE activity, and these data suggested the inhibitory effects of Hcy also.[14] The previous study demonstrated that the combination of Hcy and ZnPPR IX has led to increased activity of AChE in relation to the control, suggesting that CO is potentially very important gaseous molecule for mediation of Hcy-induced effects on cardiac AchE.[33]

Finally, the limitation of this study could be that determination of certain gasotransmitters effects was not evaluated directly, but indirectly by inhibition of their production. Data on mRNA or protein levels in the cardiac tissue, as well as cellular data would verify the findings that cardiac AChE activity is altered by homocysteine level.

CONCLUSION

It has been concluded that Hcy may alter function of rat heart in part by reduction of AChE activity; however, there are limited supportive data presented in the manuscript. This is an association study where administration of Hcy *ip* or certain gasotransmitters production inhibition was associated with up-, or downregulation of acetylcholine. This study can contribute to the clarification of these interactions.

CONFLICT OF INTEREST

None.

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AUTHORSHIP STATEMENT

Marko Djuric: performing of experimental procedures, statistical analysis, interpretation of data, preparation of the manuscript; Slavica Mutavdzin: statistical analysis, interpretation of data, preparation of the manuscript; Dragana Loncar-Stojiljkovic: interpretation of data, analysis of results, preparation of the manuscript; Sanja Kostic: statistical analysis, interpretation of data, preparation of the manuscript; Mirjana Colovic: biochemical analysis; Danijela Krstic: biochemical analysis, interpretation of data; Vladimir Zivkovic: interpretation of data, analysis of results; Vladimir Jakovljevic: interpretation of data, analysis of results; Dragan Djuric: study design, performing of experimental procedures, statistical analysis, interpretation of data, analysis of results, preparation of the manuscript.

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CONTENT

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ORIGINAL ARTICLE

Dermal Regeneration with MilliGraft® Kit of Nanofat: the Micrograft of Adipose Tissue. A Clinical Assessment Study

Fabiano Svolacchia[1], Lorenzo Svolacchia[2]

ABSTRACT

The simple filtration by means of the MilliGraft® Kit of a disaggregated lipoaspirate allows to extract the class of cells defined as progentors with characteristics of adult stem cells present in the nanofat exclusively on the basis of their dimensions. It also allows the elimination of the fibrous branches and cell membranes destroyed by the emulsion phase and obtain a population of cells deprived of the inflammatory component. This method was used in regenerative and aeshetic medicine treatments with excellent and lasting clinical results in the follow-up phase.

Key words: dermal regeneration, nanofat, mesenchymal stromal progenitor cells, microfiltrate, MilliGraft®.

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INTRODUCTION

The physiological turnover of adult tissues loses efficiency after the first third of life through chrono-aging and photo-aging due to the progressive loss of the normal cellular turnover guaranteed by adult stem cells contained in the niches of differentiated tissues. The niches are made up of specialised cells protecting the mesenchymal stromal progenitor cells (MSCs) that are continually attacked by oxygen free radicals and ultraviolet B (UVB) radiation.[1] Through the influence of these highly reactive chemical species, adult stem cells will be carried out in a phase of quiescence that will force them to remain in the Go/G1 phase, influencing and facilitating their transition in the subsequent phase of senescence.[2] The senescence that adult stem cells will meet will prevent the physiological regeneration of tissues of the tissue progenitor population. Previous studies have been conducted on tissue progenitors from the dermis.[3]

In the present clinical study, it was assumed that, in order to improve photo-aging and chrono-aging damage, the dermis could be replenished with a vital micrograft from adipose tissue that contains the progenitors with adult staminal markers and those that best embody the characteristics of adult stem cells. The adipose tissue was chosen because of the simplicity of extraction and its abundance. It was processed and disaggregated through the MilliGraft® kit that allowed to deprive the tissue of cellular debris and fibrous shoots.[4] Such a suspension was injected into the dermis. It was also hypothesised that the triglycerides derived from the fragmentation of adipocytes increased the potential of engraftment[5-7] of the side population that remains suspended in triglycerides after filtration.[8]

It was also assumed that the adult stem cells derived from adipose tissue and their secretomes were able to allow for normalisation of the extracellular matrix with neocollagenogenesis and neovasculogenesis in the dermis without being influenced by hypoxia of the recipient tissue typical for aged tissue.[4] The method adopted is

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the MilliGraft® kit, a method based exclusively on the dimensions of the vital grafts and the triglyceride vehicle[5-7] in which the micrograft is suspended.[9] With the MilliGraft® kit it was able to prepare a sample of adipose tissue according to the technique of cytometry. By means of this method the tissue is disaggregated by special devices and filtered according to the necessary measures. The flow cytometer showed then that the side population, which is the one that best embodies the values of the progenitor, is smaller than the differentiated cells, that they have a greater cytoplasmic complexity and a greater expression of the stem markers. The task of these cells is to precisely maintain the equilibrium, the tissue homeostasis and the regenerative capacity of adult tissues in an incredibly flexible process.[4]

The choice of adipose tissue was also dictated by the biological reasons for the wealth of side population contained in it even after disaggregation.[5,6] In fact, it is possible to isolate 5x103 cells from a gram of it, about 500 times more than from the bone marrow[9] with the surface expression of CD 105, 90, 73 and 44 as markers[8] typical for adult stem cells[10]. Mesenchymal stromal progenitor cells (MSCs) are a population of non-haematopoietic and multipotent cells and are able to self-renew and differentiate into mesodermal cell lines. Under the normal conditions of a cell culture, they adhere to plastics and differentiate into osteoblasts, chondroblasts and adipocytes.[11] MSCs isolated from adipose tissue can function as a source of cells for the repair and dermal regeneration affected by chrono- and photo-ageing.[12] These cells have shown not to lose their vitality during the various cultivation steps up to the passage number eight and without showing typical senescence characteristics of differentiated tissues.[13] They possess a greater proliferative capacity than the bone marrow MSCs.[14]

Therefore, the preparation of adipose tissue obtained by means of the MilliGraft® kit allowed to obtain the side population and the vascular stromal fraction exclusively according to the principle of cell size.[3] The dimensional characteristics of the MilliGraft® kit allowed for the exclusion of the inflammatory fibrous shoots and cellular debris in a reproducible way without the use of enzymes.[12] Despite of the fact that a part of these cells can be lost during the disaggregation,[15] the filtration has shown that more colonies can be obtained in culture than in the unfiltered nanofat.[16] The vascular stromal fraction is rich in pericytes and active mesenchymal cells that are able to accelerate the process of tissue regeneration1.[7, 18]

The disaggregation of adipose tissue allows for the release of lipoprotein lipase and adiponectin. The function of lipoprotein lipase is to hydrolyse triglycerides in fatty acids and glycerol and is a key enzyme for the metabolism of lipoproteins and triglycerides. Lipoprotein lipase induces an increase in retention and absorption of all classes of lipoproteins.[19,20] Consequence of this induction is an increase in energy production in cells and greater protein synthesis. Adiponectin enables the catabolism of fatty acids and inhibition of inflammatory processes by improving metabolic energy in tissues.[6] Through the mesotherapeutic supplementation of adipose-derived MSCs the formation of inflammasomes is inhibited and the regulation of the population of macrophages M1 is activated through the prostaglandin E.[27]

It should be considered that fibroblasts are the most represented cells in the dermis. They are able to accumulate triglycerides[21] and to degrade them together with cholesterol esters in two different catabolic ways.[22] Injection of a microfiltered adipose tissue through MilliGraft® kit in the dermis also enables the regulation of EGFR and ERBB[3] and to normalization of sebaceous lipogenesis. In fact, triglycerides are important compounds for the skin, produced by the sebaceous glands. It was also hypothesised that the triglycerides that carry the MSCs decrease the release of LDH and reactive oxygen species (ROS) generation.[23] According to this hypothesis, there is a greater cellular vitality, a higher metabolic activity of the cells and a higher level of ATP.[24]

Triglycerides as a vehicle for suspended MSCs also cause vasodilation and increased local blood flow.[25] For this reason, it was also hypothesised that a temporary increase in blood flow in the dermis with consequent physiological normalization of the tissue increases the replication and the plastic potential of MSCs.[26] Triglycerides used as a vehicle for MSCs promote cell membrane phospholipids in human fibroblasts.[27]

The study was aimed at evaluating the clinical results of the improvement of the skin condition and to clinically verify the therapeutic response by using a microfiltrate of the population of the adipose tissue.[3]

METHODS

A total of 124 female patients were studied and their age ranged between 28 and 72 (average age 48). They signed informed consent for the use of lipoaspirate for experimental procedures. The study was performed following the standards of the local ethics committee and in accordance with the Helsinki Declaration (2000). None of the patients presented any specific pathology other than the dermal-epidermal aging process. Once the donor area was identified, the Klein solution was injected to anaesthetise the collection site. The adipose tissue was extracted under a tumescent anesthesia that allows the extraction of a greater number of viable grafts than a local anesthesia.[28] The adipocytes were extracted through a 10 ml syringe with Luer lock and a 16-gauge needle (Figure 1).

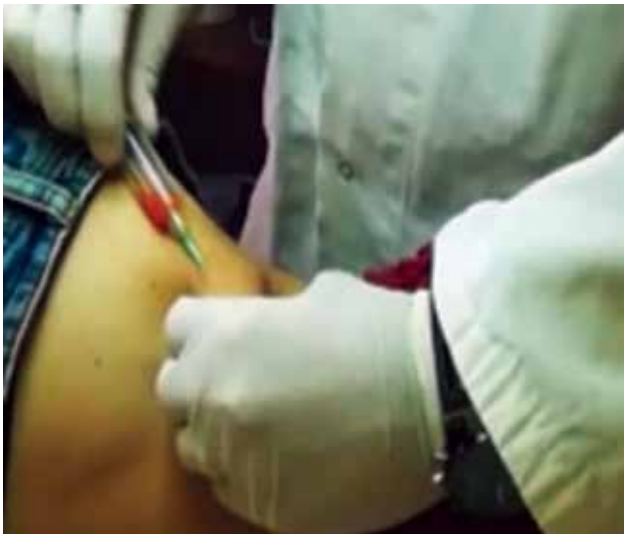

Figure 1: Tissue estraction

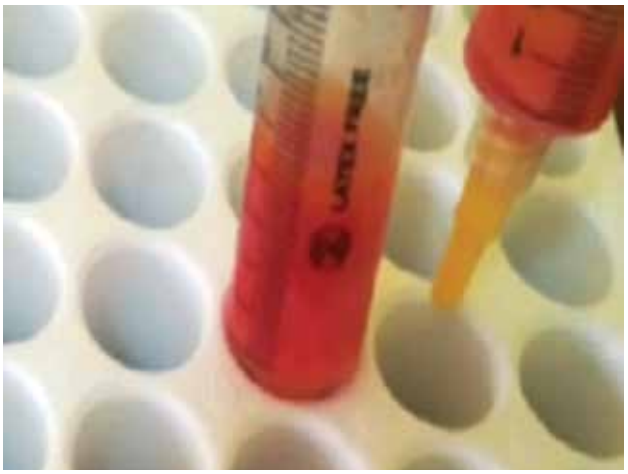

Figure 2: Decantation of fat

Figure 3: Microfiltrate

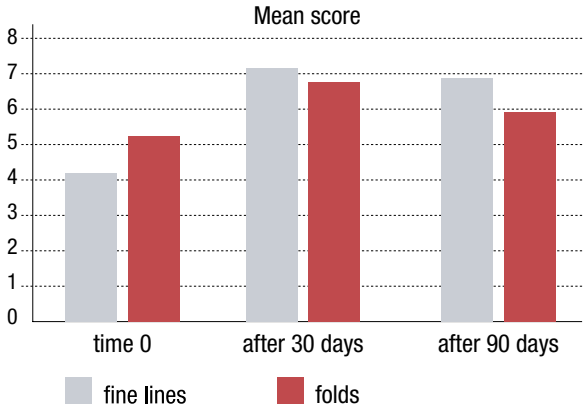

Figure 4: Clinical Mean score of treatment

An aliquot of 6/7 ml of tissue that has been decanted to eliminate anesthesia fluids was extracted (Figure 2) to obtain 5 ml of adipose tissue that was disaggregated through the MilliGraft® kit and filtered to 50 microns in order to obtain the vital side population[3,9] (Figure 3) and MicroGraft® according to the principles of cytometry. In this way the fibrous shoots and cellular debris were eliminated.[4,16] The microfiltrate was used for face, neck and décolleté treatments with 1 ml Luer lock syringes and 30 gauge needles. All the patients have shown an improvement with the treatment they have undergone and they have expressed satisfaction with the improvements achieved. Inspection visits and evaluations were made on the first day (D1, baseline) and after 30 (D30) days of treatment, with a follow-up visit after three months (Figure 4).

DISCUSION

This *in vivo* study of the MilliGraft® kit technique enabled the evaluation of a new method of dermal regeneration inspired by the cytometry method applied to a nanofat on our patients. The cytometry counts the cells in a suspension and identifies them through the markers. Because MSCs are the same as a classic lipoaspirate in the disaggregated adipose tissue, it was separated from the fibrous shoots and cell membranes of the destroyed adipocytes to make sure that a less polluted side population is achieved. The presence of MSCs is provided by the size of the filter used and the size of tissue progenitors.[3,4]

In vivo results we obtained with the MilliGraft® kit show that tissue progenitors can be isolated from the examined samples. This technique is suitable for preserving the side population thanks to the filtration measurement to which the nanofat is subjected. In this way, it was injected into the dermis of our patients through the mesotherapeutic technique, a considerable amount of viable MicroGraft deprived of the potentially inflammatory component. These MicroGrafts are able to normalise a physiological production of collagen type I and type III.[29]

Through filtration we have overcome and attenuated the biological events caused by the production of inflammatory cytokines produced by macrophages and dendritic cells present in the dermis to an increasing extent at the age[1] and triggered by fibrous shoots and by the cell membranes of the destroyed adipocytes.

The fibrous shoots and cell membranes that are injected through the nanofat technique are recognized by the TOLL-LIKE system to trigger, within that class of cells and other cells, the pro-inflammatory NF-Kb1 program interfering with the rejuvenation treatment.

The initiation of the inflammatory phenomenon through the injection of nanofat has been demonstrated[30,31] and the inflammatory state related to the dermis selects fibrotic fibroblasts with production of fibrotic collagen.[32] Through this simple procedure it will be possible to inject a nanofat deprived of the inflammatory component.

CONCLUSION

The treatment was well tolerated and the clinical results confirmed the working hypothesis on dermal-epidermal regeneration with the MilliGraft® kit procedure.

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None.

CONFLICT OF INTEREST

None.

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ORIGINAL ARICLE

Multimodal Neural Block Analgesia Versus Morphine Analgesia After Elective Knee Surgery

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ABSTRACT

Background: Total knee arthroplasty has become a standard for treatment of end-stage knee osteoarthritis. Due to intense and complex knee innervation, there is a need to improve the anaesthetic/analgesic approach to such operations. The aim of this randomised clinical trial was to compare the analgesic efficacy of the classical regimen and two of those based on the nerve blocks.
Methods: A total of 60 patients was included and subjected to elective total knee arthroplasty under the general balanced anaesthesia. They were randomised to receive postoperatively (1) only morphine 5-10 mg q6h and paracetamol 1 g q6h (MP), (2) femoral nerve block (FNB) or (3) fascia iliac compartment nerve block (FICNB). Nerve blocks were produced by a single administration of 30-40 ml of bupivacaine 0.5%. Pain intensity, duration of neural block and additional consumption of analgesics were recorded postoperatively.
Results: There were no demographic differences among the three groups of patients. Pain intensity was significantly lower in the two nerve block groups than in the MP group. The same two groups also demanded significantly less analgesics postoperatively than the MP group. Cardiovascular control was significantly better in the nerve block groups. There were no significant differences between the FNB and FICNB groups of patients regarding any of the studied parameters.
Conclusion: In comparison with the classical MP analgesia, use of FNB or FICNB after the elective total knee arthroplasty results in lower pain scores, lower systolic blood pressure and less consumption of analgesics in the immediate postoperative course.

Key words: knee arthroplasty, anaesthesia, nerve block, morphine, paracetamol.

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INTRODUCTION

Osteoarthritis (OA) is the leading form of arthritis in humans and is responsible for suffering of the 15-18% of the population.[1] According to the Danish registry of chronic diseases, the prevalence of the knee localisation of OA is 3.9%.[2] According to the Korean study performed in patients aged >50 who underwent knee radiography, prevalence of knee OA was 13% and it was positively associated with risk factors, such as older age, female gender, obesity, hypertension, low educational level, and infrequent strength exercises.[3]

It remains the main cause of the dysfunctionality of the knee joint[4] and contributes significantly to the disability retirement[5]. Its prevalence is on the rise and reflects the longer life span, sedentary lifestyle and epidemic of obesity worldwide.[4, 6-9]

OA of major joints, such as hip or knee, is associated with increasing and sometimes intolerable pain and movement disabilities10 that, with the progression of the disease, limit not only the professional performance, but also the everyday

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activities.[11] Several non-pharmacological (body weight loss, muscle strength-increasing exercises, physiotherapy) and pharmacological regimens (non-steroidal anti-inflammatory drugs po or im, intraarticular injections of viscosupplementation with hyaluronic acid, corticosteroids or biologicals) are used to treat this very painful and disabling condition.[12]

Total knee arthroplasty (TKA) remains an ultimum refugium for the patients with end-stage osteoarthritis and its prevalence is also on the rise, along with the prevalence of OA. In the UK study, the indication for TKA was made in 2.04% of people older than 55 with knee problems.[13] In Canada, for example, there was a 5-year increase in the use of TKA over the last five years of 16%.[14] It is projected that between the 2005 and 2030 the number of performed TKAs in the US will increase 673% to 3.5 million procedures annually and if the revision operations are taken into consideration, the overall increase is expected to be over eight-fold[15]. Additional explanation for this phenomenon is in the fact that TKAs, despite the increased rate of postoperative complications, represent a rational solution for the end-stage knee OA even in the population aged over 80.[16]

In Spain, a 10-year prospective cohort study in patients diagnosed with OA of the knee or hip joint revealed a significantly higher average lifetime risk for knee than for hip replacement - 30% (95% confidence interval – CI – 25-36%) versus 14% (95% CI 10-19%). Among the studied risk factors precipitating TKA, early age at the time of diagnosis of knee OA and the increased body mass index (BMI) were identified.[17] In a German study the OA prevalence among the population aged 60 or older was 21.8%, rising to 31% in those older than 80.[18]

Due to a significant list of comorbidities in patients undergoing TKA[19] on one hand and the complex innervation of the knee joint on the other,[20] postoperative analgesia following the elective knee surgery evolves towards a multimodal approach, combining nerve blocks with the conventional systemic administration of analgesics.[21,22] Among the several nerve blocks used so far,[20,23,24] the "3-in-1" femoral nerve block (FNB)[25] and the fascia iliaca compartment nerve block (FICNB)[26] have been most frequently used.

The aim of this clinical trial was to compare these two techniques with the classical systemic analgesic scheme and to compare their postoperative analgesic efficacy between themselves in patients undergoing TKA.

METHODS

A total of 60 patients of both genders older than 55, scheduled for total knee replacement were included in this randomised clinical study. They were randomised to reach equal gender distributions by means of a block-randomisation method into three groups – MP, NFB and FICNB. The study protocol had been approved by the local Ethics Committee for Trials Involving Human Subjects and followed the principles of the Declaration of Helsinki.

All patient were subjected to TKA under the general balanced anaesthesia and received postoperatively a standard analgesic treatment consisting of morphine 5 or 10 mg q6h iv (a dose of 5 mg administered to patients weighing up to 60 kg, while the 10 mg dose was reserved for those above that weight limit) and paracetamol 1 g q6h iv. In order to obtain equal gender ratio in every group, patients were block-randomised to one of three groups: (1) standard morphine/paracetamol (MP) group, (2) "3-in-1" femoral nerve block (FNB) group and (3) fascia iliaca compartment nerve block (FICNB) group.

FNB was performed in the inguinal region as a "3-in-1" block, as described elsewhere.[25,27] It included injection of 30-40 ml of a local anaesthetic bupivacaine 0.5% around the femoral nerve in the inguinal region. For FICNB a technique published earlier[26,28] was followed. A single injection 30-40 ml of bupivacaine 0.5% was administered to anaesthetise the three branches of the lumbar plexus: n. cutaneus lateralis, n. femoralis and n. obturatorius.

The following parameters were registered: basic demographics, pain intensity on the 0-10 numeric pain scale (NPS), need for extra doses of analgesics postoperatively, time to the occurrence of such a need, duration of nerve block and cardiovascular parameters.

Statistical analysis was performed by using SPSS version 17.0. Kolmogorov-Smirnov test was used to test the normality of the data dis-

tribution. For parametric data Student t-test, ANOVA and Tukey post-hoc test were chosen as optimal statistical methods. For non-parametric data Mann-Whitney U test and Kruskal-Wallace test were employed. Probability at <0.05 was considered significant.

RESULTS

The demographic characteristics of all three groups of patients are contained in Table 1.

Table 1: Demographic characteristics of patients subjected to total knee arthroplasty.

Characteristic		MP	FNB	FICNB
Number of patients		20	20	20
ASA status (N)	II	7	6	8
	III	13	14	12
Gender	Female	13	13	13
	Male	7	7	7
Age (years)	Mean ± SD	72.45 ± 9.70	72.70 ± 10.68	70.25 ± 9.50
	Range	57-90	56-88	57-85
Body weight (kg)	Mean ± SD	73.90 ± 11.58	72.80 ± 13.00 12.88	75.25 ± 10.57
	Range	56-96	55-101	57-93
Body height (m)	Mean ± SD	1.70 ± 0.07	1.69 ± 0.10	1.70 ± 0.09
	Range	1.56-1.84	1.47-1.88	1.51-1.82
BMI (kg/m^2)	Mean ± SD	25.58 ± 3.14	25.36 ± 3.96	26.17 ± 3.37
	Range	20.57-32.91	18.62-35.76	19.27-34.58
Duration of operation (min)	Mean ± SD	107.45 ± 15.00	111.25 ± 15.22	106.25 ± 17.40
	Range	87-131	83-135	80-135

MP - morphine paracetamol, FNB - femoral nerve block, FICNB - fascia iliaca compartment nerve block

All three groups of patients were similar regarding the ASA status, age, body weight and height, BMI and the duration of operation.

Mean numeric pain scores for all three groups of patients taken hourly after the end of the operation are contained in Figure 1.

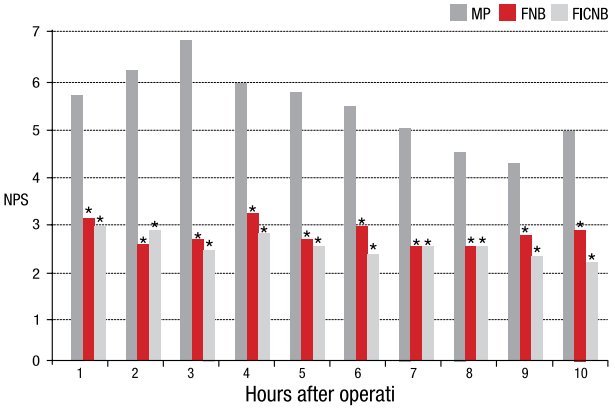

Figure 1: Effect of analgesic regimens on postoperative pain scores in patients after knee alloarthroplasty.

NPS - numeric pain scale, MP - morphine/paracetamol, FNB - femoral nerve block, FICNB - fascia iliaca compartment nerve block; *p<0.05 vs the MP group.

It is obvious that the patients in the MP had much higher pain scores than in the other two groups. This difference was the greatest three hours after the end of operation. Thereafter it started to diminish, becoming non-significant only after 9th hour. There was no difference in pain scores between the FNB and FICNB.

The time when additional analgesic was needed postoperatively, and the duration of neural blocks are given in Table 2.

In the MP group, the need for additional doses of analgesics occurred much earlier than in the other two groups, while the difference in this time was not significant when the FNB and FICNB groups were compared.

The postoperative use of morphine and paracetamol during the first 24 h postoperatively in all three groups of patients is shown in Table 3.

Table 2: Need for additional analgesics and duration of neural block in patients undergoing total knee arthroplasty.

		MP	FNB	FICNB
Time until first additional dose of analgesic (h)	Mean ± SD	4.10 ± 1.25	7.90 ± 1.12*	8.20 ± 1.58*
	Range	2-6	6-10	6-11
Duration of nerve block (h)	Mean ± SD	n/a	4.60 ± 1.19	5.10 ± 0.97
	Range	n/a	3-7	4-7

MP - morphine paracetamol, FNB - femoral nerve block, FICNB - fascia iliaca compartment nerve block
*p<0.05 vs the MP group, n/a - not applicable.

Table 3: Additional analgesic consumption in patients undergoing total knee arthroplasty during first 24 h after the end of operation.

		MP	FNB	FICNB
Morphine (mg/ patient/first 24 h)	Mean ± SD	5.50 ± 4.84	1.25 ± 2.22*	1.00 ± 2.05*
	Range	0-10	0-5	0-5
Paracetamol (g/patient/first 24 h)	Mean ± SD	4.70 ± 0.8	2.10 ± 0.85*	2.05 ± 0.94*
	Range	4-6	1-4	1-4

MP - morphine paracetamol, FNB - femoral nerve block, FICNB - fascia iliaca compartment nerve block
*p<0.05 vs the MP group.

Both in case of morphine and paracetamol, the postoperative consumption was significantly higher in the MP group in comparison with the FNB and FICNB groups. At the same time, the difference between these two groups themselves was not significant.

Changes in systolic blood pressure over the 5-hour period after the end of operation in all three groups of patients is shown in Figure 2.

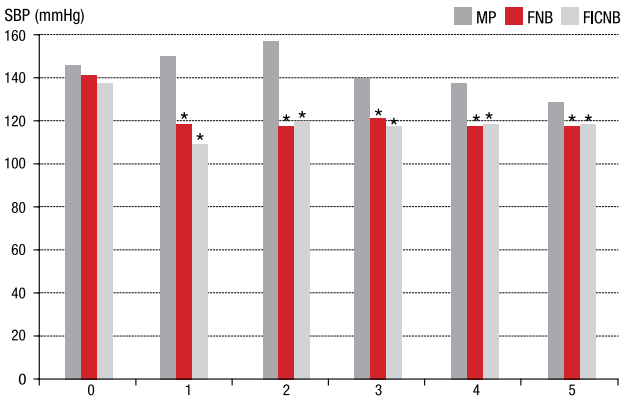

Figure 2: Changes in systolic blood pressure in three groups of patients after total knee arthroplasty.
SBP - systolic blood pressure, MP - morphine/paracetamol, FNB - femoral nerve block, FICNB - fascia iliaca compartment nerve block; *p<0.05 vs the MP group.

It is obvious that at the end of operation values of systolic blood pressure were similar in all three groups. However, in the following hours, they stayed high only in the MP group, while in the FNB and FICNB groups they significantly decreased.

Mean number of episodes of adverse effects is shown in Table 4.

Table 4: Frequency of postoperative adverse effect in patients subjected to total knee arthroplasty (number of episodes /patient).

		MP	FNB	FICNB
Nausea	Mean ± SD	2.30 ± 0.98	0.55 ± 0.69	0.55 ± 0.69
	Range	1-4	0-2	0-2
Vomiting	Mean ± SD	1.25 ± 0.97	0.10 ± 0.31*	0.15 ± 0.37*
	Range	0-3	0-1	0-1
Pruritus	Mean ± SD	0.35 ± 0.49	0.15 ± 0.37*	0.10 ± 0.31*
	Range	0-1	0-1	0-1
Sedation	Mean ± SD	0.85 ± 0.37	0.15 ± 0.37*	0.15 ± 0.37*
	Range	0-1	0-1	0-1

MP - morphine paracetamol, FNB - femoral nerve block, FICNB - fascia iliaca compartment nerve block
*p<0.05 vs the MP group.

It is obvious that postoperative nausea, vomiting, pruritus and sedation occur significantly less frequently in FNB and FICNB groups, in comparison with the MP group.

DISSCUSION

Results of the present study indicate that the two peripheral nerve block procedures, FNB and FICNB, assure significantly less frequent need for rescue analgesic medication, better analgesia, cardiovascular control and adverse effect profile when compared to the morphine/paracetamol regimen. In none of the monitored parameters any difference in efficacy or tolerability between 3-in-1 FNB and FICNB could be found.

Reduction in the pain intensity, judged according to the lower scores on the NPS in comparison with the opioid group, found in the FNB group was in accordance with the results of several other clinical trials.[29-36] Publications on the postoperative efficacy of FICNB are less abundant, but a meta-analysis unequivocally confirms the results of the present study.[37] One of the rare available clinical studies publishing the head-to-head comparison between 3-in-1 FNB and FICNB found no difference in the efficacy and safety of these two multi-modal analgesic techniques,[34] corroborating thus the current results.

Use of classical opioid-based postoperative techniques is accompanied by classical opioid adverse effects, such as postoperative nausea and vomiting (PONV), pruritus and sedation. In the present study, the decreased need for the rescue morphine administration resulted in diminished frequency and intensity of the morphine adverse effects, which paralleled the findings of the quoted clinical trials.[29-31] Present results were also similar to the ones obtained in the same clinical settings, but in patients subjected to total hip replacement.[38]

When it comes to pain control during and after TKA, five anaesthetic/analgesic techniques are being used: (1) general anaesthesia (2) spinal anaesthesia, (3) spinal and peripheral anaesthesia, (4) general and spinal anaesthesia and (5) general and peripheral anaesthesia. Although each technique has some advantages and drawbacks, it seems that the combination of intraoperative general balanced anaesthesia and postoperative peripheral anaesthesia (nerve blocks) offers best analgesia and adverse effect profile.[23] Neural blocks other than FNB and FICNB are also being used, with similar effects, such as psoas compartment block (PCB)[39] and adductor canal block (ACB).[40] These new variations of the multimodal concept of analgesia offer no additional value, in comparison with the FNB and FICNB.

Both morphine and bupivacaine, a long-acting amide local anaesthetic, affect cardiovascular system. Morphine is an opioid obtained from the plant known as opium poppy (Papaver somniferum), while the other currently used opioid analgesics are synthetic. There are many side effects of opioids[41] and one of the most common effects on the cardiovascular system is prolongation of the QT interval which can lead to torsade de pointes (TDP), kind of the ventricular tachyarrhythmia that can provokes sudden death.[42,43] The other side effects on the cardiovascular system are bradycardia, histamine release, and rhythm disturbance. Mechanism of action of prolongation of QT interval is associated with prolonged cardiac repolarisation that is initiated by rapid outflow of potassium (K+), through the cardiac rapid-rectifying K+ channel.[42]

Racemic bupivacaine (Marcaine) is potentially cardiotoxic, having depressant electrophilic effects on the heart, especially in patients with compromised cardiac function. It depresses the intracardiac conduction velocity and cardiac contractility. By blocking Na+ and K+ channels it causes the prolongation of the PR and QT intervals in the electrocardiogram. Levobupivacaine is less cardiotoxic than the racemic bupivacaine.[44] Cardiotoxicity is usually not a problem in low single doses of bupivacaine, as the ones used in this clinical trial.

CONCLUSION

Multimodal anaesthesia, combining intraoperative general balanced anaesthesia and postoperative local anaesthetic-induced peripheral nerve blocks, such as FNB and FICNB, enables better analgesia and fewer adverse effects than the morphine/paracetamol postoperative regimen.

CONFLICT OF INTEREST

None.

ACKNOWLEDGEMENTS

None.

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CURRENT TOPIC

Renin-Angiotensin and Kallikrein-Kinin Systems in Diabetic Retinopathy

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ABSTRACT

This brief review describes how two complex systems, the renin-angiotensin system (RAS) and the kallikrein-kinin system (KKS), affect the retina. It emphasises the important physiological actions of components of these systems, the protective effectiveness of angiotensin I converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) in diabetic retinopathy and suggests as well the therapeutic possibilities for treatment of diabetic retinopathy by selective activation of bradykinin receptors (B1 and B2).

Key words: RAS, KKS, ACE, angiotensin II, kinins, ACE inhibitors.

INTRODUCTION

The aim of this article is to assess a role of the renin-angiotensin system (RAS) and the kallikrein-kinin system (KKS) in diabetic retinal microvascular damage. Diabetes is a major risk for loss of vision due to retinopathy and its major complication, macular oedema. The chronic hyperglycaemia of diabetes mellitus disrupts carbohydrate, fat, and protein metabolism by deficiencies of insulin secretion, insulin action, or both. In type 1 diabetes the pancreas produces very little or no insulin. Specific autoimmune markers indicate that an autoimmune process damages or destroys pancreatic beta cells in the majority of patients (nearly 90%) with type 1 diabetes.

Type 2 diabetes, the more common form of diabetes, affects both insulin action and insulin secretion. This form of the disease frequently occurs in elderly patients, and it is the most common cause of microvascular damage that may present as coronary artery disease, nephropathy, peripheral neuropathy, and retinopathy.

Renin-angiotensin and kallikrein-kinin systems

Renin-angiotensin and kallikrein-kinin systems (Figure 1) are proteolytic cascades that operate at both systemic and local (tissue) levels.[1,2] Studies of their role in the eye have focused on distribution and participation in diseases, such as glaucoma, diabetic retinopathy, age-related macular degeneration and uveitis. This short presentation explores the therapeutic potential of some pharmacological agents that could affect diabetic retinopathy (DR).

The RAS components consists of twenty peptidases, nearly twenty angiotensin peptides, and six receptors.[3] According to the classic view, the RAS is an endocrine system. Renin, which is produced in the kidney, acts on angiotensinogen in the circulation to form a biologically inactive decapeptide, angiotensin I, which is then converted by angiotensin converting enzyme (ACE) to a potent vasoconstrictor peptide, angiotensin II. Angiotensin II stimulates aldosterone synthesis and regulates blood pressure, fluid volume, electrolytic balance, and inflammation.

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CONTENT

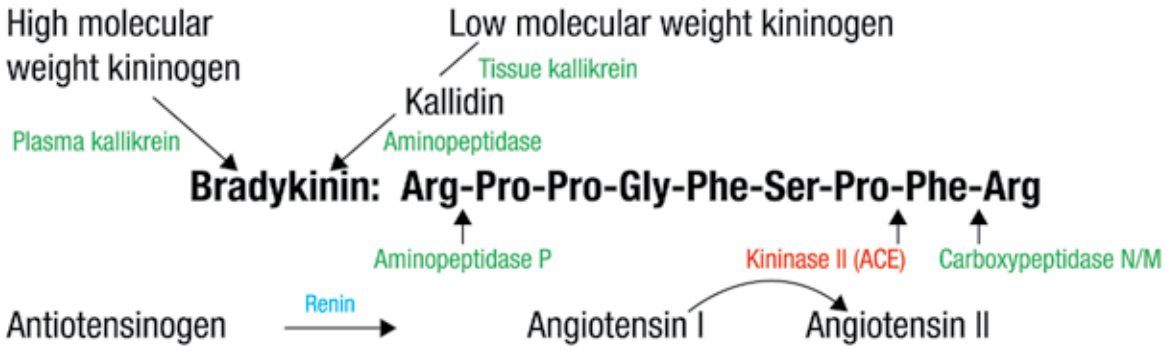

Figure 1: Peptidases and peptides of the KKS and a portion of the RAS. The enzymes in the KKS are shown in green, while renin is shown in blue; ACE, which acts in both systems, in shown in red.

This octapeptide acts through two types of receptors, AT1 and AT2. Originally it was assumed that the RAS acts as an endocrine system, but this view changed when it was shown that the RAS operates at both systemic and tissue levels.[4]

ACE was discovered in the blood, but it is also found within endothelial and epithelial cells of the lungs, kidneys, the male genital tract and other tissues, as well as within body fluids, and retinal extracts from man, guinea pig, hog, and rabbit eyes.[5] ACE converts angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) to angiotensin II by cleaving a dipeptide, His-Leu from the C-teminal end of angiotensin I. The proline (Pro7) location in the penultimate position of angiotensin II[6] prevents further cleavage by ACE. However, the enzyme also cleaves C-terminal dipeptides from various other peptide substrates, including bradykinin, kallidin, enkephalin, substance P and neurotensin. Under normal conditions, in comparison to other organs, lung tissue has the highest ACE activity and the kidney the lowest. However, in individuals with genetically high ACE activities, especially diabetic subjects, this enzyme causes higher inactivation of kinins (bradykinin and kallidin) than angiotensin II production.[7]

The components of KKS are present only in mammals.[8] Kallikreins release kinins from two substrates: high molecular weight kininogen, or Fitzgerald factor (factor XII, 100 kDa), and a low molecular weight (50-68 kDa) kininogen. Both are synthesised in the liver and are abundant in plasma. Plasma kallikrein (Fletcher factor) releases bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), while tissue kallikrein releases kallidin.[9] Both kinins act upon two types of receptors, B1 and B2. Under the most conditions, kinins act through B2 receptors, increasing vasodilatation, vascular permeability and by increasing intracellular calcium in smooth muscle and endothelium via increased nitric oxide and prostacyclin release from endothelial cells. Des-Arg9 bradykinin and des-Arg10-kallidin activate the B1 receptor, which is rapidly expressed in inflammation or tissue injury. Kinins have a short half-life, less than 15 seconds. They are inactivated by two types of kininases: kininase II or ACE and carboxypeptidases N and M and also by aminopeptidase.[10,11]

Interactions between the RAS and KKS occur at three enzyme levels: ACE, kallikrein, and prolylcarboxypeptidase (PRCP). Additional interactions between the two systems occur at B2 receptors, and also at angiotensin AT1 and AT2 receptors. These interactions indicate a degree of co-dependence, but the KKS does not always counterbalance the RAS. Sometimes both systems act in the same direction, eg during tissue injury.[12]

ACE inhibitors block angiotensin II release and catabolism of kinins (bradykinin and kallidin), but they also amplify the role of other enzymes that hydrolyse angiotensin I and kinins by increasing release of other biologically active products, such as angiotensin 1-7 and angiotensin 1-9.[13] These peptides oppose angiotensin II activity and potentiate kinin action in various tissues, especially in the heart.[14]

Renin-angiotensin and kallikrein-kinin systems in the eye

Van Haeringen in the British Journal of Ophthalmology (1996) noted that "Opthalmic literature concerning the RAS started in 1977 with a study by Igić and co-workers on the detection of ACE activity in homogenates of the retina."[15] Since then we have continued to study the role of the RAS in the eye.

CONTENT

The blood-ocular barrier prevents the passage of peptides and larger molecules, such as angiotensin I, angiotensin II, kinins, renin, angiotensinogen, kallikreins, and ACE. However, ocular tissue of various species, including humans, contains the components of both the RAS and KKS.[5,6-18] In addition, concentrations of prorenin, angiotensin I, ACE, and angiotensin II are higher in the retina, choroid and anterior uveal tract than in plasma. The local production of these RAS components in the eye is similar to tissue generation of these peptides in other organs, including the kidney, brain, adrenals, and reproductive tract. Distribution of ACE within human vascular tissues varies. Most of the vascular ACE is generally found in small muscular arteries and arterioles, but capillaries, large arteries, veins and venules have little of this enzyme. Pulmonary, renal and retinal capillaries, with high activities of ACE, are an exception to this distributive pattern.[19,20]

The components of the KKS (kallikrein, B1 and B2 receptor proteins and kinin-inactivating enzymes, are found in various parts of the eye.[21,22] Because the concentration of kallikrein is lower in plasma than in homogenates of retinal tissue, it is unlikely that the retinal enzyme is a contaminate from blood. Endothelial ACE increases in diabetes resulting in locally increased generation of angiotensin II and simultaneous decreases in kinin activities. Individuals with genetically increased ACE activities have a greater risk for cardiovascular damage, including retinopathy, neuropathy and nephropathy.

Diabetic retinopathy

Diabetic retinopathy (DR) is a frequent cause of blindness in middle-aged and elderly people. Risk factors include age (primarily 50-70 years), duration and inadequate control of diabetes, hypertension, and hyperlipidemia. In addition to DR, further diabetic eye complications include glaucoma, macular oedema and cataracts. DR may affect the peripheral retina, the macula, or both.[23] In DR major retinal damage includes varying degrees of micro-aneurisms, exudates, hemorrhages, new vessel formation, and retinal thickening. These diabetic complications are evidence of microvascular disease, but retinal neurodegeneration is also involved.

There are two types of DR - proliferative and non-proliferative. Vascular endothelial growth factor (VEGF) stimulates neovascularisation in proliferative diabetic retinopathy. It was recently shown that the retinal and vitreous RAS regulates VEGF production.[24] Increased levels of VEGF are found in the vitreous humor and in vascular tissue in the eyes of individuals with proliferative DR. This pro-angiogenic cytokine factor acts at two types of tyrosine kinase receptors involved in the regulation of angiogenesis.

Proliferative DR includes changes in retinal vessel diameters and an increase in vessel permeability. This results in leakage, although the blood retinal barrier persists in both types of DR. The proliferative type exhibits retinal neovascularization extending into the vitreous body.[25] This abnormality may obstruct the passage of light to the retina, cause haemorrhage, fibrous tissue formation, and produce vitreoretinal traction and retinal detachment. In juvenile diabetics (type 1 diabetes) DR is the major cause of blindness.

Treatment for the proliferative type DR or macular edema includes laser photocoagulation, vitrectomy, or injection of VEGF inhibitors (bevacizumab, ranibizumab, pegaptanib, aflibercept) into the vitreous. The optimal dose and dosing sequence for VEGF inhibitors remain unclear, but higher doses appear to be most effective in inducing regression of neovascularisation. Side effects of laser photocoagulations and injections of the VEGF inhibitors are the reason why other therapeutic options are preferred. Although Anti-VEGF agents are effective in many DR patients, they do not influence the pathogenesis of the retinopathy and the necessity for repeated intravitreal injections over many years poses a permanent risk of adverse effects.

Several innovative therapeutic strategies have sought to improve the treatment of DR and other ocular diseases (eg glaucoma and age-related macular degeneration). Among new lines of investigation, vasoactive peptides (angiotensin II and kinins) were considered as candidates in the pathogenesis of eye diseases. However, E G Erdös[26] showed that the rapid enzymatic inactivation of these peptides precludes their use as therapeutic agents. If these transient peptides locally influence any specific pathological conditions, only agents that block their effect or inhibit their enzymatic degradation would be useful for therapeutic purposes. This important concept eventually led to the discovery of ACE inhibitors.

Tissue renin-angiotensin and kallikrein-kinin systems in diabetic retinopathy

The RAS and KKS operate in pulmonary, systemic, and local circulations, including various ocular tissues. The peptides produced by the two systems in the retina (angiotensin II and kallidin) contribute to the regulation of neurovascular retinal function. They influence inflammatory and angiogenic signalling molecules, such as VEGF and chemokines that may participate in both physiological and pathological conditions. Since both the RAS and KKS act to maintain homeostatic regulation and meet the metabolic needs of the retinal tissue, any dysfunction within these systems may damage this highly sensitive tissue.

In addition to retinal tissue, components of the RAS are found in the vitreous humour where they can influence neovascularisation by stimulating VEGF production in proliferative DR. This poses the possibility for early therapeutic interventions, such as the reduction of VEGF and other pro-inflammatory and angiogenic cytokines. Development of a single-strand inhibitory RNA that targets a (pro)renin receptor [(P)RR] will be clinically tested in patients with DR and several other eye diseases.[24] Effectiveness of this ribonucleic acid interference molecule should be also tested in ocular inflammation and angiogenesis.

Endothelial ACE activity often increases in diabetic patients, and this may accelerate the development of nephropathy, neuropathy, and retinopathy.[7] Genetic variation of ACE activity due to insertion/deletion polymorphism also causes variability in plasma and tissue ACE activities, which may slightly increase the risk of tissue damages in non-diabetic subjects, but in diabetic patients the risk is much higher. Increased production of active ACE results in faster kinin inactivation than conversion of angiotensin I to angiotensin II, accelerating tissue damages.

For these reasons, ACE and ARBs were explored in clinical studies for potential beneficial effects on diabetic retinopathy.[27] In 21 randomized clinical trials with a total of 13,823 participants, RAS inhibitors reduced risk of progression and increased possibility of regression of diabetic retinopathy. ACE inhibitors proved to have the highest potential for regression of diabetic retinopathy, followed by ARBs, placebo, and calcium channel blockers. The conclusion was that ACE inhibitors are preferable to ARBs for treating diabetic retinopathy.

Kinins increase vasodilation, vascular permeability, and sweating. An increase in kinin activity by ACE inhibitors would seem beneficial in cardiovascular diseases; the vasodilation caused by these agents may be important for end-organ protection. Locally produced kinins in the eye might help to prevent diabetic retinopathy as well. Because kallikrein activation by gene therapy is unlikely to be easy developed for clinical use,[7] activation of B1 or B2 receptors might be a better pharmacological target. Pseudo-peptide analogues of kinins, resistant to the actions of peptidases, could reduce end/organ damage by activation of those receptors.[28,29] If receptor activation of B2 causes side effects, such as angioedema, hypotension or pain, B1 receptor activation may prove to be safer. Initial studies with synthetic pseudo-peptide analogues of kinins in diabetic rats show that prolonged (up to two weeks) treatment by B1 receptor activators produces safe and effective microvascular protection.[30,31] Although these peptide analogues can be given only intravenously or by osmotic mini pumps, further experiments should examine both B1 and B2 receptor activators in animal models of diabetic retinopathy. Such studies may help us to open the road towards discovery an orally effective bradykinin receptor agonist – perhaps the best solution for prevention and treatment of diabetic end-organ damage.

NOTE

I devote this article to Ervin G Erdös (96), my teacher at postdoctoral training in biochemical pharmacology (Oklahoma City, 1970-1972) and a good friend. We collaborated in research for several decades[2]. He visited the former Yugoslavia several times where he presented seminars (Sarajevo, Tuzla, Sombor and Belgrade) and stimulated local scientists to study metabolism and activity of vasoactive peptides of diabetic end-organ damage.

DISCLOSURE

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CONTENT

Nikolić and Rogač. Scr Med 2019;50(3):134-7. DOI:10.5937/scriptamed50-23153

CURRENT TOPIC

Quality of Life in Children With Epilepsy

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ABSTRACT

Children with epilepsy are a particularly sensitive part of population and require continuous monitoring by neuro-paediatricians, so that any behavioural and functional changes that occur during mental maturation from the side effects of the therapy, but also the consequences of the disease itself, could be timely noticed. The aim of this paper is precisely to point out the potential difference in the quality of life of children with epilepsy, through the synthesis of relevant literature, in order to alert the local professional public about the need for mutual cooperation with neuro-paediatricians, psychologists, child psychiatrists, but also social workers and teachers at school.

Key words: children, epilepsy, quality of life.

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INTRODUCTION

Contemporary epileptology is focused on shaping the definitions and classifications of epilepsy, inter alia with the aim of reducing a persistent stigma in the society around it, especially in developing countries. Epilepsy is nowadays no longer treated as a disorder, but as a disease. The latest classification, which has simplified the aetiological and clinical division of epileptic seizures, has enabled people with epilepsy and their immediate environment to better understand the diagnosis.[1, 2]

Namely, it is known that the stigma used to be related to the fear of the unpredictable and unknown, because that is how epileptic seizures once seemed. Being a chronic disease that requires the use of antiepileptic therapy over a long period of time, epilepsy can greatly change the life of the patient and his/her family. The obligation to take daily therapy itself makes a patient lead a different life than his/her environment. There are also numerous side effects of antiepileptic drugs that lead to changes in physical appearance, behaviour, psychosocial and emotional functioning. On the other hand, weaker control of the incidence of epileptic seizures, limitations in playing sports, choosing a profession, may contribute to the impression of less value for those with the disease. All this can lead to anxiety and depression.[3, 4]

Do children with epilepsy have a different lifestyle?

Since epilepsy is a chronic disease with a sometimes diverse symptomatology of the onset of seizures, coping with the diagnosis varies from one diseased to another. Potential reactions to the disease are: a regression or withdrawal reaction that manifests as a form of helplessness; depressive reaction when the patients are inactive, unmotivated, unwilling and passively expecting help, projective reaction, when they are angry and wondering why they are sick of all people, when they often blame others for their own condition; a negative reaction, when patients do not perceive the seriousness of their status, do not see a reason for fear, do not cooperate with the doctor, and thus escape from reality; an adaptation or acceptance reaction when the sick invest a large amount of energy to cope with their illness.[4, 5]

Since epilepsy can affect different spheres of

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life, questionnaires dealing with measuring the quality of life of patients, with little difference depending on the geographic area of validation, focus on the field of emotions, perception of reality, cognition, opportunities to perform daily physical activities, participate in social activities, social functioning of children and their parents. These studies are often associated with assessments of intellectual functioning, depression and suicide anxiety scales, estimates of compliance in terms of adherence to therapy, and control of seizure frequency.[6, 7]

There are significant studies that have compared the lifestyles of patients with epilepsy and healthy children but also of those with other chronic diseases. In a study done in India, epilepsy patients were found to have a lower quality of life than those with migraine and diabetes mellitus, and to cope with greater stigma in the society than patients with other chronic diseases. They are also less likely to engage in sport activities, have fewer hobbies, and are more obese than the healthy controls.[8]

In a prospective 2-year follow-up study, epilepsy was found to be associated with impaired intellectual functioning, clearly indicating that at the start of the IQ trial both control and epilepsy groups were in the same bands. What was expected was a slight delay in intellectual functioning to be found in pharmacoresistant epilepsies, with a higher frequency of seizures.[9]

A study conducted in Western Europe found that a lower quality of life in children with epilepsy was associated with a lower educational level of parents. What is unusual about the results of that study is that more frequent visits to neuro-paediatricians are associated with greater anxiety and depression in children, as well as with poor school grades.[10]

Furthermore, Taylor et al. found that children with epilepsy are less likely to initiate social contacts, they feel frustrated about going to school trips and outings, start consuming cigarettes earlier and, although at a small higher percentage, are more prone to risky behaviour.[11]

Hamama-Raz et al. showed in their study that children with epilepsy sometimes had behavioural and concentration problems. This is a reason why research that seeks to compare the quality of life of patients with epilepsy and the side effects of antiepileptic therapy is interesting, as it often remains unclear what is the cause and what is the consequence.[12]

Many studies have sought to link psychic changes in children with epilepsy, and the type and frequency of seizures. The results are similar - anxiety-depressive disorders and concentration problems are more prevalent in uncontrolled generalised tonic-clonic and partial onset seizures and what remains unknown is the cause and the effect between poorer quality of life and less active lifestyles.[13, 14]

Children with epilepsy may have significantly more trauma and post-traumatic stress disorder than healthy controls and are well known to have more psychiatric illness than healthy controls. They cope with anxiety, depression and repressed feelings, and all this also can lead to the development of psychogenic non–epiletic seizures.[15]

Epilepsy may have a big role in choosing a profession, in family planning and also in various life organisation situations. If children with epilepsy do not receive adequate support from the family and the society, they may feel insecure about their life decision-making.[16]

Surveys of children the day after experiencing seizures at school or in the society show how much stigma, shame, fear of recurrence, and feelings of rejection children with epilepsy may sometimes cope with.[12]

Some studies that place the quality of life of children with epilepsy in the context of their growing environment have come to the conclusion that their families are similar to most families affected by a stressful life situation that necessarily leads to changes in family relationships and therefore and that there is an imperative that family relationships are balanced and imbued with emotions.[13]

Most studies, on the other hand, have come to the conclusion that if children have adequate support from the society, there are no limits to their daily functioning, nor is there a difference between the quality of life compared to healthy children and teenagers.[9–14]

How to improve the quality of life in children with epilepsy?

Recognition of the weak links in the relationship of children with epilepsy and their families indicates a need to insist on a multidisciplinary approach and on the involvement of psychologists, social support, educators of school staff in addition to neuro-paediatricians. Along with the efforts of pharmaceutical companies to find the most ideal antiepileptic drug, it is clear that a sick child needs to regain self-esteem and self-love, which is something that actually truly lies within the power of us, physicians.[17]

In more developed countries there are special programmes known as "Self-management for people with poorly controlled epilepsy". The goal of these programmes is to maximise the potential that children carry, despite suffering from epilepsy. As part of the program, psychologists trained in epilepsy and the potential side effects of antiepileptic therapy are hired. In addition to them, there are social workers in the school, who provide occasional education for school staff and healthy children about epilepsy, thus reducing social stigma and supporting the family of children with epilepsy. Also, the existence of the Association of Epilepsy Patients makes it possible that patients and their families, who often encounter similar obstacles, can support one another, and exchange experiences about therapy, overcoming difficulties in school and society.17, 18 Unfortunately, there are still no such programmes in our region, but it follows from all this that the whole team, led by a neuro-paediatrician, must encourage children with epilepsy to live life without restriction.

According to numerous studies, physical activity can significantly reduce the anxiety and depression in children with epilepsy. Practicing physical activity for children with epilepsy brings numerous benefits, has the effect of reducing anxiety and depression and enhances quality of life.[17]

Contemporary epileptology, when it comes to counselling a child with sports-related epilepsy, adheres to the guidelines issued in 2016 by the International League Against Epilepsy (ILAE). Different types of sports are divided into three groups, based on the dangers that the patient and his/her teammates may cause if the attack occurs during physical activity. After a patient is classified into one of the groups, one considers the type of epilepsy, characteristics and frequency of seizures, depending on the sport the patient wants to practice. Although the guidelines are clear, it is necessary to encourage the patient to practice appropriate sports activities if there is no danger to his or her health and the environment.[18]

In addition to monitoring seizures, correcting antiepileptic therapy, and reading EEG in the outpatient clinics of the neuro-paediatrician, attention must also be paid to the psychological status of the child and his/her family. Therefore, it is recommended that in the area of professional relationships inquire about their functioning as a family, to work together to find the best solution if there are problems. [19, 20, 21]

Due to the potential side effects of antiepileptic therapy, the implementation of standardised scales relating to anxiety and depression, suicidality, cognition, behavioural changes, quality of life of the diseased children and their parents, are recommended at the beginning of the introduction of therapy and after a year. In this way, chances to miss changes that the family may not be able to verbalise or reluctantly talk about are reduced.[18, 22]

CONCLUSION

Children with epilepsy, based on relevant research on this topic, in most cases, have a lower quality of life because, in addition to the unpredictability of seizures, they struggle with anxiety, depression, and lower sense of self-esteem. Therefore, increased caution is needed for neuro-paediatricians, along with psychological monitoring and education of the environment and society to reduce the stigma that is still associated with epilepsy. All of these proposed recommendations are intended to completely monitor the patient with epilepsy during the paediatric period in order not to miss events that may lead to a decrease in quality of life. Except for being diagnosed with epilepsy, these children are no different from other children and can grow into individuals, with equal support from their healthy peers, who work, produce, and contribute to the society, as well as their healthy peers.

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CONFLICT OF INTEREST

None.

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CONTENT

Dragičević-Cvjetković et al. Scr Med 2019;50(3):138-41. DOI:10.5937/scriptamed50-22618

PROFESSIONAL ARTICLE

Effects of the Radial Extracorporeal Shock Wave Therapy (rESWT) in Patients With Calcific Tendinopathy of the Shoulder

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ABSTRACT

Introduction: Calcific tendinopathy (CT) of the shoulder is frequently a painful condition that is occurring when there are calcium deposits in the tendons of the rotator cuff. There are many options for treatment starting from therapeutic options like medicaments, physical therapy and radial extracorporeal shock wave therapy (rESWT). If conservative treatment fails, surgical treatment is the next option. The purpose of this study was to investigate the outcome effect of rESWT in patients with calcific tendinopathy.
Methods: A prospective study from February 2010 to March 2019 monitored 67 patients with CT of the shoulders of the average age of 47.06 ± 15.2 (mean± standard deviation) who were treated with a rESWT. All patients were evaluated clinically with selected radiographic evaluation of the shoulder before therapeutic intervention. The treatment protocol consisted of a sound pressure intensity of 3 bar, a wave frequency of 14/sec, a total of 2,000 waves per session. All patients performed a treatment consisting of three sessions described every seven days. The outcome parameters were VAS scale of pain and shoulder radiography before and after therapy. The Student's t-test was used in the statistical analysis.
Results: Clinical and radiographic improvement was recorded in 70% of patients in the treatment of CT of the shoulder 6 weeks after the therapy was performed with a rESWT ($p < 0.05$) in terms of reduced pain and disintegration of calcification.
Conclusion: rESWT has showed positive effects in the treatment of patients that have calcific tendinopathy of the shoulder.

Key words: rESWT, calcific tendinopathy, shoulder.

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INTRODUCTION

Calcific tendinopathy of the shoulder is often a painful condition that can be a major therapeutic challenge. This clinical entity represents one of the overuse syndrome, characterised by deposition of calcium deposits usually in the tendons of the rotator cuff (predominantly in supraspinatus muscle) with the possibility of propagation into a subchromial or subdeltoid spaces.[1] Epidemiological studies suggest that this syndrome is most common in women aged 40-60.[2] To confirm the diagnosis of calcific tendinopathy of the shoulders, most often, after a clinical examination of the shoulder, radiographs, ultrasound and magnetic resonance are used.[3] Radial extracorporeal shock wave therapy (rESWT) is one of the non-invasive treatment options for calcific tendinopathy of the shoulders with medication, physiotherapy and ultrasound-guided injection and needling.[4] If conservative management fails, surgical intervention in the form of arthroscopy and debridement is considered.[5] Some of the authors estimate the effectiveness of rESWT in patients with CT at 78-91%.[6] The mechanism of action rESWT, which is still be-

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ing studied is triple: mechanical sound waves of different intensities lead to disintegration of calcite, the analgesic effect is achieved at the level of the 'gate control system' by the hyperstimulation analgesia and the direct effects of the sound flux in the treated region is caused by neovascularisation.[7,8] Consensus on the treatment algorithm for this overload syndrome has not yet been achieved.

AIM OF THE STUDY

The purpose of this study was to investigate the outcome effect of rESWT in patients with CT in whom other conservative treatment methods did not give the expected results or improvement within 6 months of the onset of symptoms.

METHODS

In the period from February 2010 to March 2019, 67 patients with calcific tendinopathy of the shoulder were monitored by a prospective study. The research was carried out at the Institute of Physical Medicine and Rehabilitation 'Dr Miroslav Zotović' Banja Luka. A triage of patients for rESWT was performed by a physiatrist and orthopaedic surgeon during outpatient work at the Orthopaedic Department of the Institute, and after seeing the ineffectiveness of other methods of conservative treatment at last 6 months after the symptoms have been reported. After the clinical examination, all patients had radiographs of the shoulders in the AP position. The treatment consisted of three rESWT sessions that were conducted every 7 days with the following parameters: intensity of sound pressure 3 bar, frequency of waves 14 / sec, a total of 2000 waves per session. The treatment outcome parameters were VAS scaling pain and shoulder radiography before and after therapy. Patients determined the intensity of pain before and after rESWT using a visually analogue scale of pain (VAS). VAS scale of pain is a line of 10 units (from 0 to 10), where the mark is 0 - no pain and the 10 - worst possible pain. Other ratings on the scale are 2 and 3 - mild pain, 4 and 5 - moderate pain, 6 and 7 - severe pain, and 8 and 9 - very severe pain. The outcome of treatment of radiography was graded in three degrees: complete disintegration of calcification (complete healing), disintegration of calcification by more than 50% (satisfactory result) and calcification without change (failed treatment). The follow-up period was 6 weeks after the last session of the rESWT when patients came for a control exam with control radiographs that were done on the same device as the initial device.

In the statistical analysis, the parameters of descriptive statistic (mean and standard deviation) and Student's t-test were used. As a level of statistical significance the value $p < 0.05$ was taken.

RESULTS

Regarding clinical data relevant to this study, it is important that all monitored patients with CT are included in rESWT after realising that other conservative treatment methods have not led to a subjective and clinical improvement. The investigated sample of patients consisted of 43 women and 24 men of the average age of 47.06 ± 15.2 (Table 1).

Table 1: Distribution of patients with calcific tendinopathy of the shoulder treated with radial extracorporeal shock wave therapy (rESWT) by age and gender

Gender	Number	Age (mean ± standard deviation)
Male	24	50.12 ± 13.43
Female	43	44.00 ± 16.97
Total	67	47.06 ± 15.2

A statistically significant reduction in pain was recorded in all patients after 6 weeks of rESWT ($p < 0.05$) (Figure 1).

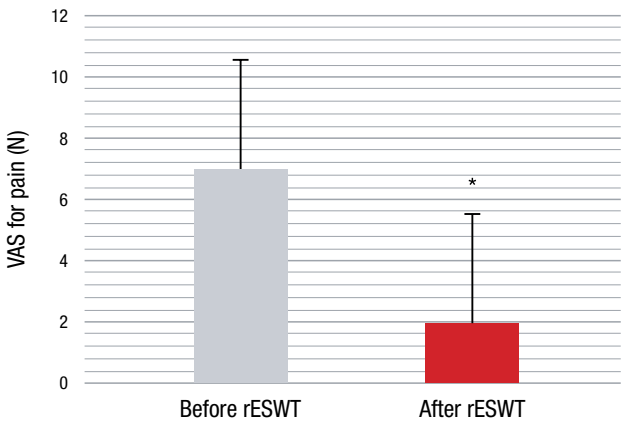

Figure 1: Outcome of treatment measured by mean values of visual analogue scale (VAS) for pain ± SD before and after radial extracorporeal shock wave therapy (rESWT). * $p<0.05$ vs before rESWT.

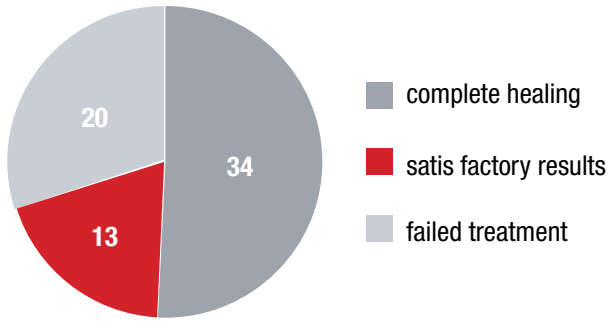

Figure 2: Radiographic outcomes of radial extracorporeal shock wave therapy (rESWT) – number of patients.

Figure 2 shows radiographic complete healing after using rESWT in a half of the patients, while the other 20% is at the level of a satisfactory result. In summary, in 70% of patients, a statistically significant positive effect of rESWT has been demonstrated ($p < 0.05$).

DISCUSSION

This prospective study was followed by 67 patients aged 30 to 60 who were treated with rESWT due to calcific tendinopathy of the shoulder. Epidemiologically, in this case, it was confirmed that CT is most common at the middle-aged women. rESWT is effective short-term treatment in patients with CT in clinically significant pain reduction and disintegration of calcification as the primary cause of damage to the shoulder function. Most of the public studies show positive outcomes for the treatment of CT by rESWT.[9-17] It is undeniable that there are also studies that have not proven that rESWT in CT treatment has a better effect than placebo.[18-20]

It is believed that shortcoming of this study is short period of follow-up, but knowing the pathophysiology of calcification in soft tissues, good results in a long-term can be expected. This is also testified by the research of other authors.[21-24] A group of Italian authors has shown the effectiveness of shock wave therapy (SWT) as a decrease in pain and a significant rate of calcification disintegration in a six-month follow-up period in patients with CT.[25] Spindler et al estimated complete healing by rESWT in three patients after two years with complete disintegration of calcite immediately after treatment.[26] Long-term follow-up studies have shown positive effects of rESWT in almost 90% patients with CT. These results are radiographically confirmed. No calcium deposits were recorded again in the rotator cuff tendons in the next two years after rESWT. It has been shown that the reduction of shoulder pain is directly related to the reduction/elimination of calcite.[21] Rompe et al (1997) reported positive effects of rESWT in more than two-thirds of patients with shoulder CT and complete or partial disintegration of the calcite in slightly more than half of patients.[27]

In the second study, Rompe et al announced that shock waves therapy was no superior to surgery in patients with calcific tendinopathy of the shoulder.[28]

The effect of different doses of rESWT was not investigated or compared in relation to surgical treatment in this study. These could be the subjects of future research.

CONCLUSION

rESWT showed positive effects in the treatment of patients that have calcific tendinopathy of the shoulder. This option of treatment should be considered in cases where other conservative treatment methods do not show any signs of improvement.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Šušak et al. Scr Med 2019;50(3):142-4. DOI:10.5937/scriptamed50-22522

CASE REPORT

Surgical Treatment of Aortic Valve Fibroelastoma: a Case Report

Stamenko Šušak[1,2], Bogdan Okiljević[1,2], Strahinja Mrvić[1], Jovan Rajić[1], Ranko Zdravković[1]

ABSTRACT

A 45-year-old man was admitted to Clinic for the first time, with symptoms of chest pain and fatigue. Computerised tomography (CT) diagnostics of the chest showed a soft tissue vegetation of approximately 5x5 mm on the left aortic coronary cusp. A double-vessel coronary disease was also diagnosed. The patient underwent surgery, a complete resection of the tumour was achieved, which was confirmed by postoperative transoesophageal echocardiography (TEE). Because of the risk of valve damage, it was decided to replace the aortic valve. A bypass from left internal mammary artery (LIMA) to left anterior descending (LAD) coronary artery (LAD-LIMA) and right coronary artery (RCA) was also performed. The patient was discharged on the 14th postoperative day with satisfactory results.

Key words: fibroelastoma, aortic valve, tumour, cardiac surgery.

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INTRODUCTION

Primary intracardiac tumours are not frequent findings. Their prevalence is below 0.3%. Intracardiac tumours develop mostly from the endocardium, rarely from the cardiac muscle or pericardium. The largest part of these tumours is benign (approximately 75%). However, their true incidence can be only assumed, because they are frequently asymptomatic.[1]

Papillary fibroelastoma is a benign tumour which originates from endocardium. Share of papillary fibroelastomas in all cardiac tumours is less than 10%. Besides, papillary fibroelastoma is the second most common cardiac benign tumour and the most common tumour of the cardiac valves. This tumour can have a wide spectrum of clinical presentations. It can be asymptomatic or can cause severe embolic complications (eg stroke, myocardial infarction). Cardiac fibroelastomas are most often diagnosed in people between the 4th and 8th decade of life and it is more often seen in men. They are mostly slowly growing tumours with multiple vascular papillary fronds, which are made of proteoglycans, elastic fibres and a lot of collagen. Macroscopically, they are small and friable masses. Their pathogenesis is unknown to this moment. Although benign, papillary fibroelastomas can cause life-threatening complications. The risk of complications can be assessed in dependence on their location, mobility and dimension.[2-4]

CASE HISTORY

A 43-year-old man started feeling chest pain and fatigue symptoms on exertion five years ago. Symptoms intensified in the last two months. Nine years ago, the patient was treated for cardiac arrhythmia caused by adenovirus. Chest X-ray and blood laboratory tests were normal. Coronary angiography showed 80% stenosis in the mid-left anterior descending coronary artery (LAD), 45% stenosis in the circumflex (Cx) artery and 50% stenosis in the right coronary artery (RCA). CT diagnostics of the chest showed a soft tissue vegetation of approximately 5x5 mm on the left aortic coronary cusp. Based on the above findings, a differential diagnosis was

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made, and it included a thrombus, myxoma, fibroelastoma and inflammatory mass. Based on the potential embolic risk, either of the mass itself or of associated thrombus and the possibility of further enlargement, symptoms at the time of diagnosis and the findings of coronary angiography, the patient was referred for surgical excision of the mass and revascularisation.

The operation was conducted on extracorporeal circulation (ECC) with the ascending aorta and the right cavoatrial cannulation. The vent was placed in the upper-right pulmonary vein, to secure the suction of the left atrium cavity. After starting ECC, aortic clamping was performed on the ascending aorta. Cold blood cardioplegic solution was injected through coronary ostia. After achieving cardiac arrest, transversal aortotomy was performed. After the aortotomy, on the left aortic cusp, a pedunculated flower-like tumour was found (Figure 1). With simple shave, the excision of the tumour was undertaken with particular care to avoid embolisation and ensure that no remnants from fragmentation of this friable tumour were left behind both locally on the cusp and near the ascending aorta and left ventricle (Figure 2). A complete resection of the tumour was achieved. The aortic valve was three-leaflet, fibrously modified and of thin leaflets. Because of the risk of valve damage, it was decided to replace the aortic valve (Figure 3). Mechanical valve No. 23 was implanted. Also, a bypass to LAD branch with left internal mammary artery (LIMA) and saphenous vein graft on RCA was performed. Total cardiac arrest time was 87 minutes, and ECC time was 100 minutes. The lesion was histologically diagnosed as a papillary fibroelastoma. After 14 days of postoperative hospitalisation, the patient was discharged with optimal results of surgical treatment.

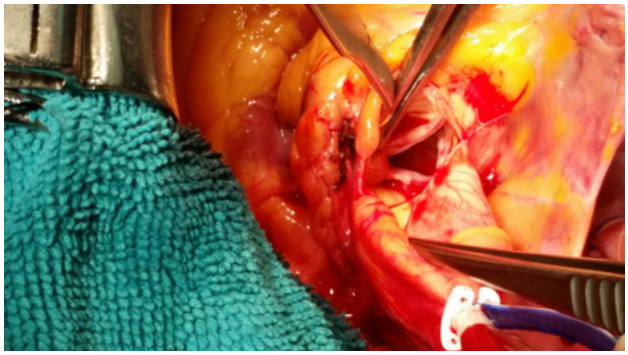

Figure 1: Tumour on the left aortic cusp

Figure 2: Tumour after resection

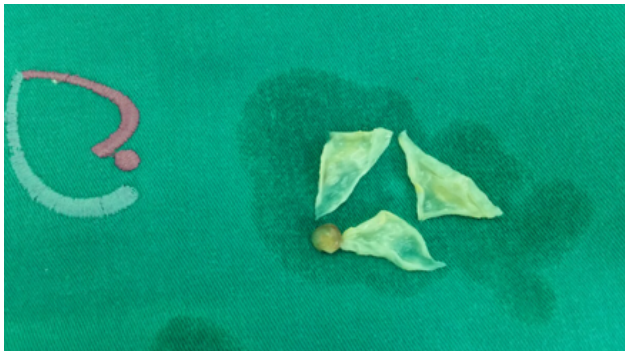

Figure 3: Tumour and aortic cusps after resection

DISCUSSION

Papillary fibroelastomas really are primary benign endocardial tumours. They originate from fibrous tissue, elastic fibres or smooth muscle cells - component that are otherwise normal constituents of endocardium. These tumours look like sea anemone - they are on short pedicle and they have multiple papillary fronds.1 Usually papillary fibroelastoma originate from the valvular endocardium (85%). It is mostly seen on the aortic (29%) and mitral valves (25%), not so often on tricuspid (17%) and pulmonary valves (13%).[5] There are several hypotheses about the development of cardiac fibroelastoma, but without unequivocally scientific evidence. The most accepted hypothesis is the microthrombus theory, which assumes that the lesions are acquired and that they arise as small thrombi on the coapting margins of the valves as a result of minor endothelial damage. Fibroelastomas are usually small tumours (with average diameter 9 to 12 mm). Although histologically benign, complications as acute valvular dysfunction, ventricular fibrillation, embolism, stroke and sudden death can be consequences of this type of tumours.[4] The diagnosis can be made by trans-

thoracic or transoesophageal echocardiography (TEE). Additional diagnostic methods, as 3D echocardiography, cardiac magnetic resonance and multi-slice spiral computed tomography, can be used for more precise identification of the tumour.[5]

The question arises, when to treat such tumours. It depends on presence of symptoms and on risk of life-threatening complications. There is an agreement that all symptomatic patients should be surgically treated. Although asymptomatic, patients with large (>1 cm) left sided mobile masses, as in this case, should also be treated surgically, because of the danger of life-threatening complications. Patients who do not meet these criteria are being closely followed up echocardiographically.[1] Early anticoagulation is mandatory in the management of these tumours. The surgical excision of tumour requires extracorporeal circulation, induced cardiac arrest and an access to the valve from which the tumour originates. The papillary fibroelastoma can be easily removed without a valve damage. Fragmentation of the tumour tissue can occur and therefore special care should be taken to avoid it. When there is a resultant valve defect after tumour excision, valve should be repaired or replaced, depending on institutional preference.[5]

The study of Gopaldas and colleagues described a case of a patient with fibroelastoma and elective coronary artery bypass grafting.[4] In this case, the valve was not replaced, and only tumour removal was done. In the present case it was decided to perform valve replacement due to the changes described in the aortic cusps.

CONCLUSION

Fibroelastoma can cause a variety of symptoms or can be asymptomatic. Several authors stated that after the diagnosis, it is necessary to operate all symptomatic and asymptomatic tumours, except the asymptomatic ones with the tumour size less than 1 cm. Such tumours should be monitored and checked regularly. The patient described in this paper had symptoms, which may have been the result of coronary artery disease, and even in that case the decision was made to remove the tumour of 5x5 mm in size.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Tables

Each table should be typed double-spaced on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully. Each table should be named in order to indicate the author's name the number of the table, (e.g. Johnson_Table 1). The place of the table in the text should be marked as in the following example:“(Table 1 near here)”.

Illustrations

Any forms of graphic enclosures are considered to be figures and should be submitted as additional databases in the System of Aseestant. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should be named in order to indicate the author's name and the number of the figure (e.g. Johnson_Figure 1). If a figure has been published, state the original source.

Captions for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. Explain the method of staining in photomicrographs. Captions should be detailed enough to allow for understanding of the content of the figure without previous reading of the text. The place of the figure in the text should be marked as in the following example:“(Figure 1 near here)”.

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