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AN INTEGRATED VIEW: NEUROADIPOCRINOLOGY OF DIABESITY

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INTEGRISANI PRIKAZ: NEUROADIPOKRINOLOGIJA DIABESITY (DIJABETES TIP 2 UDRUŽEN SA GOJAZNOŠĆU)

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ABSTRACT

Today's achievements in systems biology *and -omics sciences have facilitated a shift from studying individual molecules and tissues to characterising molecules and cells holistically. In this article, we attempt to discuss the status of a much-needed coherent view that integrates studies on neurobiology and adipobiology, as well as those on diabetes and obesity. Globally, cardiometabolic diseases (atherosclerosis, hypertension, type 2 diabetes mellitus, obesity, diabesity, and metabolic syndrome) are the most prevalent pathologies. In 2000, Astrup and Finer (Obes Rev 1: 57-59) wrote the following: "Since type 2 diabetes is obesity dependent, and obesity is the main aetiogical cause of type 2 diabetes, we propose the term 'diabesity' should be adopted." Arguably, the research field of adipobiology has witnessed three major paradigm shifts since the discovery of leptin, an adipose-derived hormone, in 1994. Various neuroendocrine and neurotrophic factors are included in the growing list of endocrine and paracrine adipose-secreted signaling proteins collectively designated adipokines. These findings open a novel field of research known as neuroadipocrinology, a component of neuroendocrinology. Adipokines, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), mediate multiple biological processes, such as food intake, immunity, inflammation, memory, mood, and metabolism. The effects on metabolism involve the maintenance of glucose, lipid and energy homeostasis as well as cardioprotection, neuroprotection, and aging. In this article, we highlight the role of metabotrophic factors (MTF) and the adipose- and nonadipose-derived biomolecules that mediate these effects. Recent results demonstrate that circulating and tissue levels of certain MTFs, e.g., adiponectin, NGF, BDNF, glucagon-like protein-1, sirtuin-1, interleukin-10, and aquaporin-7, are altered in cardiometabolic diseases, including diabesity. Overall, this may cultivate*

SAŽETAK

Današnja dostignuća u biologiji sistema i povezanim biološkim naukama omogućila su prelazak sa proučavanja pojedinačnog molekula i tkiva na holistički prikaz molekula i ćelija. Ovde pokušavamo da objasnimo koherentan prikaz koji integriše studije neurobiologije i adipobiologije, kao i one o dijabetesu i gojaznosti. Uopšteno, kardiometaboličke bolesti (ateroskleroza, hipertenzija, dijabetes melitus tip 2, gojaznost, diabesity (dijabetes melitus tip 2 udružen sa gojaznošću), i metabolički sindrom) predstavljaju najčešća oboljenja današnjice. 2000. godine Astrup i Finer (Obes Rev 1: 57-59) su napisali : "Obzirom da dijabetes melitus tip 2 zavisi od gojaznosti, a gojaznost je glavni etiološki uzrok dijabetesa tip 2, predlažemo da se termin 'diabesity' usvoji." Verovatno je polje istraživanja adipobiologije svedočilo o tri velike promene od otkrića leptina, hormona adipoznog porekla, 1994.godine. Različiti neuroendokrini i neurotrofični faktori su takođe bili uključeni u povećanje liste endokrinih i parakrinih signalnih proteina sekretovanih od strane adipocita koji zajedno čine adipokine. Ovi nalazi otvaraju novu oblast istraživanja, neuroadipokrinologiju, deo neuroendokrinologije. Adipokini, uključujući faktor rasta nerava (NGF) i neurotrofični faktor poreklom iz mozga (BDNF), posreduju u višestrukim biološkim procesima kao što su unos hrane, imunitet, inflamacija, pamćenje, raspoloženje i metaboliza. Efekti na metabolizam uključuju održavanje glukoze, lipida i energetske homeostaze, kao i kardioprotekciju, neuroprotekciju i starenje. Ovde izdvajamo ulogu metabotropnog faktora (MTF), biomolekula poreklom iz masti, kao i biomolekula koji ne vode poreklo iz masti, koji posreduju ove efekte. Nedavni rezultati pokazuju da se cirkulišući i/ili tkivni nivoi nekog MTF, na primer adiponektin, NGF, BDNF, glukagonu sličan protein-1, sirtuin-1, interleukin- 10, akvaporin-7, menjaju u kardiometaboličkim bolestima, uključujući diabesity. Uopšteno, ovo može otvoriti nov pristup u razmišljanju o dijabetesu tip 2

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a novel thinking for diabesity, herein also referred to as Homo diabesus.

udruženim sa gojaznošću, koji se takođe ovde označava kao i Homo diabesus.

Key words: *adipobiology, adipokines, diabetes, obesity, neurobiology, NGF, BDNF, metabotrophins*

Ključne reči: a*dipobiologija, adipokini, dijabetes, gojaznost, neurobiologija, NGF, BDNF, metabotrofini.*

ABBREVIATIONS

AD-Alzheimer's disease
AQP-aquaporin
BAT-brown adipose tissue
BDNF-brain-derived neurotrophic factor
MTF-metabotrophic factor
NGF-nerve growth factor
NT-neurotrophin
PPAR-peroxisome proliferator-activated receptor
Trk-tropomyosin-related kinase/receptor tyrosine kinase
UCP-uncoupling protein WAT, white adipose tissue

Thus, the task is not so much to see what no one has yet seen, but to think what nobody has yet thought about that which everybody sees.

Arthur Schopenhauer

INTRODUCTION

In the second half of the 20th century, holism (from the Ancient Greek word *holos,* meaning whole, entire, or total) led to thinking in terms of systems and their derivatives, such as systems biology. Life at both the local and systemic levels requires nutritional, immune, neurotrophic and metabotrophic support. Any dysfunction of or deficit in this support may result in a disease phenotype, such as type 2 diabetes or obesity, or a combination of the two, diabesity.

Type 2 diabetes mellitus is largely responsible for the prediction that the number of diabetics worldwide will double within a period of 30 years, increasing from 150 million people in 1995 to over 300 million by 2025 (1).

At its core, obesity may be briefly classified as the accumulation and inflammation of adipose tissue (Fig. 1), and the adipose-derived pro-inflammatory signals are disseminated to many organs of the body, leading to the subsequent development of cardiometabolic and neurodegenerative diseases (the scope of the present short review), as well as non-alcoholic steatohepatitis, polycystic ovarian syndrome, obstructive sleep apnoea, inflammatory bowel disease, thyroid-associated ophthalmopathy, cancer and many other diseases outside the scope of present review.

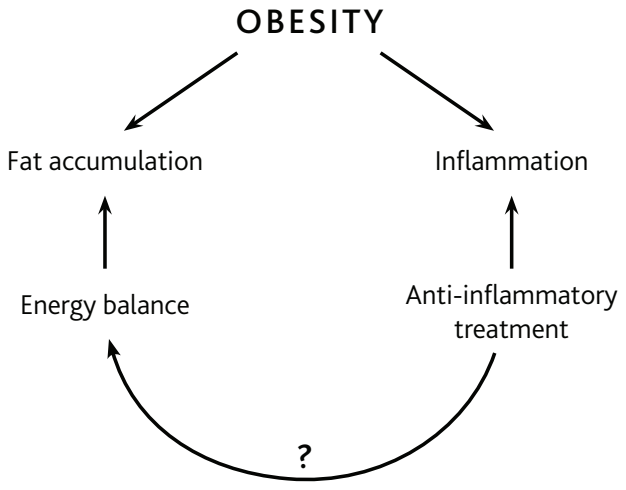

Figure 1. A drawing showing an oversimplified view of the possible pathogenesis of and therapies for obesity.

Obesity is the most prevalent disease in the world. In 2005, 800 million people were overweight (BMI 25.0–29.9 kg/m^2), and 400 million were obese (BMI over 30 kg/m^2) (1). Although the pathogenesis of obesity is not yet completely understood, there is now solid evidence that type 2 (non-insulin dependent) diabetes is strongly associated with the obese man (*Homo obesus*) (2). Therefore, diabesity (3) or *Homo diabesus* (4) has moved to centre stage as one of the most challenging biomedical and social threats, with its rising prevalence and impacts on both health and economics, in the present century. The health impact of diabesity includes a reduction of both quality of life and life expectancy due to complications such as myocardial infarction, stroke and end-stage renal disease. The burden of diabetes on the world economy has been rising in the last decade, as costs reached 376 billion dollars in 2010 and are expected to reach 490 billion dollars by 2030 (3). These latter authors wrote: "This century is the unprecedented diabetogenic era in human history. It is thus urgent to take steps including screening, prevention and early management in an attempt to control this evolving epidemic of diabesity." Furthermore, there is an "interaction" between diabesity and Alzheimer's disease, which will be highlighted below.

Adipobiology: a field marked by three paradigm shifts

One of biggest recent advances in studying cardiovascular diseases is associated with the "rediscovery" of a neglected tissue, adipose tissue.

In 1962, Thomas S. Kuhn published his book *The Structure of Scientific Revolutions* (1st edition, University of Chicago Press, Chicago, USA). Its publication was

Table 1. A paradigm shift: never before has adipose tissue been so active

FROM
Adipose tissue is a lipid and energy storage and is involved in obesity
TO
Adipose tissue is an endocrine and paracrine organ
Adipose tissue is a neuroendocrine organ
Adipose tissue is a steroidogenic organ
Adipose tissue is an immune organ
Adipose tissue is a source of and target for inflammatory mediators
Adipose tissue produces all components of the rennin-angiotensin system
Adipose tissue is therefore involved in numerous diseases beyond obesity

a landmark event in both the history and philosophy of scientific knowledge (epistemology). Kuhn challenged the then prevailing view of "normal science," which was viewed as "development-by-accumulation" of accepted facts and concepts leading often to *epistemological paralysis*, or neophobia. Kuhn argued for a model in which a period of such conceptual continuity in *normal science* was interrupted by a period of *revolutionary science,* leading to a new paradigm, an event he designated the *paradigm shift*.

At an epistemological level, adipose tissue has undergone three major paradigm shifts in the last 20 years, and has risen above the horizon and taken centre stage in a number of syndromes and that astonishes most scientists and medical doctors.

The first paradigm shift says: while considered as passive storage-release of lipids by most cell biologists and pathologists for a long period of time, adipose tissue is now considered the biggest endocrine and paracrine organ of the human body (Table 1). The discovery of leptin, an adipose-secreted hormone, published on 1 December 1994 in *Nature* 1994, 372:425–432 by Jeffrey Friedman and colleagues, marked this revolutionary event. This discovery was based on the pioneering contributions of Douglas Coleman (1931-2014). His work established the first clues regarding a genetic component to obesity. In the 1970s, Coleman conducted a series of experiments that led him to propose the existence of a *satiety factor* that would account for the development of obesity and type 2 diabetes among laboratory mice.

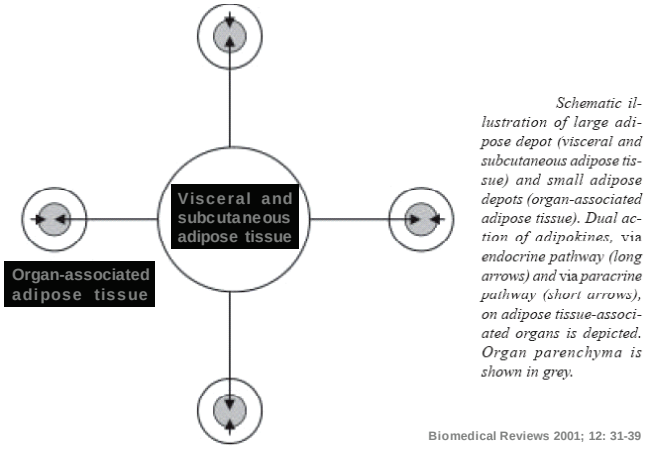

Figure 2. As indicated above/right.

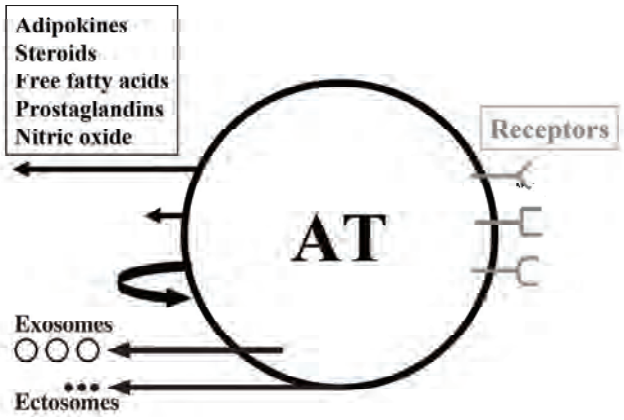

Figure 3. A drawing illustrating both the secretory and receptor nature of adipose tissue (AT) cells. At the secretory level, AT-derived signaling molecules communicate via multiple pathways, such as endocrine (arrows 1, 4 and 5, from top to bottom), paracrine (arrow 2) and autocrine (arrow 3, curved) pathways. Also depicted is that AT cells express receptors for various ligands. From (24).

The second paradigm shift derived from a study by Jeffrey Bell and colleagues (5), who have scanned nearly 800 people with magnetic resonance imaging (MRI) to obtain a map of white adipose tissue (WAT). The authors demonstrated that as many as 45 percent of women and nearly 60 percent of men have normal body mass index (BMI, 20-25 kg/m^2) scores and appear thin outside (TO), but actually have excessive levels of internal adipose tissue:, i.e., they are fat inside (FI). Therefore, they have the TOFI phenotype of body fat. The TOFI phenotype was also found among professional models. TOFI may therefore be considered an, "invisible" expression of both *Homo obesus* (2) and *Homo diabesus* (4).

The third paradigm shift features the increasing significance of brown adipose tissue (BAT) in both health and disease (see below).

Accumulation of adipose tissue in visceral and subcutaneous abdominal tissue, as well as near internal organs (Fig. 2), is a major risk factor for the development of numerous disorders, including diabesity and other related diseases. *Metaflammation* (metabolically induced inflammation) has emerged as a pivotal process in these disorders (6).

Adipose tissue is very plastic tissue and is constantly remodelled with weight gain and weight loss. It is a dynamic cellular and extracellular matrix assembly of adipocytes, fibroblasts, immune cells and matrix components and is also rich in sympathetic nerve fibres, blood vessels, and stem cells. There are two major subtypes of adipose tissue, WAT and BAT.

By sending and receiving different types of protein and non-protein signals, adipose tissue communicates with

many organs in the body (Fig. 3), therefore contributing to the control of energy, lipid and glucose homeostasis, as well as inflammation, immunity, learning and memory, among other biological functions.

In the human body, WAT stores energy and BAT dissipates energy by producing heat. BAT-mediated increases in energy expenditure are realised by uncoupling respiration from ATP synthesis via uncoupling protein 1 (UCP1), which is expressed in brown adipocytes, subsequently generating heat, a process known as adaptive thermogenesis. Animal studies have shown that the activation of BAT counteracts the effects of diet-induced weight gain and related disorders such as type 2 diabetes and metabolic syndrome: this may also be the case in humans (7). Recently, knowledge regarding WAT and BAT was enriched by information about their relatives, namely *brite* (brown in white) and *bruscle* (brown in skeletal muscle) adipocytes (8). Hence, brown adipobiology is emerging as a new focus in biomedicine.

In effect, such an adipocentric approach has revealed that although BAT is major thermogenic organ, whereas WAT is the body's largest endocrine and paracrine organ and produces multiple signaling proteins, which are collectively termed adipokines (9-12). Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are also produced by both WAT and BAT (13).

Multifunctionality of neurotrophins and adipokines

At the end of the 19th century it was envisaged by Santiago Ramon y Cajal but has not been proved that the nerves require trophic support, an idea that has never been proven. The proof was obtained through a rare combination of scientific reasoning and intuition by Rita Levi-Montalcini (1909-2012) in the early 1950s, in Saint Louis, MO, USA, when the first cell growth factor, NGF, was discovered. Levi-Montalcini was awarded the Nobel Prize in Medicine or Physiology 1986. The discovery of NGF has been embodied in a conceptual framework known as the neurotrophic theory. It reveals a pivotal role of effector (target) cells in the control of neuronal differentiation, survival and function via the production of NGF and other neurotrophic factors (14).

The neurotrophin family of proteins consists of NGF, BDNF, neurotrophin-3 (NT-3), NT-4/5, NT-6, and NT-7. Neurotrophins mediate their effects via ligation of (i) the panneurotrophin receptor, $p75^{NTR}$, and (ii) the receptor tyrosine kinases (tropomyosin-related kinase) (Trk), TrkA (for NGF), TrkB (for BDNF and NT-4), and TrkC (for NT-3) (reviewed in 12,14,15).

The past three decades have witnessed a number of breakthroughs regarding Rita Levi-Montalcini's NGF. Studies have revealed that NGF and BDNF not only are stimulators of nerve growth and survival but they also exert trophic effects on (i) immune cells, acting as immunotrophins; (ii) keratinocytes, enterocytes, and prostate and breast epithelial cells, acting as epitheliotrophins; and (iii) endothelial cells, acting as angiogenic factors (reviewed in 12,14-15).

From neurotrophins to metabotrophins

In 2003, additional phenotypic expressions of NGF were revealed, including metabotrophic actions on glucose, lipids, energy, pancreatic beta cells and cardiovascular homeostasis, and subsequently designated (analogous to neurotrophic factors and neurotrophins) as metabotrophic factors (MTF) or metabotrophins (from the Greek words *metabole* and *trophe*, meaning "nutritious for metabolism") (12,15-18), a family to which BDNF also belongs. The proof-of-hypothesis was based on results demonstrating that circulating and tissue levels of both NGF and BDNF are (commonly) decreased in atherosclerosis, metabolic syndrome (19), type 2 diabetes (20) and Alzheimer's disease (15), which currently is considered type 3 diabetes (21).

Neuroadipocrinology

As a multiplex of biological systems, life requires an interaction between its molecular and cellular components. One of the biggest recent achievements of neurobiology and adipobiology is the studies on neurotrophic factors (e.g., NGF and BDNF) and adipokines (e.g., leptin and adiponectin).

As often occurs, the framework of an initial concept of the physiological role of a newly discovered molecules extends in the light of emerging findings. This was the case with neurotrophic factors and adipokines. For instance, in the more than 30 years following the discovery of NGF, there have been few indications that it acts on non-neuronal cells. Therefore, it was remarkable when Aloe and Levi-Montalcini discovered that treatment of newborn rats with NGF caused a systemic increase in mast cells, in 1977. This seminal finding paved the way for a novel research field, neuroimmunology (22, 23 and references therein).

As indicated above (9-13), WAT is a dynamic endocrine and paracrine organ, producing a large number of adipokines. Some of them, e.g., leptin, mediate cross-talk between adipose tissue and the hypothalamus in regulating food intake and energy expenditure. However, the hypothalamus is not the only brain target for leptin, and the regulation of food intake is not this adipokine's only biological action. Rather, some adipokines support various cognitive functions and have neurotrophic activity. Current data regarding adipose-derived neuroendocrine and neurotrophic factors are summarised in Tables 2 and 3. This finding raises an intriguing question as to whether WAT may be a peripheral counterpart of the hypothalamus-hypophysis axis. Cumulatively, linking neurobiology and adipobiology resulted in neuroadipology (24), herein renamed *neuroadipocrinology*.

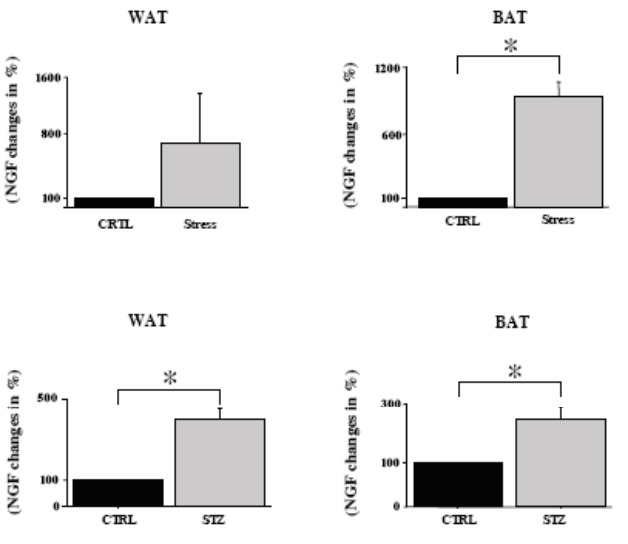

Figure 4. Changes in the amount of nerve growth factor (NGF) in white adipose tissue (WAT) and brown adipose tissue (BAT) of controls (CTRL) compared to the concentration of NGF in stressed mice (Stress) and streptozotocin-induced diabetic rats (STZ), expressed as a percentage of the controls. Note the enhanced presence of NGF in WAT and BAT in stressed mice, as well as in diabetic rats. The vertical lines in the figure indicate pooled S.E.M. derived from the appropriate error mean square in the ANOVA. * Significant differences between groups ($p < 0.05$). From (13).

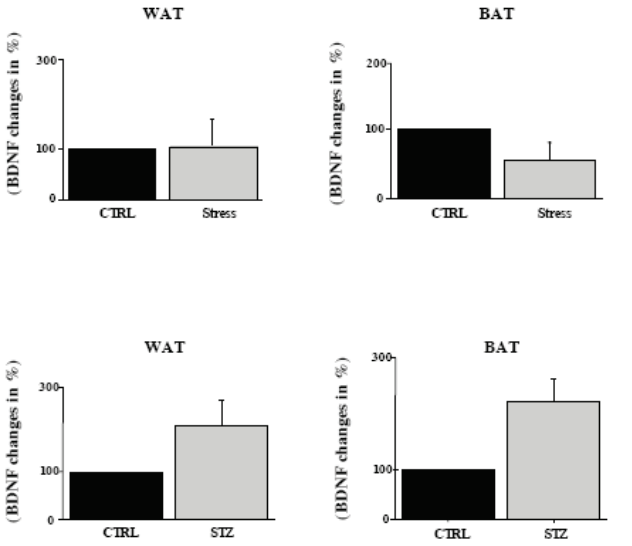

Figure 5. Changes in the amount of brain-derived neurotrophic factor (BDNF) in epicardial white adipose tissue (WAT) and brown adipose tissue (BAT) of controls (CTRL) compared to the concentration of BDNF in stressed mice (Stress) and in streptozotocin-induced diabetic rats (STZ), expressed as a percentage of the controls. The vertical lines in the figure indicate pooled S.E.M. derived from the appropriate error mean square in the ANOVA. From (13).

In an attempt to "close" the metabotrophic "loop" in cardiometabolic disease, we have measured circulating levels of NGF and BDNF in patients with acute coronary syndrome, and found that they are significantly reduced (25, cf. 26). Another study revealed altered levels of NGF in the pancreas and brain in streptozotocin-induced diabetes (27). Recently, it was demonstrated that in response to experimental stress or diabetes, the amount of NGF and BDNF was altered both in WAT and BAT (Fig. 4,5); for mast cells see Figure 6.

Table 2. Selected list of adipose-derived neuroendocrine factors

Neuropeptides
Neuropeptide tyrosine (NPY)
Substance P
Calcitonin gene-related peptide
Agouti-related protein
Adrenomedullin
Somatostatin
Kisspeptin
Neuromedin B
Neurotensin
Apelin
Nesfatin-1
Hypothalamic factors
Mineralocorticoid-releasing factors
Corticotropin-releasing hormone (CRH)
Stresscopin, urocortin (CRH-like peptides)

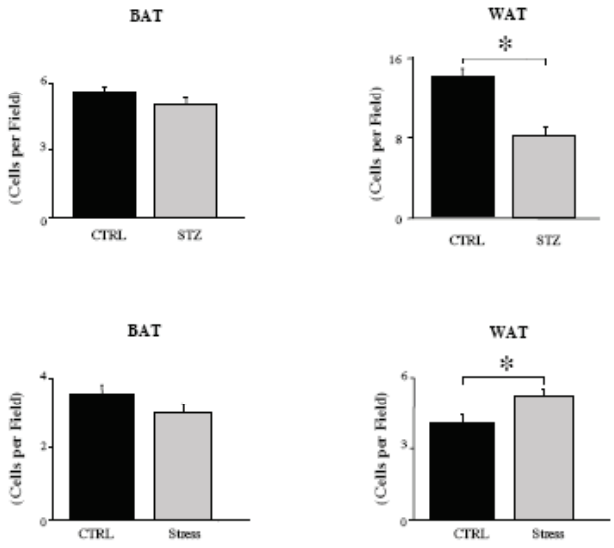

Figure 6. Changes in the number of mast cells in brown adipose tissue (BAT) and epicardial white adipose tissue (WAT) of controls (CTRL) compared to streptozotocin-induced diabetic rats (STZ) and stressed mice (Stress), expressed as a percentage of the controls. The vertical lines in the figure indicate pooled S.E.M. derived from the appropriate error mean square in the ANOVA.

PERSPECTIVE

Examples of proof-of-metabotrophic hypothesis derived from other laboratories include the following: *(i)* pancreatic beta cells secrete NGF and express its receptor TrkA, findings implicated in the pathogenesis of diabetes mellitus (28), and (ii) mutations affecting the *Bdnf* gene

Table 3. Selected list of adipose-derived neurotrophic factors

Leptin
Nerve growth factor
Brain-derived neurotrophic factor
Angiopoietin-1
Vascular endothelial growth factor
Ciliary neurotrophic factor
Glial cell line-derived neurotrophic factor
Steroids
Metallothioneins

(encoding BDNF) in mice or the *Ntr2k2* gene (encoding the high-affinity BDNF receptor TrkB) in humans are associated with hyperphagia and severe obesity (15 and references therein). Lists of selected metabotrophins (Table 4) and the metabotrophic effects of NGF and BDNF (Table 5) are provided in the aforementioned tables.

In this context, the recent discovery of (i) humanin, a mitochondria-derived peptide expressing neuro-metabotrophic effects (29,30), and (ii) irisin, a myokine/adipokine involved in the browning of WAT (31,32), may lead to the development of a novel approach in therapy for *Homo diabesus*. It may open new paths in the search for *exogenous* MTF, such as (i) small molecules that boost the secretory or signaling pathways of MTF (15) and (ii) incretin mimetics and receptor agonists, because the insulinotropic hormone, glucagon-like peptide-1 (GLP-1), and exendin-4, a GLP-1 receptor agonist, exert neuro-metabotrophic effects (33,34). Furthermore, (i) transgenic mice with Alzheimer's disease fed J147, a new compound, demonstrate improved memory, a finding correlated with reduced soluble levels of beta-amyloid and increased hippocampal levels of NGF and BDNF, in addition to the

Table 4. Selected list of endogenous metabotrophic factors*

Secretory proteins
Nerve growth factor, Brain-derived neurotrophic factor
Ciliary neurotrophic factor, Neuron-derived neurotrophic factor
Adiponectin, Irisin, Humanin, Omentin, Chemerin, Apelin, Otopetrin 1
Interleukin-10, Interleukin-1 receptor antagonist, Metalothioneins
Glucagon-like peptide-1
Intracellular proteins
Sirtuin-1, PPAR-gamma, Uncoupling protein-1 (UCP-1)
Aquaporin-7**

* Modified from (12). For references, see the text, and also (43-55).

** Discovered in 1986 by Gheorghe Benga (56) as the water channel integral membrane protein, in erythrocytes, the family of proteins designated the aquaporins (AQP) was appreciated when the Nobel Prize in Chemistry was awarded in 2003 to Peter Egre, whereas its original discovery by Gheorghe Benga has been ignored. Today, the AQP family consists of more than 10 members, AQP7 being expressed in adipocytes and related to obesity (57,58).

Table 5. Metabotrophic effects of NGF and BDNF*

NGF shares homology with proinsulin
NGF and BDNF are produced by pancreatic beta cells and exert insulinotropic effects
NGF and BDNF are trophic factors for pancreatic beta cells, and also improve beta cell transplantation
NGF up-regulates the expression of LDL receptor-related protein
NGF up-regulates the expression of PPARgamma
NGF inhibits glucose-induced down-regulation of caveolin-1
NGF improves skin and corneal wound healing
NGF may improve vascular (atheroma) wound healing
NGF rescues silent myocardial ischemia in diabetes mellitus
NGF improves diabetic erectile dysfunction
NGF and BDNF suppress food intake
Healthy lifestyle increases brain and circulating levels of NGF and BDNF
An atherogenic diet decreases brain BDNF levels
BDNF-deficient mice develop abnormalities similar to metabolic syndrome
BDNF improves cognitive processes

Modified from (15). For references, see the text, and also 36, 39, 47, 48, 50-53, 66, 67.

BDNF-responsive synaptotrophic proteins Homer-1 and Egr3 (35): (ii) an ATP-NGF complex, but not NGF itself, appears to be the active neuroprotective mediator (36): (iii) NGF is related to enhanced expression of the purinergic P2X(3) receptor (37): (iv) metformin, a widely prescribed drug for type 2 diabetes, may exert neuroprotective effects by increasing BDNF levels (38), and (v) vitamin A may exert antidiabetic effects via NGF expression (39). Likewise, the role of microRNA in diabetes development has been recognised (40, also see 41 and 42 for sirtuin-1). A possible therapeutic pathway for the management of diabesity is shown in Figure 7.

The present integrated view also suggests that understanding the precise role of MTF in the origin of *Homo diabesus* may lead to new therapies for diabesity and related diseases, including Alzheimer's disease (AD). The use of transcript clustering to identify molecular mecha-

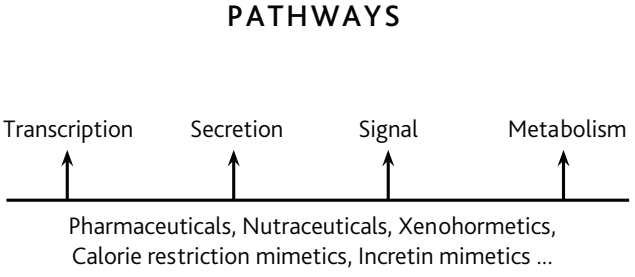

Figure 7. A drawing presenting a possible therapeutic pathway for diabesity.

nisms contributing to the early stages of AD in mice has identified changes in the insulin signaling pathway, including the down-regulation of insulin receptor substrate 4 (Irs4), an early event in AD (59). Insulin and MTF signaling are strongly associated with diabesity, which has recently been identified as a potential risk factor for AD (60-66; also see 67).

CONCLUSION

In 1999, Albee Messing published in an editorial entitled "Nestin in the liver - lessons from the brain" in *Hepatology* (29: 602-603). He wrote the following: "Most neuroscientists manage to get through each day without thinking of the liver even once… but I think that is about to change." This may also be the case for adipose tissue. Future *new thinking* in neuroadipocrinology of diabesity may lead to a deeper insight about how we can make MTF secretion and signaling work for the improvement of physical and mental quality of life of *Homo diabesus* who is expressing now in more than a trillion earthians.

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SUSCEPTIBILITY OF DIABETIC HEART TO CATECHOLAMINE-INDUCED ARRHYTHMIAS IS INDEPENDENT OF CONTRACTILE DYSFUNCTION

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OSETLJIVOST SRCA NA KATEHOLAMINIMA IZAZVANE ARITMIJE JE NEZAVISNA OD KONTRAKTILNE DISFUNKCIJE U DIJABETESU

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ABSTRACT

Background: *Diabetes is associated with myocardial electrical instability and prolongation of action potential duration that result in disturbances in the rhythm of the heart.*

Objective: *This study was undertaken to examine the role of circulating catecholamines in abnormal cardiac rhythm and contractility during different stages of diabetes.*

Methods: *Diabetes was induced in male Sprague-Dawley rats with streptozotocin (STZ; 65 mg/kg, i.v.). Epinephrine (4-128 μg/kg, i.v.) -induced arrhythmias and plasma levels of epinephrine (Epi) and norepinephrine (NE) were determined in control, 4- and 8-wk diabetic animals. Echocardiography was used to assess cardiac remodeling and contractile function.*

Results: *Although diabetes induced cardiac dysfunction, there were no significant differences in cardiac output, ejection fraction, left ventricle (LV) dimensions, LV fractional shortening between the 4- and 8-wk diabetic animals. The electrocardiogram of both diabetic groups showed deep S wave as well as changes in T wave and ST segment. In addition, prolongation of the RR interval in the 4- and 8-wk diabetic animals was seen, while prolongation of the QT and PR intervals were only seen in the 8-wk diabetic animals. The severity of Epi-induced ventricular arrhythmias, as assessed by arrhythmia score, was significantly lower in the 8-wk diabetic rats, as compared to the 4-wk diabetic animals. Circulating Epi levels were significantly decreased in the 8-wk diabetic rats, whereas NE levels were increased in the 4-wk diabetic rats.*

Conclusions: *The sensitivity of the diabetic heart to catecholamine-triggered arrhythmias may be dependent on circulating Epi rather than NE and thus it can be proposed that the increased incidence of sudden cardiac death in diabetics may not be associated with response to catecholamines.*

Key words: *diabetes, arrhythmias, cardiac dysfunction, norepinephrine, epinephrine*

SAŽETAK

Uvod: *Dijabetes je udružen sa električnom nestabilnošću miokarda i produženim trajanjem akcionog potencijala što rezultuje poremećajima srčanog ritma.*

Cilj: *Ova studija je sprovedena sa ciljem da ispita ulogu cirkulišućih kateholamina kod poremećaja srčanog ritma i kontraktilnosti miokarda tokom različitih stadijuma dijabetesa.*

Metode: *Kod muških pacova soja Sprague – Dawley dijabetes je izazvan streptozocinom (STZ; 65 mg/kg, i.v.). Aritmije izazvane adrenalinom (4 – 128 μg/kg, i.v.) i koncentracija adrenalina i noradrenalina detektovane su u kontrolnoj grupi i nakon 4. i 8. nedelje kod životinja kojima je indukovan dijabetes. Remodelovanje srca kao i kontraktilna funkcija su procenjene ehokardiografijom.*

Rezultati: *Iako je dijabetes izazvao poremećaj srčane funkcije, nije bilo značajnijih razlika u udarnom volumenu, ejekcionoj frakciji, dimenzijama leve komore, frakcionom skraćenju leve komore između životinja koje imaju dijabetes 4 i 8 nedelja. Elektrokardiogram obe grupe životinja sa dijabetesom pokazao je duboki S talas i promene u T talasu i ST segmentu. Pored toga, došlo je do produženja RR intervala kod životinja koje imaju dijabetes 4 i 8 nedelja, dok se produženje QT i PR intervala javilo samo kod životinja koje imaju dijabetes 8 nedelja. Opasnost od ventikularnih aritmija izazvanih adrenalinom, koja se procenjuje pomoću aritmija skora, bila je značajno niža kod životinja koje imaju dijabetes 8 nedelja u poređenju sa životinjama koje imaju dijabetes 4 nedelje. Nivoi cirkulišućeg adrenalina su bili značajno niži kod životinja koje imaju dijabetes 8 nedelja, dok su nivoi noradrenalina bili povišeni kod životinja koje imaju dijabetes 4 nedelje.*

Zaključak: *Osetljivost dijabetičnog srca na aritmije izazvane kateholaminima može zavisiti više od koncentracije cirkulišuceg adrenalina nego od koncentracije noradrenalina, zbog čega se može pretpostaviti da povećana incidenca iznenadnih srčanih smrti u dijabetesu ne mora biti povezana sa odgovorom na kateholamine.*

Ključne reči: *dijabetes, aritmije, poremećaji srčane funkcije, noradrenalin, adrenalin*

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ABBREVIATIONS

4DM, 4-week diabetes
8DM, 8-week diabetes
AS, arrhythmia score
CaMKII, Ca2+/calmodulin-dependent protein kinase II
cAMP, cyclic AMP
CO, cardiac output
CON, control group
ECG, electrocardiogram
EF, ejection fraction
Epi, epinephrine
FS, fractional shortening
HPLC, high performance liquid chromatography
LV, left ventricle
LVEDV, LV end-diastolic volume
LVESV, LV end-systolic volume
LVFS, LV fractional shortening
LVIDD, left ventricular internal diameter diastole
LVIDS, left ventricular internal diameter systole.
NE, norepinephrine
PKA, protein kinase A
PVBs, premature ventricular beats
PWTd, diastolic posterior wall thickness
PWTs, systolic posterior wall thickness
SR, sarcoplasmic reticulum
STZ, streptozotocin
VT, ventricular tachycardia

INTRODUCTION

The overactivation of the sympathetic nervous system is invariably seen in subjects with high risk for sudden cardiac death and elevated circulating catecholamine levels are considered to result in lethal ventricular arrhythmias and subsequent sudden cardiac death (1-4). Such arrhythomogenic effects of catecholamines are generally believed to occur, in part, by producing defects in intracellular Ca^{2+}-handling (1-4). In addition, oxyradicals, which are known to generate oxidative stress, may play a critical role in the genesis of ventricular arrhythmias that may result in sudden cardiac death (1-4). Both bradycardia and malignant ventricular arrhythmias occur in diabetic subjects (5,6). Prolongation of the action potential duration leading to myocardial electrical instability predisposes the heart to rhythm disturbances and is considered to be a main feature of cardiac dysfunction of diabetic subjects (7-10). In addition, the diabetic heart is characterized by an early asymptomatic left ventricular diastolic dysfunction followed by late systolic dysfunction (11, 12).

Although diabetic cardiomyopathy is a frequent complication of diabetes, the mechanisms are not completely understood. In view of the fact that the activation of the sympathetic nervous system is associated with the occurrence of arrhythmias as well as impaired cardiac function, the present study was undertaken to investigate if the susceptibility to catecholamine-induced arrhythmias is correlated to the stage or duration of diabetes by employing a well-established streptozotocin (STZ) rat model that clinically resembles human type 1 diabetes. Furthermore, we examined if the extent of the cardiac dysfunction is correlated to the duration of the diabetic state. This is the first study to report that the susceptibility to catecholamine-induced ventricular arrhythmias may be dependent on the stage of diabetes and that long-term diabetes may be associated with increased resistance to catecholamine-induced ventricular arrhythmias.

MATERIALS AND METHODS

Animals

All experimental protocols for animal studies were approved by the Animal Care Committee of the University of Manitoba, following the Guidelines established by the Canadian Council on Animal Care. Male Sprague-Dawley rats (200-220 g each) were kept at 12-h day/night cycle and fed rat chow and water *ad libitum*. After one week of quarantine, the rats were randomly assigned into 3 groups; control (CON), a 4-wk diabetes (4DM) and 8-wk diabetes (8DM).

Induction of experimental streptozotocin-induced diabetes

Diabetes was induced in rats by a single tail-vein injection (65 mg/kg) of streptozotocin (STZ) dissolved in 0.1 M citrate buffer, pH 4.5, as described previously (13). CON animals were injected with the vehicle only. The blood glucose levels were tested by using the Sigma kit for glucose determination (Sigma) and plasma insulin was measured using a standard radioimmunoassay technique as described elsewhere (13).

ECG parameters

Six-lead electrocardiographic (ECG) monitoring (leads I-III, augmented vector right aVR, augmented vector left aVL and augmented vector foot aVF) was used and different ECG parameters such as PQ, QRS, RR, and QT intervals were obtained from baseline recordings (AcqKnowladge 3.0.3 software). Measurement of all variables was performed in a blinded manner. No attempt was made to correct QT for heart rate because previous studies have shown that QT interval was not rate-dependent in rats (14).

Epinephrine-induced arrhythmias

Epinephrine (Epi) treatment was performed as previously described (4,15). Briefly, the tail vein of anesthetized rats was cannulated and cumulative doses of Epi given intravenously in a bolus of 4, 8, 16, 32, 64, 128 μg/kg at

	C	4DM	8DM
A. General characteristics			
Body weight (g)	555 ± 26	347 ± 11*	358 ± 17*
Plasma glucose (mM)	7.9 ± 0.4	37.3 ± 2.5*	34.6 ± 0.8*
Plasma insulin (pM)	57.8 ± 9.7	35.3 ± 3.5*	16.8 ± 1.2*#
B. Electrocardiographic parameters (msec)			
PR	0.035±0.001	0.033±0.002	0.39±0.001*
QRS	0.060±0.002	0.058±0.001	0.057±0.002
QT	0.093±0.004	0.094±0.002	0.100±0.002*
RR	0.175±0.007	0.193±0.007*	0.211±0.006*

Table 1. General characteristics and electrocardiographic parameters of the controlas well as 4- and 8-wk diabetic rats.

10 min intervals or until death of the animals. A 10-min baseline and continues ECG until the last Epi injection was recorded. Epi-induced arrhythmias including premature ventricular beats (PVBs), bigemines, salvos and ventricular tachycardias, were analyzed according to the Lambeth Conventions (16). Ventricular tachycardia (VT) was defined as a run of three or more consecutive ectopic beats. In addition, each individual animal was evaluated by means of a 6-point arrhythmia score (AS), and an assigned number corresponded to the most severe type of arrhythmia observed in that animal. AS was used for the group analysis of the severity of arrhythmias.

Echocardiography

An ultrasound imaging system (SONOS 5500 ultrasonograph (Agilent Technologies, Mississauga, ON, Canada) was used for the measurement of cardiac output, heart rate, left ventricular (LV) wall size and internal diameters during systole and diastole as well as fractional shortening (FS) and ejection fraction (EF). Echocardiographic measurements were conducted in rats anesthetized using 2.5% isoflurane in 2 l/min of oxygen. Briefly, the transthoracic short-axis measurements were performed using a 12-MHz annular array ultrasound transducer. The M- mode images of posterior wall of the LV at the level of the papillary muscle were obtained for posterior wall thickness (PWT) and chamber dimensions. Images were stored in digital format on a magnetic optical disk for analysis. LV systolic function was assessed by calculating LV fractional shortening (LVFS) using the formula (LV end-diastolic diameter - LV end-systolic diameter) x 100/LV end-diastolic diameter. Cardiac output (CO), LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) were calculated using the following formulas:

CO = (heart rate x stroke volume)/1 000, LVEDV = 7 (LV end-diastolic diameter)^3/(2.4 + LV end-diastolic diameter) and LVESV = 7 (LV end-systolic diameter)^3/(2.4 + LV end-systolic diameter). All these parameters were determined from at least 3 consecutive cardiac cycles (17).

Measurement of plasma catecholamines

Plasma from blood collected from the abdominal aorta of experimental animals was used to assess both norepinephrine (NE) and Epi levels by the Bio-Rad plasma Ca reagent kit and high performance liquid chromatography (HPLC) with electrochemical detection as previously described (4).

Heart rate (beats/min)	361 ± 5	301 ± 17*	296 ±13*
Stroke volume (ml)	0.90 ± 0.02	0.62 ± 0.07*	0.71 ± 0.04*
Cardiac output (ml/min)	247 ± 5	184 ± 15*	210 ± 14*
Fractional shortening (%)	54 ± 1	40 ± 3*	45 ± 2*
Ejection fraction (%)	84 ± 3	76 ± 3*	81 ± 1*
LV volume (ml) Systolic	0.11±0.02	0.22±0.04*	0.18±0.01*
Diastolic	0.71±0.02	0.91±0.07*	0.90±0.03*
LVID (mm) Systolic	3.47±0.19	4.35±0.35*	4.15±0.03*
Diastolic	6.79±0.08	7.39±0.21*	7.40±0.10*
PWT (mm) Systolic	2.85±0.17	2.26±0.17*	2.19±0.15*
Diastolic	2.05±0.18	1.53±0.14*	1.55±0.11*

Table 2. Echocardiographic parameters of the control, 4- and 8-week diabetic rats.

Statistical analysis

Data are expressed as means ± S.E.M. for 5-8 animals per group. One-way analysis of variance (ANOVA) followed by Duncan`s multiple test were used for comparison of differences in parametric variables among the groups. Statistical differences between two mean values were evaluated by Student`s t-test. The incidence of arrhythmias was expressed as percentage and compared by using the 2 x 2 Chi-square test. Since the episodes of arrhythmias are not normally distributed, these data were compared using Mann-Whitney test. $P < 0.05$ indicated a significant difference.

RESULTS

General characteristics and cardiac function of the 4-wk and 8-wk diabetic animals

The diabetic state of the animals was confirmed by the elevated blood glucose and marked reduction in insulin levels following STZ injection (Table 1) as reported in our previous studies (13, 18-20). Although a further decline in the insulin levels was seen in the 8-wk diabetic animals, this did not result in a further increase in blood glucose levels. Diabetes was also associated with lower body weights, with no differences between the 4-wk and 8-wk diabetic animals (Table 1). ECG revealed a prolongation of the RR intervals in both 4- and 8-wk diabetic rats without any changes in QRS complex whereas a prolongation of the PR and QT intervals was seen in the 8-wk diabetic rats only (Table 1).

Representative echocardiographic images depicting the changes in left ventricular internal diameter (LVID) are shown in Figure 1 and the analysis of the data for different parameters of cardiac performance is given in Table 2. Although the heart rate, stroke volume, CO, EF

Dose of Epi	*Incidence PVBs*			*Number of episodes PVBs*		
(μg/kg)	CON	4DM	8DM	CON	4DM	8DM
4	1/8(13%)	1/5 (20%)	0/5 (0%)	0.88±0.88	4.20±4.2	0.00±0.00
8	3/8 (38%)	4/5 (80%)	1/5 (20%)	0.75±0.41	2.80±1.83	0.20±0.20 #
16	4/8 (50%)	4/5 (80%)	3/5 (60%)	8.38±7.53	7.40±5.22	2.00±0.89*
32	7/8 (88%)	5/5 (100%)	5/5 (100%)	3.75±1.49	10.83±4.13 *	3.40±0.75#
64	8/8 (100%)	5/5 (100%)	5/5 (100%)	8.25±2.39	8.75±3.40	3.60±1.69 *#
128	8/8 (100%)	5/5 (100%)	4/5 (80%)	12.00±2.88	10.40±2.25	4.40±1.63 *#

Table 3. Incidence and number of episodes of premature ventricular beats (PVBs) induced by cumulative doses of epinephrine (Epi) in control as well as 4- and 8-wk diabetes .

The values are mean ± SEM of 5-8 experiments. The percentage of animals showing the incidence of PVBs are in brackets at each dose of Epr. CON, control; 4DM, 4-wk diabetes; 8DM, 8-wk diabetes. Significant at *P<0.05 vs. CON; # P<0.05 vs. 4DM

and FS were depressed in the 4-wk diabetic animals, further depressions in these parameters were not seen in the 8-wk diabetic animals (Table 2). Both LV volume and LVID in systole and diastole were increased in the 4-wk diabetic rats, but no further increases were seen in the 8-wk diabetic rats (Table 2). On the other hand, PWT in both systole and diastole were decreased in the 4-wk diabetic rats; however, no further changes were seen in the 8-wk diabetic animals.

ECG Parameters

Abnormalities in the T wave, ST segment and deep S wave were observed in both the 4- and 8- wk diabetic groups (Figure 2A). No ventricular arrhythmias were observed in either of the diabetic groups during stabilization. Figures 2B and 2C show representative ECG recordings following Epi injections. It can be seen that Epi triggered various types of ventricular arrhythmias and the most frequently developed arrhythmias observed were PVBs (Figure 2C).

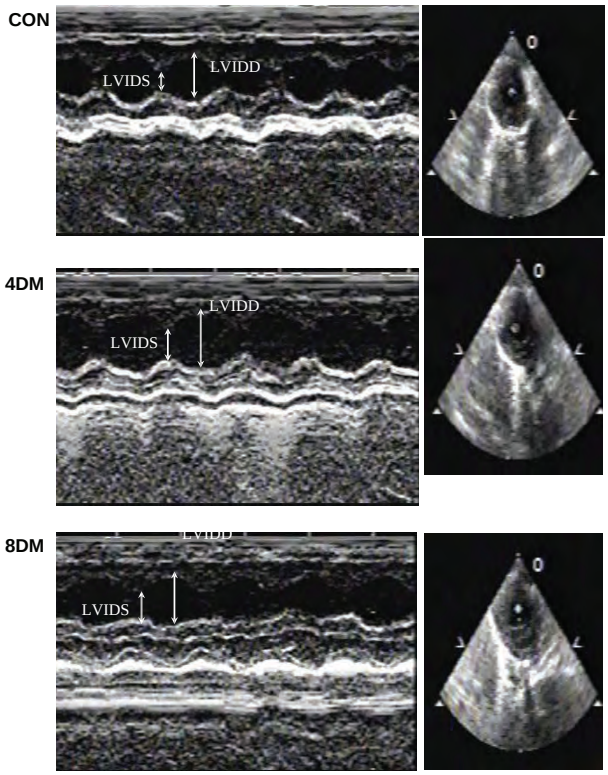

Figure 1. Baseline transthoracic echocardiographic images. 2D (left panel) and M-mode (right panel) images are shown for control (CON), 4-wk diabetes (4DM) and 8-wk diabetes (8DM). LVIDD, left ventricular internal diameter diastole; LVIDS, left ventricular internal diameter systole.

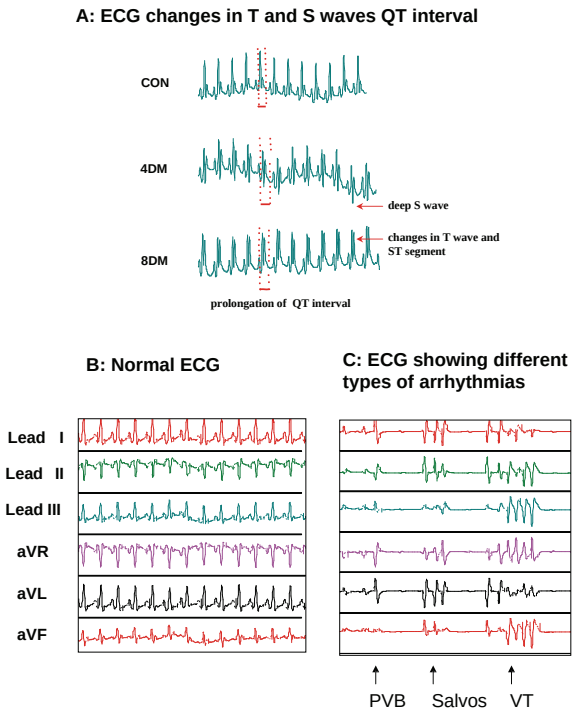

Figure 2. A. Baseline ECG recordings of lead III of the control, 4- and 8-wk diabetic animals with the indications of the T wave abnormalities, QT prolongation and deep S wave. B. Representative 6- lead ECG recording of a control animal before and C. following epinephrine administration. CON, control; 4DM, 4-wk diabetes; 8DM, 8-wk diabetes; PVBs, premature ventricular beats; VT, ventricular tachycardia.

Dose of Epi	*Number of episodes of all VA*			*Duration of VA*		
(μg/kg)	CON	4DM	8DM	CON	4DM	8DM
4	0.88±0.88	4.40±4.40	0.00±0.00	0.11±0.11	0.63±0.73	0.00±0.00
8	0.75±0.41	2.80±1.83	0.20±0.20	0.12±0.06	5.85±5.54	0.03±0.03
16	8.38±7.53	8.00±5.39	2.30±1.02	1.10±0.94	1.14±0.66	0.47±0.23
32	4.38±1.53	13.0±4.71*	3.80±1.66#	1.00±0.35	2.23±0.71	1.12±0.41
64	10.88±2.23	8.20±3.48	3.60±1.69*	2.55±0.57	1.43±0.65	0.51±0.23*#
128	21.00±5.21	12.60±2.52	4.80±1.88*#	18.09±5.25	3.06±0.82*	1.06±0.54*#

The values are mean ± SEM of 5-8 experiments. CON, control; 4DM, 4-wk diabetes; 8DM, 8-wk diabetes. Significant at *P<0.05 vs. CON; #P<0.05 vs. 4DM

Table 4. Influence of 4- and 8-wk diabetes on the number of episode and duration of all ventricular arrhythmias (VA) induced by cumulative doses of epinephrine (Epi).

Ventricular arrhythmias induced by cumulative doses of epinephrine in 4- and 8-wk diabetic animals

At low doses (4-16μg/kg) of Epi, the incidence of PVBs was lower in the 8-wk diabetes group as compared to the 4-wk diabetes group. On the other hand, lower number of episodes of PVBs were observed with high doses (32-128 μg/kg) of Epi in the 8-wk diabetic animals as compared to the 4-wk diabetic rats (Table 3). A lower sensitivity to Epi-induced ventricular arrhythmias as well as shorter duration times of arrhythmias were also observed in the 8-wk diabetic animals, as compared to the 4-wk diabetic animals (Table 4).

Arrhythmia score and plasma catecholamine levels in 4- and 8-wk diabetic rats

The overall severity of ventricular arrhythmias, expressed as AS, was significantly lower in the 8-wk diabetic animals, as compared to both 4-wk diabetic and control animals, whereas that in the 4-wk diabetic rats was similar to control values (Figure 3A). In order to determine if sympathetic nervous activity was increased in the 4- and 8-wk diabetic animals, catecholamine levels in the plasma were measured. Although Epi levels did not differ between the control and 4-wk diabetic rats, Epi levels were significantly lower in the 8-wk diabetic animals (Figure 3B). However, in contrast, NE levels were markedly higher in the 4-wk diabetes group whereas no change was seen in the 8-wk diabetic animals, as compared to control values (Figure 3C).

DISCUSSION

The present study was undertaken to investigate the association of the sensitivity of the diabetic heart to Epi and incidence of ventricular arrhythmias as well as cardiac remodeling and dysfunction in rats at different stages of diabetes. Although comparable cardiac remodeling and impaired cardiac function was seen in both the 4-wk and 8-wk diabetic animals, a prolongation of the RR interval was seen in the 4-wk diabetic animals, which was further increased in the 8- wk diabetic animals. This suggests that the depression in the conduction of the electrical impulse may be dependent on the stage of diabetes. In addition,

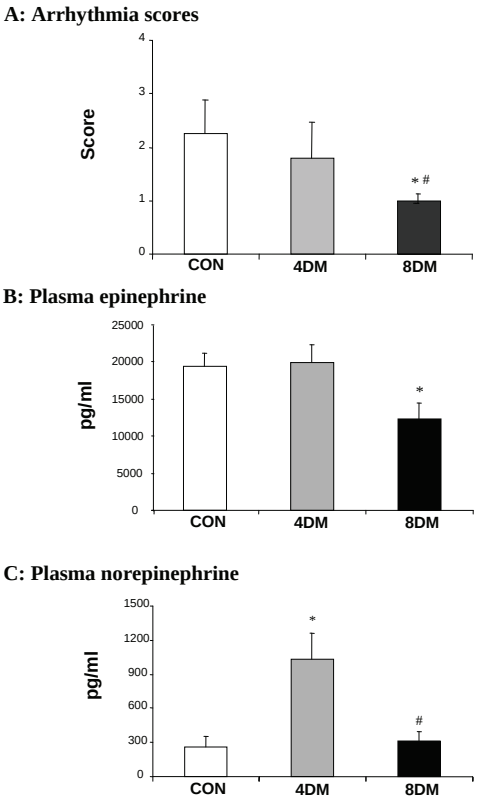

Figure 3. A. Severity of ventricular arrhythmias (arrhythmia score) and B. plasma catecholamine levels after epinephrine injections in 4- and 8-wk diabetic animals. Diabetes was induced with a single intravenous injection of STZ (65 mg/kg). The values are mean ± SEM of 5-8 experiments. CON, control; 4DM, 4-wk diabetes; 8DM, 8-wk diabetes. *P<0.05 vs. C; #P<0.05 vs. 4DM.

prolongation of the QT and PR intervals were seen only in the 8-wk diabetic animals. Similar experimental and clinical observations have also been reported by others (21-24). While a dose-independent increase in the number of PVBs was seen in the 4-wk diabetic animals, the 8-wk diabetic rats were more resistant to Epi-induced PVBs as compared to control animals. In addition, the number of catecholamine-induced arrhythmias was higher in the 4-wk diabetic rats as compared to the 8-wk diabetic rats. In fact, the 8-wk diabetic rats were resistant to Epi-induced arrhythmias, indicating that ventricular arrhythmias induced by catecholamines are dependent on the stage of diabetes, in other words more ventricular arrhythmias were seen at early phase of diabetes. While the arrhythmia score and plasma Epi levels in the 4-wk diabetic animals were comparable to those of control animals, low plasma Epi levels in the 8-wk diabetic rats were associated with a lower severity of ventricular arrhythmias. In contrast, NE levels were increased in the 4-wk diabetic animals, but unchanged in the 8-wk diabetic rats.

Heart dysfunction in chronic diabetes has been observed to be associated with Ca^{2+}- handling abnormalities in cardiomyocytes as defects in the sarcoplasmic reticular (SR) and sarcolemmal (SL) calcium transport processes have been detected in the diabetic heart (25). Indeed, defects in SL Na^+/K^+-ATPase, Na^+/Ca^{2+} exchanger, Na^+/H^+ exchange, Ca^{2+}-channels and Ca^{2+}-pump activities lead to increased concentration of cytosolic Ca^{2+} (26). The mechanism by which hyperglycaemia produces ventricular instability may be related to the increased sympathetic activity, increased cytosolic calcium content in cardiomyocytes, or both (27). Thus, it is reasonable to suggest that there is an increase in the risk of ventricular arrhythmias in early stage diabetes, which may be related to increased NE levels. On the other hand, a reduced susceptibility to ventricular arrhythmias in the 8-wk diabetic animals may be related to reduced catecholamine levels. In this regard, reduced plasma NE levels have been reported in long term human diabetes (28-31). A higher resistance against ischemia/reperfusion induced arrhythmias has also been reported (32-35) and several mechanisms have been proposed. Recently, diabetic hyperglycemia has been demonstrated to activate Ca^{2+}/calmodulin-dependent protein kinase II (CaM KII), which results in increased spontaneous SR Ca^{2+}-release events that can contribute to cardiac arrhythmias (36). Furthermore, genetic ablation of CaM KIIδ prevents high glucose induced arrhythmias (36). Accordingly the attenuation of arrhythmias in late stage of diabetes may be related to a reduction in CaM KII activity. However, some of our earlier observations (18, 37) have reported that although SR CaM KII activity is increased in the diabetic (6-wks post STZ-induced diabetes) heart, it is decreased in 10 wks sucrose-fed rats (38). Therefore, it appears that changes in CaM KII activity are biphasic in nature and dependent on the stage or severity of diabetes. Biphasic changes in CaM KII are also seen during the development of heart failure (39, 40). Consequently, some caution must be exercised in the implication of CaM KII in arrhythmias and cardiac dysfunction; thus, examination of the time course of changes in CaM KII activity as well as the development of experimentally induced arrhythmias is required before any meaningful conclusions can be made.

It is well known that excessive amounts of catecholamines play a major role in the induction of cardiac rhythm disorders (41). These effects are mediated through the activation of β-adrenoceptors-cAMP-PKA system. Furthermore, in view of the occurrence of oxidative stress in diabetes (26, 27, 42), under conditions of increased catecholamines levels, there is an increase in the formation of catecholamine oxidation products, aminochromes, which have also been linked to arrhythmogenesis (2, 3). Since the plasma levels of both Epi and NE were reduced in the 8-wk diabetic animals, there could also be a reduced production of aminochromes and therefore a reduced susceptibility to catecholamine-induced arrhythmias. The reduced susceptibility of catecholamine-induced arrhythmias could also be, in part, related to the reported decrease in β_1- and β_2- adrenoceptors in diabetic hearts (43, 44). The α_1- adrenoceptor is known to modulate intracellular Ca^{2+}- concentration through the activation of phospholipase C-mediated generation of inositol trisphosphate and diacylglycerol (20, 45). The myocardial α_1- adrenoceptor signaling system has been reported to be impaired in STZ-induced diabetic rats (46, 47). Thus, it is possible that the resistance to arrhythmias in longer stage diabetes may be related to a reduced capacity for α_1- adrenoceptor mediated increases in intracellular Ca^{2+}. It is pointed out that there is a selective reduction in myocardial Na^+/K^+-ATPase, which reduces the capacity of the heart for maintaining K^+- and Ca^{2+}- homeostasis in STZ-induced diabetes (48), and increasing the risk of arrhythmias. It is thus conceivable that stage-dependent changes in Na^+/K^+-ATPase may exist in diabetes and impacting on the susceptibility or attenuation to catecholamine-induced arrhythmias; a possibility that warrants further investigation.

CONCLUSIONS

Although cardiac function in the 4-wk diabetic animals was impaired, it did not deteriorate further in the 8-wk diabetic animals. The 8-wk diabetic rats were more resistant to ventricular arrhythmias as compared to the 4-wk diabetic rats. The increased susceptibility of the 4-wk diabetic animals to ventricular arrhythmias was associated with an increase in plasma Epi levels. The reported higher incidence of sudden cardiac death in diabetic individuals may be due to other diabetes-induced cardiovascular complications.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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EVALUATON OF THE USE OF BONE IMPLANTS AS A THERAPY FOR DEEP DEFECTS IN THE PARODONCIUM

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PROCENA TERAPIJSKIH REZULTATA NAKON PRIMENE KOŠTANIH IMPLANTATA U TERAPIJI DUBOKIH DEFEKATA PARODONCIJUMA

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* Momir Stevanović i Dušica Ćirić su podjednako (sa 50%) učestvovali u pisanju ovog rada, te se smatraju prvim autorima

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ABSTRACT

Reconstruction of infrabony defects created by the chronic inflammatory processes of parodontopathy represents a major clinical problem in paradontology.

The objective of this research was to compare and evaluate the efficiency of two bone substituents at reversing the deep infrabony defects in the paradentium after the application of a new "Biohapel" material consisting of biphasic calcium phosphate/poly DL-lactide-co-glycolide.

This study included 12 patients of both genders with an average age of 49.7 years who were suffering from clinically manifested parodontopathy. The main criteria for selecting patients were the presence of six similar infrabony periodontal defects that were at least 4 mm deep and in the lower side of the teeth on both sides of the jaw. After applying a basic causal parodontopathy treatment, defects were divided into two groups, including an experimental group (n=72) in which defects were reconstructed using the "Biohapel" bone substituent (on one side of the lower jaw) and a control group (n=72) in which infrabony defects were reconstructed using β tricalcium phosphate (βTCP) (Cerasorb®), a preparation very commonly applied in regenerative parodontopathy treatment (on the other side of the lower jaw). Markers to assess treatment efficacy were determined before surgery and 6 months after surgery by measuring the depth of periodontal pocket and the level of the junctional epithelium.

We observed statistically significant differences in the periodontal regeneration markers between the experimental and control groups (p<0.05).

Application of "Biohapel" during the surgical treatment of infrabony defects in the paradentium significantly improves the bone regeneration of the paradentium when compared with the standard application of β-tricalcium phosphate .

Keywords: *infrabony defects, bone substituents, periodontal pocket, level of junctional epithelium.*

SAŽETAK

Osnovni klinički problem u terapiji parodontopatije predstavlja rekonstrukcija infrakoštanih defekata nastalih u toku hroničnog inflamatornog procesa.

Cilj ovog istraživanja bio je da se uporede primene dva koštana substituenta i proceni uspešnost regeneracije dubokih infrakoštanih defekata parodoncijuma, posle primene novog materijala bifaznog kalcijum fosfata/poli-dl-laktid-koglikolida- "Biohapel"-a.

U istraživanje je uključeno 12 pacijenata, oba pola, prosečne starosti 49, 7 godina, sa klinički manifestnom parodontopatijom. Osnovni uslov za odabir pacijenata za studiju bilo je prisustvo šest sličnih infrakoštanih parodontalnih defekta najmanje dubine 4 mm, u predelu istoimenih donjih bočnih zuba, sa obe strane vilice. Nakon sprovedene bazične kauzalne terapije parodontopatije, defekti su podeljeni u 2 grupe: eksperimentalnu grupu (n=72), koja je rekonstruisana "Biohapel" koštanim zamenikom (jedna strana donje vilice), i kontrolnu grupu (n=72), gde su infrakoštani defekti rekonstruisani β tri kalcijum fosfatom (βTCP) (Cerasorb®-om), preparatom koji se vrlo često primenjuje u regenerativnoj terapiji parodontopatije, (druga strana donje vilice). Parametri uspešnosti terapije su određeni pre, kao i 6 meseci posle hirurškog zahvata, merenjem dubine parodontalnog džepa i nivoa pripojnog epitela.

Uočeno je da ima statistički značajne razlike između eksperimentalne i kontrolne grupe (p<0, 05), u odnosu na ispitivane parametre parodontalne regeneracije.

Primena "Biohapel"-a u hirurškoj terapiji infrakoštanih defekata parodoncijuma omogućava bolju koštanu regeneraciju parodoncijuma u odnosu na primenu β tri kalcijum fosfata koji je poznati standard u regenerativnoj terapiji .

Ključne reči*: infrakoštani defekti, koštani zamenici, parodontalni džep, nivo pripojnog epitela*

ABBREVIATIONS

A+SD - average ± standard deviation
βTCP - β tricalcium phosphate
DPP - depth of the periodontal pockets
HAP - hydroxyapatite
LJE - level of the junctional epithelium
MWRO - modified flap operation
PLGA - poly(lactic-co-glycolic acid)

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INTRODUCTION

Parodontopathy represents a multifactorial illness caused by periodontal pathogenic microorganisms, and its course and outcome are determined by the genetic predisposition and immunological response of the patient[1] . The aetiology of parodontopathy is well-known, but the treatment of this disease is very complex and dependent on many factors (1, 2). The chronic inflammatory processes observed in parodontopathy can lead to extensive damage of the periodontium. One of the most complicated symptoms of parodontopathy is the development of infrabony defects because they are extremely difficult to treat. Reconstruction of these defects is exceptionally demanding and their presence contributes to an unfavourable illness prognosis (1).

Regeneration of destroyed tissues within in paradentium (*restitutio ad integrum*) represents the main objective for parodontopathy treatment. A standard procedure in the treatment of periodontal pockets is an MWRO (modified flap operation), but it shows little success in eliminating infrabony periodontal defects; thus, to enhance the regeneration of periodontal tissues, we have assessed various bone substituents (3, 4). A novel concept for the regeneration of periodontal tissues is based on the application of biomaterials that enable regeneration of the paradentium with varied efficiency (5).

Bone tissue has the smallest regenerative potential when compared with the surrounding tissues of the paradentium, but its proper functioning is of great importance. In periodontal surgery, various materials of different origins, including biological and synthetic, have been used for implantation into bony defects in an attempt to improve the regeneration and reparation of periodontal tissue (4).

The materials used for implantation need to have particular features, including biological compatibility, be easy to use, cause minimum postoperative complications and be financially acceptable for the patient. The basic principle behind using bone substituents is the assumption that the material has an osteogenic, osteoconductive and osteoinductive potential. This material is also expected to be resorptive and completely replaced by the newly formed bone (6).

Calcium phosphate is the main constituent of bone, and crystal hydroxyapatite (HAP) provides mechanical firmness to the bone. HAP, which is identical to the HAP in bones, can be obtained synthetically from biphasic calcium phosphate to produce a HAP that is mixed with tricalcium phosphate and a polymer component of poly lactide-co-glycolide (*Biohapel*) (7). Immediately after implantation, the polymer component (poly lactide-co-glycolide) of *Biohapel* shows an exceptional ability at adhering to osteoblasts, enhancing its osteogenic potential (8). When in contact with a vital bone, the material is resorbed within several months and is replaced with a newly formed bone without causing local or systemic toxicity (9).

The objective of our research was to analyse the degree of bone regeneration in infrabony defects of the paradentium from a bone substituent based on a hydroxyapatite coated with a polymer, *"Biohapel, "* in comparison to a commonly used synthetic preparation of β tricalcium phosphate (*Cerasorb®).*

MATERIALS AND METHODS

Here, we used a controlled and blinded trial based on a *split mouth method.* Our study compared two implant materials, including *Biohapel* in granules of 50-650 nanometres and ßTCP (*Cerasorb®,* Curasan, Germany) in granules of 63-250 micrometres. This research was approved by the Ethical Committee of the Faculty of Dentistry in Belgrade (number 123/2).

The clinical study included patients without systemic diseases and allergies, patients who haven't used antibiotics for at least 6 months prior to the start of the study, and non-smokers. The study excluded patients with terminal illness, pregnant women and nursing mothers and patients with acute inflammation of the paradentium. The study included 12 patients with bilateral infrabony defects of the paradentium at least 4 mm deep on the side teeth of the lower jaw. Each participant signed a consent form to be involved in the study.

Two weeks before surgery all patients received a basic treatment for the affected paradentium consisting of instruction about maintaining adequate oral hygiene, removing all soft and firm deposits from the teeth, correction of bad fillings and other iatrogenic factors.

The surgical procedure began with the local application of lidocaine and epinephrine in a ratio of 1:100, 000. After an intrasulcular incision, the full thickness of the cheek flap and lingual flap was lifted. After treating the periodontal pockets by debriding the granulation tissue and surface of the root, random infrabony defects from one side of the lower jaw in the region of first molars and first and second premolars on the mesial and distal side were reconstructed by applying *Biohapel* (experimental group n=72). The other side of the lower jaw in a region of equivalent teeth was reconstructed using β tricalcium phosphate (*Cerasorb®*) (control group, n=72). The implanted material was firmly packed into intra-bony defects with a sterile amalgam rammer. Finally, the mucoperiosteal flaps were repositioned and sutured primarily with single interdental sutures (*Ethicon®,* Mersilk 4-0, USA).

The condition of the paradentium was evaluated using the depth of the periodontal pockets (DPP) and the level of the junctional epithelium (LJE) as clinical parameters. All parameters of the affected paradentium were verified before surgical treatment and 6 months after surgery. Measurements were performed using a millimetre graduated pigtail explorer (PCP-UNC 15, *Hu-Friedy*, Leimen, Germany), and the obtained values were recorded in millimetres. Patients had a follow-up visit on the first day after surgery and again on the seventh day post-surgery to have their stitches. Subsequently, follow-up visits occurred

once a month up for six months to follow the degree of tissue healing after the surgery. Data were analysed using the statistics package SPSS (version 18.0). The results are presented as the average ± standard deviation (A+SD). A value of $p<0.05$ was considered statistically significant.

RESULTS

After treatment, the results obtained from both groups showed a statistically significant reduction in the examined parameters (Table 1). Analysis of the depth of the periodontal pocket and the level of junctional epithelium six months after the surgery showed that there was a statistically significant difference after the application of the two tested materials (Figures 1 and 2). There was a statistically significant reduction in the DPP and LJE of the *Biohapel* group when compared with the *Cerasorb* group (Table 2). No complications were recorded during the postoperative period.

Table 1.

	Before surgery A±SD (мм)	6 months after surgery A±SD (мм)	Wilcoxon test
DPP *Biohapel*	6, 32±1, 23	2, 76±0, 52	p =0, 000
LJE *Biohapel*	4, 86±0, 92	2, 83±0, 61	p =0, 000
DPP *Cerasorb*	6, 54±1, 26	3, 36±0, 84	p =0, 000
LJE *Cerasorb*	4, 97±0, 96	3, 26±0, 71	p =0, 000

Abbreviations: DPP - depth of a periodontal pocket, LJE - level of junctional epithelium, A±SD - average ± standard deviation, p – statistical significance

DISCUSSION

Our results show that application of the bone substituent Biohapel is a significantly improved treatment for deep infrabony defects of the paradentium whencompared with synthetic β tricalcium phosphate. Numerous studies have previously shown a significant reduction in the depth of infrabony periodontal defects six or more months after the application of various bone substituents (3, 10, 11, 12, 13). Lukovic and associates conducted a clinical study that showed *Cerasorb* and Bioss have similar efficacies as a treatment for deep infrabony pockets (14). Saini and associates found limited improvement using beta tricalcium phosphate to treat infrabony defects and determined that beta tricalcium phosphate in combination with biologically active factors provides a significantly better result (15). Jansen and associates showed that the main shortcoming of beta tricalcium phosphate is its fast resorption and that priority in the treatment of bony defects should be given to autogenous transplants (16). Elyan and associates showed that application of the nanocrystal hydroxyapatite as a treatment for infrabony

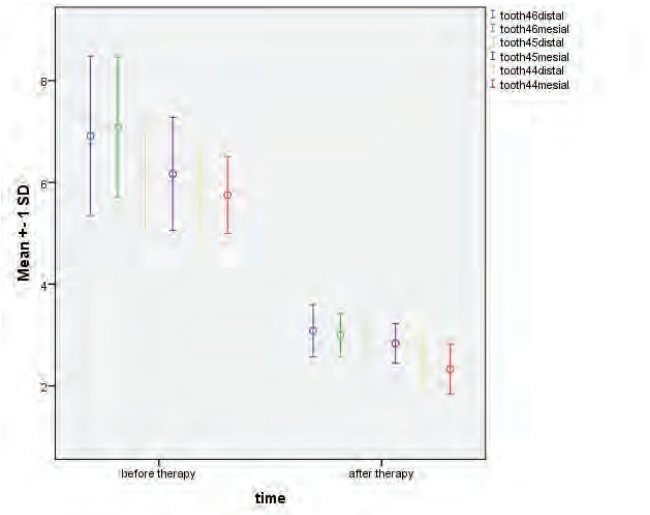

Figure 1. Probing depth of periodontal pocket before and after application of *Biohapel.*
Abbreviations: SD-standard deviation.

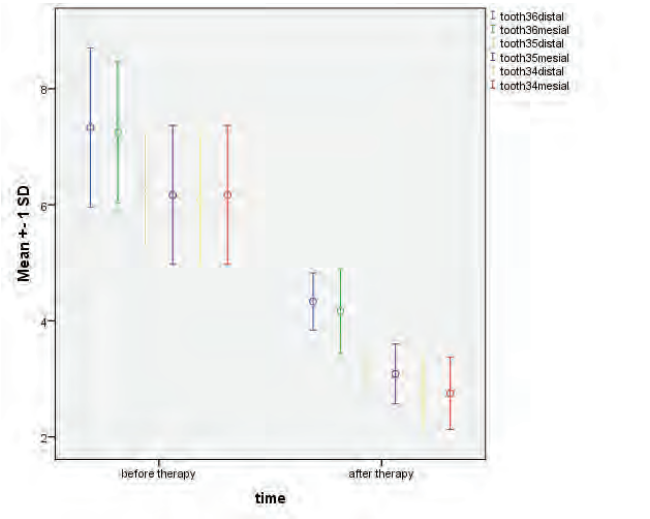

Figure 2. Probing depth of periodontal pocket before and after application of *Cerasorb.*
Abbreviations: SD-standard deviation.

Table 2.

Reduction of DPP 6 months after *Biohapel* treatment	72	3,56±0,96
Reduction of DPP 6 months after *Cerasorb* treatment	72	3,18±0,92
Mann-Whitney U test p =0,036		
Reduction of LJE 6 months after *Biohapel* treatment	72	2,03±0,77
Reduction of LJE 6 months after *Cerasorb* treatment	72	1,70±0,85
Mann-Whitney U test p =0,020		

Abbreviations: n – number of infrabony periodontal defects, A±SD - average ± standard deviation, ;p – statistical significance, DPP - depth of a periodontal pocket, LJE - level of junctional epithelium

defects led to a significant reduction in the depth of infrabony defects and had a stimulating effect on angiogenesis and osteogenesis. This same study also showed that the nanocrystal hydroxyapatite easily adhered to the fibroblasts and osteoblasts of the periodontal ligament (17). Porosity, surface structure, particle size and chemical features have are key characteristics that dictate the regenerative and osteoconductive potential of bone substituents (18). Thus, the greater treatment efficacy of *Biohapel* that we observed was most likely a consequence of the specific features of the material. *Biohapel* is the first nanocrystal material coated with a polymer component that has been used for the treatment of deep defects of paradentium, and its polymer component strongly adheres to the surrounding cells that are involved in tissue regeneration. Therefore, the size of the *Biohapel* particles and the PLGA polymer component provide this newly synthesised material with advantageous features. Additional research is needed to determine the long-term efficacy of *Biohapel*. In addition, a histological analysis of human preparations could determine any distinct characteristics of *Biohapel* as a treatment for infrabony periodontal defects. Our study undeniably shows that the application of biphasic calcium phosphate/poly DL-lactide-co-glycolide produces significant levels of bone regeneration and is a viable treatment option for infrabony defects of the paradentium.

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THE EFFECTS OF VIBROACOUSTICALLY INDUCED MICROVIBRATIONS ON ARTERIAL BLOOD PRESSURE AND OXIDATIVE STRESS IN RATS

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EFEKTI VIBROAKUSTIČKI IZAZVANIH MIKROVIBRACIJA NA ARTERIJSKI KRVNI PRITISAK I OKSIDACIONI STRES KOD PACOVA

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ABSTRACT

Vibroacoustics, a scientific field that has been intensively studied for the last thirty years, uses the properties of sound waves (infrasound, ultrasound, noise and music) to induce vibrations that, like a sound wave, may have both useful and harmful effects. The aim of this study was to examine the effects of vibroacoustically induced microvibrations on arterial blood pressure and markers of oxidative stress in the blood. The experiments were performed on Wistar male rats that had a 180-200 g body mass and were divided into control and experimental groups (6 rats in each). In the experimental group, microvibrations were induced using the Vitafon vibroacoustic apparatus (Vitafon, St. Petersburg, Russian Federation), which delivers sound waves of varying frequencies by a process called "phoning". Up to 60 minutes of phoning time was delivered to the kidney and liver using 4 different regimens that included a 5-minute stabilisation time; up to four 10-minute phoning regimens, with 5-minute breaks between each single regimen, at a 30 Hz-18000 kHz frequency range;, and 2.8 μm-12.3 μm microwave amplitudes. After the completion of a phoning regimen, animals were sacrificed and the oxidative stress markers were measured in blood samples (O_2^-, H_2O_2, nitrites, lipid peroxidation index, superoxide dismutase, catalase, and glutathione) and compared with the values of markers in the control group. Systolic arterial pressure was analysed after the acute application of up to four different regimens of vibroacoustic microvibrations. Systolic arterial pressure decreased significantly during the administration of the second regimen in comparison to the control group. Systolic arterial pressure returned, almost completely, to the initial value after the administration of the third and fourth regimens. There was no significant change in diastolic arterial pressure after the acute administration of up to four different regimens, although the pressure decreased slightly after the first and second regimens and returned to the initial value during the administration of the third and fourth regimens. Analysis of oxidative stress markers showed a statistically significant change in the catalase level. No statistically significant differences were found in the other oxidative stress

SAŽETAK

Vibroakustika je naučna oblast koja se intezivno razvija u poslednjih trideset godina, koristi mogućnost zvuka (infrazvuk, ultrazvul, buka i muzika), izaziva vibracije koje kao i zvuk može imati korisne ili štetne posledice. Cilj ovog rada je bio da se ispita uticaj vibroakustično indukovanih mikrovibracija na arterijski krvni pritisak i markere oksidacionog stresa u krvi pacova. Eksperimenti su izvedeni na pacovima Vistar soja, telesne težine 180-200 g, podeljenih u kontrolnu i eksperimentalnu grupu od po 6 životinja. U eksperimentalnoj grupi, mikrovibracije su indukovane pomoću vibroakustičnog aparata (Vitafon, Sankt Peterburg, Rusija) sa ukupno 60 minuta u četiri različita aplikaciona režima (5-minuta-stabilizacija, 10-minuta "foniranje" režim i na svakih 5-minuta pauza između signalnih režima, opseg frekvencija 30Hz - 18000 kHz, amplituda mikrotalasa 2,8 μm–12,3 μm, foniranje na jetru i bubreg). Nakon završetka eksperimentalnog protokola, životinje su žrtvovane i markeri oksidacionog stresa su analizirani u uzorcima (O_2^-, H_2O_2, nitriti, indeks lipidne peroksidacije, superoksid dismutaza, katalaza, glutation) i poređeni sa vrednostima kontrolne grupe. Analizom vrednosti sistolnog arterijskog pritiska posle akutne primene vibroakustičkih mikrovibracija različitog režima delovanja zabeležen je statistički značajan pad sistolnog arterijskog pritiska prilikom primene drugog režima u odnosu na kontrolu. Uočava se da se sistolni arterijski pritisak posle primene vibroakustičkih mikrovibracija u trećem i četvrtom režimu vratio na gotovo početnu vrednost. Analizom vrednosti dijastolnog arterijskog pritiska posle akutne primene vibroakustičkih mikrovibracija različitog režima delovanja nije zabeležen statistički značajan pad dijastolnog arterijskog pritiska niti u jednom režimu delovanja. Prilikom primene režima tri i četiri vrednosti dijastolnog arterijskog pritisaka su se vratile gotovo na početnu vrednost. Dobijeni rezultati su pokazali statistički značajnu razliku u vrednostima katalaze, dok nije bilo promene u vrednostimaostalih analiziranih parametara.

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markers analyzeanalysed. Further research is needed to clarify the physiological effects of low compared to high frequencies of vibroacoustically induced microvibrations and their possible therapeutic significance.

Key words: *vibroacoustics, arterial blood pressure, oxidative stress, microvibrations*

Dalje istraživanje je neophodno kako bi se definisali efekti niskih naspram efekata visokih apliciranih frekvencija, odnosno mogući terapijski značaj registrovanih efekata.

Ključne reči: *vibroakustika, arterijski krvni pritisak, oksidacioni stres, mikrovibracije*

ABBREVIATIONS

CAT - catalase
GSH - reduced glutathione
MAP - mean arterial pressure
RBCs - red blood cells
SEM - standard error mean
SD - standard deviation
SOD - superoxide dismutase
TBA - thiobarbituric acid
TBARs - index of lipid peroxidation
TCA - trichloroacetic acid

INTRODUCTION

Rohracher found that the body surface of humans, or more generally homeotherms, produced constant vibrations, which he named microvibrations (1). The most significant observation of his intensive work is that the sources of these microvibrations are the heart rate and vascular and muscle activities, which produce microvibrations in the infrasound and sound range, respectively. Rohracher further showed that maintenance of microvibrations in an organism demands considerable muscle engagement, or energy consumption and that the amplitude of microvibrations is a sensitive psychophysical measure of muscle tension and total body activity. For example, in a healthy human (or other homeothermic animal), the amplitude of these microvibrations is 1-5 international units at rest, with a frequency range of 6-12 Hz/sec (vibrations per second). Rohracher demonstrated that it is possible to detect microvibrations that, originating in the striated muscle system, over the whole body; contractions of striated muscles have a manifold magnification of the amplitude of microvibrations but do not affect their frequency; and microvibrations are constant during the registration of frequency.

Although Rohracher examined two variables, amplitude and frequency, his discoveries on frequency were of vital importance for his conclusions. The fact that muscles produce movements of various amplitudes had been known, but the finding that they show constant periodic movements with a constant frequency had significant and far-reaching implications. Based on Rohracher's studies, three important facts of microvibration frequency have been defined:

1. It is always within a range of 6-12 cycles per second
2. It is constant with any measurement
3. It varies between measurements that are taken on different occasions in the same subjectand is and is random and not correlated with other variables, as observed by that were Rohracher.

Several studies have been done performed that support Rohracher's conclusions (2, 3, 4, 5, 6, 7). Based on these studies, the aim of this paper was to examine the acute effects of vibroacoustic microvibrations on arterial blood pressure and markers of oxidative stress in blood.

MATERIAL AND METHODS

Experimental protocol

The experiments were performed on Wistar albino rats, aged 8 weeks, with a body weight of 230-250 g. The rats were placed in experimental and control groups, with and n=6 in each group, and. Experiments were carried out on each animal individually. All research procedures were carried out in accordance with the Declaration of Helsinki (last updated in 2005) and principles of Good Laboratory Practice (GLP) and were approved by the Ethical Committee for the Welfare of Experimental Animals, Faculty of Medical Sciences, University of Kragujevac. Baseline measurements were obtained for all of the parameters tested in rats without prior exposure to vibroacoustically induced microvibrations, which were used as controls. Rats were exposed to acute vibroacoustically induced microvibrations of defined amplitude and frequency using two vibroacoustic device emitters applied to the skin in the topographical area of the liver and kidney. The following settings on the vibroacoustic device (*Vitafon, St. Petersburg, Russian Federation*) were used: regimen 1, lower frequency of the 1st subrange within the limits 30–60 Hz; regimen 2, upper frequency of the 1st subrange within the limits 1–3 kHz; regimen 3, lower frequency of the 2nd subrange within the limits 0.3–0.8 kHz; regimen 4, upper frequency of the 2nd subrange within the limits 9–18 kHz; number of microvibration frequency subranges, 2; length of a single cycle of microvibration frequency change, 80–160 sec; amplitude of microvibra-

tion at the lowest frequency for settings 1 and of 3, 2.8–5.4 μm and for settings 2 and 4, 6–12.3 μm; and period of impulse modulation, 0.5–1.2 sec. The duration of vibroacoustically induced microvibration stimulation was 60 min. divided into individual 10 min. regimens, with 5 min. breaks between each.

Haemodynamic measurements

All animals were anaesthetised (35 mg/kg sodium pentobarbital; i.p.). The mean arterial pressure (MAP) was determined directly through the femoral artery catheter (PE-50, Clay-Adams, Parsippany, NY, USA) using a low-volume displacement transducer (P23 Db, Statham, Oxnard, CA, USA) and was recorded on a direct writing recorder.

Measurement of oxidative stress parameters in rat blood

Rats were anesthetizised with ether and sacrificed using cervical dislocation. For both control and experimental groups, $n_{1/2}$=12. Blood was collected in tubes (12x100), with 50 I.J. heparin/ml of blood, and kept frozen at -20°C until used for biochemical measurements. The following parameters of redox status were determined spectrophotometrically from the blood samples: index of lipid peroxidation (measured as TBARS), SOD, CAT and GSH. The presence of thiobarbituric acid reactive substances (TBARs) was used to estimate the degree of lipid peroxidation in plasma by adding 1% thiobarbituric acid (TBA) in 0.05 M NaOH to an aliquot of plasma followed by a 15 min. incubation at 100°C and reading at 530 nm. Distilled water was used as a blank probe. A TBA extract was obtained by combining 0.8 mL of plasma and 0.4 mL of trichloroacetic acid (TCA), incubating the sample on ice for 10 minutes and centrifuging the sample for 15 min. at 6000 rpm, as described previously (8). To calculate the activity of endogenous antioxidants, hhaemoglobin was measured according to the Drabkin method (9). Isolated red blood cells (RBCs) were washed three times with 3 volumes of ice-cold 0.9 mmol/L NaCl and hemolysate containing approximately 50 g Hb/L prepared. Superoxide dismutase (SOD) activity was determined by the epinephrine method. A 100 μL sample of lysate was mixed with 1 mL of carbonate buffer followed by addition of 100 μL of epinephrine. Detection of SOD was performed at 470 nm. (10). Catalase (CAT) activity was determined according to Beutler (11). Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove haemoglobin. The sample (100 μL) was mixed with 50 μL of catalase buffer and 1 mL of 10 mM H2O2. Detection of CAT was performed at 360 nm. Distilled water was used as a blank probe. The level of reduced glutathione (GSH) was determined by the oxidation of GSH with 5, 5-dithiobis-6, 2-nitrobenzoic acid using the Beutler method (12). The concentration of oxidative stress parameters is expressed as nanomoles per millilitre of red blood cells (RBCs).

Statistical analysis

Statistical analysis of experimental data included the following basic descriptive statistics: the mean value (X), standard deviation (SD) and standard error mean (SEM). For testing the normality of distribution parameters, the *Kolmogorov-Smirnov* test was used. To test the statistical significance of the results and to confirm the hypothesis, the following statistical tests were used: Student's t-test (parametric test), for dependent and independent variables and the Mann Whitney U test, for differences between the parameters. A database analysis of the results was performed using software package SPSS 10th 0 (*SPSS Inc., Chicago, IL, USA*). Ap < 0.05 was considered statistically significant.

RESULTS

Acute effects of vibroacoustic microvibrations of a specified amplitude and frequency on the arterial blood pressure of rats in vivo

Acute effects of vibroacoustic microvibrations of a specified amplitude and frequency on the arterial blood pressure of rats in vivo

An analysis of systolic arterial pressure after the acute application of vibroacoustic microvibrations using different regimens over the rat's body (5-minute single phoning time, with 5-minute breaks between single regimens) showed a statistically significant decrease of systolic arterial pressure during the administration of the second regimen compared to control ($p < 0.05$). A visible decrease in pressure was noted during the administration of the first regimen, but this was not statistically significant. Systolic arterial pressure returned to a nearly baseline level after the administration of the third and fourth regimens of vibroacoustic microvibration (Figure 1).

Diastolic arterial pressure did not decrease significantly after the acute administration of vibroacoustic microvibra-

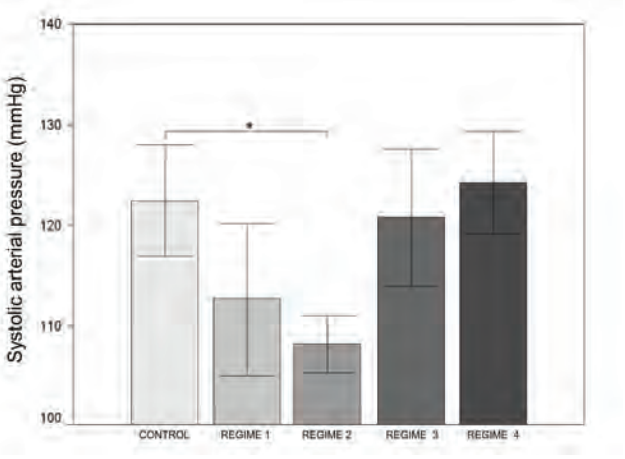

Figure 1. Systolic arterial pressure in rats after acute applications of vibroacoustic microvibrations using 4 various regimens (* p<0.05)

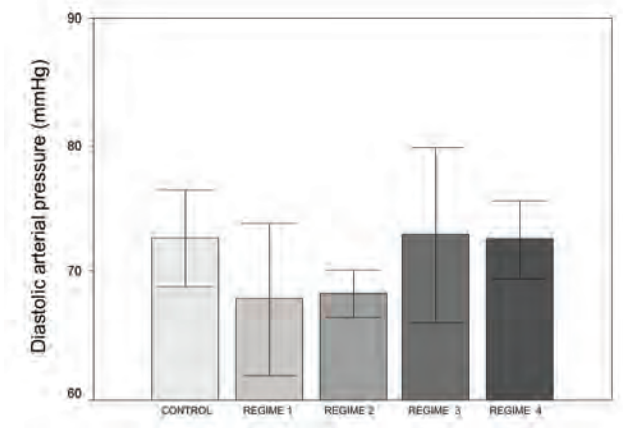

Figure 2. Diastolic arterial pressure in rats after the application of vibroacoustic microvibrations using 4 different regimens

tions using different regimens over the rat's body (5-minute single phoning time, with 5-minute breaks between single regimens). There was a trend, however, towards decreased diastolic arterial pressure during the first and second regimens, which returned to nearly baseline levels during the administration of the third and fourth regimens (Figure 2).

Acute effects of vibroacoustic microvibrations o a specified amplitude and frequency on oxidative stress parameters in the rat blood

Analysis of oxidative stress parameters after the acute application of vibroacoustic microvibrations using 4 different regimens (5-minute single phoning time, with 5-minute breaks, total phoning time 60 minutes) showed a statistically significant increase in the levels of the antioxidative enzyme catalase (CAT). In contrast, TBARs, SOD and GSH levels decreased slightly however this was not statistically significant (Table 1).

DISCUSSION

Research into the effects of vibrations and sound on human physiology has been the focus of vibroacoustic science for several years because of its significance in the environment (i.e. i.e., the ecology of labour) and in medicine. This research is especially important given the knowledge of the potentially harmful effects of high intensity vibrations on both labourers who work with vibrating machines and devices and the cardiovascular and nervous systems , as well as the harmful effects of high intensity sound on the environment. On the other hand, there are many beneficial effects of vibrations and/or low frequency sound waves, as has been shown recently, with important research being done conducted to study the vibratory sensitivity of people and potential applications of infrasound, ultrasound, noise and music.

Based on his research, Olav Skille (13) pointed to three universal effects of sound and/or music induced vibrations on humans: a. low frequency vibrations can have a relaxing effect, while high frequencies can increase tension; b. "rhythmic" music can excite while non-rhythmic music can have a sedating effect; and c. loud music can lead to aggression, and quiet music can act to sedate.

Vibroacoustic devices, which produce sound-induced vibrations and stimulate humans, were developed withover the last 30 years. Vibroacoustic therapy can be used in clinical settings. For example, music that causes sedation and/or pulse sinusoidal waves with low frequencies (between 20 Hz and 70 Hz) can be applied through a bed or armchair. Vibroacoustic therapy is currently used in the treatment of decubiti, to decrease arterial blood pressure, to reduce the heart rate, to improve circulation in post-operative treatment, and for stress-induced diseases. Reports on the improvement of circulatory beds in lower limbs and positive change of skin colour in patients treated with vibroacoustic therapy are anecdotal evidence of the benefits of this therapy (14). A hydrodynamic pump has been shown by Russian authors, to cause muscle fibres to tremble with sound oscillations, i.e., "a muscle sings at sound frequencies". According to this concept, microvibrations are a physical agent that helps organisms by reducing peripheral resistance in capillary networks and increasing venous blood flow. It has been shown that the role of microvibrations in the pump-like functioning of vascular vessels of the venous and lymphatic systems leads to the unidirectional flow of both blood and lymph. The frequency of smooth muscle trembling in the vascular walls improves the efficiency of venous and lymphatic pumping as well as the amplitude of movement, i.e., oscillations of muscle tissue appears to align with the diameter of the lumen in venous and lymphatic vessels. By applying different regimens of a vibroacoustic stimulator, vibroacoustic waves of various shapes, frequencies, amplitudes and time length can be used to synchronise their energetic stimulation on vessels that can have many different diameters. Each blood or lymphatic vessel will have its own optimum frequency and characteristic energy wave based on its unique diameter. Another important characteristic is a reduction of resistance due to blood circulation. It is assumed that at certain frequencies, vibroacoustic microvibrations decrease the friction between blood layers, thus reducing viscosity and vascular resistance, leading to an increase of "shear stress", which is the main physiological stimulus for the production of nitric oxide (no).

As discussed, there are certain effects of low frequency sound applications on the human cardiovascular system. In a study performed on an Apollo mission, astronauts using infrasound treatment found no electrocardiographic disturbances when 21 male subjects aged 21 and 23 were stimulated by sounds ranging betweenfrom 2 Hz-12 Hz, with an intensity of 119-144 decibels, in thea simulation chamber. The heart rate increased in 6 subjects by more than 6 beats per minute during maximum stimulation, but in 5 subjects, the heart rate decreased (15). Respiratory function was evaluated by pneumographic impedance and was normal in all subjects exposed to low frequency stimu-

Parameter (X±SE)	Control	Phoning	Test and Significance
TBARS	7.28±1.03	7.25±0.81	T test p=0.984
SOD	2589.87±1423.93	2017.36±1095.60	Mann Whitney p=0.937
CAT	32.04±4.98	51.29±1.70	T test p=0.010**
GSH	68051.66±3689.26	62066.66±2824.83	T test p=0.227

Table 1. Oxidative stress parameters after the acute application of vibroacoustic microvibrations using 4 different regimens (duration: 60 minutes) (** p<0.01).

li, while it increased in 6 subjects when a 140 decibel sound was applied. In this study, no discomfort, disorientation, mental confusion, tiredness or decline of mental capacities was found due to the applied vibrations.

In a second study, 40 pilots, divided into three groups, were treated with infrasonic frequencies of 14 Hz, 16 Hz and 50 Hz, respectively; the results showed occasional changes of blood pressure, decreased vigilance and somewhat prolonged reaction time. This study showed that the application of sound in the low infrasound range could impact the working environment and affect efficiency, vigilance and subject behaviour (16). Other studies conducted on healthy people using a frequency of 16Hz have not shown significant changes of the heart rate, however, in many cases, the infrasound led to increased diastolic blood pressure and marked reduction of systolic blood pressure (17).

In our study, various regimens of vibroacoustic and acutely induced microvibrations (up to 60 minutes, frequency range 30 Hz-18000 Hz, amplitude range 2.3 μm-12.3 μm) were applied to evaluate their effects on arterial blood pressure and oxidative stress parameters in rats.

We measured arterial blood pressure during periods of acute vibroacoustic microvibrations on the body of rats using four different regimens. Systolic arterial pressure, measured after acute application of different regimens of vibroacoustic microvibrations on the body of rats (5-minute phoning per single regime with 5-minute breaks), decreased significantly during the administration of the second regimen in comparison to the control group. A visible decrease in pressure was noted during the application of the first regimen, but this was not statistically significant. Systolic arterial pressure nearly returned nearly to baseline after the third and fourth regimens of vibroacoustics microvibrations administration.

Diastolic arterial pressure, measured after acute administration of vibroacoustic microvibrations of four different regimens on the body of rats (5-minute phoning per single regime with 5-minute breaks), did not decrease significantly during any regimen, although the pressure tended to decrease during the first and second regimens and returned nearly to baseline after the third and fourth regimens.

Three markers of oxidative stress (TBARS, SOD and GSH), measured after the acute application of four different regimens of vibroacoustic microvibrations on the body of rats (5-minute phoning per single regime with 5-minute breaks, total phoning time 60 minutes), decreased slightly, but this was not statistically significant. In contrast, the antioxidative enzyme catalase (CAT) increased significantly following the administration of vibroacoustic microvibrations (Table 1).

CONCLUSION

An acute application of vibroacoustic microvibrations in rats *in vivo* at low frequencies and amplitudes leads to a significant decrease in systolic arterial pressure (12%) and a trend towards decreased diastolic arterial pressure.

An acute application of vibroacoustic microvibrations in rats *in vivo* using 4 different regimens (total time 60 minutes, 5 minutes per single regime with 5-minute breaks after each) showed a slight decrease in certain markers of oxidative stress; however, this was not statistically significant. In contrast, the there was as statistically significant increase in the catalase (CAT) level.

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THE INFLUENCE OF ANTIPSYCHOTICS ON THE QUALITY OF LIFE OF PATIENTS WITH SCHIZOPHRENIA IN A LONG-STAY PSYCHIATRIC FACILITY

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UTICAJ ANTIPSIHOTIKA NA KVALITET ŽIVOTA PACIJENATA SA SHIZOFRENIJOM KOJU SU TRAJNO SMEŠTENI U ZAVODU ZA SMEŠTAJ ODRASLIH LICA „MALE PČELICE" KRAGUJEVAC

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ABSTRACT

Introduction: *Many factors concomitantly influence the quality of life of patients with schizophrenia in a long-stay psychiatric facility. The appropriate selection of antipsychotics and the intensity of their adverse effects exert a significant influence on the quality of life of these patients. The aim of this study was to identify the influence of antipsychotic-related factors on the quality of life of patients with schizophrenia.*

Methods: *The study included 102 beneficiaries at the Institute for Accommodation of Adults "Male Pčelice" in Kragujevac. The patients were interviewed on in one day using the questionnaire issued by the World Health Organization. The specified data were obtained from the health files of the beneficiaries. We performed a comparison between patients receiving only atypical antipsychotics, typical antipsychotics or a combination thereof.*

Results: *The patients who were receiving only atypical antipsychotics demonstrated better physical health quality of life scores in comparison to those who received combined antipsychotics (77.14 vs. 68.57; U = 332.0; p = 0.02). A statistically significant difference in the mental health quality of life domain was observed between groups of patients receiving various antipsychotic treatments (31.96 vs. 55.27 vs. 49.46; c2 = 7.02; p = 0.03).*

Conclusion: *Patients in a long-stay psychiatric facility who received atypical antipsychotics demonstrated a better quality of life in comparison to those who received typical antipsychotics, possibly due to the superior safety profile of atypical antipsychotics and a greater feeling of individual contentment.*

Key words: *Quality of life, chronic schizophrenia, quality of life domains, questionnaires*

SAŽETAK

Uvod: *Na kvalitet života osoba koje boluju od shizofrenije utiče mnogo faktora istovremeno. Kod pacijenata u institucionalnom smeštaju adekvatan izbor antipsihotika i intezitet neželjenih dejstva lekova ima veliki uticaj na kvalitet života ovih pacijenata. Cilj ove studije je bio da se utvrdi uticaj faktora vezanih za antipsihotike na kvalitet života pacijenata koji boluju od shizofrenije.*

Metode: *Istraživanjem je obuhvaćeno ukupno 102 korisnika Zavoda za smeštaj odraslih lica „Male Pčelice" Kragujevac. Korisnici su ispitani u samo jednom izabranom danu korišćenjem upitnika Svetske zdravstvene organizacije. Određeni podaci su prikupljeni iz zdravstvenih kartona korisnika. Poređene su grupe korisnika koji koriste samo apičan ili tipičan antipsihotik ili kombinaciju ove dve grupe antipsihotika.*

Rezultati: *Korisnici koji koriste samo atipičan antipsihotik imaju bolji skor fizičkog zdravlja od korisnika koji koriste kombinaciju antipsihotika (77,14 vs. 68,57; U = 332,0; p = 0,02). Postoji statistički značajna razlika u skoru domena psihičkog zdravlja u zavisnosti od grupe antipsihotika koji je korisniku propisan (31,96 vs. 55,27 vs. 49,46; c2 = 7,02; p = 0,03).*

Zaključak: *Korisnici ustanove za dugotrajan smeštaj psihijatrijskih bolesnika koji primaju apitične antipsihotike imaju bolji kvalitet života u odnosu na pacijente koji primaju tipične antipsihotike, verovatno zbog manje izraženih neželjenih dejstava atipičnih antipsihotika i boljeg osećaja subjektivnog zadovoljstva.*

Ključne reči: *kvalitet života, hronična shizofrenija, domeni kvaliteta života, upitnici*

Abbreviations
used in the paper:
QoL - Quality of life

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INTRODUCTION

The quality of life (QoL) of patients with schizophrenia depends on their social environment to a great extent. Patients residing in their own homes, outside of hospitals, exhibit improved QoL, which can be measured impartially using scales; however, a personal feeling of improvement is stronger in institutionalised patients, primarily due to the feeling of safety (1). In institutionalised patients, individual elements of QoL depend strongly on the intensity of negative symptoms, anxiety and depression (2).

Institutional accommodation of patients with schizophrenia is not a decisive driver of lower QoL per se; its effects are combined with the severity of patient symptoms, environmental support, educational level and concept of the illness (3). Proper selection of antipsychotics also exhibits a strong, positive influence on the QoL of these patients (4); however, the positive effects of medication are often absent or are not observed in the early stages of schizophrenia (5). In addition, the adverse effects of antipsychotics can considerably reduce patient QoL (6).

Because many factors concomitantly influence institutionalised patients with schizophrenia, the identification of interactions between these factors is crucial. The aim of this study was to identify the influence of antipsychotics-related factors on the quality of life of patients with schizophrenia.

MATERIALS AND METHODS

The study was conducted at the Institute for Accommodation of Adults "Male Pčelice" in Kragujevac. The Institute for Accommodation of Adults in Male Pčelice is a stationary institution for patients with chronic psychiatric disorders, who are cared for by a multi-disciplinary team, including psychiatrists, psychologists, general physicians and other medical staff. This institution accommodates patients, whose families cannot provide adequate care and who originate, from all of the regions in Serbia. The total capacity of the institution is 890 beds.

This study was designed as a cross-sectional study, and the patients were interviewed in a single day. The factors that influenced the QoL of the patients with schizophrenia were analysed. The patients' quality of life was evaluated using a special questionnaire designed by the World Health Organization, for which approval was obtained (7). The World Health Organization Quality of Life Scale Brief Version (WHOQOL-BREF) is used for patients with schizophrenia, and this scale has been validated (8). The WHOQOL-BREF is based on four domain structures: physical health, psychologicapsychological healthl, social relationships and environment. This scale contains a total of 26 questions. The domain scores are scaled in the positive direction (i.e., higher scores denote higher quality of life). The mean score of items within each domain is used to calculate the domain score. The transformation method converts the domain scores to a 0-100 scale. The study population was comprised entirely of patients who were diagnosed as suffering from schizophrenia according to the tenth International Classification of Diseases (ICD – 10) (9).

The research study enrolled 102 beneficiaries of this institution. The exclusion criteria were a patient's inability to participate in the study and adequately answer all of the questions, illiteracy and visual disorders that rendered reading the questionnaire impossible. Each patient received information about study participation in both written and oral forms. Study participation was voluntary, and the patients were included in the study after their approval.

Next, the patients were given the questionnaire to fill out; the obtained data remained protected. A portion of the study data, such as sex, age, marital status, education, diagnosis, length of stay at the institution, type of antipsychotic medication, administered antipsychotic and antipsychotic dosage, was obtained from the patients' health files.

The study was approved by the Ethical Committee of the Institute for Accommodation of Adults "Male Pčelice" in Kragujevac.

Statistical data processing

The obtained data were first processed using methods for descriptive statistics. Continuous data are presented as measures of central tendency (mean value) and distribution (standard deviation), and the categorical variables are shown in percentages and absolute numbers. Differences in the continuous variable values between groups were evaluated using non-parametric tests (Mann-Whitney and Kruskal-Wallis), and differences in the distribution of the categorical variable values were tested by using the Chi-square method. The maximum acceptable probability for the null hypotheses was 0.05. The commercial program SPSS for Windows 19 was used for data analysis.

RESULTS

One hundred and two patients (63 males and 39 females) were interviewed in a single day. Their baseline characteristics (psychiatric diagnoses and prescribed antipsychotic therapy with dose regimens and defined daily doses) are shown in detail in Table 1. The quality of life scores observed among the various patient subgroups and differences among the groups are shown in Table 2 if they achieved significance; the remaining comparisons are described in the following text.

No statistically significant differences were found for the physical health domain scores based on sex (74.29 vs. 71.43; U = 1217.5; p = 0.939), educational level (57.42 vs. 48.82 vs. 49.63; c2 = 1.72; p = 0.423), marital status (74.29 vs. 74.29, U = 1168.5; p = 0.497), age (52.27 vs. 51.45 vs. 50.70; c2 = 0.018; p = 0.991), diagnosis (48.35 vs. 54.27 vs.

Observed parameters		Obtained values
Sex n (%)	Males	63 (61.8 %)
	Females	39 (38. %)
Highest degree of education n (%)	Primary school	30 (29.4 %)
	Secondary school	53 (52.0 %)
	University	19 (18.6 %)
Marital status n (%)	Lives alone	59 (57.8 %)
	Separated	43 (42.2 %)
Age n (%)	18-40 years	15 (14.7 %)
	40-60 years	77 (75.5 %)
	> 60 years	10 (9.8 %)
Diagnosis n (%)	Paranoid schizophrenia	36 (35.3 %)
	Hebephrenic schizophrenia	31 (30.4 %)
	Residual schizophrenia	20 (19.6 %)
	Permanent possessive mental illness	9 (8.8 %)
	Schizoaffective mental disorder	6 (5.9 %)
Length of stay at institution n (%)	< 15 years	69 (67.6 %)
	> 15 years	33 (32.4 %)
Type of antipsychotic n (%)	Typical	13 (12.7 %)
	Atypical	75 (73.5 %)
	Combined	14 (13.7 %)
Prescribed antipsychotic n (%)	Haloperidol	13 (12.7 %)
	Risperidone	53 (52.0 %)
	Risperidone and haloperidol	14 (13.7 %)
	Clozapine	22 (21.6 %)
Number of antipsychotics per patient n (%)	One antipsychotic	88 (86.3 %)
	Two antipsychotics	14 (13.7 %)
Daily dosage n (%)	Once per day	16 (15.7 %)
	Twice per day	64 (62.7 %)
	Three times per day	22 (21.6 %)
Defined daily dose n (%)	< DDD	62 (60.8 %)
	> DDD	40 (39.2 %)

Table 1. General characteristics of patients. DDD- Defined daily dose

44.55 vs. 55.89 vs. 72.67; c2 = 5.08; p = 0.279), length of stay at the institution (65.71 vs. 74.29; U = 154.5; p = 0.067), daily antipsychotics dosage (42.59 vs. 54.46 vs. 49.36; c2 = 2.217; p = 0.330) or defined daily dose (52.27 vs. 50.31; U =1192.5; p = 0.744).

No statistically significant differences were forin the mental health domain scores were obtained based on sex (70.00 vs. 73.33; U =1110.5; p = 0.414), educational level (56.78 vs. 46.84 vs. 56.16; c2 = 2.770; p = 0.250), age (55.53 vs. 50.87 vs. 50.30; c2 = 0.333; p = 0.846), marital status (70.00 vs. 70.00; U = 1253.5; p = 0.919), diagnosis (49.57 vs. 51.11 vs. 54.08 vs. 48.89 vs. 60.42; c2 = 0.934; p = 0.920), length of stay at the institution (20.53 vs. 24.94; U = 188.0; p = 0.293), number of prescribed antipsychotics (51.82 vs. 49.46; U = 587.5; p = 0.781) or defined daily dose (49.83 vs. 54.09; U = 1136.5; p = 0.476).

Parameter	Value	Physical health domain score	Mental health domain score	Statistical test and significance
Antipsychotics group	Atypical Typical/atypical combination	77.14 68.57		U = 332.0; p = 0.02
Risperidone vs. risperidone+haloperidol	Risperidone Risperidone + haloperidol	74.29 68.57		U = 230.5; p = 0.03
Number of prescribed antipsychotics	One antipsychotic Two antipsychotics	74.29 68,57		U = 414.5; p = 0.049
Daily antipsychotics dosage	Once per day Three times per day		22.66 15.16	U =106.5; p = 0.03
Antipsychotics group **Risperidone vs. Haloperidol** **Clozapine vs. Haloperidol**	Atypical Typical Typical/atypical combination Risperidone Haloperidol Clozapine Haloperidol		55.27 49.46 31.96 36.40 21.69 21.16 12.65	c2 = 7.02; p = 0.03 U = 73.5; p = 0.01 U = 73.5; p = 0.01

Table 2. Significant differences in the quality of life scores observed in the study.

No statistically significant differences were observed for the social health domain scores based on sex (66.67 vs. 73.33; U = 1166.5; p = 0.666), educational level (55.77 vs. 48.39 vs. 53.45; c2 = 1.32; p = 0.516), marital status (66.67 vs. 73.33; U = 1031.0; p = 0.103), age (50.70 vs. 52.05 vs. 48.50; c2 = 0,143; p = 0,931), diagnosis (46.89 vs. 59.56 vs. 47.28 vs. 46.33 vs. 59.33; c2 = 4.38; p = 0.357), length of stay at the institution (19.83 vs. 25.27; U = 177.5; p = 0.191), antipsychotics group (39.27 vs. 53.67 vs. 51.21; c2 = 2.69; p = 0.260), type of prescribed antipsychotics (39.27 vs. 52.47 vs. 51.21 vs. 56.57; c2 = 2.99; p = 0.392), number of prescribed antipsychotics per patient (73.33 vs. 70.00; U = 612.0; p = 0.969), daily antipsychotics dosage (39.59 vs. 55.30 vs. 49.11; c2 = 3.88; p=0.144) or defined daily dose (50.64 vs. 52.84; U = 1186.5; p = 0.711).

No statistically significant differences were in the environmental domain scores were found based on sex (62.50 vs. 67.05; U = 1058.0; p = 0.239), marital status (50.52 vs. 52.85; U = 1210.50; p = 0.693), age (55.27 vs. 51.47 vs. 46.10; c2 = 0.58; p = 0.748), diagnosis (50.26 vs. 46.24 vs. 53.83 vs. 60.50 vs. 64.83; c2 = 3.24; p = 0.518), type of antipsychotics (46.23 vs. 52.86 vs. 49.11; c2 = 0.67; p = 0.716), length of stay at the institution (21.27 vs. 24.58; U = 199.0; p = 0.431), type of prescribed antipsychotics (46.23 vs. 52.86 vs. 49.11; c2 = 1.39; p = 0.709), number of prescribed antipsychotics per patient (51.88 vs. 49.11; U = 582.5; p =0.744), daily antipsychotics dosage (42.16 vs. 54.78 vs. 48.75; c2 = 2.59; p =0.274) or defined daily dose (52.33 vs. 50.21; U = 1188.5; p = 0.723).

A statistically significant difference in the environmental domain scores was observed based on the beneficiaries' educational status (i.e., between those who completed primary school or acquired a university degree) (71.25 vs. 65.00; U = 172.0; p = 0.02).

DISCUSSION

Numerous studies of the benefits of atypical antipsychotics in the treatment of schizophrenia, including reduced side effects, cognitive improvement, less pronounced extrapyramidal syndrome and better medication tolerance, have led to the increased administration of these antipsychotics in comparison to typical antipsychotics. Various literature reports have discussed the quality of life of patients treated with typical and atypical antipsychotics.

In summary, the results of our study indicated that the physical and mental health domain scores were dependent on the prescribed antipsychotic. The patients who received atypical antipsychotics alone demonstrated better scores for physical and mental health than those who were administered combined antipsychotics or only typical antipsychotics. Zaghdoudi et al presented similar results. The authors reported that the QoL of patients who were prescribed atypical antipsychotics was better than that of patients who received typical antipsychotics due to less frequent side effects from the atypical antipsychotics, especially extrapyramidal syndrome (10). Zhang et al also observed that the introduction of atypical antipsychotics into the therapy of patients with chronic psychiatric disorders resulted in positive effects on patient quality of life, due to their superior safety profile (11). This statement was also confirmed by the fact that, in our study, the patients who were administered risperidone alone exhibited better physical health scores than those who were prescribed both risperidone and haloperidol. A study conducted by Midori et al found that patients who received atypical antipsychotics reported improved feelings of individual satisfaction, due to a reduction in side effects, and general

contentment, in comparison to the patients who received typical antipsychotics (12).

The patients who were administered risperidone or clozapine alone exhibited better mental health scores than those who were prescribed haloperidol. Additionally, patients who received only one antipsychotic medication demonstrated better physical health scores than those who received two antipsychotics. When compared to patients who were administered typical antipsychotics, those who received atypical antipsychotics required noticeably fewer interventions with anticholinergic and anxiolytic medications to treat side effects (13). The treatment of patients who had suffered from psychiatric diseases for a long time revealed that the administration of atypical antipsychotics, in comparison to typical antipsychotics, increased the probability of complete remission (14).

The results of our study indicated that the mental health scores of patients who were administered antipsychotics once per day were higher than those of patients who received antipsychotics three times per day. Regarding compliance, another study demonstrated that the patients who were administered atypical antipsychotics exhibited better tolerance of their side effects and were therefore more accepting of their medication (15). The patients who were excluded from therapy with typical antipsychotics and were administered atypical antipsychotics such as risperidone and olanzapine demonstrated improvement in their general symptoms of schizophrenia and amelioration of undesirable motoric effects (16). A statistically significant difference in the environmental domain score was observed based on the patients' educational level, between patients who had only completed primary school and those who had obtained an academic education.

In contrast to these findings, other papers deny the benefits of atypical antipsychotics. In their study, Jones et al did not observe any superiority of atypical antipsychotics in regards the reduction of treatment costs, improvement of QoL and alleviation of symptoms in comparison to typical antipsychotics (17). Loffler et al reported that no significant differences in the individual improvement of the patients were found between typical and atypical antipsychotics treatments (18). Diaz et al suggested that there are no differences in the response to therapy between patients who were administered typical or atypical antipsychotics (19).

The limitations of our study include the relatively small number of patients and single centre analysis. Because the study was cross-sectional, we were unable to observe the influence of previous therapeutic protocols and metabolic changes associated with the use of atypical antipsychotics.

The results of this study indicate that patients in a long-stay psychiatric facility who are administered atypical antipsychotics exhibit better QoL in comparison to those who receive typical antipsychotics, possibly due to the improved safety profile of atypical antipsychotics and greater feelings of individual contentment. To more successfully monitor the effect of antipsychotics in cases of schizophrenia, doctors and other medical staff should pay attention to individual feelings of contentment among their patients in addition to performing an impartial evaluation. An improved understanding of antipsychotic medications is necessary to improve therapeutic strategies in the treatment of schizophrenia.

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HEPATO-RENAL SYNDROME: ETIOPATHOGENESIS, DIAGNOSIS AND TREATMENT

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HEPATO-RENALNI SINDROM: ETIOPATOGENEZA, DIJAGNOZA I LEČENJE

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ABSTRACT

Hepatorenal syndrome (HRS) involves reversible renal failure in patients with advanced cirrhosis or acute liver failure. The aim of the study was to determine the pathogenetic mechanisms of the development of hepatorenal syndrome and to emphasise the clinical importance of early detection and timely treatment of patients with this condition. The one-year incidence rate of hepatorenal syndrome in patients with liver cirrhosis is 18-20%. The risk factors for the development of hepatorenal syndrome include the following: spontaneous bacterial peritonitis, gastrointestinal bleeding, nephrotoxic drugs, diuretics, non-steroidal anti-inflammatory drugs, and hyponatraemia. The primary plan of treatment is a liver transplantation, while a secondary plan of treatment is the use of a vasoconstrictor in conjunction with albumin. Early diagnosis and prompt appropriate treatment can significantly reduce the mortality rate of patients with hepatorenal syndrome.

Key words: *liver, kidney, risk factors, liver transplantation*

SAŽETAK

Hepatorenalni sindrom (HRS) predstavlja reverzibilnu bubrežnu insuficijenciju kod bolesnika sa uznapredovalom cirozom ili akutnom insuficijencijom jetre . Cilj rada je da utvrdi patogenetske mehanizme razvoja hepatorenalnog sindroma i da ukaže na klinički značaj ranog otkrivanja i pravovremenog lečenja bolesnika sa hepatorenalnim sindromom. Jednogodisnja stopa hepatorenalnog sindroma kod bolesnika sa cirozom jetre iznosi 18-20%. U faktore rizika za nastanak hepatorenalnog sindroma spadaju: spontani bakterijski peritonitis, gastrointestinalno krvarenje, nefrotoksični lekovi, diuretici, nesteroidni antiinflamatorni lekovi, hiponatriemija. Primarni plan lečenja je transplantacija jetre, dok sekundarni plan lečenja je primena vazokonstriktora u kombinaciji sa albuminima. Rano dijagnostikovanje i pravovremena primena odgovarajućeg lečenja znatno smanjuju stopu smrtnosti bolesnika obolelih od hepatorenalnog sindroma.

Ključne reči: *jetra, bubreg, faktori rizika, transplantacija jetre*

ABBREVIATIONS

HRS – hepatorenal syndrome
NO – nitric oxide
RAAS – renin - angiotensin - aldosterone system
SBP – spontaneous bacterial peritonitis
TIPS – transjugular intrahepatic portosystemic shunt

INTRODUCTION

Hepatorenal syndrome (HRS) refers to reversible renal failure in patients with advanced cirrhosis or acute liver failure (1-6). It can occur rapidly, over 48 hours, or gradually, over a week or two (7-9). The incidence of HRS in patients with cirrhosis of the liver is approximately 18-20% during the first year, and it increases to 39% after 5 years (10, 11). HRS may occur spontaneously. In half of the patients with HRS, one or more precipitating factors can scause HRS, including: bacterial infection, particularly spontaneous bacterial peritonitis (57%), gastrointestinal bleeding (36%) and therapeutic paracentesis (7%)(10, 12). HRS is the most common complication in patients with liver cirrhosis and ascites, and there areits morbidity and mortality rates are high (13-15). Only 3.5% of patients recover spontaneously from hepatorenal syndrome (3).

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Even if its pathogenesis is not fully explained, the main etiological factor is renal hypoperfusion, which results from vasodilatation and vascular resistance in the lesser splanchnic region (4).

It is interesting that the histological appearance of the kidneys is normal in hepatorenal syndrome, which makes this syndrome a unique pathophysiological disorder of the renal circulation in which there are no elements of morphological kidney damage. Therefore, the renal insufficiency is reversible, and it occurs from functional disorder of the circulation, rather than direct morphological kidney damage, as evidenced by the fact that after liver transplantation, kidney function returns to normal.

The type of HRS predominantly determines the outcome and survival of patients. There are two types of hepatorenal syndrome, type 1 and type 2; the two types are pathophysiology similar, while the clinical features and prognosis vary (16). Hepatorenal syndrome type 1 is characterised by rapid, progressive reduction of the effective circulating volume, which is due to the extreme intestinal arterial vasodilation and a decrease in the cardiac output, which is usually preceded by spontaneous bacterial peritonitis. Hepatorenal syndrome type 2 is caused by a gradual and stable reduction of the glomerular filtration rate and it is common in patients with relatively intact liver function. As defined by Salerno et al, HRS type 1 is indicated by a laboratory increase in the serum creatinine above 133 mmol/l (1.5 mg/dl) in patients with liver cirrhosis and ascites in the absence of hypovolemic shock, nephrotoxic drugs or primary kidney disease. In type 2 HRS, the serum creatinine is doubled (over 100%) compared to baseline to more than 221 mmol/l (2.5 mg/dl) (17, 18). The increase in the serum creatinine can be acute (type 1 HRS) or gradual (type 2 HRS) (19).

Type 2 HRS is more common in clinical practice than type 1 (20). The expected survival rate for type 1 HRS is approximately 2 weeks, while this period is much longer, approximately 6 months, in type 2 HRS (12). While the survival of the patients with type 2 is considerably longer than forthat of patients with type 1, it is still shorter than for patients who do not have HRS.

Pathogenesis

Although HRS as a clinical entity was first described 50 years ago, the pathogenesis of this syndrome has not yet been fully characterizised (21). HRS is the final stage of a series of disturbances in the kidney and is accompanied by deterioration of liver function and portal hypertension (21).

The main feature of hepatorenal syndrome is renal vasoconstriction, although the pathogenesis of this process has not been previously explained. A number of mechanisms are associated with this syndrome, including increased activity of systemic and renal vasoconstriction, leading to reduced renal perfusion and a decrease in the glomerular filtration rate. On the basis of the disturbance, a vasodilation splanchnic circulation is expressed, reducing the effective circulating volume and hypotension with consequent activation of the sympathetic system, renin-angiotensin system, and vasopressin (22). Entotelin, adenosine and leukotriene L4 also play an important role in addition to being the main vasoconstrictors (renin - angiotensin - aldosterone composition (RAAS) and the sympathetic nervous system). All of these vasoconstrictors lead to renal vasoconstriction. Local formation of kidney vasodilators, mainly prostaglandins and nitric oxide (NO), is weakened (12, 23, 24).

The splanchnic circulation is resistant to vasoconstrictors for the continuous production of local vasodilators such as NO. In the splanchnic circulation, the creation of vasodilators is maintained at a high level, and the response to the effects of endogenous vasoconstrictor systems becomes weaker (1-6).

The clinical picture

HRS has non-specific symptoms and signs, which makes early identification and diagnosis more difficult. Because most patients with HRS have chronic liver disease, it is important to note the signs, including the following: palmar erythema, a leukonychia, asterixis, and clubbing fingers (hand), icterus sclera, spider nevi, foetor hepaticus, xanthelasma, and gynecomastia (head), caput medusae, hepatosplenomegaly, ascites, and paraumbilical herniation (abdomen), pubic hair loss and atrophic testes (genitals), and, and peripheral oedema and clubbing fingers (extremities).

The symptoms and signs of disease in HRS and chronic liver diseases overlap, and they include the following: arterial hypotension (middle arterial pressure values of approximately 80 mmHg or lower), oliguria, tachycardia, jaundice, hepatic encephalopathy, and ascites (25).

In the final stage of HRS, the patient is comatose and hypotensive with a urine output of less than 100 ml in 24 hours. In more than 80% of the patients, death occurs in a few days to a maximum of 12 weeks (12, 21, 26). The cause of death is a terminal defect of the liver cells rather than renal failure (21).

Laboratory analyses show hyponatraemia, serum levels below 130 mmol/l with an incidence of approximately 21.6% for HRS (27). Patients with liver cirrhosis and hyponatraemia are at high risk of developing HRS (10). Several studies have shown a positive correlation between hyponatraemia and hepatic encephalopathy. A low level of serum sodium and increased level of amonium lead to major electroencephalographic changes, resulting in the development of hepatic encephalopathy (28). Hyponatraemia predicts poor prognosis, and the median survival in patients with liver transplantation is less than 6 months (29). Hyponatraemia affects the patients' quality of life. A recent study has shown that a low level of Na^+ is an independent predictive factor of the quality of life of patients with cirrhosis (30).

Diagnosis

The diagnosis of the disease is sometimes extremely difficult due to the lack of a specific test or pathognomonic marker of the disease. The diagnosis of hepatorenal syndrome is based on the exclusion of other diseases that reduce the rate of glomerular filtration in the absence of other causes of chronic renal disease.

Table 1 Criteria for the diagnosis of HRS - International Ascites Club -2007 (17):

- Cirrhosis of the liver with ascites
- Creatinine in the serum > 1.5 mg/dL (133 mmol/l)
- Absence of shock, absence of current or recently completed treatment with nephrotoxic drugs, absence of parenchymal kidney disease and fluid loss
- No stable improvement in the renal function after at least 2 days (48 h) (reduction of the serum creatinine of less than 1.5 mg/dl or an increase in the creatinine clearance of more than 40 ml/per min) after the completion of a diuretic and after the application of albumin and an intravenous (iv) solution (1 g/kg TT/a day dose of albumin up to a maximum of 100 g/a day),
- Proteinuria of less than 500 mg per day
- Normal renal ultrasound findings
- The number of red blood cells in the urine is less than 50, and microhematuria

Risk factors

If there are precipitating factors that lead to the development of HRS, it is necessary to eliminate them in a timely fashion. These factors include spontaneous bacterial peritonitis, gastrointestinal bleeding, nephrotoxic drugs, diuretics, non-steroidal anti-inflammatory drugs, and hyponatraemia. The most important risk factor for the development of HRS is bacterial infection, particularly spontaneous bacterial peritonitis (31, 32). HRS develops in approximately 30% of patients who have SBP (31). The treatment of SBP includes infusion of albumin and antibiotics, reducing the risk of developing HRS and improving survival (31). Potassium-sparing diuretics should be excluded from treatment to avoid hyperkalaemia (33).

Liver transplantation

Liver transplantation is the method of choice for type 1 and type 2 HRS; the survival rate is approximately 65% for type 1 and 80% for type 2 (20, 34). A slightly lower survival rate is noted compared to patients with cirrhosis without HRS because of the presence of renal insufficiency, which represents a major predictor of an unfavourable outcome after liver transplantation (35, 36).

Over the past century, liver transplantation has been successfully performed in only a few patients with HRS because most patients died before transplantation due to the rapid disease progression in type 1 HRS.

In a prospective study of 15 patients with HRS type 1 who were candidates for transplantation, 12 patients had contraindications to liver transplantation, and the remaining three died while waiting for transplantation (37).

Without liver transplantation, the HRS prognosis is unfavourable. A study was conducted on 68 type 1 HRS patients who were candidates for a liver transplantation. All patients were treated medically with various combinations of the following: albumin, vasopressors, midodrine, octreotide and haemodialysis. The results showed that the median survival was 13 days for the entire group. Early treatment can increase the survival rate of HRS (38).

The main problem with liver transplantation is the long waiting time and short-term survival of these patients. The one-year and four-year survival rates of patients with HRS who undergo liver transplantation are 71% and 60%, whereas in patients with liver transplantation without HRS, the one-year and four-year survival rates are 83 and 70% (39). However, 10% of patients require dialysis after transplantation (1).

A new approach to the treatment is suggested, the so-called "Treatment of bridge healing ", which is meant to reduce the number of patient deaths during the wait for liver transplantation. The treatment of bridge healing "consists of a combination of terlipressin 4-6 mg/a day with albumin, which enables to bridge, that is to overcome the period of waiting until liver transplantation" (40, 41). However, few patients with HRS undergo transplantation.

The correction of renal hypovolaemia

Given that HRS lab values are similar to those observed pre-renal azotaemia, previous attempts were made to begin treating hypovolaemia with infusions (saline or dextran). Because this form of treatment was not successful, it was abandoned. Today, the main way that hypovolaemia is corrected is by increasing albumin to 50 g/day in combination with administering vasoconstrictors (42).

Drug treatment

Many medications have been used to treat hepatorenal syndrome in the past, but vasoconstrictors have had the-best effects. These drugs cause vasoconstriction of blood vessels of the splanchnic region and reperfusion of the renal arteries (43, 44). The most common side effects of treatment are cardiovascular or ischemic complications, which occur with an average frequency of 12% in treated patients (24, 45). Various types of studies have tested the efficacy of vasoconstrictors.

A retrospective study was performed on 59 patients with HRS type 1; after a combination of vasoconstrictor and albumin was administered, there was a greater than 10 mmHg increase in middle arterial pressure. The respondents had improved treatment efficacy, a favourable response to liver transplantation and a reduced need for dialysis. (46).

A meta-analysis of six randomised controlled studies in which patients who had been on various vasoconstrictor drugs in combination with or without albumin were monitored. The authors reported that mortality was reduced by 18% compared to the control groups of people who did not undergo therapy with vasoconstrictors (15). A meta-analysis of four randomised controlled studies showed that patients who were treated with terlipressin with or without albumin were 3.8 times more likely to recover (heal) from HRS and 2 times more likely to have improved renal function compared to patients who were not treated with vasoconstrictors (15). Despite all of these encouraging results regarding the use of vasoconstrictors with or without albumin, these drugs are effective in reducing mortality for 15 days, without significant effects at 1, 3, and 6 months. Vasoconstrictor therapy is effective in 46 to 48% of patients (15).

Vasoconstrictors are analogues to vasopressins (ex. Terlipresin), analogues to somatostatins (Octreotide), and agonists of the α-adrenergic receptors (Midodrine) in combination with albumin infusion (43, 44) .

Terlipressin is the most effective and most widely used vasoconstrictor. Terlipressin, vasopressin's analogue, acts on the two types of receptors, V1 and V2. V1 receptors are found in the smooth muscles of blood vessels, and through these receptors, vasopressin causes vasoconstriction. V2 receptors are found in the renal tubules, and, through these receptors, this hormone acts as an antidiuretic. Terlipressin has agonistic effects on V1 receptors and partial agonistic effects on the renal V2 receptors. It affects the V1 receptors of the intestinal vasculature, causing dominant vasoconstriction in the mesenteric circulation compared to the renal arteries. However, its effects on the serum concentrations of Na are controversial. Terlipressin is most commonly used in Europe because it reduces the chances of ischemic complications.

Today, terlipressin, according to general recommendations, represents the most effective vasoconstrictor in the treatment of HRS type 1. There are reports on the significantly higher efficacy of combination therapy with terlipressin and albumin (14, 45)., Although this combination is also used in type 2 HRS, there is still limited information on the use of terlipressin on these patients (47, 48).

The protocol for the treatment with terlipressin involves an initial dose of 0.5 - 1 mg/4 - 6 h via i.v. or continuous i.v. infusion 2 mg/day. If the creatinine level has not fallen by 25% on the third day of treatment, the dose is increased to 2 mg/4 h or 12 mg/day by continuous intravenous infusion. In the case of failure to maintain a central venous pressure of 10 - 15 mm H_2O, the initial dose of albumin is 1 gr/kg for two days up to a maximum 100 g/day (23).

Treatment continues until there is no improvement in the laboratory values, which is normally not more than 2 weeks. In almost 59% of patients with type 1 HRS, there is complete (reduction of serum creatinine < 133 mmol/l) or partial (reduction of serum creatinine > 50% with values > 133 mmol/l) healing (49).

Alternative vasopressors are rarely used because they have not been adequately investigated, and there are few studies on these drugs (50).

Alpha-adrenergic agonists have an advantage over terlipressin because their price is lower, but they are less efficient. Midodrine is an alpha agonist with that has an advantage of being the only agent that can be administered orally (2.5 to 75 mg/8 h).

Norepinephrine is applied by continuous infusion at a dose of 0.5 - 3 mg/h according to the level of arterial blood pressure. Unfortunately, the number of patients treated with noradrenaline is small, and there are no randomised comparative studies for assessing its efficacy.

Previously, dopamine and prostaglandins were posited as potential vasodilators in the literature, but their application has not been accepted in clinical practice.

Transjugular intrahepatic portosystemic shunt

A transjugular intrahepatic portosystemic shunt (TIPS) is a percutaneously created connection within the liver parenchyma between the portal and systemic circulation. A TIPS is set to reduce the portal pressure in patients with complications that are associated with portal hypertension.

The aim of a TIPS placement is to redirect the blood flow in the hepatic veins, reducing the pressure gradient between the portal and systemic circulation.

According to currentthe present study, the use of a TIPS is effective in the treatment of ascites and leads to improvement in renal function. It can be used as a bridge therapy while patients are waiting for a liver transplantation (51, 52).

CONCLUSION

HRS is one of the most serious complications of liver disease and is most common in patients with decompensated liver cirrhosis. The survival time of these patients is short, and spontaneous recovery is very rare. The only therapy for HRS is liver transplantation. The aim of new studies will be the search for better diagnostic and therapeutic procedures.

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GRADE III CORONARY ARTERY PERFORATION FOLLOWING PCI AND UNUSUAL STENT GRAFT DELIVERY SYSTEM

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PERFORACIJA KORONARNE ARTERIJE TIP III TOKOM PCI REŠENA NEUOBIČAJENIM PLASIRANJEM STENT GRAFTA

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ABSTRACT

Coronary perforations are rare but vicious complications of PCI procedures. Ellis type III coronary artery perforation represents the most severe form and demands an urgent solution. It is often necessary to perform pericardiocentesis and utilizise multiple interventional techniques to seal the perforation. Polytetrafluoroethylene (PTFE)-covered stent implantation has become one of the most frequently used percutaneous solutions, but disadvantages of this intervention are its high profile and low device flexibility. In our case, we attempted to improve the deliverability of the PTFE stent graft alone by mounting it on a bare metal stent used as a stent graft carrier.

Key words: *PTFE stent, coronary artery perforation, pericardiocentesis, cardiac tamponade, coronary disease, intraoperative complications, angioplasty*

SAŽETAK

Koronarne perforacije su retke ali izuzetno neugodne komlikacije perkutanih intervencija. Perforacije koronarnh arterija trećeg stepena po Elisu predstvaljaju najozbiljniju formu perforacija i zahtevaju hitno zbrinjavanje. Često je neophodno uraditi perikardiocentezu i primeniti brojne interventne tehnike kako bismo rešili perforaciju. Stentovi prekriveni politetrafluoroetilenom(PTFE) postali su jedno od najčešće korišćenih perkutanih rešenja, ali su njihove mane visoki profil i slaba fleksibilnost. U našem slučaju, pokušali smo da poboljšamo plasiranje PTFE stenta montiranjem na metalni stent, koji smo iskoristili kao nosač.

Ključne reči: *PTFE stent, perforacija koronarne arterije, perikardiocenteza, srčana tamponada, koronarna bolest, intraoperativne komplikacije, angioplastika*

ABBREVIATIONS

PCI- percutaneous coronary intervention
PTFE- polytetrafluoroethylene
NSTEMI- non ST segment elevation myocardial infarction
LVH- left ventricular hypertrophy
LAD- left anterior descending artery
D1- first diagonal branch
S1- first septal branch
RCA- right coronary artery
SC- semi compliant balloon
DES-drug eluting stent
BMS-bare metal stent
CPR- cardiopulmonary resuscitation
CABG- coronary artery bypass graft

CASE REPORT

A 68 year old female was admitted to the cath lab due to complaints of postinfarction angina. She suffered an anterior NSTEMI 5 months prior to presentation. Her risk factors for cardiovascular disease were as follows: hypertension, diabetes, dyslipidemia, and a history of smoking. An electrocardiogram showed sinus rhythm, with a rate of 75 bpm, as well as signs of LVH and a reduced R in V2. Echocardiography revealed a reduced ejection fraction - 40% and anterior wall hypokinesia. Angiography was performed, showing that the LAD was diffusely atherosclerotic and calcified, with tight stenosis approaching 90% of the vessel lumen immediately after the D1 and S1 branches (Figure1).

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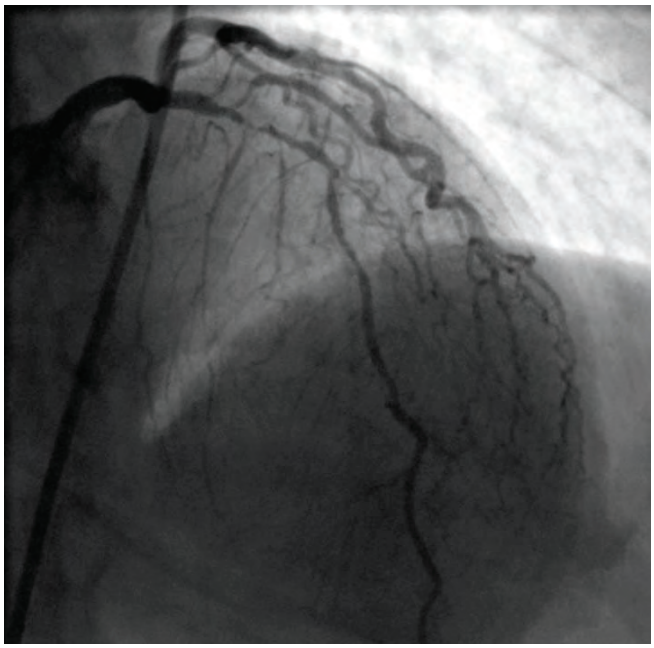

Figure 1.

The RCA provides collaterals to the LAD. The initial operator decided to perform an ad hoc PCI of the LAD. The lesion was accessed with an SC balloon with dimensions of 2.0x20 mm, using pressure up to 12 atm. Quantitative coronary analysis assessed the artery diameter as beingapproximately 2.5 mm, but the operator decided to implant a Sirolimus DES with dimensions of 3.0x33 mm, at a pressure of 14 atm, distally. Next, a Zotarolimus DES with dimensions of 3.5x26 mm, at 16 atm, was placed proximally, but overlapped the distal device. Immediately following stent deployment, frank contrast was noted streaming into the pericardial space, representing an Ellis grade III perforation at the distal aspect of the stent (Figure 2).

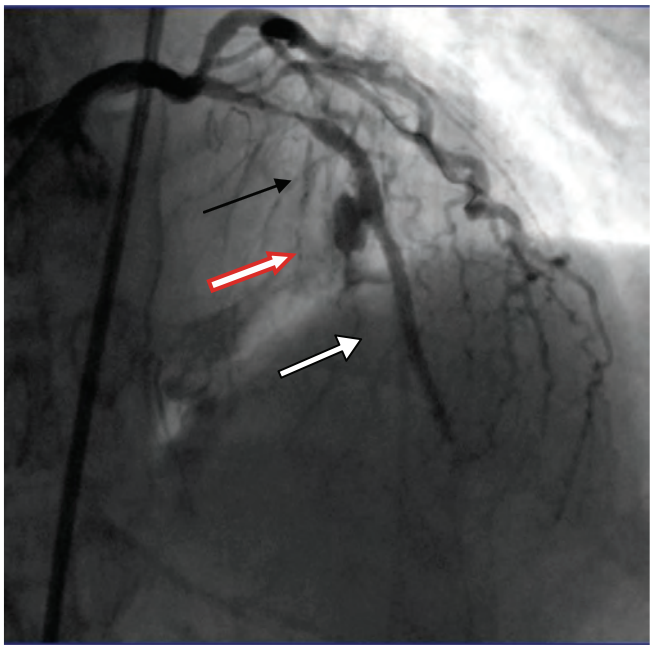

Figure 2. The white arrow indicates the position of the distal stent (Sirolimus 3.0x33 mm), and the black arrow points to the proximal stent (Zotarolimus 3.5x26 mm). The red arrow points to frank contrast streaming into the pericardium.

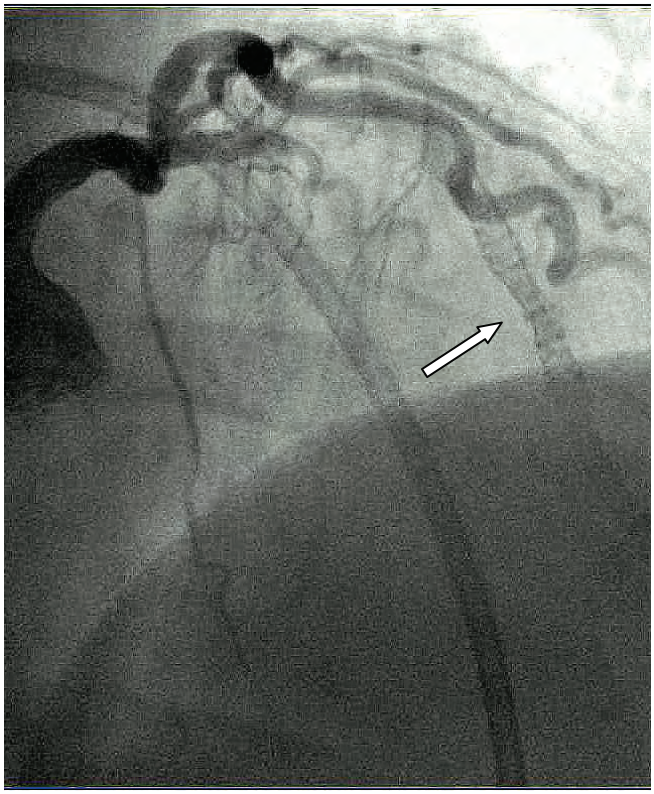

Figure 3. The white arrow points to inadequate expansion in the distal part of the polytetrafluoroethylene (PTFE) Jostent.

The stent balloon was immediately re-inflated to seal the leak. The operator was afraid mayof losing the stent graft when trying to place it over a proximal unexpanded stent on heavily calcified tissue. We did not have a premounted PTFE stent, so we used a bare metal stent (BMS), Flexmaster F1, (Abbott Vascular Laboratories, Redwood City, California), with dimensions of 3.5x26 mm, as a stent graft delivery system. The idea was to increase friction between the stent graft and the carrier. We manually modified and pressed the stent graft onto the BMS carrier to reduce the chances of losing the stent when crossing the heavily calcified, long lesion. The stent balloon was inflated throughout the preparation of the PTFE stent. After balloon disinflation, respiratory arrest occurred, and CPR was performed. A pericardial drain was immediately inserted, and 450 ml of haemorrhagic fluid was evacuated. After haemodynamic stability was established, we implanted the stent graft mounted on the BMS used as its delivery system, opening it with a high pressure of 18 atm. Despite this elevated pressure, inadequate expansion in the distal portion of the 2.5x19 mm polytetrafluoroethylene (PTFE) Jostent, to which we applied manual shaping, was observed (Figure 3).

Stent thrombosis eventually occurred, and the remainder of the procedure was clinically uneventful, with no signs of further pericardial leakage. The patient was haemodynamically stable, as only a mild rise of cardiospecific enzymes was observed. A control echo was performed the next day, following the pericardial drain removal. The patient was discharged 3 days after the procedure.

DISCUSSION

Ellis et al. defined grade III coronary perforation as extravasation of blood through a frank perforation (≥1 mm) or into an anatomic cavity chamber on coronary angiography [1]. Previous studies reported that the incidence of grade I to III coronary perforations ranges from 0.1% to 3.0% [2,3]. In 2011, Rasha Al-Lamee et al. conducted a study focused only on grade III coronary perforations, and they reported that this remains rare, with an incidence of 0.23% [4]. Additionally, in this study, the superiority of covered stent implantation over prolonged balloon inflation in reaching haemostasis was shown, and multiple methods of treatment were required in an attempt to achieve haemostasis in 39.3% [4] of patients. Grade III is the most dangerous form of perforation, associated with rates of cardiac tamponade as high as 40%, and the need for emergency CABG is reportedly between 20% and 40% [5,6]. In our case, we did not manage to achieve adequate haemostasis by prolonged balloon inflation; therefore, prompt implantation of a PTFE stent, along with a pericardial drain, was required to resolve this iatrogenic complication.

CONCLUSION

This approach is proposed as an alternative technique that may be used in cases where other treatment options for coronary perforation are either unavailable or are potentially not successful. BMS was successful as a stent carrier in our case, but a disadvantage of utilizing this technique is stent malposition due to forced manual shaping, as well as consecutive stent thrombosis. Pericardial drainage is mandatory to resolve threatening haemodynamic instability, and should be performed before stent implantation. Although this grade of perforation remains rare, interventional cardiologists should be aware of the risk factors and have the necessary skills to perform multiple methods of treatment.

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Author. (Year of publication). Article title. *Journal Title. Volume* (issue), range of pages. DOI.

Example:

Nikora, V. (2006). Hydrodynamics of aquatic ecosystems: spatial-averaging perspective. *Acta Geophysica*, 55(1), 3-10. DOI: 10.2478/s11600-006-0043-6.

b. Journal Article (two or more authors)

Format:

Author1, Author2 & Author3. (Year of publication). Article title. *Journal Title. Volume* (issue), range of pages. DOI.

Example:

Cudak, M. & Karcz J. (2006). Momentum transfer in an agitated vessel with off-centred impellers. *Chem. Pap.* 60(5), 375-380. DOI: 10.2478/s11696-006-0068-y.

c. Journal article from an online database

Format:

Author(s). (Year of publication). Article title [Electronic version]. *Journal Title. Volume* (issue), range of pages. Retrieved date of access, from name of database. DOI.

Example:

Czajgucki Z., Zimecki M. & Andruszkiewicz R. (2006, December). The immunoregulatory effects of edeine analogues in mice [Abstract]. *Cell. Mol. Biol. Lett.* 12(3), 149-161. Retrieved December 6, 2006, from PubMed database on the World Wide Web: http://www.pubmed.gov. DOI: 10.2478/s11658-006-0061-z.

d. Newspaper article (no author)

Format:

Article title. (Publication date). Journal Title. page.

Example:

Amazing Amazon region. (1989, January 12). New York Times, p. D11.

e. Encyclopedia article

Format:

Author. (Year of publication). Article title. In Encyclopedia title (volume number, pages). Place of publication: Encyclopedia name.

Example:

Bergmann, P. G. (1993). Relativity. In The new encyclopedia britannica (Vol. 26, pp. 501-508). Chicago: Encyclopedia Britannica.

4. Other formats

a. Web page

Format:

Author/Sponsor. (last update or copyright date). *Title*. Retrieved date of access, from URL.

Example:

Walker, J. (1996, August). *APA-style citations of electronic resources*. Retrieved November 21, 2001, from http://www.cas.usf.edu/english/walker/apa.html

b. Lecture note

Format:

Author(s). (Date of presentation). *Lecture title*. Lecture notes distributed in the unit, at the name of the teaching organisation, the location.

Example:

Liffers, M. (2006, August 30). *Finding information in the library*. Lecture notes distributed in the unit Functional Anatomy and Sports Performance 1102, University of Western Australia, Crawley, Western Australia.

c. Patent

Format:

Author. (Year). Patent number. The location. Issue body.

Example:

Smith, I. M. (1988). U.S. Patent No. 123,445. Washington, D.C.: U.S. Patent and Trademark Office.

d. Standard

Format:

Issue body. (Year). Standard name. Standard number. The location.

Example:

Standards Association of Australia. (1997). Australian standard: Pressure equipment manufacture. AS4458-1997. North Sydney.

e. Video

Format:

Producer, P. P. (Producer), & Director, D.D. (Director). (Date of publication). Title of motion picture [Motion picture]. Country of origin: Studio or distributor.

Example:

Zhang, Y. (Producer/Director). (2000). Not one less [Motion Picture].China: Columbia Pictures Industries, Inc.

f. Audio recording

Format:

Songwriter, W. W. (Date of copyright). Title of song [Recorded by artist if different from song writer]. On Title of album [Medium of recording]. Location: Label. (Recording date if different from copyright date).

Example:

Taupin, B. (1975). Someone saved my life tonight [Recorded by Elton John]. On Captain fantastic and the brown dirt cowboy [CD]. London: Big Pig Music Limited.

g. Mailing list

Format:

Author. (Exact date of posting). Subject line of message. Message posted to followed by name of mailing list, archived at followed by address for the archived version of the message

Example:

Hammond, T. (2000, November 20). YAHC: Handle Parameters, DOI Genres, etc. Message posted to Ref-Links electronic mailing list, archived at http://www.doi.org/mail-archive/ref-link/msg00088.html

h. Computer software

Format:

Author(s). (Year). Title [computer software]. The location: Company.

Example:

Ludwig, T. (2002). PsychInquiry [computer software]. New York: Worth.

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DOI:10.2478/s11533-007-0023-3.

Book:

Jones, E., Farina, A., Hastorf, A., Markus, H., Miller, D., & Scott, R. (1984). *Social stigma: The psychology of marked relationships*. New York: W. H. Freeman.

Chicago style[2]

Article in a journal:

Spitzer, Steven. Review of *The Limits of Law Enforcement*, by Hans Zeisel. *American Journal of Sociology* 91 (1985): 726-29; DOI:10.2478/s11533-007-0023-3.

Book:

Lloyd, Donald A., and Harry R. Warfel. *American English and Its Cultural Setting*. New York: Alfred A. Knopf, 1956.

Harvard style[3]

Article in a journal:

Conley, TG & Galenson, DW 1998, 'Nativity and wealth in mid-nineteenth century cities', *Journal of Economic History*, vol. 58, no. 2, pp. 468-493, DOI:10.2478/s11533-007-0023-3.

Book:

Hodgson, A 1998, *Accounting theory*, John Wiley & Sons, Brisbane.

Oxford style[4]

Article in a journal:

KHOO, G.K. Accounting for leases. *The Chartered Accountant in Australia*, 46(5): Nov. 1975: 19-23: DOI:10.2478/s11533-007-0023-3.

Book:

GIBBS, Graham. *Teaching students to learn: a student-centred approach*. Milton Keynes, Open University Press, 1981.

MLA style[5]

Article in a journal:

Joyce, Michael. "On the Birthday of the Stranger (in Memory of John Hawkes)." Evergreen Review 5 Mar. 1999. 12 May 1999 http://www.evergreenreview.com/102/evexcite/joyce/nojoyce.html. DOI:10.2478/s11533-007-0023-3.

Book:

Bird, Isabella L. A Lady's Life in the Rocky Mountains. New York, 1881. Victorian Women Writers Project. Ed. Perry Willett. 27 May 1999. Indiana U. 4 Oct. 1999

IEE style[6]

Article in a journal:

I.E. Sutherland, R.F. Sproull, and R.A. Schumaker, "A Characterization of 10 Hidden-Surface Algorithms," *ACM Computing Surveys*, Mar. 1974, pp. 1-55, DOI:10.2478/s11533-007-0023-3.

Book:

W.M. Newman and R.F. Sproull, *Principles of Interactive Computer Graphics*, McGraw-Hill, 1979, p. 402.

Vancouver style[7]

Article in a journal:

You CH, Lee KY, Chey WY, Menguy R. Electrogastrographic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology 1980;79:311-4; DOI:10.2478/s11533-007-0023-3.

Book:

Eisen HN. Immunology: an introduction to molecular and cellular principles of the immune response. 5th ed. New York: Harper and Row; 1974.

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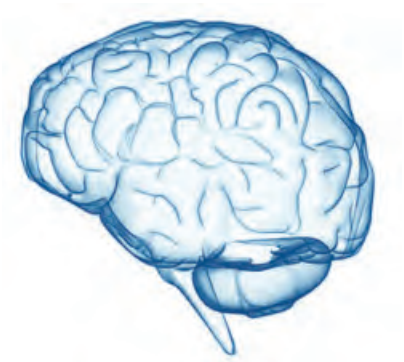

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