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ARTYKUŁ ORYGINALNY / *ORIGINAL ARTICLE*

Histological assessment of myocardium in lethal ethanol intoxication

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Abstract

Background: The pathological mechanism of sudden death in healthy persons following incidental ethanol intoxication has not yet been fully elucidated and might be underlain by cardiogenic causes.

Aim: Histological assessment of the myocardium in lethal ethanol intoxication. The analysis was based on a histological assessment of specimens of the myocardium obtained from the hearts of 30 deceased males within the age range 29–45 years.

Methods: The material for the study was taken from the myocardium of the anterior wall of the left ventricle and interventricular septum of the heart. The fixation material was first examined according to the standard histological procedure and subsequently subjected to a morphometric examination, which assessed the number of cardiomyocytes, their area, circumference, and circular deviation.

Results: The examination showed an increase in the area and circumference of cardiomyocytes, as well as fragmentation and segmentation of cardiomyocytes with a significant enlargement of cell nuclei. Additionally, it revealed the presence of lymphocytic cells in several cases.

Conclusions: The obtained findings indicate a harmful influence of alcohol on the myocardium.

Key words: myocardium, ethanol, lethal intoxication

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INTRODUCTION

Diseases of the cardiovascular system constitute some of the most frequent causes of death in the adult population of economically developed countries, including Poland [1]. The steadily growing rate of alcohol consumption goes hand in hand with an increased number of sudden deaths of apparently healthy persons [2]. The majority of studies have so far focused on the evaluation of the impact of chronic alcohol consumption on the viscera, with special attention paid to the liver. However, the influence of acute incidental alcohol intoxication on the myocardium and, in particular, of the consumption of high-percentage alcohol in large quantities within a relatively short period of time have not yet been scrutinised. Therefore, the pathological mechanism leading to the sudden death which sometimes occurs after an incidental ethanol intoxication is not precisely known [3–5]. The dysfunction of the circulatory and respiratory centres in the brainstem is believed to be one of the causes of sudden death. It is also supposed that some typical cardiogenic causes (the pathology of cardiomyocytes, cell skeleton and extracellular matrix, as well as alterations in the coronary vessels and coagulation system) may significantly participate in the mechanism of death [6–8]. Also, the electrolytic disturbances accompanying hypoxia of the myocardium may play an important role because they lead to electric instability of the heart, which may result in death caused by ventricular tachycardia or ventricular fibrillation [9]. The aim of the study was a histological assessment of the myocardium in lethal ethanol intoxication.

METHODS

The study, approved by the Bioethical Commission of the Medical University of Bialystok (No. R-I-002/129/2008), was based on a histological assessment of specimens of the myocardium obtained from the hearts of 30 deceased males within

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the age range 29–45 years, who died after an incidental ethanol intoxication (hereafter referred to as the alcohol-exposed group). Autopsies were carried out in the Department of the Forensic Medicine at the Medical University of Bialystok. The results of the post-mortem autopsies conducted in the studied group of males did not reveal other causes of death. The subjects had not been previously treated. The medical history provided by their relatives excluded the occurrence of the following illnesses: chronic ischaemic cardiac disease, cardiac infarction, cardiomyopathy, arterial hypertension, cerebral stroke, diabetes, chronic renal disease, and alcohol and drug abuse. Before death, the studied males had occasionally (2–3 times per month) consumed moderate amounts of alcohol. The only explicable factor triggering their sudden death was an alcohol intake confirmed by its concentration in the blood and urine samples. The control group (n = 10) comprised the myocardium collected from deceased males within the age range 20–30 years, victims of a violent death, whose blood and urine did not reveal the presence of alcohol (hereafter referred to as the control group). The direct cause of their death was a post-traumatic intracranial haemorrhage. Prior to the lethal traumatic injury, the males in the control group had been healthy. Their post-mortem examination did not reveal any significant lesions in the other viscera.

Alcohol concentration in the blood and urine of the deceased patients was evaluated by means of the gas chromatography method and the use of a flame-ionisation detector. The hearts were opened according to the standard procedure, blood and blood clots were washed out, and approximately 1 cm of the ascending aorta and the pulmonary trunk were included. The hearts were weighed during the autopsy with a precision to the nearest tenth of a gram. The material for the histological examination was obtained from the myocardium of the anterior wall of the left ventricle and interventricular septum of the researched hearts. Three specimens of the myocardium of the left ventricle were collected from each heart: from the superior, middle, and inferior part of the anterior wall of the left ventricle at a distance of 1 cm from the anterior interventricular sulcus, which is the place of the course of the anterior interventricular branch of the left coronary artery. Specimens were also collected from the muscular part of the interventricular septum in its superior, middle, and inferior part. All the specimens were of the size 10 × 10 × 1 mm. The obtained material was fixed in 10% buffered formalin. After 24-h fixation, the material was embedded in paraffin blocks and cut on the microtome into 6-mm paraffin sections that were subsequently stained with haematoxylin and eosin according to the standard histological procedure. Afterwards, the material was subjected to a histomorphometric analysis performed in the Department of Human Anatomy of the Medical University of Bialystok with the use of an Olympus B×41 microscope equipped with an Olympus DP20 camera, and computer software for the histomorphometric analysis — Olympus Cell D Imaging Software for Life Science Microscopy. The histomorphometric examination in the alcohol-exposed group and control group was carried out at a magnification of ×400. Transverse sections of three randomly chosen microscopic fields were examined focusing on the following morphometric parameters of cardiomyocytes: surface area, circumference of myocardium fibres, and the number of myocardium fibres. The shape of cardiomyocytes (circular deviation — CD) was assessed by applying the formula CD = 4 μA/C2. Mean values for the examined parameters were defined in the alcohol-exposed group and control group. The results were then analysed and elaborated by means of the computer programme Statistica 10PL. Statistical analysis was performed with the t-test (statistical significance) and the U Mann-Whitney test. Statistical significance was accepted for $p < 0.05$.

RESULTS

As far as the alcohol-exposed group was concerned, the concentration of ethanol in the analysed blood samples stood at between 2.5 and 4.4‰ [3.73 ± 0.33], whereas in the analysed samples of urine it amounted to between 2.6 and 5.4‰ [4.19 ± 0.72]. Nothing more than trace amounts of endogenous alcohol (less than 0.5‰) were revealed in the control group. Table 1 depicts the statistical differences in alcohol concentration in the alcohol-exposed group and control group. No significant difference in the heart weight was seen between the examined groups. The mean heart weight in the alcohol-exposed group was 352 ± 52 g, whereas in the control group it stood at 348 ± 58 g. Statistically significant differences between the groups concerned the number, area, and circumference of their cardiomyocytes (Fig. 1). A lower number of cardiomyocytes in the microscopic field in transverse sections and a hypertrophy of individual cardiomyocytes with a significant enlargement of cell nuclei (Fig. 2) was observed in the alcohol-exposed group. The said cardiomyocytes exhibited also an increased area and circumference of fibres. Additionally, fragmentation and segmentation of individual cardiomyocytes with a significant enlargement of cell nuclei was observed in 26 cases. The number of cardiomyocytes was higher in the control group (Fig. 3). There were no statistically significant differences between the alcohol-exposed group and the control group with regard to the parameter of CD. The presence of a small number (1–3) of lymphocytic cells was noticed in several cases of the microscopic fields. No morphological features typical of cardiomyopathy were seen in either group.

DISCUSSION

The most frequent causes of sudden cardiac death (SCD) in the general population are either ventricular arrhythmias or electromechanical dissociation of the heart. It is estimated that ventricular arrhythmias such as ventricular fibrillation or stable

Table 1. Concentration of ethanol in urine and blood and heart weight

		Concentration of ethanol [‰]		Heart weight [g]
		In urine	In blood	
Alcohol-exposed group (n = 30)	Mean	4.19	3.73	352
	Standard deviation	0.72	0.33	52
	Minimum	2.6	2.5	310
	Maximum	5.4	4.4	375
Control group (n = 10)	Mean	0.5	0.5	348
	Standard deviation	–	–	58
	Minimum	–	–	290
	Maximum	–	–	368

Concentration of ethanol in urine–Alcohol-exposed group–Control group: $p < 0.05$
Concentration of ethanol in blood–Alcohol-exposed group–Control group: $p < 0.05$
Heart weight–Alcohol-exposed group–Control group: $p > 0.05$

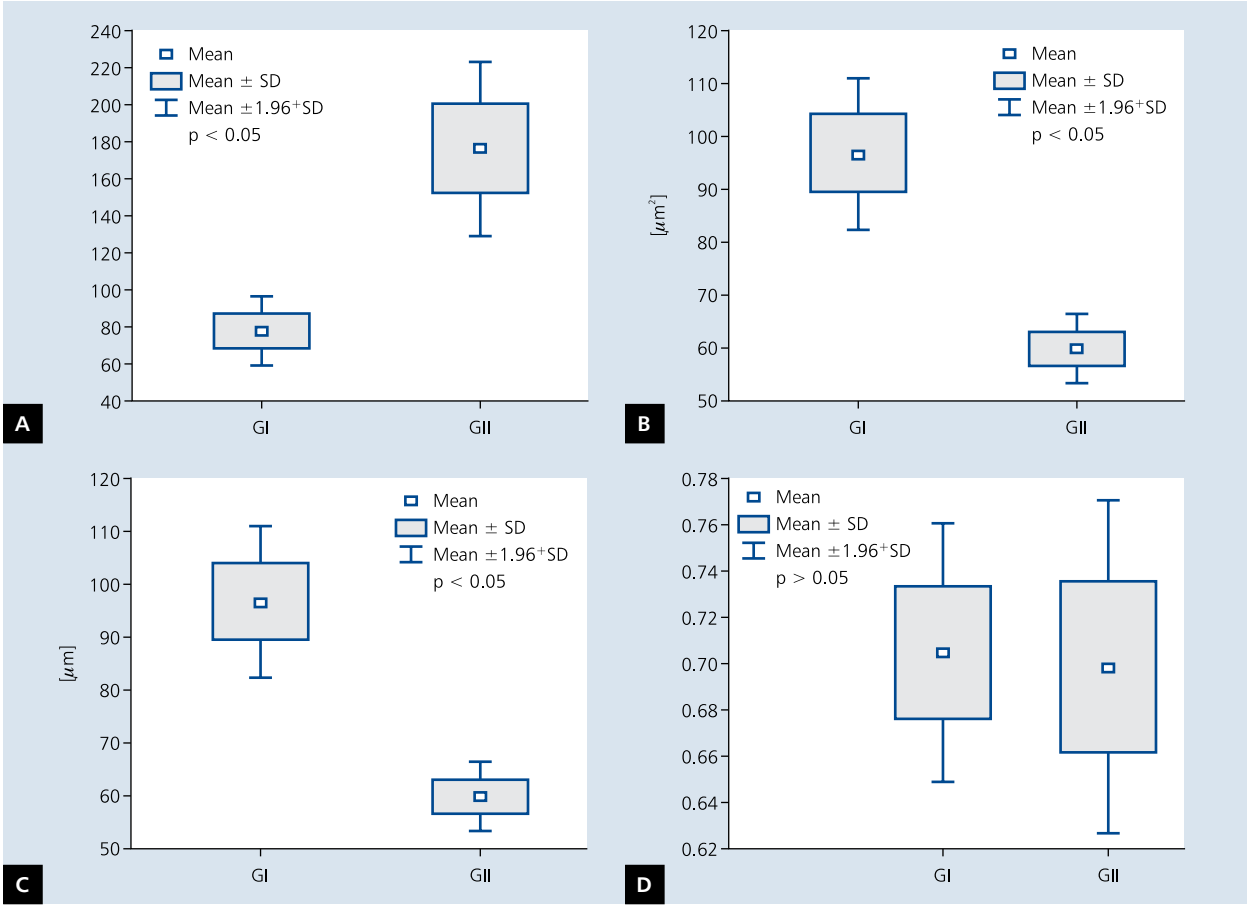

Figure 1. Values of the analysed morphometric parameters in the alcohol-exposed group (GI) and control group (G II); **A**. Number of cardiomyocytes in the microscopic field; **B**. Area of cardiomyocytes in the transverse section [μm^2]; **C**. Circumference of cardiomyocytes in the transverse section [μm]; **D**. Circular deviation

ventricular tachycardia are responsible for 60–80% of SCD cases, whereas electromechanical dissociation, atrioventricular block and sinusal arrest account for 20–40% of them. In 4% of cases, SCD may be the first symptom of post-alcoholic dilated cardiomyopathy [9, 10]. Despite the enlargement of cardiomyocytes observed in our study, no morphological features of cardiomyopathy were found in the examined heart structures. Among the factors inducing an arrhythmogenic

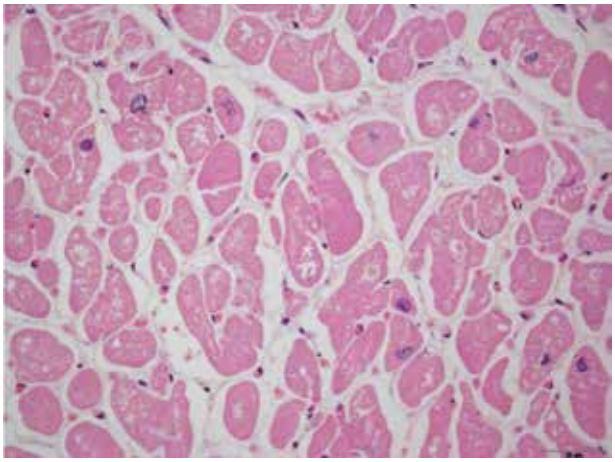

Figure 2. Transverse section of cardiomyocytes in the alcohol-exposed group (HE, ×400). Visibly lower number of cardiomyocytes in the microscopic field and hypertrophy of individual cardiomyocytes with a significant enlargement of cell nuclei

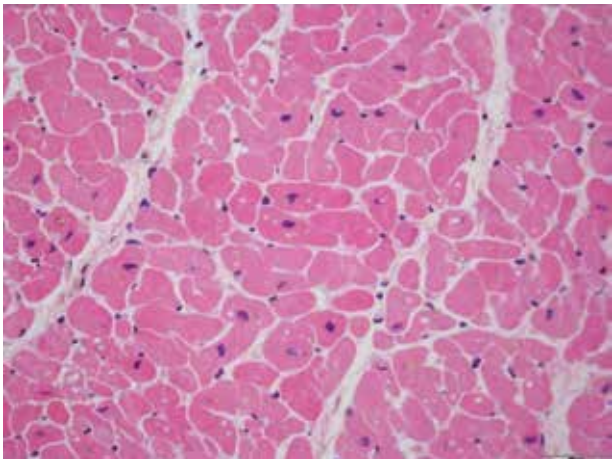

Figure 3. The transverse section of normal cardiomyocytes in the control group (HE, ×400)

environment in the myocardium the following are usually noted: structural alteration resulting from hypoxia, inflammatory process, toxic action of various chemical substances including ethanol and haemodynamic factors, disturbance of the ionic balance, and disturbance of the regulation of the autonomic nervous system [11–14]. The results of studies carried out in vitro and on animals suggest that alcohol may activate the apoptosis of cardiomyocytes, severely impair the function of mitochondria and the sarcoplasmatic reticulum, affect the expression of sarcomeric proteins, and result in disturbances of calcium metabolism in cardiomyocytes, which may produce serious consequences. In the group of males who died after an incidental ethanol intoxication we observed the following types of significant alterations in the myocardium: hypertrophy of individual cardiomyocytes with a significant enlargement of cell nuclei, and a lower number of cardiomyocytes in the microscopic field in transverse sections under ×400 magnification. It is worth underlining that Figures 2 and 3 convey a false visual illusion of the two different magnifications owing to the fact that the cardiomyocytes examined in the alcohol-exposed group had an increased area and circumference of fibres (Fig. 1) compared to the cardiomyocytes in the control group (Fig. 3). No statistically significant differences were found with regard to CD. Fragmentation and segmentation of individual cardiomyocytes with a significant enlargement of cell nuclei was observed in 26 subjects from the alcohol-exposed group. In seven subjects from this group we observed the presence of a small number (1–3) of lymphocytic cells in the microscopic fields. The higher the ethanol concentration found in the blood and urine of a deceased person, the more intensive the disturbances in the examined histopathological specimens. In the control group, no significant pathology of the histomorphological structure of the examined samples of the myocardium was observed. Our study suggests that the toxic influence of alcohol depends on its dose, but according to other authors it is not a linear dependence [15]. In addition, as we examined only a group of males, we cannot refer to another interesting effect that is widely known, i.e. an increased sensitivity to the toxic influence of alcohol in females. Furthermore, previous studies concerning the influence of alcohol on the human organism focused primarily on persons addicted to alcoholic beverages [16–18], which makes any comparison between their results and ours difficult to make.

CONCLUSIONS

The obtained results indicate a harmful influence of alcohol on the structure of myocardium. The observed destructive alterations in the myocardium may induce circumstances conducive to an electric destabilisation of the heart, which may result in sudden death in the mechanism of arrhythmia.

Conflict of interest: *none declared*

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KAMPANIA „ZASTAWKA TO ŻYCIE” („*VALVE FOR LIFE*”)

Inicjatywa *European Association of Percutaneous Cardiovascular Interventions* (EAPCI), *European Society of Cardiology* (ESC), Polskiego Towarzystwa Kardiologicznego (PTK) i Asocjacji Interwencji Sercowo-Naczyniowych PTK

Mimo poprawy spadku umieralności w Polsce w dalszym ciągu choroby układu sercowo-naczyniowego są główną przyczyną zgonów. Jeżeli ta sytuacja nadal się utrzyma, liczba zgonów w 2020 r. przekroczy 200 tysięcy.

Choć polska kardiologia plasuje się w czołówce najlepszych w Europie, zgony z powodu chorób układu sercowo-naczyniowego dotyczą prawie połowy naszego społeczeństwa. Posiadamy obecnie jeden z najwyższych w Europie i na świecie odsetek leczenia ostrych zespołów wieńcowych za pomocą zabiegów przezskórnej angioplastyki wieńcowej (PCI) na milion mieszkańców. W ciągu kilku lat wzbogaciliśmy się o kolejne kardiologiczne placówki (160 ośrodków, w tym 148 dyżurujących w systemie 24/7) oraz nowych specjalistów. Nadal jednak pojawiają się obszary, które choć mają duże możliwości terapii, to zastosowanie nowych metod leczenia w naszym kraju jest zbyt małe. Mowa tu m.in. o metodach terapii osób cierpiących na ciężkie zwężenia zastawki aortalnej, u których zabieg chirurgiczny wiąże się z wysokim ryzykiem. Ocenia się, że 30–40% pacjentów z ciasnym zwężeniem zastawki aortalnej nie kwalifikuje się do leczenia chirurgicznego. Rozwiązaniem dla tych chorych jest zabieg TAVI, czyli przezcewnikowa implantacja zastawki aortalnej.

Niestety ta metoda leczenia nie należy do powszechnych w Polsce. W 2013 r. wykonano 381 zabiegów TAVI, a w 2014 r. już 453, co stanowi zaledwie 11,7 zabiegów na milion osób. Średnia w krajach „starej unii” to 50 na milion osób, w Niemczech ponad 100. Aby osiągnąć średnią unijną w naszym kraju, powinno się wykonywać 2000 zabiegów TAVI rocznie. Choć dochód narodowy na mieszkańca w Niemczech jest wyższy 3,6-krotnie w porównaniu z Polską, liczba zabiegów TAVI jest tam aż 22 razy większa. Przy czym Polska w ostatnich latach rozwija się bardzo dynamicznie, notując realny dodatni wzrost PKB, przekraczający ten obserwowany w wielu państwach Europy Zachodniej. Tak słabe wyniki w naszym kraju potwierdzają fakt, że możliwości, jakie posiadamy, nie są adekwatne do znanych nam realiów życia. Bez odpowiednich środków wsparcia niektórych metod leczenia i poszerzania świadomości społecznej wśród osób chorych oraz ich bliskich polska medycyna nie może liczyć na większe zmiany w obszarze zwalczania chorób układu sercowo-naczyniowego.

Europejska inicjatywa “*Valve For Life*” jest w Polsce koordynowana przez prof. Dariusza Dudka i prof. Adama Witkowskiego, przy współpracy prof. Zbigniewa Kalarusa, Prezesa PTK oraz prof. Jarosława Kazimierczaka, konsultanta krajowego w dziedzinie kardiologii. Więcej szczegółów znajdziecie Państwo na oficjalnej internetowej stronie kampanii: **www.zastawkatozycie.pl**.

Ocena histologiczna mięśnia sercowego po śmiertelnym zatruciu alkoholem etylowym

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Streszczenie

Wstęp: Przyczyny nagłego zgonu po spożyciu śmiertelnej dawki alkoholu etylowego nie są dokładnie znane. W patomechanizmie zgonu może być brany pod uwagę czynnik kardiogenny.

Cel: Celem pracy była ocena histologiczna mięśnia sercowego po śmiertelnym zatruciu alkoholem etylowym. Badaną grupę stanowiło 30 zmarłych mężczyzn w wieku 29–45 lat. Badania autopsyjne przeprowadzono w Zakładzie Medycyny Sądowej Uniwersytetu Medycznego w Białymstoku.

Metody: Materiał do badań pobrano z wolnej ściany lewej komory i z przegrody międzykomorowej serca. Po utrwaleniu materiał poddano standardowym procedurom histologicznym. Badania morfometryczne wykonano w Zakładzie Anatomii Prawidłowej Uniwersytetu Medycznego w Białymstoku.

Wyniki: W badanej grupie stwierdzono: wzrost powierzchni i obwodu badanych kardiomiocytów, fragmentację i segmentację kardiomiocytów z istotnym powiększeniem jąder komórkowych oraz w pojedynczych przypadkach nacieki z komórek limfocytarnych.

Wnioski: Obserwowane zmiany w obrębie mięśnia sercowego mogą się przyczyniać do destabilizacji kardiomiocytów i w efekcie być przyczyną zgonu w mechanizmie arytmogennym.

Słowa kluczowe: kardiomiocyty, etanol, śmiertelne zatrucie

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