VERSITA

Bull Vet Inst Pulawy 57, 269-274, 2013
DOI: 10.2478/bvip-2013-0047

INFLUENCE OF DOCOSAHEXAENOIC ACID OBTAINED FROM NEW GENERATION OF EGGS ON THE REPOLARISATION OF VENTRICLES IN PIGS WITH EXPERIMENTAL TACHYCARDIOMYOPATHY

AGNIESZKA NOSZCZYK-NOWAK, URSZULA PASŁAWSKA, JÓZEF NICPOŃ, ADRIAN JANISZEWSKI, PIOTR SKRZYPCZAK[1], JAROSŁAW POPIEL, ŁUKASZ BOBAK[2], AND TADEUSZ TRZISZKA[2]

Department of Internal Diseases with Horse, Dog and Cat Clinic
[1]Department and Clinic of Surgery, Faculty of Veterinary Medicine
[2]Department of Animal Products Technology and Quality Management,
Faculty of Life Sciences and Technology,
Wroclaw University of Environmental and Life Sciences, 50-366 Wroclaw, Poland
agnieszkann@poczta.onet.pl

Received: December 17, 2012 *Accepted: May 3, 2013*

Abstract

The influence of docosahexaenoic (DHA) acid obtained from a new generation of eggs on ventricular electrical function of 24 pigs with experimentally induced tachycardiomyopathy was described. Two groups were formed, 12 pigs had experimentally evoked tachycardiomyopathy and were getting standard feed (control group TIC). Twelve pigs with tachycardiomyopathy received feed with an addition of a dietary supplement containing phospholipids isolated from new generation of eggs at a dose of 1,000 mg of DHA/animal/24 h (experimental group TIC). Electrophysiological study was carried out from an external programmer immediately after implantation of the pacemaker. All the tests were carried out in general anaesthesia. After 8 weeks of fast ventricular pacing at 170 bpm in pigs receiving phospholipids obtained from eggs, a statistically significant shortening of ventricular refraction time was observed during sinus rhythm and also during the ventricular pacing of 130 bpm, and 150 bpm in the group of pigs fed standard feed. The ventricular refraction time in the sinus rhythm was significantly longer after 12 weeks of fast pacing and in the pacing at 130 bpm and 150 bpm it was significantly longer after 8 weeks of fast pacing. Phospholipids containing high percentage of n-3 polyunsaturated fatty acids (PUFA) obtained from new generation of eggs may contribute to the shortening ventricular refraction period after its oral administration. The n-3 PUFA obtained from a new generation of hen eggs may be an alternative to fish oil source of DHA and other polyunsaturated fatty acids.

Key words: pigs, heart, polyunsaturated acid, repolarisation period, tachycardiomyopathy.

In 1976, after the publication of a report indicating that high consumption of sea fish diminishes the risk of contracting cardiovascular diseases, there was an increased interest in therapeutic applications of polyunsaturated fatty acids (PUFA) in prevention and treatment of these diseases (1). Numerous experiments on animal models and randomised tests on people with heart diseases, which aimed at defining the therapeutic role of PUFA in cardiovascular disease, have been conducted since that moment. Despite of many tests on animal models and cell cultures, the mechanism of antiarrhythmic action of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has not been completely explained and therefore, experimental and population tests on various groups of people suffering from cardiovascular system diseases are performed. At the same time, the search for sources of these fatty acids, other than fish oils has begun because of the patients' intolerance to large amounts of such oils, which is needed to reach a therapeutic DHA dose.

The aim of the study was to define the influence of DHA obtained from new generation of eggs on repolarisation of ventricles in pigs with an experimental tachycardiomyopathy.

Material and Methods

Twenty four pigs were used in the experiment: 12 pigs with experimentally evoked tachycardiomyopathy getting standard feed (control group TIC) and 12 pigs with tachycardiomyopathy fed a diet supplement containing phospholipids isolated from new generation of eggs at a dose of 1,000 mg of DHA/animal/24 h for 12 weeks (15 g of product from eggs) (experimental group TIC). Tachycardiomyopathy was evoked by

means of one-chamber pacemaker IDENTITY ADx DR (St. Jude Medical, USA) with a ventricular electrode TENDRIL STS (St. Jude Medical, USA) pacing at 170 bpm according to the procedures described by Pasławska *et al.* (12).

The material consisted of specially designed eggs obtained from hens of the Lohmann Brown line. Laying hens were fed standard feed *ad libitum* supplemented with a mixture containning: 3% fish oil, 1.5% of dried algae, 2% of Humokarbowit and Humobentofet humic preparations, and 0.01% of vitamin E. The eggs were subjected to a process of separating the yolks, which were then spray dried. Drying was carried out at a temperature of inlet air of 185°C ± 5°C to the drying chamber with an outlet air temperature of 70°C ± 2°C. The extraction of the powder was carried out in a tank equipped with a mechanical stirrer while maintaining the ratio of solvent yolk dilution at 1:6 m: v. A uniform suspension process was carried out for 70 min. After the separation of alcohol from the residue using a filter press, it was evaporated in vacuum of 150 mbar (1.5 x 104 Pa) at 50°C. The crude phospholipids were analysed to determine purity, fatty acids profile, and content of the basic groups of the phospholipids.

The phospholipids were converted to fatty acid methyl esters (FAME) as follows: 50 mg of sample were dissolved in 4 mL of 0.5 M methanolic NaOH solution and heated under reflux for 2 min. After that, 4 mL of BF_3-MeOH (14%) complex were added and the mixtures were heated again under reflux for the next 2 min. After cooling, the mixtures were extracted with 6 mL of hexane and the organic layers were washed with saturated NaCl solution. Hexane extracts were dried over anhydrous magnesium sulphate and analysed directly by gas chromatography. In order to determine the fatty acid profile chromatographic analysis (GC/MS) was performed with the use of gas chromatographer 6890N coupled with mass spectrometer GC5973 (Agilent Technologies, Poland). The HP 88 column was used and the flow of carrier gas (He) was at a level of 1.0 mL/min. Injector temperature was set at 230°C, and the detector at 240°C. The temperature programme was established within the following time frame: 100°C/hold 0.5 min then 3°C/min to 180°C, hold 17 min and 5°C/min to 210°C, hold 45 min. The content of fatty acids in phospholipid fractions is presented in Table1.

Table 1
Fatty acid composition in phospholipids from egg yolk (%)

Fraction	Percentage
Acetone insoluble matter (AI)	73.00
Phosphatidylcholine (PC)	81.72
Phosphatidylethanolamine (PE)	18.27
Fatty acids	
C14:00	0.40
C14:01	0.16
C16:00	26.36
C16:01	2.52
C17:00	0.24
C18:00	14.05
C18:01	29.66
C18:02	13.08
C18:03	3.12
C20:02	0.18
C20:03	0.12
C20:04	2.41
C20:05	0.58
C22:06	**7.12**
ω-3	10.82
ω-6	15.79
ω6/ω3	**1.46**
saturated	41.05
unsaturated	**58.95**
MUFA	32.34
PUFA	26.61

The content of basic groups of phospholipid fractions was measured with high performance liquid chromatography (HPLC) (Agilent 1200 Series HPLC Systems, purchased from Agilent Technologies, Poland). Ten milligram samples were dissolved in 1 mL of 2-propanol and analysed directly by liquid chromatography. HPLC was used to define the content of phosphatidylocholine (PC) and phosphatidyletha-nolamine (PE) in the extracts isolated from egg yolk. For this purpose, 1% solution of extract of phospholipids in acetonitrile/methanol (2:1 v/v) after filtration was subjected to chromatographic analysis. The eluent was prepared with acetonitrile, methanol with the addition of 2% phosphoric acid and the flow was set at 1.25 mL/min. The column SUPELCOSIL™ LC-Si 15 cm×4.6 mm 3 μm particle size was heated at 35°C. The content of the basic groups of phospholipid fractions is presented in Table 1.

The animals were qualified as healthy before the onset of the examinations based upon earlier morphological and biochemical blood tests. The following biochemical parameters were determined: activity of alanine aminotransferase (ALT) and asparate aminotransferase (AST), as well as the levels of urea, creatinine, Na^+, K^+, total Ca, Mg^{2+}, and Cl^-. Morphological blood tests were performed in an Micros ABX Vet Animal Blood Counter (ABX Diagnostics). Biochemistry tests were performed in Konelab Prime 30i (Thermo Scientific).

Before the pacemaker implantation, all pigs underwent an ECG test (BTL 08 SD) with a great attention paid to the period of repolarisation (QT, QTc) and an echocardiographic test (ALOKA 4000 + with 5 MHz sector heads). Animals with arrhythmias were excluded. In the echocardiographic test from right transthoracic echocardiography, the following parameters were assessed: left ventricular diameter in diastole (LVIDd) and systole (LVIDs), interventricular septum thickness in diastole (IVSd) and systole (IVSs), and left ventricular free wall in diastole (LVPWd) and systole (LVPWs). Ejection fraction (EF) and fractional shortening (SF) were calculated automatically. Electrophysiological study (EPS) was carried out from an external programmer immediately after implantation of the pacemaker. EPS protocol included the following: pacing with a single premature impulse (8+1) during the sinus rhythm and with a single double (8+2), triple (8+3) premature impulses during pacing rhythm at 130 bpm and 150 bpm, and also a pacing during a frequency of 180/min for one min. This pacing was used to provoke of arrhythmias. All the tests were performed in general anaesthesia. The pigs were premedicated by intramuscular injection of a mixture of 0.1 mg/kg of midazolam (Midanium 5 mg/mL, Polfa, Poland), 0.02 mg/kg of medetomidine (Cepetor 1 mg/mL. CP-Pharma Handelsges), and 8 mg/kg of ketamine (Bioketan 100 mg/mL Vetoquinol Biowet, Poland). Anaesthesia was induced by an intravenous bolus injection of 2 mg/kg propofol (1% Propofol, MTC/LCT Fresenius Kabi). Fourteen days after implantation (healing time), the pacemaker was programmed in the VVI 170 bpm mode, ECG test, heart ultrasonography, and EPS from the pacemaker were carried out after 4, 8, and 12 weeks of DHA administration. The experiment was approved by the National Bioethical Committee.

Results

All pigs developed tachycardia induced heart failure. Its presence was confirmed by the appearance of the following characteristic symptoms: diminished eagerness to move, gradual fall of physical capacity, earlier appearance of dyspnoea, and changes of echocardiographic parameters during the tests carried out at 4, 8, and 12 weeks of the experiment. Cardiac enlargement and the fall of the shortening and ejection fractions were observed in echocardiography in both groups of pigs. No statistically significant changes in ECG parameters were observed between the tested groups of animals during the respectively compared time of the experiment (Table 2). No changes in the tested morphological and biochemical parameters of blood were observed during the experiment

After 8 weeks of fast ventricular pacing in pigs getting phospholipids from new generation of eggs, statistically significant shortening of time of ventricular refraction both in the sinus rhythm and also in the ventricular pacing at 130 bpm and 150 bpm was observed (Table 3). Significantly shorter refraction time was also noticed in the 12^{th} week of phospholipids administration compared to the test run in the 4^{th} week but it was not statistically different from the refraction time defined in the 8^{th} week of phospholipids administration. In the group of pigs fed standard feed, the ventricular refraction time in the sinus rhythm was significantly longer after 12 weeks of fast stimulation and in the pacing at 130 bpm and 150 bpm it was significantly longer after 8 weeks of fast pacing. The tendency of prolonged refraction time was also observed in the 12^{th} week of the experiment. Still, the results did not gain a statistical significance compared to the results observed in the 8^{th} week of the experiment.

In EPS run in the group getting phospholipids immediately after implantation of the pacemaker and after 4 weeks of fast ventricular pacing, a sustained ventricular tachycardia in one pig, a non-sustained ventricular tachycardia in two pigs and ventricular fibrillation interrupted by a defibrillation in one of the animals were evoked (Fig. 1).

After 8 and 12 weeks of phospholipid supplementation, it was impossible to evoke arrhythmia in any of the animals. In the group getting standard feed ventricular tachycardia was provoked in two pigs and it was evoked during the whole period of the experiment.

Discussion

The unique characteristic of the study is the source of unsaturated omega 3 acids, *i.e.* the new generation of eggs, rather than fish oil previously investigated by other research groups (2-4, 8, 14).

Table 2
Data of echocardiography and ECG examination in both groups

Parameters	Control group TIC			Experimental group TIC		
	After 4 week	After 8 week	After 12 week	After 4 week	After 8 week	After 12 week
Ejection fraction (%)	67 ±3.56	42.5 ±7.45	36 ±20.1	64 ±4.45	44.5 ±10.45	40 ±15.5
Shortening fraction (%)	37 ±4.4	24.6 ±7.4	20.3 ±10.6	34 ±2.9	22.9 ±6.1	21.7 ±8.2
LA/Ao	1.56 ±0.25	2.1±0.2	2.42 ±0.25	1.61 ±0.18	1.9 n±0.29	2.39 ±0.24
LVIDd (cm)	5.67 ±0.45	6.57 ±0.39	7.01 ±0.9	5.48 ±0.41	6.3 ±0.4	6.55 ±0.73
LVIDs (cm)	3.55 ±0.21	4.67 ±0.6	5.01 ±1.1	3.48 ±0.36	4.8 ±0.76	5.45 ±1.6
P time (ms)	68 ±9.3	98 ±11.2	118 ±11.6	67 ±9.1	96 ±13.4	114 ±9.2
PQ (ms)	120 ±20.4	168 ±14.5	181 ±18.1	123 ±21.4	170 ±12.86	179 ±16.3
QRS (ms)	65 ±17.1	80 ±11.6	81.2 ±14.2	63 ±18.9	76 ±10.8	79.6 ±14.6
QT (ms)	345 ±42	360 ±26.1	356 ±34.4	356.7 ±39	358 ±24.6	326 ±43.2
QTc (ms)	426 ±43.1	435.5 ±36.7	408 ±54.3	425.3 ±34.9	418 ±32.6	392.5 ±47.6
HR	84.4 ±5.6	83 ±5.8	81 ±6.3	86.4 ±4.6	83.4 ±6.1	86.8 ±10.5

LVIDd - left ventricular internal end-diastolic diameter, LVIDs - left ventricular internal systolic diameter, HR - heart rate

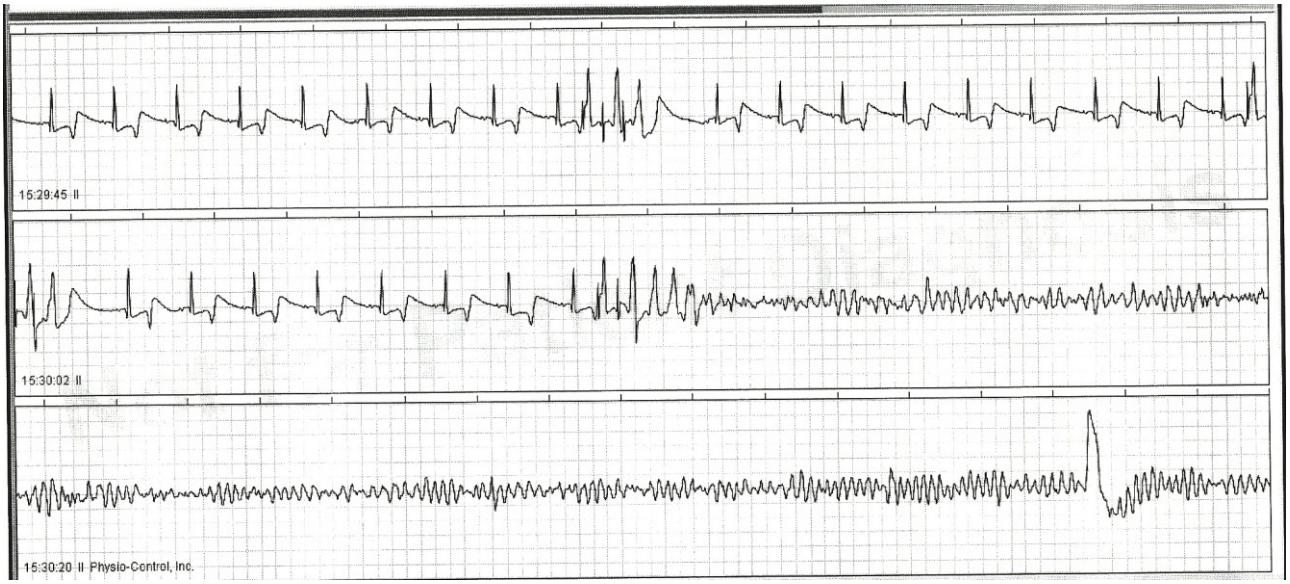

Fig. 1. Ventricular fibrillation in pigs after ventricular pacing at 150 bpm 8+3 cycle

Phospholipids isolated from the new generation of eggs are devoid of a specific taste and smell, which makes them significantly different from unsaturated fatty acids, obtained from fish oils. The animals were taking the phospholipids willingly in the amount required by the experimental protocol during the whole period of the experiment, which took 12 weeks. Another distinguishing feature of the study is the long duration of the experiment-12 weeks, which is one of the longest described periods of observation to date. Furthermore, it should be emphasized that the experiment was performed on home swine with inducted chronic heart failure, which is an expensive model, however it is considered to be the closest animal model of human chronic heart failure.

Development of chronic heart failure (tachycardiomyopathy) in the tested animals was proved by control tests showing a significant enlargement of left ventricle and left atrium and the fall of shortening fraction and ejection fraction. Expected prolongation of P wave time, PQ interval, QRS complex, and duration time of OT and OTc related with a myocardial remodelling were observed.

Despite the fact that the results of recently published studies indicate that the influence of administered unsaturated omega 3 acids on retardation of the development or reoccurrence of supraventricular arrhythmia was overestimated (3, 4, 14), it does not change the fact that some antiarrhythmic influence has been documented (5, 13).

Table 3
Data from electrophysiological study

	Control group TIC			Experimental group TIC		
Parameters [ms]	After 4 week	After 8 week	After 12 week	After 4 week	After 8 week	After 12 week
VERP sinus rhythm 8+1	308 ±18.4*	336 ±21.7^	360 ±23.4*. ^^	293 ±19.1#. ##	244 ±21.4#. ^	237 ±16.4##. ^^
VERP 130 bpm 8+1 cycle	250 ±21.2*' **	298 ±14.4*. ^	300 ±12.2**. ^^	271 ±29.3#.##	234 ±31.2#. ^	184 ±54.2##. ^^
VERP 130 bpm 8+2 cycle	191 ±47.5*' **	241 ±24.5*. ^	250 ±20.6**. ^^	206 ±34.0#. ##	174 ±21.4#. ^	158 ±13.7##. ^^
VERP 130 bpm 8+3 cycle	171 ±26.7*' **	210 ±25.1*. ^	220 ±24.4**. ^^	172 ±31.4#. ##	156 ±20.4#. ^	Below 152##. ^^
VERP 150 bpm 8+1 cycle	232 ±17.2*' **	250 ±17.1*. ^	260 ±29.2**. ^^	242.1 ±21.4#. ##	221 ±30.6#. ^	175 ±39.7##. ^^
VERP 150 bpm 8+2 cycle	172 ±27.4*' **	210 ±29.4*. ^	226 ±26.4**. ^^	180.6 ±20.3#. ##	167 ±31.4#. ^	157 ±31.4##. ^^
VERP 150 bpm 8+3 cycle	158 ±18.4*"**	210 ±25.2*. ^	220 ±21.5**. ^^	159 ±19.8#. ##	156 ±11.4#. ^	Below 156 ##. ^^

VERP-refraction period of ventricle; * # ^ significant difference

In order to establish whether the phospholipids originating from new generation of eggs indicate a similar antiarrhythmic influence, an invasive electrophysiological test was used.

In the EPS protocol used, a pacing with a single premature impulse on the sinus rhythm and on two frequencies of an pacing at 130 bpm (460 ms cycle) and 150 bpm (400 ms cycle) was applied in order to establish the ventricular refractory period. To evoke arrhythmia, a pacing with two (8+2) and three (8+3) premature impulses was applied during the pacing at 130 bpm and 150 bpm and fast ventricular pacing at 180 bpm for 1 min. A significant shortening of VERP after 8 weeks of phospholipids administration was observed and it was still present at the 12th week of the experiment. Earlier studies revealed that the unsaturated fatty acids, especially DHA, reversibly modulate the operation of ion channels and ion exchangers in the cardiomyocyte cell membranes influencing the action potential time (7, 8). Verkerk *et al.* (16) observed shortening of action potential period of cardiomyocytes isolated from swine ventricles after 8 weeks of application of a diet rich in fish oil. This was possibly related to the increased density of I_{K1}, I_{Ks} potassium channels and decreased density of Na^+-Ca^{2+} I_{NCX} ion exchanger and calcium channels of L $I_{Ca.L}$ type (15, 16). In the experiment, the swine received phospholipids orally for a long period, which shows a chronic direct influence of unsaturated fatty acids on the ventricular refraction time. Its shortening progressed together with the duration of the experiment. This phenomenon indicates that the cardioprotective influence of the tested phospholipids is possible with longer oral DHA administration.

A different influence of DHA on the atrial effective refraction period (AERP) was observed. Cunha *et al.* (4) in a test carried on dogs, did not observe any changes in AERP after intravenous administration of EPA+DHA during a sinus rhythm or during an ventricular pacing immediately after PUFA administration. They observed, however, prolongation of AERP after 3 h of fast atrial stimulation 400 bpm. The differences in the obtained influence on the cardiac muscle refraction time may result from different ways of n-3 PUFA administration, different times of exposition to DHA, and/or a different reaction of atrial and ventricular cardiomyocytes caused by a different course of potential activation of atrial and ventricular cardiomyocytes (11). Atrial repolarisation depends on I_{to} and I_{Kur} sodium channels, which is why the I_{Kur} inhibition may prolong the refraction of the atrium but not the ventricles (15). Cunha *et al*. (4) administered DHA+EPA intravenously, leading to an acute exposure to DHA and EPA during which the blockade of sodium channels dominated, resulting in shortening the activation potential and atrial repolarisation.

In the conducted experiment, the decreased ability to evoke ventricular dysrhythmia in swine supplemented with phospholipids was observed. More studies are, however, needed for several reasons.

At first, the examined population was too small to draw a conclusion on efficient antiarrhythmic function of phospholipids obtained from new generation of eggs. One of the potential mechanisms of antiarrhythmic action of n-3 PUFA is related to the influence on potassium and sodium channels. Increasing of I_{Ks} potassium current in the repolarisation phase may contribute to the shortening of the action potential duration similarly to a decreased I_{NCX} density. The change of I_{K1} density may cause earlier repolarisation and more stabilised resting membrane potential (6), which may protect from the occurrence of late secondary depolarisations (16). Shorter duration of action potentially may also protect from arrhythmias *via* the re-entry mechanism. Many experiments present an antiarrhythmic tendency of n-3 PUFA related to the

duration time of action potentials and creation of delayed and early after depolarisations (1, 7, 8, 10, 16).

To conclude, phospholipids obtained from the new generation of eggs, containing a high percentage of n-3 PUFA, especially DHA, may contribute to the shortening of the ventricular refraction period after oral administration. n-3 PUFA obtained from hen eggs may be an alternative source of DHA and other polyunsaturated fatty acids to fish oil and they can be a basis for dietary supplement or bio-preparations.

Acknowledgments: The study was performed during the realisation of project No. POIG.01.03.01-00-133/08- "Innovative technologies of production of bio-preparations based on new generation of eggs (OVOCURA)". The project is co-financed by the European Regional Development Fund within the Innovative Economy 2007-2013 Operational Programme.

References

1. Ander B.P., Weber A.R., Rampersad P.P., Gilchrichst J.S., Pierce G.N., Lukas A.: Dietary flaxeed protects against ventricular fibrillation induced by ischemia-reperfusion in normal and hiperxholesterolemic rabbits. J Nutr 2004, **134**, 3250-3256.
2. Bang H.O., Dyerberg J., Hjoorne N.: The composition of food consumer by Grenland eskimos. Acta Med Scand 1976, **200**, 69-73.
3. Bianconi L., Calò L., Mennuni M., Santini L., Morosetti P., Azzolini P., Barbato G., Biscione F., Romano P., Santini M.: n-3 polyunsaturated fatty acids for the prevention of arrhythmia recurrence after electrical cardioversion of chronic persistent atrial fibrillation: a randomized, double-blind, multicentre study. Europace 2011, **13**, 174-181.
4. Cunha D., Hamlin R.L., Billman G.E., Carnes C.A.: n-3 (omega-3) polyunsaturated fatty acids prevent acute atrial electrophysiological remodeling. Br J Pharmacol 2007, **150**, 281-285.
5. Jahangiri A., Leifert W.R., Patten G.S., McMurchie E.J.: Termination of asynchronous contractile activity in rat atrial myocytes by n-3polyunsaturated fatty acids. Mol Cell Biochem 2000, **206**, 33-41.
6. Kang J.X.: Reduction of heart rate by omega-3 fatty acids and the potential underlying mechanisms. Front Physiol 2012, **3**, 416.
7. Leaf A., Albert C.M., Josephson M., Steinhaus D., Kluger J., Kang J.X., Cox B., Zhang H., Schoenfeld D.: Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 2005, **112**, 2762-2768.
8. Leaf A., Xiao Y.F., Kang J.X., Billman G.E.: Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. Pharmacol Ther 2003, **98**, 355-377.
9. Leifert W.R., McMurchie E.J., Saint D.A.: Inhibition of cardiac sodium currents in adult rat myocytes by 3-n polyunsaturated fatty acids. J Physiol 1999, **520**, 671-679.
10. MacLeod J.C., Macknight A.D.C., Rodrigo G.C.: The electrical and mechanical response of adult guinea pig and rat ventricular myocytes to ω3 polyunsaturated fatty acids. Eur J Pharmacol 1998, **356**, 261-270.
11. Moreno C., Macias A., Prieto A., Cruz A., Gonzalez T., Valenzuela C.: Effects of n-3 polyunsaturated fatty acids on cardiac ion channels. Front Physiol 2012, **3**, 245.
12. Pasławska U., Gajek J., Kiczak L., Noszczyk-Nowak A., Skrzypczak P., Bania J., Tomaszak A., Zacharski M., Sambor I., Dzięgiel P., Zyśko D., Banasiak W., Jankowska E.A., Ponikowski P.: Development of porcine model of chronic tachycardia-induced cardiomyopathy. Int J Cardiol 2011, **153**, 36-41.
13. Rennison J.H., Van Wagoner D.R.: Impact of dietary fatty acids on cardiac arrhythmogenesis. Circ Arrhythm Electrophysiol 2009, **2**, 460-469.
14. Sandesara C.M., Chung M.K., Van Wagoner D.R., Barringer T.A., Allen K., Ismail H.M., Zimmerman B., Olshansky B.: A randomized, placebo-controlled trial of Omega-3 fatty acids for inhibition of supraventricular arrhythmias after cardiac surgery: the fish trial. J Am Heart Assoc 2012, **1**, 1-8.
15. Tomargo J., Caballero R., Gomez R., Valenzuela C., Delpon E.: Pharmacology of cardiac potassium channels. Cardiovasc Res 2004, **62**, 9-33.
16. Verkerk A.O., Ginnekan A.C., Berecki G., Ruijter H.M., Schumacher C.A., Veldkamp M.W., Baartscheer A., Casini S., Opthof T., Hovenier R., Fiolet J.W., Zock P.L., Coronel R.: Incorporated sarcolemmal fish oil fatty acids shorten pig ventricular action potentials. Cardiovasc Res 2006, **70**, 509-520.
17. Xiao Y.F., Kang J.X., Morgan J.P., Leaf A.: Blocking effect of polyunsaturated fatty acids on Na+ channels of neonatal rat ventricular myocytes. Proc Natl Acad Sci 1995, **92**, 11000-11004.