

EVALUATION OF THE POTENTIAL ANTIARRHYTHMIC EFFECT OF FISH MEAT DURING MYOCARDIAL ISCHEMIA–REPERFUSION IN RATS

Nikolett Morvay¹, Judit Péter-Szűcsné², Ljiljana M. Kostadinović³, Olivera M. Đuragić³,
Bojana M. Kokić³, Istvan Csengeri⁴, Istvan Lepran^{1*}

¹Department of Pharmacology, Faculty of Medicine, University of Szeged, Hungary

²Institute of Animal Sciences and Wildlife Management, Faculty of Agriculture,
University of Szeged, Hungary

³University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia

⁴Research Institute for Fisheries, Aquaculture and Irrigation (HAKI) Szarvas, Hungary

*Corresponding author:

E-mail address: lepran.istvan@med.u-szeged.hu

ABSTRACT: The potential antiarrhythmic effect of dietary fish meat feeding was investigated in rats. Fish meat, harvested from carps fed experimental foods enriched with essential fatty acids, was mixed with a commercial rat food and fed to rats for 4 weeks. At the end of the feeding period in anesthetized rats myocardial ischemia–reperfusion induced arrhythmias were induced by occlusion of the coronary artery for 6 min, followed by reperfusion. Supplying fish meat, rich in n–3 type polyunsaturated fatty acids, significantly decreased the incidence of myocardial ischemia–reperfusion induced arrhythmias in the rat, and increased the survival rate. These results confirm previous investigations that feeding fish oil or fish meat may offer cardioprotective effect, and consumption of the farmed fish, like carp, may result in similar protective effects to the marine food.

Key words: rats, myocardial ischemia-reperfusion, fish meat diet, cardioprotection

INTRODUCTION

Cardiovascular diseases are the leading cause of death both in developed and developing countries (WHO, 2008). In most cases ischemic heart disease, acute myocardial infarction and associated ventricular arrhythmias stand in the background. The restoration of blood flow is commonly used therapy in case of acute myocardial infarction. Early reperfusion after a short lasting myocardial ischemia, however, can result in arrhythmias, tissue injury and cell death, called reperfusion injury (Yellon and Hausenloy, 2007).

Several investigations emphasize the importance of n–3 polyunsaturated fatty acid (PUFA) consumption and its cardioprotective effects (Psota et al., 2006), both in

primary and secondary prevention of cardiovascular complications (Saravanan et al., 2010). These human studies about the beneficial effects of PUFA feeding are supported by *in vivo* investigations in rats (Leprán et al., 1981; McLennan et al., 1990; Leprán and Szekeres, 1992; Zhu et al., 1994; Földes et al., 2006), marmosets (Charnock, 1994) and dogs (Oskarsson, 1993). Long chain n–3 PUFA has also triglyceride lowering effect, and decrease the synthesis of inflammatory eicosanoids and cytokines (reviewed by Calder, 2004).

The human body cannot synthesize these essential fatty acids, thus we need to take with consuming food rich in PUFA. Marine foodstuff, rich in eicosapentaenoic and docosahexaenoic acids, is the main source

of n–3 fatty acids for human beings. However there are many continental countries without the opportunity to consume fresh marine food, thereby people suggested to eat farmed fish more, e.g. the carp. The aim of the present investigations was to study the effects of feeding carp meat, harvested from fishes after using different fish food composition, on myocardial ischaemia–reperfusion induced arrhythmias in rats.

EXPERIMENTAL

Animals and diet

The experiments were performed on male Sprague–Dawley rats weighing between 250–300 g. The animals were housed 5 to a cage, and fed carp meat enriched chow as follows. All carp–meat enriched rat food (RF2, RF3, RF4, RF5) was prepared using frozen fish fillets mixed with corn flake in a ratio of 3:1 of fish meat and corn flake. This fillet mixture was then grinded and pelleted after mixing with commercial rat chow ingredients. Rat food RF1 was prepared by using commercial fish meal with added lard to equalize the energy contents. The final experimental rat diets contained approximately 10% of fish flesh.

Fish flesh used for preparing rat food (RF2–RF5) was taken from carps, fed on experimental extruded expanded foods (carp food, CF2–CF5), enriched with different oils (linseed oil; fish oil and *Mortierella alpine* oil, containing 40% arachidonic acid (ARA)) or eicosapentaenoic (EPA) and docosahexaenoic (DHA) ethyl esters. The amounts of the added oils were as follows: CF2: 6.0% Linseed oil (LSO); CF3: 3.5%

LSO + 1.5% Arachidonic acid (ARA) + 1% DHA; CF4: 6.0% Fish oil (FO); CF5: 4.5% FO + 1.5% ARA. Detailed data of fish food analysis and other observations obtained during the fish feeding experiments will be presented elsewhere.

The weekly portions of the rat foods were sealed under vacuum in plastic bags and stored in frozen form until use. The control rats (CF) were fed a standard rat chow (CRLT/N rodent food, Bonafarm, Bábolna, Hungary; containing 20.0% crude protein, 4.0% crude fat and 4.3% crude fiber), supplemented with 10 weight% of pork fat, rich in saturated fatty acids. Rats were fed for 4 weeks with the experimental diets. During this time the animals allowed to eat the experimental food and to drink tap water *ad libitum*. The rat experiments were performed according to the protocol reviewed by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Hungary.

Composition of the rat foods

Proximate composition and metabolizable energy contents of the experimental rat foods, RF1 to RF5, as determined by standard laboratory methods (AOAC, 2005), are presented in Table 1.

Biological assessment of modified fish meat in rats

Blood samples were taken for the measurement of plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol using commercial laboratory tests (Roche Diagnostics, Switzerland).

Table 1.

Proximate composition (g/kg) and metabolizable energy content of the rat foods

Composition	Rat foods				
	RF 1	RF 2	RF 3	RF 4	RF 5
Dry matter	876	868	870	874	877
Crude protein	234	244	241	233	238
Crude fat	55	62	54	52	60
Crude fiber	31	34	36	35	32
Crude ash	73	68	65	65	64
N–free extract	483	460	474	489	483
Starch	264	291	286	310	321
ME (MJ/kg)	16.0	16.2	16.1	16.0	16.3

Myocardial ischemia–reperfusion induced arrhythmias in anesthetized rats

Animals were anaesthetized with pento-barbitone and the left carotid artery was cannulated for measuring the blood pressure. The trachea was cannulated for artificial ventilation (Harvard Ventilator, Model 603) and the chest was opened in the fourth intercostal space. The heart was exposed and a loose loop of atraumatic silk was placed around the left main coronary artery, approximately 2 mm from its origin. Both ends of the ligature were led out of the thoracic cavity through flexible tubing (Leprán and Szekeres, 1992). The standard electrocardiogram (lead II, ECG) and the changes in blood pressure were recorded continuously and displayed after A/D conversion (Chart5, ADInstruments, United Kingdom). After finishing the preparation the animals were allowed to stabilize for 10 min, then the loose loop of the coronary artery ligature was tightened and fixed by clamping on the silk and thus regional myocardial ischemia was produced for 6 min and then followed by reperfusion.

The survival rate and the incidence of arrhythmias were analysed in accordance with the Lambeth Conventions (Walker, 1988), i.e. ventricular fibrillation (VF), ventricular tachycardia (VT) and other types of arrhythmias, including single extrasystoles (VEB), bigemina, and salvos. The duration of VF, VT and other types of arrhythmia attacks were also measured. An

arrhythmia score was used, including the incidence and duration of different arrhythmias by giving a grade to each animal (Leprán and Szekeres, 1992).

Statistical analysis

The survival rate was compared by using the χ^2 -method with Yates–correction. All other parameters were expressed as mean $\pm$ standard error of the mean (SE) and after analysis of variance were compared by means of the modified ‘t’-statistical method.

RESULTS AND DISCUSSION

The consumption of the amount of experimental rat food was not different from that of the control rat food. The final body weight of the animals after 4-week dietary period was not different among the different experimental groups. The plasma triglyceride, total cholesterol, high density lipoprotein cholesterol and the low density lipoprotein cholesterol concentrations were also not significantly different after feeding different experimental diets in rats. The baseline heart rate and blood pressure also did not differ among the experimental groups studied (data not shown).

Coronary artery occlusion in rats produced a slowly developing regional myocardial ischemia that induced various arrhythmias within 4–5 min (Table 2). There were no significant differences in the survival rate, incidence of arrhythmias during myocardial ischemia in different diet fed groups, as compared to the control.

Table 2.
Survival rate and the incidence of arrhythmias during 6 min myocardial ischemia in anesthetized rats

Incidence of ischemia induced arrhythmias												
Group	N	Survived		None		VF		VT		Other		Score
		n	%	n	%	n	%	n	%	n	%	
CF	16	15	94	3	19	1	6	8	50	12	75	1.75±0.36
RF 1	15	11	73	5	33	4	27	8	53	8	53	2.13±0.65
RF 2	15	12	80	8	53	4	27	6	40	4	26	1.67±0.64
RF 3	13	12	92	2	15	1	8	9	69	6	46	1.62±0.42
RF 4	15	15	100	5	33	0	0	7	46	9	60	1.20±0.26
RF 5	14	14	100	5	36	1	7	7	50	9	64	1.00±0.30

N = total number of animals in a given group; n = number of animals showing the given response. None = no arrhythmia occurred; VF = ventricular fibrillation; VT = ventricular tachycardia; Other = other types of arrhythmias, including ventricular extrasystoles, bigemina.

Table 3.

Survival rate and the incidence of arrhythmias during reperfusion after 6 min myocardial ischemia in anesthetized rats

Incidence of reperfusion induced arrhythmias												
Group	N	Survived		None		VF		VT		Other		Score
		n	%	n	%	n	%	n	%	n	%	
CF	15	5	33	0	0	13	87	15	100	11	73	5,40±0.27
RF 1	11	9	82*	2	18	4	36*	7	64*	6	55	3.09±0.62*
RF 2	12	9	75	1	8	7	58	8	67*	10	83	3.92±0.57*
RF 3	12	7	58	1	8	8	67	11	92	7	58	4.17±0.61
RF 4	15	5	33	1	7	13	87	13	87	8	53	5.13±0.46
RF 5	14	8	57	0	0	9	64	14	100	12	86	4.43±0.47

For abbreviations see Table 2. Asterisk denotes statistically significant difference compared to the control rat food (CF) fed animals (P<0.05)

Reperfusion after 6 min myocardial ischemia induced rapidly developing, severe arrhythmias (Table 3). RF1 and RF2 diets significantly decreased the incidence of reperfusion induced arrhythmias after myocardial ischemia.

The other diets offered somewhat less protection. Although the survival rate after feeding RF4 diet was significantly lower and the incidence of ventricular fibrillation was higher after feeding RF4 diet as compared to the other RF diets, the overall survival during myocardial ischemia and reperfusion did not change significantly (e.g. RF1=60%, RF2=60%, RF3=54%, RF5=57 vs. RF4=33%, no significant difference), due to the higher survival during coronary artery occlusion in the RF4 group.

Preliminary findings from parallel investigations on the composition of fish meat after different fish food suggest that fish meat used for preparing the rat food of the RF4 group was originated from carp group fed on fish oil fortified feed showed the best growth, however, producing the leanest fish fillet.

This is represented by the smallest amount of crude fat in RF4 (52 g/kg, Table 1) in the present investigations. As a result, this group of rats were fed smaller amount of fish fat, in spite of adding the same weight percentage of fish meat to the rat food.

The detailed analysis of fish meat and the changes in the composition of rat tissue as a result of dietary treatment needs further investigations.

CONCLUSION

We may conclude that as a result of the 4-week long feeding period in rats there were no significant differences among the five dietary groups, i.e. feeding fish meat after different fish food composition, as concerning the body weight, the baseline electrocardiogram and blood pressure, the blood lipid parameters, or the response to 6 min myocardial ischemia and 5 min reperfusion in rats.

These data suggest that each of the present experimental diets (RF1–RF3, RF5) contained enough polyunsaturated fatty acids to evoke protection against the development of myocardial ischemia–reperfusion induced arrhythmias in anaesthetized rats, as compared to the control, saturated fatty acid rich diet.

Supplying fish meat, rich in n–3 type polyunsaturated fatty acids, significantly decreased the incidence of myocardial ischemia–reperfusion induced arrhythmias in the rat, and increased the survival rate. These results confirm previous investigations that feeding fish oil or fish meat may offer cardioprotective effect.

The present results support that consumption of farmed fish, like carps, may result in similar protective effects to the marine food, thereby offering a good alternative to human dietary suggestions for continental countries, not having fresh marine food. However, the observed differences in the effects of fish meat containing diets in the present investigations

are warning, that when using oil enriched foods care should be taken both about the quantity and the balanced availability of the long chain n–6 and n–3 fatty acids in the diet.

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ЕВАЛУАЦИЈА ПОТЕНЦИЈАЛНОГ АНТИАРИТМИЧНОГ ЕФЕКТА РИБЉЕГ МЕСА ЗА ВРЕМЕ ИСХЕМИЈЕ МИОКАРДА-РЕПЕРФУЗИЈЕ КОД ПАЦОВА

Nikolett Morvay¹, Judit Péter-Szűcsné², Ljiljana M. Kostadinović³, Olivera M. Djuragić³,
Bojana M. Kokić³, Istvan Csengeri⁴, Istvan Lepran^{1*}

¹Department of Pharmacology, Faculty of Medicine, University of Szeged, Hungary

²Institute of Animal Sciences and Wildlife Management, Faculty of Agriculture,
University of Szeged, Hungary

³Универзитет у Новом Саду, Институт за прехранбене технологије у Новом Саду,
21000 Нови Сад, Булевар цара Лазара бр. 1, Србија

⁴Research Institute for Fisheries, Aquaculture and Irrigation (HAKI) Szarvas, Hungary

Сажетак: У овом раду је истраживан потенцијални антиаритмични ефекат исхране рибљим месом код пацова. Рибље месо шарана, храњеног експерименталном храном обогаћеном есенцијалним масним киселинама, мешано је са комерцијалном храном за пацове и давано експерименталним пацовима у току 4 недеље. По истеку овог периода, пацови су анестезирани и индуковане су аритмије изазване исхемијом миокарда–реперфузијом тако што је коронарна артерија блокирана 6 минута, да би потом била примењена реперфузија. Рибље месо, богато у n–3 полинезасићеним масним киселинама, значајно је смањило појаву аритмија изазваних исхемијом миокарда–реперфузијом и повећало постотак преживљавања. Ови резултати потврђују претходна истраживања да исхрана рибљим уљем или рибљим месом могу да пруже кардио–заштиту и да конзумирање гајене рибе, као што је шаран, може резултирати у сличним заштитним ефектима које пружају плодови мора.

Кључне речи: пацови, исхемија миокарда–реперфузија, оброк са рибљим месом, кардио–заштита

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