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Paraoxonase Activity and Concentration of Indicators of Lipids Status in the Serum of Cardiac Patients

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A B S T R A C T

In this research, we measured the activity of paraoxonase (basal and activated) enzyme, and components of lipid status components (total cholesterol, LDL cholesterol, HDL cholesterol and Apo A I) in the serum of patients, undergoing bypass surgery. We also tested how the applied EKC affected changes of defined indicators. Measuring of all the given parameters was conducted prior to the operation, 90 minutes, 1.5 hour, 6 hours, 24 hours and 72 hours, on 29 patients (11 of them did undergo myocardium revascularization with the application of EKC, while the rest of them did not). Activity of paraoxonase (both basal and activated) changes significantly during the postoperative period, in relation to pre-operative values, $p < 0.05$. Total cholesterol concentration is reduced in both examined groups, regardless of the application of EKC. This trend is also accompanied by LDL cholesterol concentration. On the other hand, HDL cholesterol concentration during post-operative period does not indicate any significant statistical change in relation to pre-operative values, while we noticed difference with regard to EKC application, 90 minutes after surgery. This change of lipid status indicator is partly due to heparin, a stimulator of lipoprotein lipase that was applied during the surgery. Our conclusion is that lipid profile changes significantly after the bypass surgery, mostly regardless of the application of EKC.

Key words: *paraoxonase activity, lipids status, cardiac patients.*

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Introduction

Paraoxonase, aryldialkylphosphatase is a serum esterase, synthetized in liver. It is a catalyzor for hydrolysis of organic-phosphoric compounds, like paraoxon, for example. It is capable of hydrolyzing esters of phosphatic and phosphitic acid, as well as esters of aromatic carboxyl acids. When it comes to its structure, human serum paraoxonase is a richly glycosylated protein, molecular mass of 45 kDa. It is bonded by its N-thermal hydrophobic domain to Apo A I apolipoprotein HDL particles, a high-density lipoprotein. Determining paraoxonose activity can be useful for discovering resistance to atherosclerosis development. Namely, it has been discovered that paraoxonase takes part in the inhibition of low-density lipoprotein particles' oxidation; therefore, it is considered to be the independent factor of protection against atherogenesis; that is why most of information given in the literature relate the changes in the activity of this enzyme with cardiac disorders[1–3].

Changes in the paraoxonase activity, conditioned by the surgery, were measured after kidney transplantation[4].

Our examining encompassed 29 patients, who have undergone bypass surgery. Only 18 of them did not undergo revascularization of myocardium, supported by extracorporal circulatory system, while the rest of them did.

Apart from paraoxonase activity (both activated and inactivated), we also determined lipid status indicators in the serum of these patients: LDL cholesterol, HDL cholesterol and Apo A I.

We were interested in how the values of given indicators vary the first few days after the surgery, and whether or not we can conclude that the intensity and direction of the changes depends on the use of extracorporal circulation.

Patients and Methods

Upon obtaining Hospital Ethics Committee approval and informed consent from patients, 29 patients undergoing the operative procedure of aortocoronary bypass between November 2001 and February 2002 were enrolled in this prospective clinical study. Inclusion criteria were triple coronary disease, age 60–70 years, and no use of antioxidant medication (allopurinol, mannitol, capropril and deferoxamine) or anti-inflammatory drugs (corticosteroids, acetylsalicylic acid and its derivatives). Patients were divided into two groups: EKC(+); n = 11 on conventional myocardial revascularization with CPB support, and EKC(–); n = 18 submitted to open heart procedure of myocardial revascularization without extracorporal circulation. Exclusion criteria included left ventricular ejection fraction < 40%, chronic inflammatory disease, diabetes mellitus, and previous cardiosurgical procedure.

Anesthesiology technique and monitoring

All patients received premedication with morphine (Morphine Merck®, Merck KgaA, Darmstadt, Germany), 10 mg i.m. one hour preoperatively. The general anesthesia protocol was the same for all patients and included induction with 0.1 mg/kg midazolam (Dormicum®, F. Hoffman-La Roche Ltd., Basle, Switzerland), 25 μg/kg alfetanyl (Rapifen®, Janssen Pharmaceutica, Beerse, Belgium) and 0.5 mg/kg i.v. atracurium (Tracrium®, The Wellcome Foundation Ltd., London, UK). Upon orotracheal intubation, patients were connected to mechanical ventilation (tidal volume of 12 ml/kg and frequency of 12 inspirations/minute) (Cato, Dräger, Lübeck, Germany). Anesthesia was maintained by a mixture of nitrogen and oxygen (60%:40%) and isoflurane (Forane®, Abbott Laboratories S.A., Abbott Park, IL, USA) of 1–1.3 minimal alveolar concentration.

Patient monitoring consisted of electrocardiography (ECG), pulse oximetry, and capnography. Invasive arterial pressure was monitored after radial artery catheter puncture (Arrow International, Reading, PA, USA). Using Seldinger technique and medial approach, a 3-luminal central venous catheter (Arrow International, Reading, PA, USA) was introduced through the right internal jugular vein for the right atrium pressure monitoring. The pressure curves and numerical values were monitored on the screen (Hewlett Packard Viridia CMS, Hewlett Packard, Boeblingen, Germany).

Operative technique

Upon access to the heart through medial sternotomy and pericardiectomy following previous graft preparation, extracorporal circulation is established by the aorta and right atrium double-stage cannulation. Artificial ventricular fibrillation is provoked, the aorta is clamped, and Buckberg cardioplegic solution is injected into the aortic bulb. During the creation of anastomosis, retrograde cardioplegia through the cannula in coronary sinus is used. Before the creation of proximal anastomoses, warm blood is administered retrogradely, and the aortic clamp is released upon rhythm restitution.

In a procedure without use of the extracorporal circulation device, access to the heart is the same, whereby exposure of the target vessels and stabilization of the myocardial wall are achieved by use of Octopuss 3 and Starfish vacuum stabilizers (Medtronic Inc., Minneapolis, MN, USA). Coronary circulation is ensured by the placement of an appropriately sized intracoronary shunt. Distal anastomoses are created by the continuous suture technique with Prolen 7–0, and proximal anastomoses with Prolen 6–0.

Blood samples were taken prior to surgery (1 day before) and after surgery (90 minutes, 6 hours, 24 hours and 72 hours).

The blood was centrifuged for 15 minutes/3000 r.p.m. in Heraus Minifuga T. centrifuge. Serum was stored at –40 °C, till the analysis.

Paraoxonase assays were performed in the absence of sodium chloride (basal activity), and in the presence of 1 mol/L NaCl (NaCl stimulated activity)[5,6]. Initial rates of hydrolysis of paraoxon (O,O-diethyl-O-p-nithrophenylphosphate; Sigma Chemical Co, London, UK) were determined by measuring liberated p-nitrophenol at 405 nm at 37 °C on a Tehnicon RA-1000 autoanalyzer (Bayer, Milan, Italy).

The basal assay mixture included 2.0 mmol/L paraoxon and 2.0 mmol/L of Ca-chloride in 0.1 mol/L Tris-HCl buffer, pH 8.0. To 350 μL the reagent mixture 10 μ of serum was added. For the NaCl-stimulated assay, 1 mol/L NaCl was added into above described mixture. Phenylacetate was used as a substrate to measure arylesterase activity[7]. Initial rates of hydrolysis were determined spectrophotometrically by detecting the increase in phenol concentration at 270 nm. The reaction mixture contained 2.0 mmol/L phenylacetate (Sigma) and 2.0 mmol/L Ca-chloride in 0.1 mol/L Tris-HCl buffer, pH 8.0.

The total cholesterol, LDL and HDL cholesterol, Apo A I have been measured on the Olympus AU 2700 analyzer, and we also used the original putty by the same manufacturer.

Correction of plasma dilution

Post-operative concentration values of all measured biochemical indicators in the serum were corrected according to McColl et al., the following formula:

corrected concentration = measured concentration x (l – Htc after surgery)/ (1 – Htc prior to surgery)[8].

Statistical analysis

The results obtained (of absolute concentration or activity) were processed by

the standard statistical methods using statistical program Sigmastat (Version 2.0, Jandel Co.). The nonparametric test, Mann-Whitney, was used to test differences of two studied groups. The Friedman repeated measures analysis of variance on ranks was performed to test differences between preoperative and post-operative values. The $p < 0.05$ value is considered to be statistically significant.

Results

Table 1 shows absolute values of POX activities (basal activity and NaCl- stimulated activity) and HDL cholesterol expressed by median and range. By means of Mann-Whitney test, we compared values of the given parameters at the same point of determining group of patients who did not undergo EKC, and those who did.

Based on the obtained values, we can conclude that the measured basal activity paraoxsonase statistically differ only in the measuring point 24 hours after surgery, depending on whether the myocardium revascularization was made with or without extracorporal circulation.

Furthermore, by applying Friedman repeated measures analysis of variance on ranks we compared activities of basal POX at post-operative points, to pre-operative activity. The results obtained indicate that at all post-operative measuring points, the basal POX activities statistically significantly differ, compared to pre-operative activities values ($p < 0.05$).

By using Mann-Whitney test, we have concluded that activity of NaCl-stimulated POX differs at the measuring point 24 hours after surgery, only in those patients that have undergone EKC, and those that have not.

By Friedman repeated measures analysis of variance on ranks, we have also compared activities of NaCl-stimulated post-operative measuring points to pre-operative activities. At all points of measuring catalytic concentrations NaCl-stimulated POX after surgery, we have found statistically significant change, in relation to pre-operative activity ($p < 0.05$).

Table 1 shows the result of statistical significance of differences in HDL cholesterol concentration at the individual measuring point, between groups of patients that have undergone EKC, and those that have not. Considering the fact that statistic significance limit has the value $p < 0.05$, it is obvious that the concentration of HDL in the examined groups of patients differs only at the first post-operative measuring point, i.e. 1.5 h after surgery. This is, at the same time, the only measuring point at which HDL cholesterol concentration values differ, with regard to pre-operative concentration.

Table 2 shows absolute values of the overall cholesterol concentration, LDL cholesterol and Apo A I, given in median and range. By using Mann-Whitney test, we compared values of the given parameters at the same measuring point, of the patients that have undergone EKC and those that have not. The value $p < 0.05$ is determined as the border level of significance. The statistically significant difference, related to the application of EKC, has not been found at none of the measuring points. By Friedman repeated measures analysis of variance on ranks, we have compared values of the overall cholesterol concentration after surgery with those before the surgery. At all post-operative measuring points the value of the overall cholesterol concentration statistically differs significantly from the pre-operative value.

We could say that the trend of the total cholesterol follows, to some extent, LDL cholesterol; i.e. at all measuring points after the surgery, we have discovered (by using Friedman repeated measures analysis of variance on ranks

TABLE 1
ACTIVITY OF POX (BASAL AND NaCl-STIMULATED), AND CONCENTRATION OF HDL CHOLESTEROL IN THE SERUM OF THE EXAMINEES

	POX basal activity (U/L)			POX NaCl-stimulated activity (U/L)			HDL cholesterol (mmol/L)		
Time	EKC (+) N = 11	EKC (–) N = 18	p	EKC (+) N = 11	EKC (–) N = 18	p	EKC (+) N = 11	EKC (–) N = 18	p
Pre-operative	290 (135–1,245)	315 (135–1,020)	0.605	576 (345–2,880)	640 (275–2,265)	0.574	0.68 (0.48–1.31)	0.84 (0.61–1.02)	0.116
After 1.5 h	218 (115–940)	335 (197–715)	0.096	483 (250–2,050)	705 (420–1,5359)	0.088	0.60 (0.38–0.90)	0.73 (0.41–0.98)	0.015
After 6 h	225 (125–1,020)	370 (145–755)	0.121	495 (270–2,190)	785 (310–1,695)	0.126	0.66 (0.51–0.95)	0.80 (0.64–1.05)	0.065
After 24 h	230 (100–955)	380 (180–665)	0.041	475 (210–2,165)	385 (835–1,515)	0.046	0.79 (0.46–1.03)	0.90 (0.71–1.33)	0.076
After 72 h	225 (105–825)	360 (175–839)	0.101	488 (200–1,975)	790 (385–1,500)	0.101	0.74 (0.38–1.15)	0.77 (0.56–1.27)	0.323

TABLE 2
OVERALL CONCENTRATION OF TOTAL CHOLESTEROL, LDL CHOLESTEROL, AND APO A I IN THE SERUM OF EXAMINEES

	Total cholesterol (mmol/L)			LDL cholesterol (mmol/L)			Apo A I (g/L)		
Time	EKC (+) N = 11	EKC (–) N = 18	p	EKC (+) N = 11	EKC (–) N = 18	p	EKC (+) N = 11	EKC (–) N = 18	p
Pre-operative	4.74 (2.95–7.13)	5.62 (2.76–7.68)	0.291	2.78 (1.82–4.61)	3.8 (1.39–5.57)	0.357	1.09 (0.80–1.77)	1.17 (1.02–1.48)	0.185
After 1.5 h	3.74 (2.54–5.95)	5.04 (2.30–5.67)	0.157	2.38 (1.41–4.50)	3.28 (1.41–4.36)	0.208	0.91 (0.67–1.31)	1.06 (0.88–1.36)	0.056
After 6 h	3.54 (2.14–6.10)	4.29 (2.21–5.47)	0.164	2.19 (1.14–4.25)	2.89 (1.33–4.12)	0.381	0.96 (0.73–1.29)	1.03 (0.84–1.48)	0.157
After 24 h	2.84 (1.69–4.71)	3.95 (2.05–4.82)	0.088	1.65 (0.71–2.85)	2.49 (1.05–3.18)	0.065	0.93 (0.57–1.17)	0.99 (0.85–1.47)	0.111
After 72 h	2.98 (1.95–3.85)	3.41 (2.18–4.64)	0.076	1.74 (0.81–2.81)	2.05 (1.07–2.88)	0.056	0.87 (0.67–1.05)	0.99 (0.76–1.47)	0.111

(Dunnett method) statistically significant difference with regard to pre-operative LDL concentration.

Using the same method, we have discovered that the measured concentration of apolipoprotein A I, at all post-operative points, statistically differs from pre-operative measuring points.

Discussion

For most of its part, the research, relating lipid status with heart surgery, is a study-observation of statine therapy flavostatine, lovostatine, paravastatine, probucole) during longer post-operational period (weeks, months).It is the maintenance of high concentration of ateroprotective HDL and consistent LDL/HDL concentration ratio, ranging from 2.2–2.4, that postpones formation of atherosclerotic lesions in the transplant segment[9,10].

We were interested in whether the changes of lipid status components (total cholesterol, LDL cholesterol, Apo A I) are visible the first few days after surgery, and whether the application of extracorporal circulation can cause differences in dynamics changes. It is the application of cardiac-pulmonary bypass during a heart surgery, with the blood components being exposed to non-physiological surface, that takes part in the activation of complements, sequestration of leukocytes in lungs, and the increased volume of lipid peroxidation[11]. The development of system oxidative stress increases reperfusion of highly oxygenized blood, which is the result of ishemia.

By measuring paraoxonase activity, we wanted, to some extent, to support the obtained results of the parameters given above, since that enzyme is related to HDL particle. The authors have pointed to the lowered POX activity in patients that have suffered myocardium heart attack, 72 hours after they experienced chest pain[12]. Paraoxonase is an enzyme, susceptible to genetic polymorphism, which is based on substitution by Arg aminoacid, at Gln point, at position 55 of polypeptide chain. Furthermore, the POX activity depends on the concentration of calcium ions that is why, in order to define its activity, we have avoided use of EDTA, which forms complexes with Ca-ions (all parameters have been measured in serum).

There is no literature regarding the change of POX activity after heart surgery. Activity of that hydrolase was determined only in patients that have undergone kidney transplantation[4], and after laparoscopic colecistoctomy[13].

It is the reduced POX/HDL and POX Apo A I ratio that causes reduction of antioxidative HDL capacity, which can be risk factor for atherosclerosis development in transplanted patients[4].

Significantly reduced concentration of HDL-cholesterol has been described in patients after AIM, with the simultaneous increase of the overall I LDL cholesterol concentration[14].

Most likely, it is the use of heparin during surgery that is partly responsible for reduction of LDL cholesterol concentration, which results in reduction of the overall cholesterol concentration[15].

Miida et al. have measured changes in the overall cholesterol, trigliceride and apolipoproteine A I, B and E concentration, in the patients with acute myocardium heart attack, who have undergone urgent coronary angiography, within the period of 6 hours after heart attack[16].

According to them, the reduction of cholesterol and trigliceride concentration is partly due to the use of heparin during the surgery. Furthermore, in this research the authors have divided patients into 2 groups: those with the low initial HDL concentration (with statistically significant increase of HDL concentration

discovered after the surgery), and those, whose initial HDL amount came within referent scope, and there was no significant increase of concentration after the surgery. This can be partly explained by the fact that heparin stimulates the release of LPL enzyme, a lipoprotein lipase, which helps the hydrolyze of triglyceride-rich lipoproteins (VLDL, IDL, LDL). Kekki et al. suggest that it is the activity of LPL that provides lipid components for HDL, during the lipolyze of triglyceride-rich lipoproteins[17].

Another state of increased risk from coronary disease development is familiar hypercholesterolemy. One of the ways to treat these patients is HELP (heparin-induced extracorporal lipoprotein precipitation). This method is based on LDL precipitation with heparin, at low pH, with the absence of two-valent cations[7].

The bypass surgery is closely related to the use of heparin, which keeps the blood liquid. This is especially important for EKC patients, considering that the contact between the blood and alien surface can activate the coagulation cascade, by activating Hageman's factor.

The results of this research have indicated the significant change of the lipid profile after the bypass surgery, which is mostly independent of the EKC use.

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AKTIVNOST PARAOKSONAZE I KONCENTRACIJA INDIKATORA LIPIDNOG STATUSA U SERUMU KARDIOLOŠKIH BOLESNIKA

S A Ž E T A K

U ovom istraživanju mjerena je aktivnost paraoksonaze (bazalne I aktivirane), te komponenti lipidnoga statusa (ukupni kolesterol, LDL kolesterol, HDL kolesterol i Apo A I) u serumu bolesnika podvrgnutih ugradnji premosnice. Mjerenja svih parametara načinjena su prije operacije, 90 minuta, 1.5 sat, 6 sati, 24 sata i 72 sata nakon operacije kod 29 bolesnika (kod njih 11 je revaskularizacija miokarda tijekom operacije načinjena uz potporu izvantjelesnog krvotoka, a kod ostalih nije korištena EKC). Aktivnost paraoksonaze (bazalne i aktivirane) značajno se mijenja tijekom postoperativnog perioda u odnosu na predoperativne vrijednosti ($p < 0.05$). Koncentracija ukupnog kolesterola smanjuje se u obje ispitivane skupine, neovisno o primjeni EKC. Taj trend prati i koncentracija LDL kolesterola. S druge strane, koncentracija HDL kolesterola tijekom postoperativnog perioda se ne mijenja značajno u odnosu na predoperativne vrijednosti tijekom postoperativnog perioda. Navedene promjene pokazatelja lipidnoga statusa mogu djelomično biti uzrokovane primjenom heparina tijekom operacije. On može stimulirati lipoprotein lipazu. Može se zaključiti da se profil lipida značajno mijenja nakon ugradnje premosnica, uglavnom neovisno o primjeni EKC.