Evaluation of Lipid Peroxidation and Antioxidant Status in Myocardial Tissue and Coronary Sinus Blood of Patients Undergoing Cardiopulmonary Bypass

Kardiyopulmoner Baypas Uygulanan Hastalarda Miyokard Dokusu ve Koroner Sinüs Kanında Lipid Peroksidasyon ve Antioksidan Durumun Değerlendirilmesi

Hüray İŞLEKEL[1] Baran UĞURLU[2] Eyüp HAZAN[2]

Nurten SAYDAM[1] Okay SAYDAM[1] Öztekin OTO[2]

Gül GÜNER[1]

Abstract

Reperfusion of the ischemic heart of the patient on cardiopulmonary bypass (CPB) causes generation of oxygen derived free radicals which play an important role in post-ischemic tissue damage. These free radicals are removed by intracellular and extracellular scavenger enzymes and low molecular weight antioxidant substances. The present study was undertaken to evaluate the major antioxidant status markers, namely; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH Px), glutathione reductase (GR) activities, as well as oxidized glutathione (GSSG), reduced glutathione (GSH) concentrations and GSH/GSSG ratio in myocardial tissue and plasma obtained from coronary sinus blood samples of patients undergoing CPB (n=19). All samples were also analyzed for thiobarbituric acid reactive substances (TBARS) levels for lipid peroxidation assessment. Myocardial tissue samples from the right atrial free wall of patients were obtained before aortic cross clamping (basal status) (T0) and just after aortic declamping (beginning of reperfusion) (T 1). Blood samples were obtained from the coronary sinus of heparinized patients under total CPB at six different intervals: before aortic cross clamping (basal status) (T0), immediately after aortic cross clamping, while the first antegrade cold blood cardioplegia was administrated (beginning of ischemia) (T1), while the second antegrade cold blood cardioplegia was administrated (app. 20 min after clamping) (T2), before aortic declamping, while warm blood cardioplegia was administrated (T3), just after aortic declamping (beginning of reperfusion) (T4), 20 min after declamping (late reperfusion) (T5). In myocardial tissue, as compared to the basal status, while SOD activities and GSSG concentrations were found significantly elevated in T1, significant decreases were observed in GSH Px and GR activities as well as the GSH/GSSG ratio in the same period ($p<0.05$). In plasma, compared to the basal status, SOD activities were significantly decreased in T1, T2 and T4; CAT activities were significantly decreased in T1, increased in T4 and T5, GSH Px activities were significantly decreased both in T1 and T2. While the GSH/GSSG ratio was found significantly elevated in T2 compared to only T1, GSH concentrations were augmented in T2 when compared to both T0 and T1. Significant lipid peroxidation is determined neither in myocardial tissue nor in plasma at all time intervals. It can be concluded that; myocardial tissue and coronary sinus plasma antioxidant mechanisms are changed without evident lipid peroxidation in patients on cardiopulmonary bypass undergoing coronary bypass surgery using hot shot perfusion in combination with cold blood cardioplegia; however the antioxidant status changes observed in the plasma do not completely reflect the antioxidant capacity of the myocardial tissue.

Keywords : lipid peroxidation, antioxidant status, coronary sinus plasma, myocardial tissue, cardiopulmonary bypass

Özet

Kardiyopulmoner baypas altındaki hastalarda iskemik kalbin reperfüzyonu, post-iskemik doku hasarında rol oynayan oksijen kaynaklı serbest radikallerin oluşumuna yol açmaktadır. Bu serbest radikaller hücre içi ve dışı antioksidan enzimler ve moleküller tarafından ortadan kaldırılırlar. Bu çalışmada kardiyopulmoner baypas altındaki hastalarda (n=19) miyokard dokusu ve koroner sinüs kan

1 Dokuz Eylül University, School of Medicine, Departmens of Biochemistry İnciraltı, 35340 İZMİR
2 Dokuz Eylül University, School of Medicine, Departmens of Thoracic and Cardiovascular Surgery, İnciraltı, 35340 İZMİR

örneklerinde temel antioksidan durum belirteçleri olan; süperoksid dismutaz (SOD), katalaz (KAT), glutatyon peroksidaz (GSH Px), glutatyon redüktaz (GR) aktiviteleri yanısıra okside glutatyon (GSSG), redükte glutatyon (GSH) konsantrasyonları ve GSH/GSSG oranı incelendi. Ayrıca tüm örneklerde lipid peroksidasyon göstergesi olarak tiyobarbitürik asid ile reaksiyona giren madde miktarları belirlendi. Miyokard doku örnekleri hastaların sağ atrium serbest duvarından aortik kros klemp öncesinde (bazal durum) (T0) ve kros klempin kaldırılmasından hemen sonra (reperfüzyon başlangıcı) (T1) alındı. Kan örnekleri ise kardiyopulmoner baypas altında, heparinize edilmiş olan hastaların koroner sinüslerinden altı değişik zamanda elde edildi: aortik kros klempleme öncesinde (bazal durum) (T0), kros klemplemeden hemen sonra, ilk antegrad soğuk kan kardiyoplejisi verilirken (iskemi başlangıcı) (T1), ikinci antegrad soğuk kan kardiyoplejisi verilirken (klemplemeden yaklaşık 20 dk sonra) (T2), kros klempin kaldırılmasından önce sıcak kan kardiyoplejisi verilirken (T3), kros klempin kaldırılmasından hemen sonra (reperfüzyon başlangıcı) (T4), kros klempin kaldırılmasından 20 dk sonra (geç reperfüzyon) (T5). Miyokard dokusunda; bazal değere (T0) göre, T1 de SOD aktivitesi ve GSSG konsantrasyonu anlamlı yüksek bulunurken, GSH Px ve GR aktiviteleri ve GSH/GSSG oranı azalmış olarak belirlendi ($p<0.05$). Plazmada; bazal değere (T0) göre, SOD aktivitesi T1, T2 ve T4 zamanlarında anlamlı düzeyde azalmış, KAT aktivitesi T1 de azalırken T4 ve T5 zamanlarında artmış, GSH Px aktivitesi ise hem T1 hem de T2 de azalmış olarak belirlendi ($P<0.05$). GSH/GSSG oranı T2 de T1 e kıyasla artmış olarak belirlenirken, GSH konsantrasyonu T2 de hem T1 hem de T0 a göre yüksek bulundu ($p<0.05$). Miyokard dokusunda ve plazmada bazal değerler ile karşılaştırıldığında anlamlı lipid peroksidasyon ürünü varlığı hiç bir zaman için saptanmadı. Sonuç olarak, koroner bypass operasyonlarında kardiyopulmoner baypas altında soğuk kan kardiyoplejisi yanısıra hot shot sıcak kardiopleji uygulanan hastalarda miyokard dokusu ve koroner sinüs plazmasında belirgin bir lipid peroksidasyonu olmaksızın antioksidan mekanizmalar etkilenmektedir, ancak plazma antioksidan durumundaki değişiklikler miyokard dokusununun antioksidan kapasitesini tamamen yansıtmamaktadır.

Anahtar kelimeler : antioksidan durum, lipid peroksidasyonu, koroner sinüs plazması, miyokard dokusu, kardiyopulmoner baypas.

INTRODUCTION

Cardiopulmonary bypass (CPB) is a widely used operative technique in cardiovascular surgery (1). However, oxygen free radicals produced during CPB due to ischemia-reperfusion and those generated by the "whole body inflammatory response" contribute to the morbidity and mortality after cardiac surgery. Oxygen free radicals can damage biological molecules including proteins, lipids and nucleic acids; it is particularly peroxidation of lipid membranes which results in disturbance of cell structure and function (2). Although the vascular endothelial cells and activated neutrophils are implicated in part as free radical source in ischemia reperfusion process during CPB, most of the free radicals are generated intracellularly by electron leakage from the mitochondrial electron transport chain and cellular membranes. Starting from the onset of reperfusion and induce biomembrane peroxidation (3-5). Thus the intracellular antioxidant capacity of the tissue affected from ischemia reperfusion is particularly important for the primary endogenous defense against free radical induced injury (6). The myocardium contains abundant superoxide dismutase (SOD), and therefore the superoxide formed upon return of molecular oxygen will be rapidly dismutated to hydrogen peroxide. A selenoenzyme, glutathione peroxidase (GSH Px) and catalase (CAT) are the unique enzymes to degrade hydroperoxides and therefore act in concert with SOD. However, in heart muscle, since catalase is at low concentrations, hydrogen peroxide is mainly reduced to water by GSH Px using reduced glutathione (GSH). In this process an equivalent of glutathione disulfide (GSSG) is produced and recycling of GSH through NADPH-dependant glutathione reductase (GR) must be achieved for the protection to be maintained (7,8).

There is a considerable number of studies investigating the oxidative stress in coronary sinus plasma during cardiac surgery with CPB. In most of these studies some of the antioxidants and/or lipid peroxidation end products are assessed (1,2,5,9-19). Although the results of these studies reveal the altered antioxidant capacity during CPB, conflicting experimental data have been published, probably due to the various cardioplegia techniques employed and/or the differences between sampling times used in the studies.

Little is known about the changes of the intracellular antioxidants and lipid peroxidation levels in myocardial tissue, as well as to which extent the changes of these antioxidants and lipid peroxidation product contents in coronary sinus plasma may reflect the status of the myocardial tissue during CPB. To date there is a limited number of studies evaluating the lipid peroxidation and the antioxidant status of the myocardial tissue (11,16,20-22). Furthermore, we could not find any report in the literature on the cor-

relation between the myocardial tissue and the coronary sinus plasma values of the oxidative stress parameters.

This study was carried out to investigate the changes of the myocardial tissue and plasma antioxidant defense systems against oxidative stress and lipid peroxidation extent during CPB. With this aim, we determined the activities of major antioxidant enzymes (SOD, CAT, GSH Px and GR), in addition to GSH, GSSG concentrations and the GSH/GSSG ratio in tissue and coronary sinus plasma. As an index of lipid peroxidation we measured thiobarbituric acid reactive substances (TBARS) levels.

MATERIALS AND METHODS

Patients and samples

Nineteen coronary bypass patients operated under CPB in the Department of Thoracic and Cardiovascular Surgery, Dokuz Eylül University, School of Medicine from May 1997 to April 1998 were included in this study. Their mean (±SD) age was 65.47 ± 4.45 and the woman/man ratio was 8/11. Informed consent was obtained from all patients and the study was approved by our local ethics committee.

In order to obtain homogenous groups and to apply the same surgical technique, the study group was chosen from elective coronary bypass patients, with no recent myocardial infarction history, left main coronary artery disease and accompanying pathologies such as diabetes mellitus, chronic renal insufficiency or cerebrovascular disease. All of the patients were operated by the same surgical team. Median sternotomy under general anesthesia was applied to all patients. Then with routine aortic and bicaval canulation cardiopulmonary bypass is established. During the operation, antegrade cold blood cardioplegia (8-10°C, 1000 mL: 300 mL blood, 700 mL Abbott PlegisolB) was administrated twice; the first one being just after the aortic cross clamping and the second one approximately twenty minutes after the first one. Before cross clamp release, warm pump blood (37°C, 500 mL) was induced to all patients. All patients were heparinized 20-30 minutes before the onset of CPB with a dose of 3mg/kg heparin in order to maintain four times increased activated clotting time (ACT) during the whole procedure. Blood samples from the coronary sinus and myocardial tissue samples from the right atrial free wall were obtained at the intervals shown in Table I.

All blood samples were centrifuged at 3000 rpm for 10 min. The plasma obtained, and the tissue samples washed with isotonic saline solution were stored at -70°C before analysis.

Tissue Preparation

10 % tissue homogenates were prepared in 50 mM phosphate buffer solution (pH 7.4). The homogenates were centrifuged at 100 000 x g for 60 min to obtain the cytosolic fraction. All of the parameters except TBARS were assayed in the cytosolic fraction. TBARS concentration was determined in 10% tissue homogenates prepared in 150 mM ice cold KCl.

Biochemical analysis

SOD Determination

The method used for superoxide dismutase activity assay is a slight modification of an indirect inhibition assay developed by Sun et al (23). Xanthine-xanthine oxidase is utilized to generate a superoxide flux. Nitroblue tetrazolium reduction by O^{-}_{2} to blue formazon is determined at 560 nm spec-

Table I a: Tissue sampling times

T0	Under total CPB, before aortic cross clamping (basal status)
T1	Under total CPB, just after aortic declamping (beginning of reperfusion)

Table I b: Plasma sampling times

T0	Under total CPB, before aortic cross clamping (basal status)
T1	Under total CPB, immediately after aortic cross clamping, while the first antegrade cold blood cardioplegia was administrated (beginning of ischemia)
T2	Under total CPB, while the second antegrade cold blood cardioplegia was administrated (app. 20 min after clamping)
T3	Under total CPB, before aortic declamping, while warm blood cardioplegia was administrated
T4	Under total CPB, just after aortic declamping (beginning of reperfusion)
T5	Under total CPB, 20 min after declamping (late reperfusion)

trophotometrically. The SOD in the samples competes for O^-_2, inhibiting the reaction rate of O^-_2 with the indicator scavenger (NBT). The percentage of this inhibition is the basis on which the amount of activity is estimated. The results were expressed as U/mL for plasma and U /mg protein for tissue.

CAT Determination

Catalase activity was determined using the method of Goth (24) which is a spectrophotometric assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdate. The results were expressed as U/mL for plasma and U /mg protein for tissue.

GPX Determination

Glutathine peroxidase activity was determined with a modified procedure of Paglia et al (25) and Lawrence et al (26). In this method, reduced glutathione (GSH) concentration is kept constant with the addition of exogenous glutathione reductase (GR) and NADPH. GSH is oxidized to GSSG in the presence of GPX in the sample. GSSG is again reduced to GSH with GR catalysis. NADP production is followed with the absorbance decrease at 340 nm. The results were expressed as U/L for plasma and nmol/min/mg protein for tissue.

GR Determination

In the presence of GSSG and NADPH as substrate and coenzyme respectively, glutathione reductase in the sample catalyses NADP production. The reaction is followed with the absorbance decrease at 340 nm (27). The results were expressed as U/L for plasma and nmol/min /mg protein for tissue.

Glutathione Determination

Reduced and oxidized glutathione levels was determined according to the method of Teare et al (28). GSSG is reduced to GSH with GR and NADPH. The colored product formed with the reaction of GSH and DNTB (5,5' Ditiyo-bis 2-nitrobenzoic acid) is determined at 412 nm spectrophotometrically. The results were expressed as µmol/L for plasma and nmol /mg protein for tissue.

TBARS determination

TBARS determination was made in tissue homogenates and plasma using the methods of Uchiama (29) and Stocks (30), respectively. Malondialdehyde in the samples reacts with thiobarbituric acid. The color formation is assessed spectrophotometrically at 535 nm. The results were expressed as mol/L for plasma and nmol /mg protein for tissue.

Protein Determination

In tissue samples, total protein concentration in the cytosols and the homogenates were determined by Lowry's (31) method.

Statistical analysis

Results were expressed as mean ± standard error of mean. Differences between the paired time groups were analyzed by Wilcoxon Matched-Pairs Signed-Ranks test. Differences were considered significant at a probability level of $p<0.05$.

RESULTS

Statistically significant increase in tissue SOD activity and GSSG levels is observed with reperfusion compared to the basal status ($p<0.05$). Although the catalase activity and TBARS levels were also increased in the same period, this was not significant. With reperfusion, tissue GPX, GR activities and the ratio of reduced to oxidized glutathione were significantly diminished compared to the basal status ($p<0.05$), while the decrease in GSH levels alone was not statistically significant (Table II).

The paired differences in plasma SOD, CAT, GSH Px, GR activities and GSH, GSSG, TBARS levels and GSH/GSSG ratio in various paired time

Table II : The differences in paired time groups for tissue SOD; CAT; GSH Px; GR activities, GSH, GSSG, TBARS concentrations and GSH/GSSG ratio

Paired Times	Paired Differences							
	SOD (U/mg protein)	CAT (U/mg protein)	GSH Px (nmol/min/mg protein)	GR (nmol/min/mg protein)	GSH (nmol/mg protein)	GSSG (nmol/mg protein)	GSH/GSSG	TBARS (nmol/mg protein)
T0-T1 (n=19)	-4.24±1.77*	-0.27±0.38	7.99± 2.32*	14.18±4.93*	0.16±0.84	-0.02±0.01*	8.69± 3.25*	-0.14±0.06

*p<0.05

(+) positive values represent a decrease

(-) negative values represent an increase

groups are shown in Table III and Table IV. Statistically significant decrease is observed in plasma superoxide dismutase activities just at the beginning of ischemia, 20 min after ischemia while the first cardioplegic solution is given and at the beginning of reperfusion when compared to the basal status ($p<0.05$). In the blood, collected just after the administration of hot cardioplegic solution, superoxide dismutase activities seems to be significantly increased in comparison to the values belonging to the time of 20 min ischemia ($p<0.05$).

During the CPB, plasma catalase activities show a significant decrease at the beginning of ischemia, as compared to the basal status ($p<0.05$). Although an increase is observed in the activity of this enzyme with the beginning of reperfusion and 20 min reperfusion periods compared to the basal status, a significant decrease was observed just at the beginning of reperfusion when compared to the activity obtained just before the reperfusion and hot cardioplegia application ($p<0.05$).

In comparison to the basal status, glutathione peroxidase activities were significantly decreased with the beginning of ischemia and 20 min after ischemia ($p<0.05$).

Reduced glutathione is found significantly increased in plasma 20 min after ischemia while the first cardioplegic solution is given as compared to the basal status and to the beginning of ischemia ($p<0.05$).

The ratio of reduced to oxidized glutathione is significantly increased 20 min after ischemia while the first cardioplegic solution is given as compared to the beginning of ischemia ($p<0.05$).

There were no statistically significant differences between all these paired time groups for glutathione reductase, oxidized glutathione and TBARS plasma values.

Table III: The differences in paired time gruops for plasma antioxidant enzyme activities and TBARS levels

Paired Times		Paired Differences				
	n	SOD (U/mL)	CAT (U/mL)	GSH Px (U/L)	GR (U/L)	TBARS (μ mol/L)
T 0-T 1	19	2.48 ± 1.04*	1.02 ± 0.52*	13.05 ± 4.68*	- 1.95 ± 4.88	0.18 ± 0.12
T 0-T 2	19	1.87 ± 0.97*	0.75 ± 0.45	12.00 ± 6.44*	- 6.82 ± 5.35	0.09 ± 0.11
T 0-T 3	19	2.72 ± 1.51	- 0.12 ± 0.17	2.63 ± 3.76	- 7.87 ± 7.33	0.18 ± 0.17
T 0-T 4	19	3.16 ± 1.26*	- 1.86 ± 0.99*	3.22 ± 3.13	- 4.27 ± 5.51	0.16 ± 0.12
T 0-T5	19	3.14 ± 1.31	- 1.88 ± 0.71*	1.36 ± 4.41	- 6.73 ± 6.52	0.16 ± 0.09
T 1-T 2	19	0.48 ± 0.42	- 0.02 ± 0.02	- 1.61 ± 3.17	- 3.76 ± 3.01	- 0.08 ± 0.10
T 2-T 3	19	- 0.13 ± 0.31*	- 1.25 ± 0.70	- 9.53 ± 4.98	- 3.00 ± 7.24	0.04 ± 0.11
T 3-T 4	19	- 0.12 ± 0.384	2.11 ± 1.65*	0.33 ± 2.68	0.67 ± 5.97	- 0.06 ± 0.05
T 4-T 5	19	- 0.05 ± 0.18	0.52 ± 0.45	- 2.77 ± 3.28	- 2.55 ± 6.86	0.06 ± 0.12

*$p<0.05$
(+) positive values represent a decrease
(-) negative values represent an increase

Table IV: The differences in paired time gruops for the plasma glutathione parameters (GSH, GSSG, GSH/GSSG)

Paired Times		Paired Differences		
	n	GSH (μ mol/L)	GSSG (μ mol/L)	GSH/GSSG
T 0-T 1	19	0.30 ± 0.26	0.01 ± 0.02	1.06 ± 1.16
T 0-T 2	19	- 0.83 ± 0.35*	- 0.01 ± 0.03	- 3.57 ± 1.71
T 0-T 3	19	- 0.65 ± 0.38	0.01 ± 0.02	-2.18 ± 1.51
T 0-T 4	19	- 0.56 ± 0.32	- 0.01 ± 0.03	- 0.32 ± 1.31
T 0-T 5	19	0.29 ± 0.32	0.01 ± 0.02	0.88 ± 1.26
T 1-T 2	19	-0.98 ± 0.33*	- 0.01 ± 0.02	- 4.30 ± 1.57*
T 2-T 3	19	0.29 ± 0.39	- 0.01 ± 0.03	1.93 ± 2.28
T 3-T 4	19	- 0.45 ± 0.34	0.02 ± 0.02	-1.27 ± 1.99
T 4-T 5	19	0.25 ± 0.38	0.02 ± 0.02	1.08 ± 1.70

*$p<0.05$
(+) positive values represent a decrease
(-) negative values represent an increase

Biyokimya Dergisi
Ekim-Kasım-Aralık/ 1999
Sayı : 4, Cilt : 24

DISCUSSION

It has been shown that in patients undergoing cardiopulmonary bypass, termination of ischemia by the resumption of coronary perfusion, although necessary for myocardial recovery, can result in a paradoxical extension of ischemic damage, the so-called ischemia-reperfusion injury. A large body of evidence indicates that this injury is mediated by oxygen-derived free radicals, which are maximally produced at the onset of myocardial reperfusion (5). These free radicals may initiate lipid peroxidation by attacking the susceptible cellular components, especially the cell membrane which consists of lipids containing unsaturated double bonds. Lipid peroxides disturb the structure of cell membrane causing changes in permeability and possibly leading to cellular death. It is also known that cells have an elaborate defense system against free radicals, consisting of antioxidant enzymes and low molecular weight substances. These antioxidants help to keep the steady state concentrations of active oxygen at acceptable levels under physiological conditions, and their inhibition can induce a prooxidant state (32).

The results of our study on myocardial tissue parameters can be interpreted as follows: We have shown that, in myocardial tissue, most of the intracellular antioxidants change with reperfusion, in patients on cardiopulmonary bypass undergoing coronary bypass surgery. The increase in the tissue activity of superoxide dismutase, the unique antioxidant enzyme to dismutase the superoxide radical, generated the first in ischemia-reperfusion period might be explained with the transient substrate induction of this enzyme by the superoxide radicals presumably produced with reperfusion of myocardium (33).

The demonstration that tissue glutathione is oxidised to GSSG, and the decrease in the ratio of reduced to oxidized glutathione prove that intracellular antioxidant defenses are actively engaged during reperfusion of the heart. It is known that GSH, directly or indirectly as a cosubstrate of glutathione peroxidase, plays an essential protective role against oxygen reactive species. This mechanism results in increased formation of glutathione disulphide (13). This might explain our data on, the decrease in the glutathione peroxidase activity, the major enzyme that metabolizes hydrogen peroxide produced either by SOD reaction or other sources, by using reduced glutathione as cosubstrate (6). The concentration of reduced glutathione might be also diminished related to the decreased activity of glutathione reductase, the enzyme which accomplishes the recycling of reduced glutathione from glutathione disulphide. The reduction in both glutathione peroxidase and glutathione reductase activities might also be due to being overwhelmed by sudden generation of free radicals upon reperfusion as Kilgore et al have explained (4).

In our study catalase activities in myocardial tissue did not change in the time course of cardiopulmonary bypass. It is known that catalase is a peroxisomal enzyme which catalyses the two-electron dismutation of hydrogen peroxide to molecular oxygen and water as glutathione peroxidase does (7,8). The unchanged catalase activities in our study might be related to two factors: firstly, the low concentration of this enzyme making the detection of change in activity impossible technically; secondly, the peroxisomal localization, providing safe environment for catalase keeping it from reacting with hydroperoxides produced out of the peroxisomes (8).

The slight and statistically insignificant increase in tissue TBARS levels reveals that the myocardium is protected well from lipid peroxidation with cellular and extracellular antioxidants or with the cardioprotection techniques used during cardiopulmonary bypass, or both.

There is a very limited number of studies investigating the cardiopulmonary bypass-induced oxidative stress in myocardial tissue (11,16,20-22). The results of a study designed to assess the activities of glutathione peroxidase, glutathione reductase, glutathione transferase, copper/zinc-containing and manganese-containing superoxide dismutase and catalase in right atrial myocardium of patients undergoing coronary bypass surgery, reveal both the induced activation of the studied enzymes and the TBARS increase in the samples obtained 5 min. after aortic cross clamping when compared to those of precross clamping (16). This is somewhat a similar study to ours considering the biochemical parameters examined in the tissue and the localization of the biopsy material collected; however both the cardioplegic solution used and the sample collection

times differs from ours; the second sample representing only the ischemia, but not ischemia and reperfusion as in our study. Thus, although our finding on the superoxide dismutase activity increase observed in the right atrial tissue samples obtained after cross clamp release as compared to basal status seems parallel to the result Mezzetti's study, the comparison of the data found in these two studies need to be precautiously evaluated due to the above-mentioned reasons. In another study performed on 20 patients who underwent open-heart valve replacement under cardiopulmonary bypass, the effects of myocardial preservation is examined in radial artery blood with the activity assays of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), while in biopsy specimens from the right atrium, activities of superoxide dismutase, creatine kinase and levels of malondialdehyde were studied. This investigation is the only similar one in the literature to ours, firstly, being designed to study the effects of ischemia and reperfusion both in blood and in myocardial tissue and secondly, reporting unchanged lipid peroxidation between the ischemic period and the reperfusion period (11). The unchanged superoxide dismutase activities observed in Yuan's study are not parallel with our results, probably due to the different reperfusion periods and the cardioplegia techniques applied.

The results of our study on the plasma antioxidant enzymes, SOD, CAT and GSH Px, can be interpreted as follows: An explanation for the interaction of these three enzyme activities is brought by Mantha et al (34). According to this; the activities of GSH Px is inhibited by superoxide radicals, while SOD is inhibited by hydrogen peroxide. Thus, in our study probably the increased superoxide radicals in plasma inactivated GSH Px leading to an increase in hydroperoxides which cause an inhibition of SOD activity. The decrease in plasma catalase activities observed immediately after aortic clamping might be explained in the same way, however it is not easy to bring an explanation both to the increased catalase activities found in the later periods and the increased reduced glutathione levels observed 20 min after ischemia as compared to the basal status and to the beginning of ischemia.

Our data on the three glutathione parameters (GSH, GSSG and GSH/GSSG ratio) are evaluated as follows: Reduced glutathione is found significantly increased in plasma 20 min after ischemia as compared to the basal status and to the beginning of ischemia. Although these results are partly in good correlation with the increase in GSH/GSSG levels observed in plasma samples obtained in the same periods, no significant change is observed in plasma GSSG levels in the identical periods of GSH increase. Parallel with the increase in oxidized glutathione levels in myocardial tissue with reperfusion, elevated GSSG levels might be expected in the plasma due to the active transport of intracellularly generated GSSG across the cell membrane The unchanged plasma GSSG levels might be either due to the ability of the cell to produce equivalents for GSSG reduction or the adequate GSH levels in the plasma to reduce GSSG (13). Plasma glutathione reductase activities also did not change during the whole procedure in spite of a decrease in tissue activity, assayed in the samples representing reperfusion when compared to those of the basal status. Furthermore, plasma GSH/GSSG change is not correlated with those of the tissue samples belonging to the identical periods.

When the data on the myocardial tissue are analyzed for a possible correlation with plasma: Regarding the activities of superoxide dismutase and catalase in addition to the glutathione redox status, the inconsistencies between plasma and tissue values increase might be explained by the differences in the antioxidant capacities of plasma, particularly the erythrocytes and the myocardial tissue or the dilution of plasma caused by the cardioplegic solutions employed or a combination of these (1). The unchanged TBARS values across the all paired time groups are in good correlation with the tissue results and reveals that a significant lipid peroxidation did not take place in plasma in the time course of CPB.

The results of several studies carried on patients undergoing cardiopulmonary bypass with various cardioplegic techniques applied for myocardial protection have evidenced that, blood values of various antioxidants is either changed or remain unchanged and lipid peroxidation markers might be seen in the course of the operation (5,9-11,15,17,35-37). Biagioli et al (9) have found that, plasma glutathione redox status is not changed with continuous warm blood cardioplegia in patients who underwent coronary artery bypass surgery with cardiopulmonary bypass. Mezzetti et al (5) have also observed that significant release of reduced and oxidized glutathione and lipid

peroxidation products is not seen in plasma 20 min. after aortic cross clamp removal with antegrade warm blood cardioplegia. Inal et al (1) have reported elevated erythrocyte catalase and glutathione reductase, but unchanged glutathione peroxidase activities in early and late reperfusion periods, during cardiopulmonary bypass established on patients undergoing open heart surgery for various cardiac pathologies. In another study investigating the effects of cardiopulmonary bypass on erythrocyte antioxidant status and lipid peroxide level, superoxide dismutase activities were found reduced significantly, while lipid peroxide levels were increased with reperfusion as compared to the values obtained before cardiopulmonary bypass (15). In a study performed on 24 patients undergoing coronary artery bypass grafting, total plasma antioxidant status were found significantly lowered even after 72 h. following cardiopulmonary bypass compared to the preoperative values, while lipid peroxidation concentration was found significantly elevated only 1.5 h. after the cessation of CPB (17). In another study, in patients undergoing elective coronary artery surgery, Cohen et al (2) have determined decreased total plasma antioxidant status in peripheral venous blood samples obtained 1 h after the end of cardiopulmonary bypass as compared to the preoperative values both with intermittent aortic cross clamping with fibrillation, and antegrade cold crystalloid cardioplegia. In the same study, lipid peroxidation measurements were higher in the same time interval when compared to the preoperative values. The results of these studies indicate that, plasma antioxidant capacity might be changed to counterbalance the oxidative burst following ischemia and reperfusion and lipid peroxidation end products might be seen in the plasma, in patients undergoing cardiopulmonary bypass. While some of our findings related to the plasma antioxidants are in line with those of the above studies, differences in the cardioplegia techniques employed and the sample collection times may be responsible for the inconsistencies between some other data.

The most meaningful finding in our study is lack of correlation between most of the investigated myocardial tissue and coronary sinus plasma values. Furthermore, although in myocardial tissue, the changes observed in single parameters could be correlated with each other according to the widely accepted rules of the oxidative stress, it is not easy to bring an explanation for the interaction of some of the parameters studied in the plasma. The vascular endothelial cells and activated neutrophils are implicated in part as free radical sources in ischemia-reperfusion process during CPB. The infiltration of neutrophils into the ischemic zone begins within 60 min. after the onset of ischemia and increases progressively for up to 90 min. after reperfusion (4). However, in our study the sample collection times do not coincide with the mentioned periods. Thus, the more meaningful results obtained in the tissue, which represents mostly the intracellular oxidant-antioxidant processes better than the plasma, might be related to the dominantly intracellular origin of the reactive oxygen species during cardiopulmonary bypass. Another criticism could be related first to the dilution of plasma by cardioplegic solutions employed for myocardial protection which may cause false changes in the values of studied antioxidants and lipid peroxide end products, and second the scavenging capacity of antioxidants localized in erythrocytes where the free radicals probably diffuse.

In conclusion, the changes in the myocardial tissue and the coronary sinus plasma antioxidant enzyme activities as well as glutathione redox status without evident lipid peroxidation during cardiopulmonary bypass procedure could reveal that; oxygen derived free radicals presumably generated by the ischemia reperfusion of the heart or by a nonspecific effect of the perioperative state or a combination of these are well balanced by the elaborate cellular and extracellular antioxidant defense systems, and the heart is well protected with hot shot perfusion in combination to cold blood cardioplegia as Buckberg has claimed (38-40).

It can also be stated that; the changes in the antioxidant parameters of the coronary sinus plasma do not completely reflect the antioxidant status of the myocardial tissue. Thus in patients undergoing cardiopulmonary bypass, to evaluate the oxygen free radical scavenging capacity of the myocardium effected from possible ischemia reperfusion injury, it is more valuable to investigate the antioxidant status of the myocardial tissue in addition to plasma.

REFERENCES

1. Inal, M., Alataş, Ö., Kanbak, G., Akyüz, F., Sevin, B. (1999) Changes of antioxidant enzyme activities during cardiopulmonary bypass. J. Cardiovasc. Surg. 40, 373-6.
2. Cohen, A.S., Hadjinikolau, L., McColl, A., Richmond, W., Sapsford, A. R., Glenville, E. B. (1997) Lipid peroxidation, antioxidant status and troponin-T following cardiopulmonary bypass A comparison between intermittant crossclamp with fibrillation and crystalloid cardioplegia. Eur. J. Cardio-thorac. Surg. 12, 248-53.

3. Rice-Evans, C.A., Burdon, R.H. (1994;) Free Radical Damage and its Control, pp.1- 31.Elsevier Science Press, England.
4. Kilgore, K. S., Lucchesi, B.R. (1993) Reperfusion injury after myocardial infarction: The role of free radicals and the inflammatory response. Clin. Biochem. 26, 359-70.
5. Mezzetti, A., Calafiore, M.A., Lapenna, D., Deslauriers, R., Tian, G., Salerno, A.T., Verna, M.A., Bosco, G., Pierdomenico, S.D., Caccurullo, F. (1995) Intermittant antegrade warm cardioplegia reduces oxidative stress and improves metabolism of the ischemic-reperfused human myocardium. J. Thorac. Cardiovasc. Surg. 109, 787-95.
6. Gutteridge, J.M.C. (1995) Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem. 41 (12), 1819-28.
7. Rice-Evans, C.A., Burdon RH (1994) Free Radical Damage and its Control, Elsevier Science Press, England, pp. 113-29.
8. Rice-Evans, C.A., Burdon RH (1994) Free Radical Damage and its Control, Elsevier Science Press, England, pp. 43-65
9. Biagioli, B., Borrelli, E., Maccherini, M., Bellomo, G., Lisi, G., Giomarelli, P., Toscano, M. (1997) Reduction of oxidative stress does not affect recovery of myocardial function: warm continious versus cold intermittant blood cardioplegia. Heart. 77(5), 465-73.
10. Isomura, T., Hisatomi, K., Sato, T., Hayashida, N., Ohishi, K. (1995) Interrupted warm blood cardioplegia for coronary artery bypass grafting. Eur. J. Cardiothorac. Surg. 9(3), 133-8.
11. Yuan, S.M. (1997) Effects of myocardial preservation on enzyme levels in serum and myocardium: a clinical study comparing cold crystalloid versus warm blood cardioplegia. Chung Hua I Hsueh Tsa Chih (Taipei) 59(1), 21-7.
12. Starkopf, J., Zilmer, K., Vihalemm, T., Kullisaar, T., Zilmer, M., Samarutel, J. (1995) Time course of oxidative stress during open-heart surgery. Scan J Thorac Cardiovasc. Surg. 29(4), 181-6.
13. Pala, M.G., Paolini, G., Paroni, R., De Veechi, E., Gallorini, C., Stefano, P.L., Di Credico G., Zuccari, M., Galli, L., Agape, V. (1995) Myocardial protection with and without leukocyte depletion: a comparative study on the oxidative stress. Eur. J. Cardiothorac. Surg. 9(12), 701-6.
14. Tao, S., Calza, G., Lerzo, F., Virgone, A., Camassa, N., Panizzon, G., Brunelli, L., Moretti, R., Grasso, P., Ghiggeri, G.M (1994) Activation of the intracellular glutathione system by oxidative stress during cardiopulmonary bypass and myocardial perfusion. Perfusion. 10(1), 45-50.
15. İnal. M., Alataş, Ö., Kural T., Sevin, B. (1994) Oxygen free radicals in erytrocytes during open heart operation. J. Cardiovasc. Surg. 35, 147-50.
16. Mezzetti, A., Lapenna, D., Pierdomenico, S.D., Giammarco, G., Bosco, G., Di Ilio, C., Santarelli, P., Calafiore, M.A., Caccurullo, F. (1993) Myocardial antioxidant defenses during cardiopulmonary bypass. J. Card. Surg. 8(2), 167-71.
17. Mc Coll, A.J., Keeble, T., Hadjinikolaou, L., Cohen, A., Aitkenhead, H., Glenville, B., Richmond W. (1998) Plasma antioxidants: evidence for a protective role against reactive oxygen species following cardiac surgery. Ann. Clin. Biochem. 35(Pt 5), 616-23.
18. İnselmann, G., Kohler, K., Lange, V., Silber, R., Nellessen, U. (1998) Lipid peroxidation and cardiac troponin T release during routine cardiac surgery. Cardiology. 89(2), 124-9.
19. Prasad, K., Kalra, J., Bharadwaj, B., Chaudhary, A.K. (1992) Increased oxygen free radical activity in patients on cardiopulmonary bypass undergoing aortocoronary bypass surgery. Am. Heart J. 123, 37-45.
20. De Vecchi, E., Pala, M.G. Di Credico, G., Agape, V., Paolini, G., Bonini, P.A., Grossi, A., Paroni, R. (1998) Relation between left ventricular function and oxidative stress in patients undergoing bypass surgery. Heart. 79(3), 242-7.
21. Janssen, M., van der Meer, P., de Jong, J.W. (1993) Antioxidant defenses in rat, pig, guinea pig, and human hearts:comparison with xanthine oxidoreductase activity. Cardiovasc. Res. 27(11), 2052-7.
22. Kim, K.B., Chung, H.H., Kim, M.S., Rho, J.R. (1994) Changes in the antioxidative defensive system during open heart operations in humans. Ann. Thorac. Surg. 58(1), 170-5.
23. Sun, Y., Oberley, L.W., Li,Y. (1988) A simple method for clinical assay of superoxide dismutase. Clin. Chem. 34(3, :497-500.
24. Goth, L. (1991) A simple method for determination serum catalase activity and revision of reference range. Clin. Chim. Acta. 196, 143-52.
25. Paglia, D.E.,Valentine,W.N. (1967) Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med.. 158-69.
26. Lawrence, R.A. (1976) Glutathione peroxidase activity in selenium-deficient rat liver. Biochem. Biophy. Res. Commun. 71(4), 957-8.
27. Massey, V. (1965) On the reaction mechanism of yeast glutathione redutase. J. Biol. Chem. 11, 4470-80.
28. Teare, J.P. (1993) Automated spectrophotometric method for determining oxidized and reduced glutathione in liver. Clin. Chem. 39/4, 686-9.
29. Uchiama, M., Mihara, M. (1977) Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271-78.
30. Stocks, J., Dormandy, TL. (1971) The autoxidation of human red cell lipids induced by hydrogen peroxide. Br. Haemotol. 20, 95-111.
31. Lowry, O.H., Roseburg, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem.193, 265-75
32. Seven, A., Civelek, S., İnci E., İnci, F., Korkut, N., Burçak, G. (1999) Evaluation of oxidative stress parameters in blood of patients with laryngeal carcinoma. Clin. Biochem. 32. 369-373.
33. Stanimirovic, D:B:, Micic, D.V., Markovic, M., Spatz, M., Mrsulja, B.B. (1994) Therapeutic window for multiple drug treatment of experimental cerebral ischemia in gerbils. Neurochem. Res. 19, 189-94.
34. Mantha, S.V., Kalra, J., Prasad, K. (1996) Effects of probucol on hypercholesterolemia induced changes in antioxidant enzymes. Life Sciencws 58(6), 503-509.
35. Quinlan, G.J., Westermann, S.T., Mumby, S., Pepper, J.R., Gutterdge, J.M. (1999) Plasma hypoxanthine levels during cristalloid and blood cardioplegias: warm blood cardioplegia increases hypoxanthine levels with a greater risk of oxidative stress. J. Cardiovasc. Surg. (Torino) 40(1), 65-9.
36. Grech, E.D., Baines, M., Steyn, R., Faragher, E.B., Page, R.d., Fabri, B.M., Ramsdale, D.R., Rashid, A. (1995) Evidence that continious normothermic blood cardioplegia offers better myocardial protection than intermittant hypothermic cardioplegia. Br. Heart J. 74(5), 517-21.
37. Ferrari, R., Alfieri, O., Cerullo, S., Ceconi, C., Cargnoni, A., Marzollo, P., Pardini, A., Caradonna, E., Visioli, O. (1990) Occurrence of oxidative stress during reperfusion of the human heart. Circulation 81(1), 201-11.
38. Buckberg, G. D. (1991) Myocardial temperature management during aortic clamping for cardiac surgery: Protection preoccupation, and perspective. J. Thorac Cardiovasc Surg. 102(6), 895-903.
39. Buckberg, G. D. (1991) Warm versus cold blood cardioplegia: a self-imposed and counterproductive dilemma. Ann. Thorac. Surg. 56(5), 1007-1010.