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Comparison of hydroxyl radical generation in patients undergoing coronary artery bypass grafting with and without cardiopulmonary bypass

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Abstract
We measured the hydroxyl radical (·OH) generation in fourteen patients undergoing coronary artery bypass grafting (CABG), of whom seven patients underwent on-pump CABG with cardiopulmonary bypass (CPB) and seven patients underwent off-pump CABG without CPB. To detect ·OH generation, we measured the urinary excretion of ·OH products of creatinine (Cr), creatol (CTL; 5-hydroxycreatinine) and methylguanidine (MG) with HPLC using the one point sampling and collected urine during and after the operation. The urinary CTL value corrected urinary Cr value of on-pump CABG significantly increased about 3–5 times from the beginning of CPB to 4 h after operation compared to the baseline value before CPB in both the collected urine and the one point sampling urine. The urinary MG/Cr value in both groups did not change significantly. Significantly increased ·OH generation was found during and soon after on-pump CABG.

Keywords: *Hydroxyl radical, creatol, methylguanidine, oxidative stress, coronary artery bypass grafting, cardiopulmonary bypass*

Introduction

Cardiopulmonary bypass (CPB) is an artificial heart and lung machine used to assist and completely take over the cardiac and lung functions of the patient undergoing cardiac surgery. It consists of a membrane oxygenator, tubing for the circuits and roller and centrifugal pump units. The blood flow produced by the CPB is non-pulsatile, hyperoxygenated and slightly hypothermic. Most open-heart surgeries are performed with an empty and arrested heart induced by an aortic clamp and the perfusion of high dose potassium solution to the coronary arteries. Therefore, ischemic reperfusion occurred in the coronary and pulmonary circulation after the release of aortic clamp. These factors, related to the CPB induced whole body inflammatory response and oxidative stress, lead to organ damages [1–3]. Although, oxidative stress in cardiac surgery has been reported [4,5], the strength of the oxidative stress and its time course in the whole body are still unclear because of the difficulties of direct measurement of radical species. Thus, we evaluated the oxidative stress of a patient undergoing CPB measuring the urinary creatol (CTL: 5-hydroxycratinine) [6–8] and methylguanidine (MG) [8–10], which are oxidative products of creatinine (Cr).

The oxidized synthesis pathway into MG, which was known as a uremic toxin, was proposed by Aoyagi et al. [10,11]. They reported the MG synthesis by hydroxyl radical (·OH) *in vitro* as well as activated

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human leukocytes [12] and isolated rat hepatocytes [13]. CTL is the direct ·OH adduct of Cr first isolated by Nakamura and Ienaga [6]. Cr is an intrinsic substance that is distributed equally in the body as the substrate. Due to their low molecular weight, CTL and MG are rapidly excreted into the urine. For these reasons, urinary CTL and MG make it possible to observe almost directly the ·OH generation in the whole body as an oxidative stress marker [8]. In the present study, we measured the CTL and MG in urine samples with one point sampling and collection at certain times of patients undergoing coronary artery bypass grafting (CABG) to evaluate the ·OH generation. Additionally, by comparing the off- and on-pump CABG, we attempted to evaluate the effects of CPB in ·OH generation.

Subjects and methods

Patients

After approval by the ethics committee of Tsukuba Medical Center Hospital, written informed consent was obtained from all patients. Fourteen patients who underwent primary elective CABG were studied. Seven patients underwent CABG using CPB on the potassium arrested heart (on-pump CABG). The other seven patients underwent CABG without CPB on the beating heart (off-pump CABG). These patient's operative data are shown in Table I.

On-pump CABG

All patients approached through the mediansternotomy. After preparation of the bypass grafts and systemic heparin administration (2–3 mg/kg), CPB was established with ascending aortic perfusion and right atrial venous drainage. Anastomoses of the bypass grafts were performed on the arrested heart after aortic cross clamping and the infusion of potassium enriched cardioplegic solution to coronary artery. After all anastomoses were completed, the aortic cross clamp was released and heart began to beat again. The core temperature of the patients was maintained at above 34°C during the operation.

The CPB circuit consisted of heparin-coated tubing and an open hard shell reservoir, (CAPIOX® (HP); TERUMO®), membrane oxygenater (CAPIOX®SX (HP); TERUMO®), centrifugal pump (CAPIOX® (HP) centrifugal pump; TERUMO®) and roller pumps for suction. The CPB circuit was primed with 5000 U heparin, 1000 mg ascorbic acid, 60 ml 7% sodium bicarbonate, 200 ml 20% D-mannitol and 700–1000 ml normal saline. The perfusion flow index was maintained at 2.7 L/kg/min/m^2.

Off-pump CABG

All patients approached through the mediansternotomy. The activated clotting time (ACT) was maintained above 300 s by the systemic administration of

Table I. Patients data.

Variable	On-pump (n=7)	Off-pump (n=7)
Age (y/o) (range)	65.9 ± 6.5 (59–76)	64.9 ± 7.5 (55–76)
Gender (M/F)	6/1	5/2
Height (cm) (range)	159.9 ± 8.2 (145–170.3)	159.6 ± 8.5 (145–170)
Weight (kg) (range)	63.4 ± 6.5 (59–76)	65.9 ± 6.5 (59–76)
CCS (range)	2.0 ± 0.8 (1–3)	2.9 ± 0.9 (2–4)
Previous MI	5 (71.4)	2 (28.6)
LVEF (%) (range)	60.9 ± 10.2 (43–72)	57.9 ± 12.2 (40–67)
LMT lesion	3 (42.9)	2 (28.6)
Preop. IABP (%)	0 (0.0)	2 (28.6)
DM (%)	3 (42.9)	3 (42.9)
HT (%)	4 (57.1)	5 (71.4)
HL (%)	4 (57.1)	5 (71.4)
Medication (%)		
Beta blockers	2 (28.6)	3 (42.9)
Ca antagonist	2 (28.6)	3 (42.9)
Nitrates	5 (71.4)	5 (71.4)
Bypass branches (range)	3.9 ± 0.9 (3–5)	2.4 ± 1.0 (1–4)
Vessels grafted (%)		
LAD	7 (100.0)	7 (100.0)
RCA	6 (85.7)	1 (14.3)
Cx	6 (85.7)	4 (57.1)
Additional op. (%)	1 (14.3) (MVP)	0 (0.0)
CPB (min)	169.0 ± 46.2	
AXC (min)	137.7 ± 37.5	

M, male; F, female; CCS, Canadian Cardiovascular Society; MI, myocardial infarction; LVEF, left ventricular ejection fraction; LMT, left main trunk; Preop., pre-operative; IABP, intraaortic balloon pumping; DM, diabetes mellitus; HT, hypertension; HL, hyperlipidemia. LAD, left anterior descending branch; RCA, right coronary artery; Cx, circumflex branch; op., operation; CPB, cardiopulmonary bypass; AXC, aortic cross clamp; min, minutes.

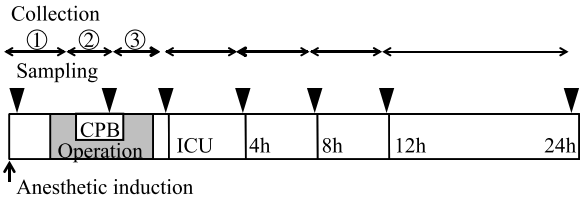

Figure 1. Urinary collection and sampling schedule. (1) pre-CPB or grafting: from anesthetic induction to the beginning of CPB (to the beginning of grafting in off-pump CABG); (2) during CPB or grafting: during CPB (during grafting in off-pump CABG); (3) post-CPB or grafting: from the termination of CPB (from the completion of grafting in off-pump CABG) to the end of operation (CPB, cardiopulmonary bypass; ICU, intensive care unit).

1.5–2.0 mg/kg heparin. An Octopus 3® Heart Stabilizer and Starfish® Heart Positioner (Medtronic Co. Inc., Minneapolis) were used for heart stabilization. Left internal mammary artery (LIMA) to left anterior descending branch (LAD) anastomosis was performed, maintaining the coronary blood flow with intraluminal shunt tube. On the other hand, bypass grafting to the circumflex and right coronary artery branches was performed by intercepting the coronary blood flow with a proximal snare. One anastomosis was completed within 15 min. Proximal anastomoses to the ascending aorta were performed under partial aortic clamp.

Sample collection

A schematic diagram of the urinary collection and the one point sampling time schedule are shown in Figure 1. Urine was sampled from a balloon catheter inserted into the patient's bladder at the points of anesthetic induction, aortic declamp (grafting end in off-pump CABG), ICU arrival, 4, 8, 12 and 24 h after the operation. Urine was also collected following time-intervals from anesthetic induction to the beginning of CPB (to the beginning of grafting in off-pump CABG), during CPB (during grafting in off-pump CABG), from the termination of CPB (from the completion of grafting in off-pump CABG) to the end of operation, from ICU arrival to 4 h after operation, from 4 to 8 h after operation, from 8 to 12 h after operation and from 12 to 24 h after operation. Urinary collection was performed with cooling. After measuring urinary volume, samples were immediately frozen below −30°C until assaying.

Analytical method for CTL, MG and Cr

After deproteinization of the urinary samples by adding trichloroacetic acid (TCA) (final concentration 10% (v/w) TCA) and centrifugation at 3000 rpm for 10 min, the supernatant was used for assay. Urinary MG was determined by HPLC using the automated guanidino compound analyzer, with 9,10-phenanthrenequinone (PQ) as a fluorogenic reagent [8]. Urinary CTL was also determined by HPLC using a modified guanidino compound analyzer, which was previously described by Nakamura and Ienaga [11]. Urinary and serum creatinine was determined with the automated analyzer using an enzymatic method. Other clinical data were also determined with the automated analyzer in the laboratory of Tsukuba medical center hospital.

Statistical analysis

Data are presented as means ± SD. Statistical analysis was performed by one way ANOVA and Fisher's PLSD for the *post hoc*-test. Student's *t*-test was also used for comparison between groups. The differences were considered statistically significant when the calculated *P* value was less than 5%.

Results

Changes in urine volume

The urine volume increased significantly between that during CPB to 4 h after the operation in on-pump CABG patients, but not in off-pump CABG patients (Figure 2.).

Changes in urinary Cr excretion and serum Cr

Changes of urinary Cr excretion and serum Cr concentration are shown in Figure 3(a) and (b). The urinary Cr excretion did not change significantly in on-pump CABG patients, but significantly decreased after completion of graft anastomoses in off-pump CABG patients. Serum Cr did not change significantly during this study period until 36 h after operation.

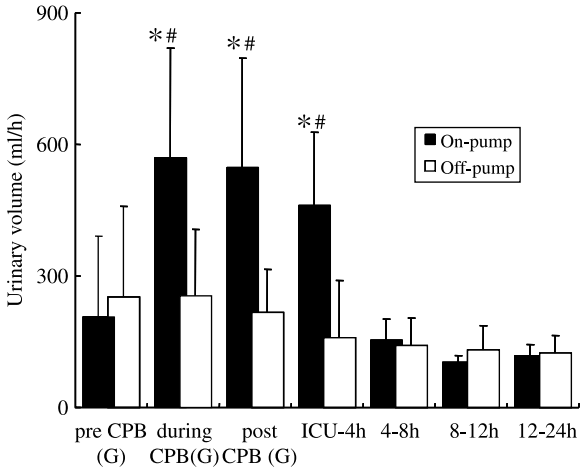

Figure 2. Changes in urine volume. $*P < 0.05$ vs pre-CPB data in ANOVA and *post hoc*-test. $\#P < 0.05$ vs off-pump CABG group in Student's *t*-test (CPB, cardiopulmonary bypass; G, grafting; ICU, intensive care unit).

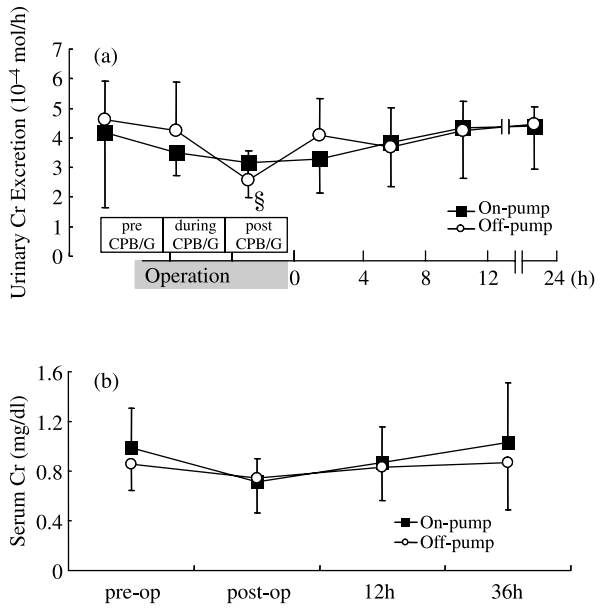

Figure 3. Changes of urinary Cr excretion (a) and serum Cr (b). $^{\S}P < 0.05$ vs pre-grafting data in ANOVA and *post hoc*-test (CPB, cardiopulmonary bypass; G, grafting; ICU, intensive care unit; op, operation).

Changes of urinary CTL/Cr value in collection and one point sampling of urine

To exclude the influence of the alteration of renal function, the molar ratio of the urinary CTL value corrected by the Cr of urine collection and one point sampling are shown in Figure 4(a) and (b), respectively. The CTL value corrected Cr value in the collected urine of on-pump CABG patients increased significantly from the pre-CPB value as a baseline value (3.70 ± 2.07 mmol/mol) to 359% (12.17 ± 4.71 mmol/mol), 362% (13.91 ± 4.92 mmol/mol) and 288% (11.7 ± 5.55 mmol/mol) in the periods during CPB, post-CPB in operation and that 4 h after operation, respectively, as shown in Figure 4(a). On the other hand, the CTL/Cr value in off-pump CABG patients was increased about 1.5 times (from 4.41 ± 2.01 to 7.39 ± 3.03 mmol/mol) compared with the pre-grafting data only in the post-grafting period.

Urinary CTL was also measured in the point-sampling urine shown in Figure 4(b). Urinary CTL/Cr value in on-pump CABG patients significantly increased from the baseline (2.95 ± 3.65 mmol/mol) to 517% (14.76 ± 4.82 mmol/mol), 523% (16.53 ± 8.55 mmol/mol) and 329% (9.91 ± 5.13 mmol/mol) at the points of aortic declamp, ICU arrival and 4 h after operation, respectively. In off-pump CABG patients, though the significant increase of the urinary CTL/Cr value was also detected at the points of grafting end, ICU arrival, 4 h after operation and 12 h after operation, even the maximum increase at aortic declamp was less than 50% of the value in on-pump CABG patients.

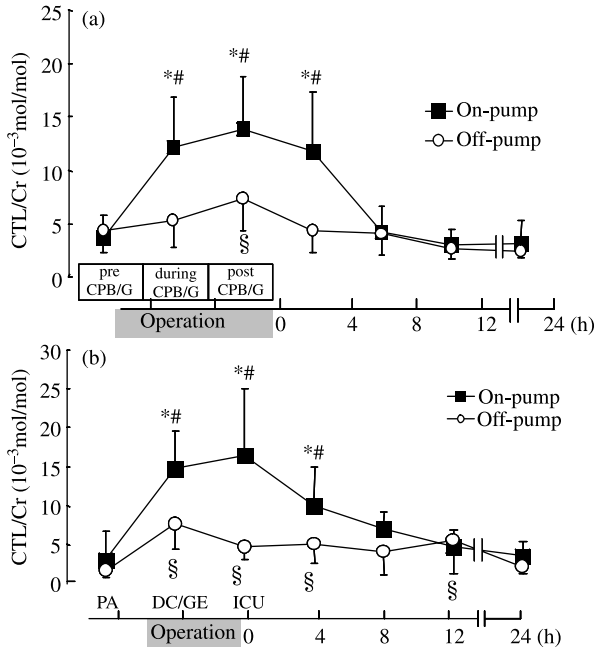

Figure 4. Changes of urinary CTL/Cr value in collection (a) and one point sampling (b) of urine. $^{*}P < 0.05$ vs pre-CPB data and $^{\S}P < 0.05$ vs pre-grafting data in ANOVA and *post hoc*-test. $\#P < 0.05$ vs off-pump CABG group in Student's *t*-test (CPB, cardiopulmonary bypass; G, grafting; ICU, intensive care unit; PA, post-anesthetic induction; DC, declamp; GE, grafting end).

Changes of the urinary MG/Cr value in one point sampling of urine

The urinary MG/Cr value was determined in the samples of the one point sampling of urine shown in Figure 5. The MG/Cr value in the patients undergoing on- and off-pump CABG did not change significantly. There was only one patient undergoing on-pump CABG whose MG/Cr value increased at the time of ICU arrival and 4 h after operation, respectively, compared with the post-anesthetic induction value.

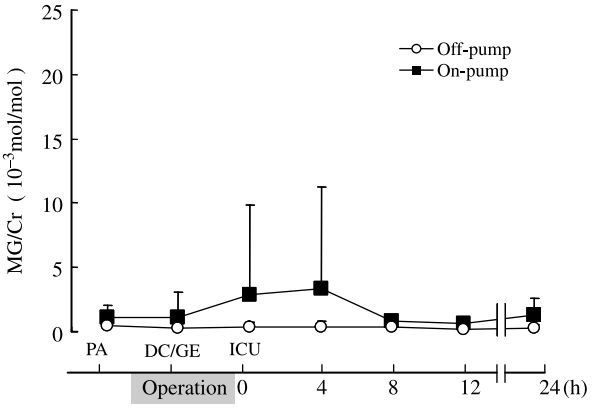

Figure 5. Change of urinary MG/Cr value in one point sampling of urine (ICU, intensive care unit; PA, post-anesthetic induction; DC, declamp; GE, grafting end).

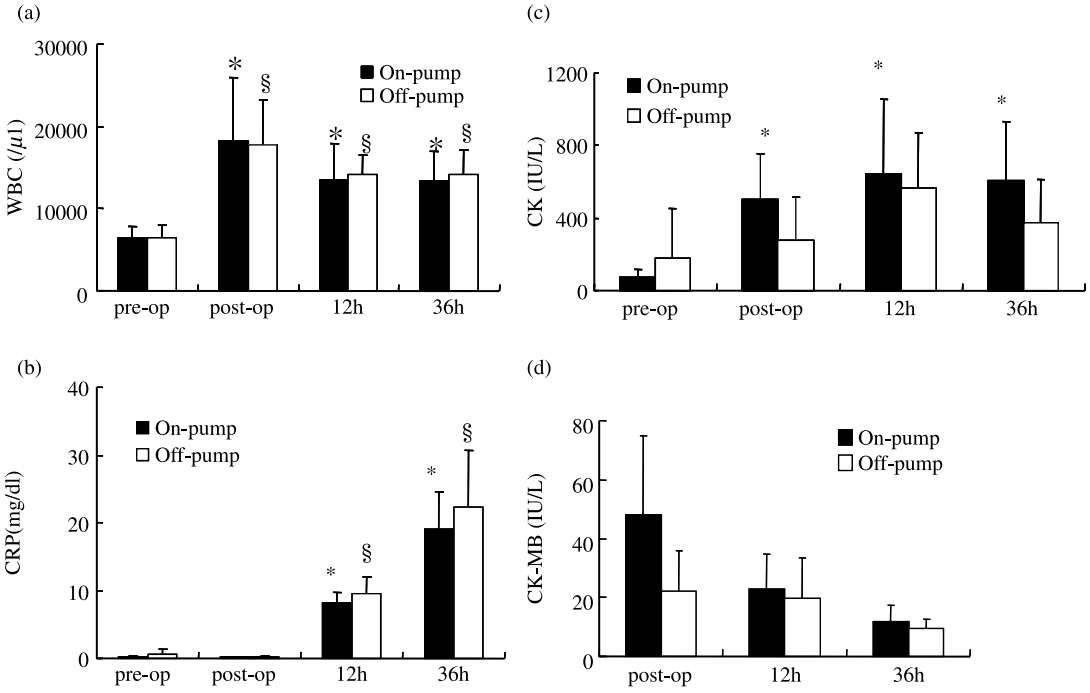

Figure 6. Changes of clinical data in serum. $\star P < 0.05$ vs pre-CPB data and $^{\S}P < 0.05$ vs pre-grafting data in ANOVA and *post hoc*-test (op, operation).

Changes of clinical data in the serum

White blood cell counts (WBC) and C-reactive protein (CRP) were measured as the inflammatory markers shown in Figure 6(a) and (b). Both rose significantly after operation, but no differences were recognized between the on- and off-pump patients.

Creatine kinase (CK) and creatine kinase MB isoenzyme (CK-MB) which was derived from the myocardium are measured as myocardial injury markers caused by ischemic reperfusion as shown in Figure 6(c) and (d). CK rose significantly after the operation in the on-pump group, but did not rise significantly in the off-pump group. CK-MB was elevated after the operation, but there was no significant difference between the groups. Because the pre-operative data of CK-MB was not measured, it was not clear whether post-operative CK-MB elevation was statistically significant.

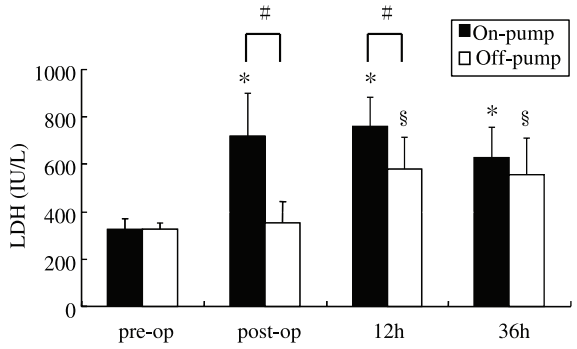

Figure 7. LDH in serum. $\star P < 0.05$ vs pre-CPB data and $^{\S}P < 0.05$ vs pre-grafting data in ANOVA and *post hoc*-test. $\#P < 0.05$ vs off-pump CABG group in Student's *t*-test (op, operation).

LDH in serum

Lactate dehydrogenase (LDH) was measured as a clinical marker of hemolysis. It increased significantly after operation in both groups. There were significant differences between the groups (Figure 7).

Discussion

In the present study, we demonstrated increased ·OH generation based on an increased ·OH marker, CTL during CPB. ·OH is thought to be a major contributor from Cr to CTL oxidation steps in the pathway to MG [6–8]. Due to CTL and MG having a low molecular weight and high renal clearance, it is excreted rapidly as soon as it is generated through the normal kidney [8].

We found that there was a significant increase of ·OH continued from the beginning of CPB to 4 h after operation in patients who underwent on-pump CABG compared to off-pump CABG. We also found that there was a small increase of CTL in off-pump patients that probably occurred because of surgical trauma. The cause of increased ·OH generation in on-pump CABG patients may be explained as follows. Generally, blood contact with a non-physiological surface and shear stresses in the CPB circuit induces explosive leukocyte activation,

which results in the release of large amounts of oxygen-free radicals [14,15]. Released superoxide anion formed hydrogen peroxide (H_2O_2) through dismutation. Shear stress in CPB causes hemolysis [15], which carries the risk of plasma iron overload and being a potential risk factor for oxidative stress [16]. Even a small amount of free iron and H_2O_2 induce ·OH generation by the Fenton reaction. Indeed, several investigators suggested the increased generation of H_2O_2 and the superoxide anion during and after CPB [17–20]. On the other hand, if nitric oxide (NO) production increases during and after CPB [21,22], ·OH may be generated through peroxynitrite ($ONOO^-$).

Essential differences between on- and off-pump CABG are the CPB related events as follows; blood contact with a non-physiological surface, shear stresses, non-pulsatile flow and hypertensive oxygen perfusion over 400 mmHg and so on. It was considered that the increased ·OH generation in CPB patients is the result of the above several factors. On the other hand, there may be no distinct differences about ischemic reperfusion of the heart and lung in our setting of research. Because of the "partial CPB" mode generally used in on-pump CABG, a part of systemic venous return remained and the pulmonary blood flow was maintained. Furthermore, the intercept of coronary blood flow by the temporary snaring of the target vessels in off-pump CABG induced slight partial myocardial ischemia. There was no significant difference related to the surgical trauma between the on- and off-pump patients because almost the same surgical procedures were used, except for the use of CPB.

On the other hand, MG, which is also an oxidative stress marker *in vivo*, was not increased significantly in both on- and off-pump CABG except for one. Because CTL had a low concentration, MG, which was converted from the CTL generated at a low concentration in off-pump CABG patients. However, in the turned on-pump CABG patients, CTL may be excreted rapidly before changing to MG due to the excessive urine excretion during and after the operation. There was only one case of increased MG generation that underwent on-pump CABG. The patient had decreased renal function. However, there was another patient with decreased renal function without increased MG. Their creatinine clearances (C_{Cr}) were around 30 ml/min that is about 30% of normal value. The CTL level of the patient with increased MG was increased by three times of that of the patient without increased MG excretion. Therefore, the oxidative stress in patient with renal dysfunction could be evaluated including MG content as well as CTL.

It is well known that CPB induces a whole body inflammatory response that is associated with the activation of complement, kinin-kallikrein, fibrinolysis, neutrophils, platelets, other coagulation factors, endothelial cells, the arachidonic acid cascade and cytokine release [1,2]. Extremely increased vascular permeability and serious multiple organ dysfunction that the surgeon often encounters during and after cardiac surgery with CPB are also possibly influenced directly and indirectly by free radical formation [23,24]. Therefore, measurement of free radical formation is effective for the investigation to the possibility of improvement of whole body inflammatory response syndrome with cardiopulmonary bypass.

Conclusions

Urinary CTL excretion significantly increased from the beginning of CPB to the 4 h after operation in patients undergoing on-pump CABG compared with off-pump CABG. These findings indicate the increased ·OH generation during these periods. Off-pump CABG has the advantage of the reduction of oxidative stress. The urinary CTL/Cr value is useful marker for oxidative stress in cardiac surgery.

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