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Original Article

Analysis of Reactive Oxygen Metabolites (ROMs) after Cardiovascular Surgery as a Marker of Oxidative Stress

Yuji Kanaoka[a]*, Ei-ichirou Inagaki[b], Souhei Hamanaka[b], Hisao Masaki[b], and Kazuo Tanemoto[b]

[a]*Division of Vascular Surgery, Department of Surgery, Jikei University, School of Medicine, Minato-ku, Tokyo 105-8461, Japan, and*
[b]*Division of Thoracic and Cardiovascular Surgery, Department of Surgery, Kawasaki Medical School, Kurashiki, Okayama 701-0192, Japan*

The transient systemic low perfusion that occurs during cardiovascular surgery leads to oxidative stress and the production of free radicals. A systemic increase of various markers of oxidative stress has been shown to occur during cardiopulmonary bypass (CPB). However, these markers have not been adequately evaluated because they seem to be reactive and short-lived. Here, oxidative stress was measured using the free radical analytical system (FRAS 4) assessing the derivatives of reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP). Blood samples were taken from 21 patients undergoing elective cardiovascular surgery. CPB was used in 15 patients, and abdominal aortic aneurysm (AAA) surgery without CPB was performed in 6. Measurements of d-ROMs and BAP were taken before surgery, 1 day, 1 week, and 2 weeks after surgery, and oxidative stress was evaluated. The d-ROM level increased gradually after cardiovascular surgery up to 2 weeks. Over time, the d-ROM level after surgery involving CPB became higher than that after AAA surgery. This difference reached statistical significance at 1 week and lasted to 2 weeks. The prolongation of CPB was prone to elevate the d-ROM level whereas the duration of the aortic clamp in AAA surgery had no relation to the d-ROM level. The BAP was also elevated after surgery, and was positively correlated with the level of d-ROMs. In this study, patients who underwent cardiovascular surgery involving CPB had significant oxidative damage. The production of ROMs was shown to depend on the duration of CPB. Damage can be reduced if CPB is avoided. When CPB must be used, shortening the CPB time may be effective in reducing oxidative stress.

Key words: oxidative stress, reactive oxygen metabolites (ROM), extracorporeal circulation (ECC)

Recently, it has become clear that arteriosclerosis, cardiovascular disease, neurological disorders, and various other diseases are associated with oxidative damage caused by reactive oxygen species (ROS) and free radicals. However, because there are a great number of free radicals and ROS and they have a very short half-life, they have been difficult to measure and evaluate. There is also a question about which markers best indicate the general condition of oxidative stress. Instead of directly measuring ROS or free radicals, it has become possible to measure the hydroperoxide concentration in the blood induced by active oxygen or free radicals using a colorimetric method. The measured concentration is considered to be directly proportional to the quantity of reactive oxygen metabolites (ROMs) affected by active ROS and

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*Corresponding author. Phone:+81-3-3433-1111; Fax:+81-3-5472-4140
E-mail:yujikana@msn.com (Y. Kanaoka)

free radicals. Therefore, measuring the ROMs enables quantitative evaluation of the condition of oxidative stress throughout the human body [1].

At the same time, minimizing the invasiveness of surgical treatments has been an ongoing goal of medical research, and the degree of invasiveness has been discussed in relation to the subject of oxidative stress levels [2-5]. The condition of oxidative stress after extracorporeal circulation (ECC) has been reported [6-8], and ischemia-reperfusion is considered to be the most common cause of increased oxidative stress [8]. From this viewpoint, we compared the invasiveness of cardiovascular surgery with aortic clamping using ECC, which is generally considered the most invasive form of surgery, and aortic surgery with aortic clamping but without the use of ECC, in terms of the oxidative stress level as indicated by d-ROMs. The extent of ischemia-perfusion in the ECC group was much greater than that in the AAA group. We compared an ECC group and AAA group because no other operation except open repair of AAA was eligible for an operation with aortic clamping but without the use of ECC. We herein report our results.

Patients and Methods

Patients. The patients underwent elective cardiovascular surgery from April to October, 2006. The reactive oxygen metabolites (ROMs) were measured preoperatively and 1 day, 1 week and 2 weeks postoperatively using an ROS and free radical automatic analyzer FRAS 4 (Diacron International, Italy). Measurements of ROMs were taken in cases of surgery with aortic clamping using ECC (ECC group) and also in cases of infrarenal abdominal aortic aneurysm (AAA) surgery with aortic clamping without ECC (AAA group). Informed consent was obtained from all patients.

Operation. In the ECC group, anesthesia was induced with propofol, fentanyl and pancuronium, and maintained with isoflurane inhalation. Artificial respiration was controlled to maintain FiO_2 at approximately 0.3-0.6, PaO_2 at approximately 100-200 mmHg, and PCO_2 at approximately 40 mmHg. 300 IU/kg of heparin was administered, and after confirming the ACT to be greater than 400 sec, ECC was established. The priming volume of the ECC was 1,100 ml, which was filled with lactated Ringer's solution. During aortic clamping, ECC was adjusted to maintain a cardiac index of 2.1 L/min/m^2, and cold blood cardioplegia was delivered anterogradely every 30 min.

In the AAA group, while anesthesia was performed in the same manner as in the ECC group, laparotomy was performed on 4 patients, and surgery was performed on 2 patients using an extraperitoneal approach. Aortic clamping was performed after 100 IU/kg of heparin was administered and the ACT was confirmed to be greater than 200 sec.

Measurements using markers for oxidative stress (d-ROM test): In all cases, blood was collected from the arterial line of the radial artery during surgery and from the peripheral veins after surgery. After blood was drawn, 20 μl of whole blood was mixed with an acetic acid buffer solution of pH 4.8 in a pipette to stabilize the hydrogen ion concentration. In an acidified medium, bivalent and trivalent iron from the protein component of the blood ionized and worked as a catalyst to break down hydroperoxide groups in the blood into alkoxyl and peroxy radicals to become free radicals. These were then transferred into a cuvette containing colorless chromogen (N. N. diethylparaphenylenediamine), which is oxidized by free radicals and changes into a radical cation with a magenta color. The density of the magenta color reflects the concentration of hydroperoxides in the blood, which is proportional to the quantity of ROMs. In this method, the magenta color is measured using a photometer (505 nm) after centrifuging for 1 min in order to measure the quantity of hydroperoxide. Since the concentrations of various hydroperoxides in the blood were measured, the values are reported in the arbitrary unit U. CARR [1].

Biological antioxidant potential (BAP) was also simultaneously measured. When the salt of a trivalent iron $FeCl_3$ is solved in a given colorless solution containing a chelation acid derivative, it turns red as a result of the action of the trivalent iron Fe^{3+} ions, but is decolorized by the addition of blood plasma due to the reduction of bivalent iron Fe^{2+} ions caused by the action of antioxidants. In theory, the antioxidant potential of blood plasma can be evaluated by measuring the degree of decolorization using a photometer. In practice, 100 μl of blood is centrifuged for 90 sec, after which the amount of trivalent iron that is deoxidized in 5 min is measured in units of μmol/l [1].

Values are shown as means ± standard deviation, with $p < 0.05$ in Welch's t-test considered to indicate statistical significance. The correlation (linear dependence) between 2 variables, aorta cross clamping time (or extracorporeal circulation time), and the d-ROM increasing rate was measured by the Pearson product-moment correlation coefficient.

Results

The ECC group included the following 15 cases: 9 cases of surgery for valvular disorders (2 cases of redo surgery), 3 cases of valve replacement and coronary artery bypass surgery (1 case of redo surgery), 2 cases of thoracic aortic aneurysm surgery (1 case of redo surgery) and 1 case of left atrial myxoma extirpation; of these, 12 patients were male and 3 were female. The ECCs were used for 152.9 ± 42.4 min and the aortic clamping time was 111.9 ± 34.9 min.

On the other hand, in the AAA group, all 6 were cases of graft replacement for infrarenal AAAs, and all the patients were male. In the AAA group, the aorta was clamped below the renal artery for 82 ± 16.6 min. The age was 59.3 ± 14.8 years old in the ECC group and 72.2 ± 5.8 years old in the AAA group, with a significantly greater age in the AAA group. No significant difference was seen between the 2 groups in terms of preoperative comorbid diseases such as high blood pressure, diabetes and renal dysfunction, but there were more patients who smoked in the AAA group (Table 1).

All patients were discharged from the hospital without any complications.

ROMs and BAP were measured in all 15 patients in the ECC group and 6 patients in the AAA group. No significant difference was observed in the preoperative d-ROM and BAP values. In all cases, a postoperative increase in ROMs was seen, and a gradual increase until the second week was seen in the ECC group (Fig. 1). Because most patients were discharged from the hospital, measurements were taken in only 3 patients during the third week, all of whose ROMs values had decreased since the second week but still remained higher than the preoperative values in the ECC group. In the AAA group, ROM values increased postoperatively, but the degree of increase was low and the values began to decrease after 2 weeks. In addition, although the values were higher than the preoperative values, the difference was not significant in the AAA group. The values

Table 1 Patient profiles of the two groups

	ECC group (n = 15)	AAA group (n = 6)	
Age	59.3 ± 14.8	72.2 ± 5.8	$p < 0.05$
Gender	female 3 (20%)	female 0	
	male 12 (80%)	male 6 (100%)	ns
Height (cm)	164 ± 7.1	162.8 ± 4.6	ns
Body weight (kg)	64.5 ± 10.1	63.8 ± 3.9	ns
BMI	23.8 ± 2.4	24.1 ± 1.3	ns
Hypertension	9 (60%)	4 (66.7%)	ns
Hyperlipidemia	1 (6.7%)	1 (16.7%)	ns
Diabetes	1 (6.7%)	1 (16.7%)	ns
Renal dysfunction	2 (13.5%)	1 (16.7%)	ns
Smoking	1 (6.7%)	3 (50%)	$p < 0.05$
ECCT (min)	152.9 ± 42.4	–	
AoCCT (min)	111.9 ± 34.9	82.0 ± 16.6 (infrarenal)	
Preoperative d-ROMs (U. CARR)	339.8 ± 113.5	344 ± 52.8	ns
Preoperative BAP (μm)	2,135.6 ± 395.8	2,011.2 ± 109.2	ns

ECCT, extracorporeal circulation time; AoCCT, Aorta cross-clamping time; d-ROMs, derivatives of reactive oxygen metabolites; BAP, biological antioxidant potential. The numbers are shown as mean ± SD.

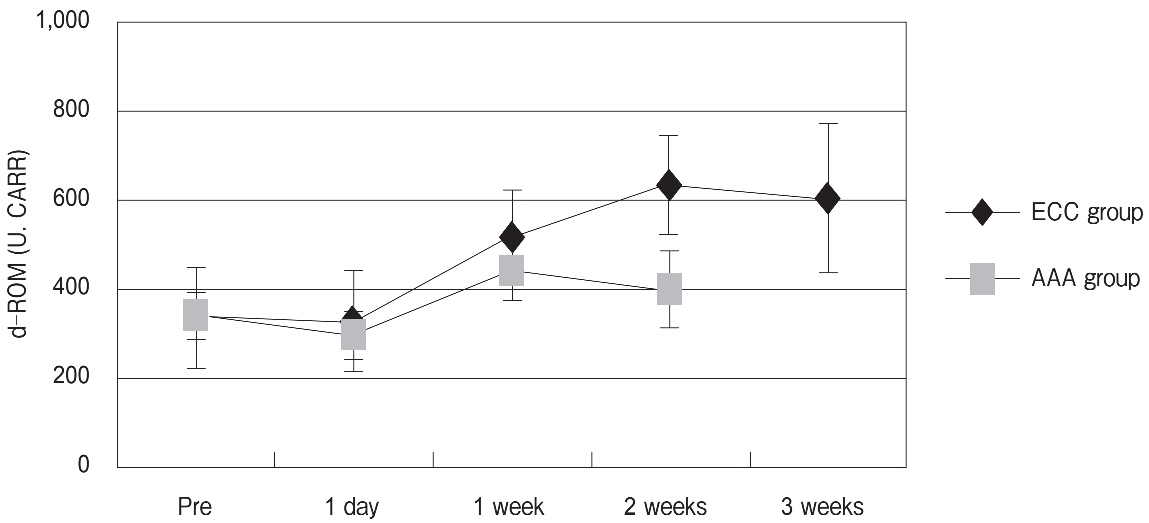

Fig. 1 Perioperative change of d-ROMs.
A gradual increase until the second week was seen in the ECC group. On the other hand, the ROMs values also increased postoperatively in the AAA group but the degree of increase was low and the values began to decrease after 2 weeks.

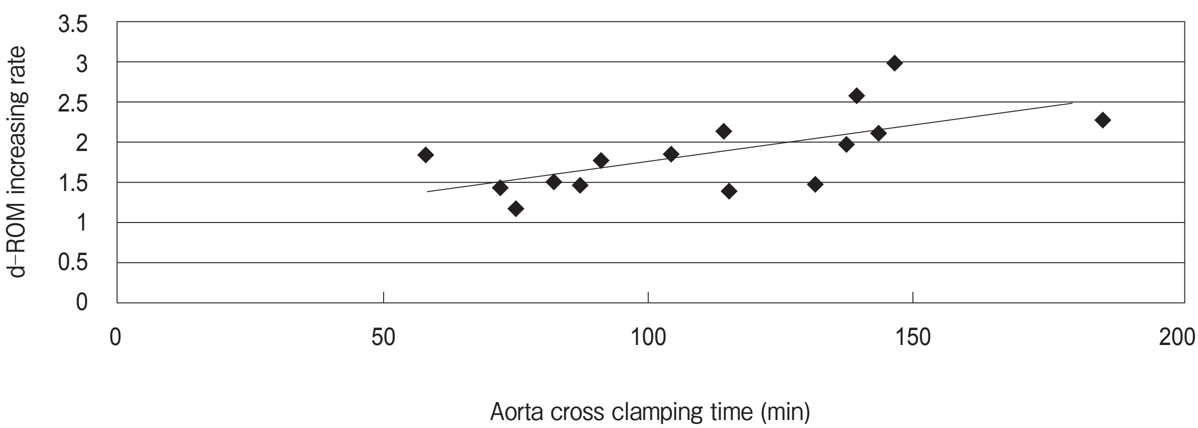

Fig. 2 Correlation of d-ROM increase with aorta clamping time in ECC group.
Rate of increase in ROMs value: maximal value of d-ROMs/preoperative d-ROMs value. $y = 0.0092x + 0.8456$. The determination coefficient (R value) = 0.649 $p = 0.009$. Prolonged aortic clamping time tended to result in a greater rate of increase in the d-ROM value in the ECC group.

after one and 2 weeks were significantly lower in the AAA group than in the ECC group.

In addition, prolonged aortic clamping and ECC time tended to result in a greater rate of increase in ROM values (maximal value of d-ROMs/preoperative d-ROMs value) in the ECC group. The determination coefficients (R values) for aortic clamping time and extracorporeal circulation time were 0.649 and 0.525, respectively, and were considered significant; the rate of ROM increase tended to be positively correlated with aortic clamping time and ECC time (Fig. 2 and 3). Meanwhile, there was no correlation observed between aortic clamping time and the rate of ROM increase in the AAA group (Fig. 4).

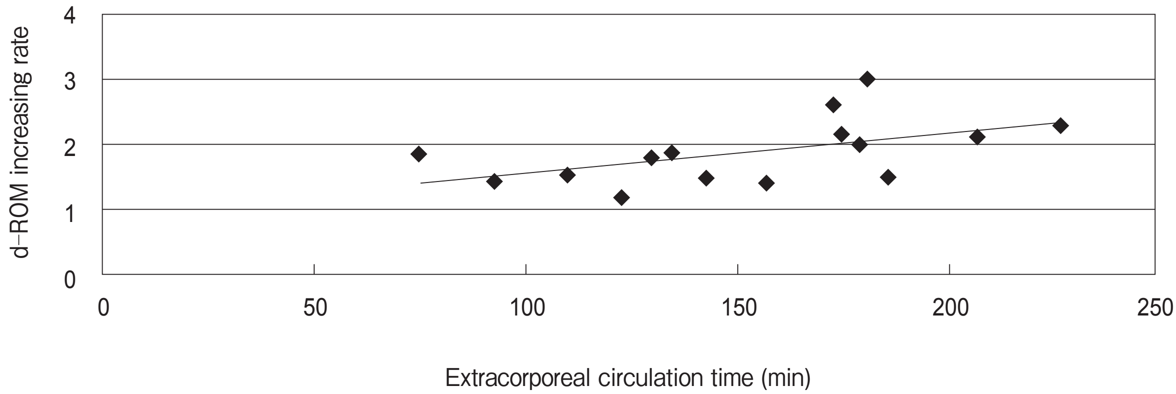

Fig. 3 Correlation of d-ROM increase with aorta clamping time in ECC group.
Rate of increase in ROMs value: maximal value of d-ROMs/preoperative d-ROMs value. $y=0.0061x+0.9379$. The determination coefficient (R value)$=0.525$ $p=0.044$. Prolonged ECC time tended to result in a greater rate of increase in the d-ROM value in the ECC group.

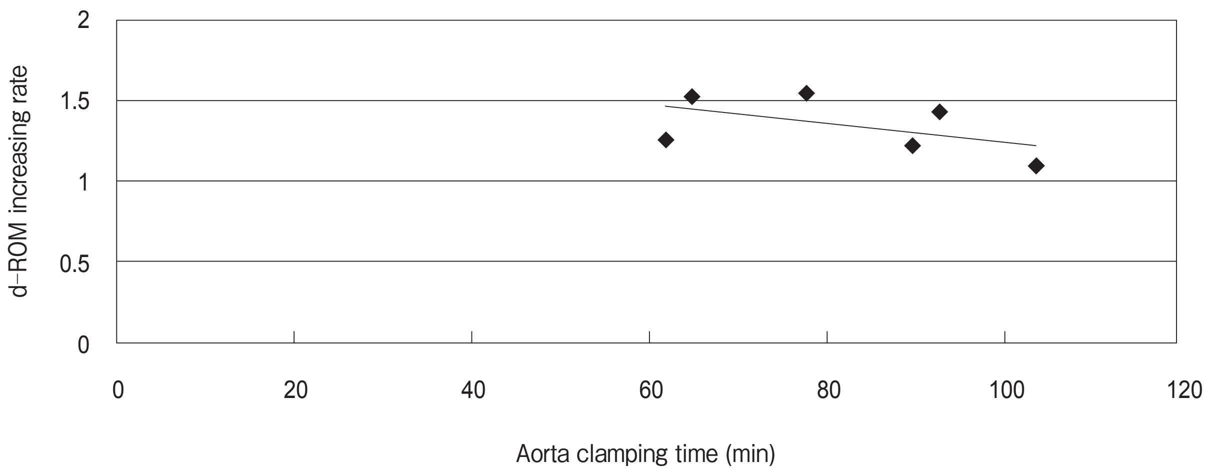

Fig. 4 Correlation of d-ROM increase with aorta clamping time in AAA group.
There was no correlation observed between aortic clamping time and the rate of ROM increase in the AAA group. $y=-0.0056x+1.8152$. The determination coefficient (R value)$=-0.511$ $p=0.300$.

BAP values increased along with an increase in postoperative d-ROM. The BAP values in the ECC group were also higher than in the AAA group, a difference that became significant 2 weeks after surgery (Fig. 5). BAP tended to increase along with an increase in ROMs, and positive correlation was seen (Fig. 6).

Discussion

Recently, it has become clear that arteriosclerosis, cardiovascular diseases and various other diseases are associated with oxidative damage caused by ROS and free radicals. Oxidative stress is a condition in which the oxidative response dominates the antioxidant response due to disruption in the balance between oxidation and antioxidative responses in the body, and is induced by advanced age, infection, ischemia-reperfusion, smoking, and various other factors. However, since there are a great number of free radicals and ROS and most of them have a very short half-life, they have been difficult to even measure and evaluate. Various types of markers have been used to evaluate oxidative stress, including lipid peroxide,

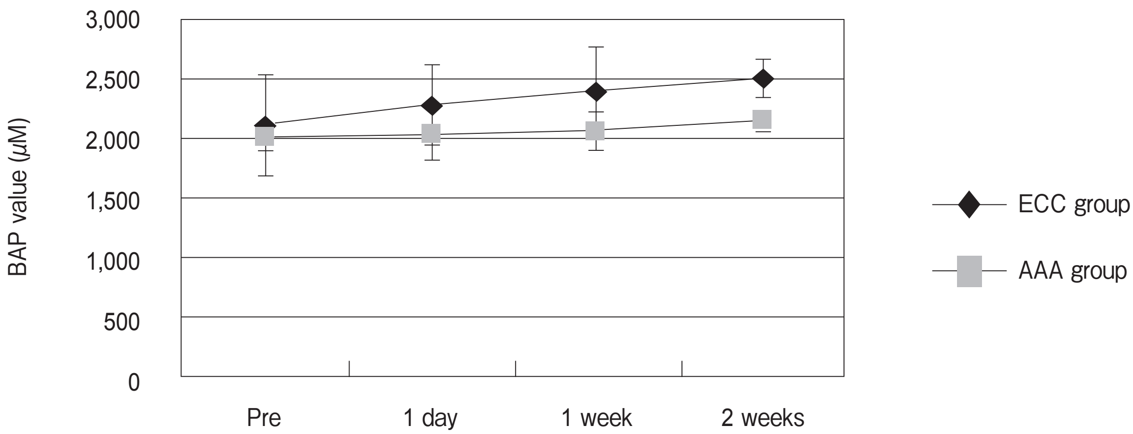

Fig. 5 Perioperative change of BAP.
BAP, biological antioxidant potential. BAP increased along with an increase in postoperative d-ROM. The BAP values in the ECC group were also higher than in the AAA group, which became significant 2 weeks after surgery.

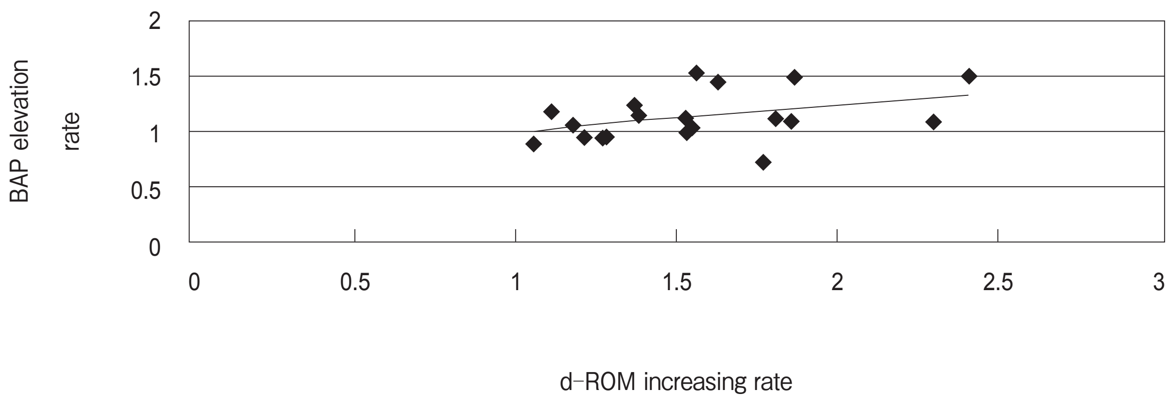

Fig. 6 Correlation of d-ROM elevation with BAP elevation.
BAP tended to increase along with an increase in d-ROM levels. $y = 0.175x + 0.8622$ The determination coefficient (R value) = 0.470. $p = 0.042$.

4-hydroxynonenal, malondialdehyde (MDA), iso prostaglandin, 8-hydroxyguanine (8-OhdG), thymine glycol, carbonyl protein, dopa, hydroxyleucine and hydroxyvaline. Recently, noninvasive analyses of *in vivo* free radical responses have been conducted using electron spin resonance (ESR) or Overhauser effect MRI and spin probe methods [9, 10]. Rather than measuring the ROS and free radicals directly, the free radical automatic analyzer (FRAS 4) that we used for this study measures the hydroperoxide concentration in the blood using the colorimetric method. The quantity of hydroperoxide is considered to be directly proportional to the quantity of ROMs, which are affected by ROS and free radicals [1]. We considered this method effective as its results were reproducible. ROM values are considered to increase due to smoking, diabetes and dialysis [11], but because only a few smokers were represented in this data, no significant difference in preoperative values between smokers and non-smokers (343.2 ± 102.1 vs. 334.8 ± 57.3) was observed. The values were high in dialyses patients, but no difference in the ROM values was observed in other preoperative conditions. We intend to conduct further studies on a greater number of subjects in the future.

At the same time, due to several reports in which the degree of invasiveness of a surgical treatment was discussed in relation to oxidative stress level, [2-6] various markers are currently measured to evaluate postoperative oxidative stress, but it has been difficult to evaluate oxidative stress levels objectively because the half-life of these compounds is short. The values increase perioperatively and postoperatively, but become normalized in a couple of days, according to many reports [7, 12]. It remains a question as to whether it is possible to evaluate the overall condition of oxidative stress by measuring a single marker.

In this study, measurements of oxidative stress indicated that the postoperative d-ROM values remained high for 2 weeks. This finding contrasted with conventional reports stating that ROM values become normalized perioperatively or postoperatively within one to 2 days [7, 12]. Although many patients were discharged from the hospital 2 weeks after surgery and measurements were taken in only 3 patients after the third week, the postoperative values decreased from the previous values in all patients, and a subsequent decline was indicated. The d-ROM values increased more significantly in surgery with ECC than without it. In addition, ECC time and aortic clamping time tended to be positively correlated with the rate of ROM increase, indicating that prolonged ECC time and aortic clamping time increased oxidative stress.

It has been reported that free radicals are produced as a result of ischemia-reperfusion [8]; in the case of CBP, from the occurrence of ischemia-reperfusion in organs exposed to non-physiological circulation during ECC. However, there is a report of a case in which retrograde warm blood cardioplegia collected from the coronary sinus after injection of a cardioplegia was effective in suppressing an MDA increase, but the MDA level was still high; therefore, it was considered that oxidative stress increased by myocardial ischemia-reperfusion [1]. On the other hand, there is also a report in which the markers for oxidative stress did not differ between blood collected from the coronary sinus and blood collected from peripheral veins [8]; hence, the source of ROM production is considered to be the entire body rather than the heart. In this study, the increase in ROMs was greater in surgery using extracorporeal circulation than in abdominal aortic aneurysm surgery in which there is true ischemia-reperfusion in the lower body. Moreover, although no correlation was observed between aortic clamping time and the increase in ROMs in the abdominal aortic aneurysm surgery, prolongation of extracorporeal circulation tends to cause an increase in ROMs when ECC is used. These facts were considered to indicate that implementation and withdrawal of extracorporeal circulation leads to exposure of the entire body, including the heart, to non-physiological circulation and reperfusion. Furthermore, it is said that a high oxygen concentration promotes postischemic reperfusion injuries [14], and in particular, that aortic clamping causes non-pulsatile flow and high oxygen loading; therefore, the degree of non-physiological circulation is increased with the use of ECC.

Furthermore, an increase in BAP, as well as ROMs, was observed in many cases after surgery, indicating that antioxidants existing in the blood attempt to inhibit an increase in ROMs once they increase. However, there is a report in which the antioxidant potential decreased during surgery, during aortic clamping, and after declamping, which was interpreted as an overload of oxidant stress [15, 16]. However, there is another report in which the antioxidant potential increased immediately following surgery [17]; hence, this matter still remains controversial.

Reactive oxygen metabolites are affected by hemoglobin level, body temperature, *etc.* During surgery, hemoglobin decreases due to dilution and a drop in body temperature; thus, it is difficult to assess reactive oxygen metabolites. In addition, they are considered to be affected to some degree even following surgery. d-ROM values temporarily decreased after the AAA surgery in this study, but this was assumed to be caused by the decreased hemoglobin level. We would like to examine more cases in the future in order to study the relationship between oxidative stress and complications, especially to determine whether a decrease in antioxidant potential causes complications and whether antioxidant administration is effective.

Conclusion. In cardiovascular surgeries associated with surgical stress, especially ischemia-reperfusion, there is an increase in reactive oxygen metabolites. In all subjects ECC, d-ROM values remained elevated for about 2 weeks; generally, the antioxidation action also increased with the ROM values. In surgery without the use of extracorporeal circulation, the increase of d-ROM values was slight, whereas in surgery using an extracorporeal circulation, the d-ROM values gradually increased for 2 weeks, and the increase was significant. The conventional markers to evaluate oxidative stress have a short half-life, and do not seem to reflect real oxidative stress. The reactive oxygen metabolites showed a tendency to increase with the prolongation of extracorporeal circulation time and aortic clamping time. From the viewpoint of oxidative stress, surgery without the use of extracorporeal circulation is less invasive. Our data suggests that it is best to keep the extracorporeal circulation time as short as possible.

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