Role of Oxygen Metabolite in the Oxygen Paradox in Hypoxic Myocardium[1]

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=Abstract=The study was conducted to confirm the hypothesis that cytotoxic oxygen free radicals are involved in myocardial cellular damage upon reoxygenation of hypoxic hearts(oxygen paradox), and to identify which species of oxygen radicals might be responsible for the damage.

Oxygen paradox was induced in isolated, Langendorff preparations of rat heart by hypoxic, cardioplegic perfusion(90 min) followed by oxygen-repleted perfusion(20 min). The releases of cytosolic enzymes(creatine phosphokinase, CPK: lactic dehydrogenase, LDH) and a lipid peroxidation product, malondialdehyde (MDA) into the coronary effluent were assayed as the indicators of myocardial cellular damage. To confirm the generation of oxygen radicals during the process of oxygen paradox, H_2O_2 relaesed into the coronary effluent was detected using a fluorescent dichlorofluorescin assay.

The releases of CPK, LDH and MDA into the coronary effluent were abruptly increased by reoxygenation in isolated hypoxic rat hearts. The concentration of H_2O_2 detected in the coronary effluent was also markedly elevated upon reoxygenation. The increases of enzymes and a lipid peroxidation product were prevented to various degrees by scavengers of O_2^- (superoxide dismutase, SOD, 10,000 U), H_2O_2 (catalase, 25,000 U) and OH· (dimethylsulfoxide, DMSO, 10%), and by an iron-chelator deferoxamine (0.5 mM). H_2O_2 in the coronary effluent increased dramatically with the adminstration of SOD in the perfusion solutions, while it was never detected when catalase was given.

It was concluded from these results that the generation of oxygen free radicals increased during the process of oxygen paradox in hypoxic hearts, and that the hydroxyl radical might play a major contributing role in the development of the myocardial cellular damage through the lipid peroxidation of cell membranes.

Key words: **Hypoxic myocardium, Oxygen paradox, Oxygen free radical, Lipid peroxidation**

INTRODUCTION

It is well known that reoxygenation of a previously hypoxic heart results in significant myocardial damage rather than improvement. This phenomenon has been termed the oxygen paradox (Hearse *et al.* 1973). The oxygen paradox in experimental animals shares similar pathophysiologic findings with the reperfusion injury which results during reperfusion of ischemic myocardium by coronary by-pass surgery or thrombolytic therapy in human patients(Hess and Manson 1984).

The mechanisms responsible for the cellular damage in the hypoxic-reoxygenated myocardium have not been clearly established. Recently, possible involvement of toxic oxygen radicals(superoxide

anion, O_2^- ; hydrogen peroxide, H_2O_2; hydroxyl radical, $OH\cdot$) has been suggested to play a crucial role in the development of myocardial injury in the process of the oxygen paradox. This is supported by observations that exogenous scavengers of oxygen radicals significantly reduce functional and biochemical indices of damage in isolated hypoxic-reoxygenated animal hearts(Gaudel and Duvelleroy 1984; Guarnieri *et al.* 1980; Myers *et al.* 1985). However, although these studies showed significant cardioprotective effects of oxygen radical scavengers, it was only indirect evidence of oxygen radical involvement, because they did not find the production of oxygen radicals directly in myocardial tissue. Furthermore, these scavenger studies provided little information about which oxygen radical species might be responsible for the myocardial damdage. The purpose of present study was to confirm the oxygen radical generation during the course of oxygen paradox and to identify particular species of oxygen radical which might play a major role in the mycocardial damage. For that purpose, H_2O_2 released into the coronary effluent was measured by a sensitive fluorometric method using fluorescent dichlorofluorescin diacetate, and cardioprotective effects of various selective exogenous oxygen radical scavengers and other intervention(iron chelator) were reevaluated. The primary indices of myocardial damage were releases of intracellular enzymes (creatine phosphokinase and lactic dehydrogenase) and a lipid peroxidation product, malodialdehyde into the coronary effluent.

MATERIALS AND METHODS

Adenosine diphosphate(ADP), adenosine monophosphate(AMP), catalase, creatine phosphate(CP), glucose-6-phosphate dehydrogenase (G-6-PDH), hematin, hexokinase, nicotinamide adenine dinucleotide phosphate(NADP), reduced nicotinamide dinucleotide(NADH), sodium pyruvate, superoxide dismutase(SOD), thiobarbituric acid were purchased from Sigma Chimcal Co.. 2′,7′-dichlorofluorescin diacetate(DCFDA) was obtained from Eastman-Kodak, dimethylsulfoxide(DMSO) was from Merck, deferoxamine was from Ciba-Geigy, and the other chemicals were reagent grade.

Isolated heart preparations and induction of oxygen paradox

Sprague-Dawley rats of either sex, weighing 150-200 g were heparinized intraperitoneally (100

iu). Forty-five min after the administration of heparin, the heart was quickly removed and was perfused through the aorta cannluated with a stainless steel cannula attached to a Langendorff perfusion apparatus at a constant perfusion pressure of 100 cm H_2O. The perfusion solution was Krebs-Henseleit bicarbonate buffer(K-H solution) containing(in mM) NaCl 118, $NaHCO_3$ 27.2, KCl 4.8, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1, $CaCl_2$ 1.2 and glucose 11.1, and was saturated with a 95% O_2-5% CO_2 gas mixture yielding a pH value of 7.4 at 37°C. The heart was kept in a humidified chamber maintained at 37°C during the perfusion. The pulmonary artery was incised to allow complete drainage of the coronary effluent. After 20 min of controlled perfusion for wash-out of the residual blood and equilibration, the heart was subjected to 90 min of hypoxic perfusion followed by 20 min of reoxygenated perfusion. Hypoxic and reoxygenated perfusion were done with cardioplegic solution in which K^+ concentration had been increased to 15 mM with a corresponding decrease in Na^+ concentration. The hypoxic solution in which glucose had been replaced by mannitol(substrate-free solution) was equilibrated with 95% N_2-5% CO_2. The reoxygenated cardioplegic solution was saturated with 95% O_2-5% CO_2 gas mixture. The reason why using the cardioplegic solution, instead of normal K-H solution, was to prevent the unexpected release of intracellular enzymes due to the occurrence of irregular spasmodic beating during hypoxic or reoxygneated period and so to provide a more reproducible experimental condition(Hearse *et al.* 1973). Some control hearts were perfused with oxygenated K-H solution for 2 hrs. At the end of each experiment, the hearts were removed, and after having blotted excess fluid, the tissues were weighed.

Indices of myocardial damage

Release of intracellular enzymes, creatine phosphokinase(CPK) and lactic dehydrogenase(LDH) into the coronary effluent was used as indicator of myocardial cellular damage. Coronary effluents were collected at indicated time intervals during hypoxic and reoxygenated perfusion. After measurement of the volume, the samples were kept in ice until the assays were completed(within 8 hrs).

CPK activity was assayed by UV-spectrophotometrically(Bergmeyer 1974). The reaction mixture(pH 6.9) contained imidazole 100 mM, glucose 20 mM, $MgCl_2$ 10 mM, ADP 1 mM, AMP 10 mM,

CP 20 mM, NADP 0.7 mM, cystein HCl 10 mM, hexokinase 0.94 U/ml and G-6-PDH 0.48 U/ml. The reaction was started by the addition of 0.05 ml of coronary effluent sample into 2.95 ml of the reaction mixture, and the rate of change of optical density at 25°C and 340 nm was measured with UV-spectrophotometer(Perkin-Elmer Model 139).

LDH activity was assayed by UV-spectrophotometry(Bergmeyer 1974). 0.5 ml of the sample was added into 2.5 ml of the reaction mixture contained 48 mM phosphate buffer(pH 7.5), 0.6 mM pyruvate and 0.18 mM NADH. The rate of change of optical density was measured by UV-spectrophotometer at 25°C and 340 nm.

The degree of lipid peroxidation in myocardial tissue was also estimated. A lipid peroxidation product, malondialdehyde(MDA) released into the coronary effluent was measured by thiobarbituric acid method (Yagi 1982). 2.4 ml of the coronary effluent was added into 0.6 ml of 1:1 mixture of 0.67% thiobarbituric acid and glacial acetic acid. The reaction mixture was placed on boiling water bath for 60 min and then cooled to room temperature. After cooling, the absorbance was measured at 532 nm with UV-VIS spectrophotometer(Perkin-Elmer Model 139). The MDA released was expressed as nmole/min/g wet wt using the molar extinction coefficient of 1.52×10^5/M/cm(Placer *et al.* 1966).

H₂O₂ assay

As measured by H_2O_2 released into the coronary effluent, the production of oxygen radical during the process of oxygen paradox was observed. The concentration of H_2O_2 was assayed spectrofluorometrically using 2′,7′-dichlorofluorescin diacetate(DCFDA)(Cathcart *et al.* 1983). 0.2 ml of coronary effluent collected at intervals during hypoxic and reoxygenation periods was mixed with 3 ml of a reaction mixture contained sodium phosphate buffer 25 mM(pH 7.2), DCFDA 0.01 mM and hematin 3.2μM. The mixture was incubated for 50 min at 50°C. After cooling to room temperature, the fluorescence was measured with spectrofluorometer(Perkin-Elmer model 1000) at the excitation wavelength of 452 nm and the emission wavelength of 550 nm. Just prior to the assay, DCFDA was converted to dichlorofluorescin. 0.5 ml of 1 mM DCFDA in ethanol was added to 2.0 ml of 0.01 N NaOH and allowed to stand at room temperature for 30 min. The hydrolysate was then neutralized with 10 ml of 25 mM sodium phosphate buffer (pH 7.2) and stored on ice. The

hematin solution was prepared by dissolving 1 mg of hematin in 0.5 ml of 0.2 N NaOH and then diluting to 100 ml with 25 mM sodium phosphate buffer(pH 7.2). This solution was made fresh each day.

Administration of oxygen radical scavenger

To assess the possible involvement of oxygen radicals in the development of myocardial damage, cardioprotective effects of exogenous, specific oxygen radical scavengers and other interventions were evaluated. Among the scavengers studied, superoxide dismutase(SOD) which dismutates superoxide anion(O_2^-) enzymatically, and catalase which degrades H_2O_2 were administered through the aortic cannula by use of a infusion pump. The infusion continued for 50 min starting from 60 min of hypoxic period to the end of reoxygenation at a rate of 0.5 ml/min. Total amount of enzymes administered were SOD, 10,000 U and catalase, 25,000 U. A OH· scavenger, dimethylsulfoxide(DMSO) was dissolved in perfusion solution at a final concentration of 10%, and administered for 25 min from 5 min before oxygen repletion to the end of reoxygenation.

To deduce a particular oxygen radical species which may play a major role in the pathogenesis of the oxygen paradox, the effect of a highly specific iron chelator, deferoxamine was also evaluated. Deferoxamine (5 mM) was infused through the aortic cannula at a rate of 0.5 ml/min for 50 min starting from 60 min of hypoxic perfusion to the end of reoxygenated perfusion. It's final concentration in perfusates was about 0.5 mM.

RESULTS

Effects of oxygen radical scavenger and deferoxamine

Coronary flow. In the control hearts that were perfused with oxygenated K-H solution, coronary flow was maintained constant (about 11 ml/min/g wet wt) throughout 2hr perfusion period. Coronary flow changes occured during hypoxia and oxygen repletion are shown in Table 1. In untreated hearts, coronary flow during hypoxia decreased gradually with time. At 90 min of hypoxia, the flow was 63% of prehypoxic value. Oxygen repletion following 90 min of hypoxia further decreased the flow to a 50% of prehypoxic value at the end of reoxygenation period(20 min). However, in the scavenger treated hearts, coronary flows during both hypoxia and reoxygenation, although reduced from prehypoxic values, were considerably greater than those of un-

Table 1. Effects of oxygen radical scavengers on coronary flow during hypoxia and reoxygenation in isolated rat heart

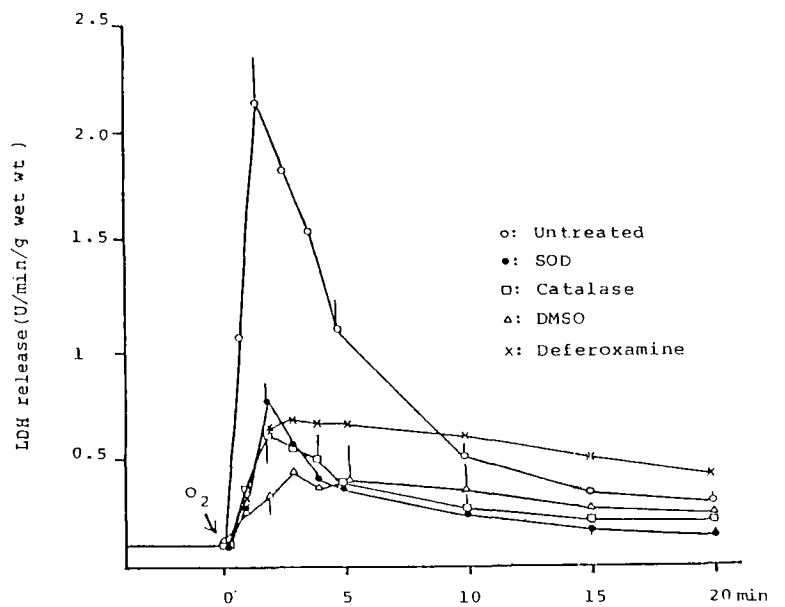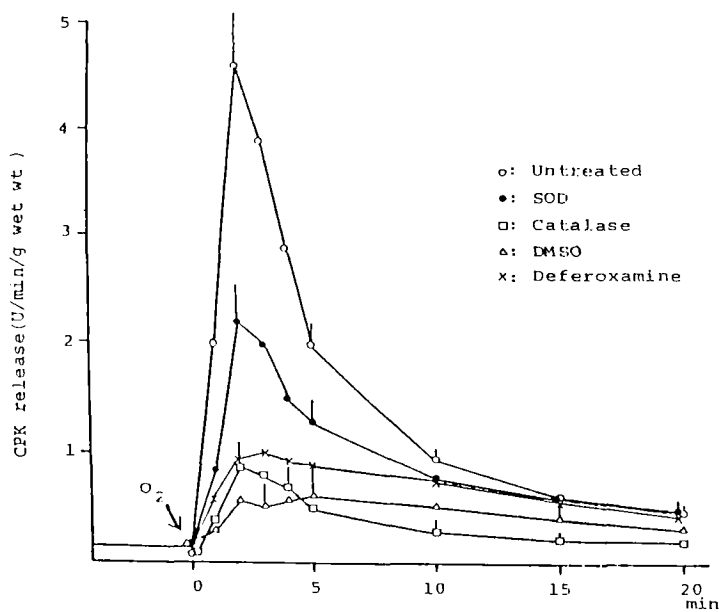| Conditions	No. of Animal	Control Perfusion	Hypoxia(min)		Reoxygenation(min)			
			60	90	2	5	10	20
Untreated	12	11.4±0.6	8.8±0.5	7.2±0.3	6.4±0.2	5.7±0.2	5.6±0.2	5.8±0.2
SOD (10,000 U)	7	11.0±1.1	10.1±0.5*	8.4±0.5*	7.9±0.3**	7.1±0.4**	7.1±0.4**	7.2±0.4**
Catalse (25,000 U)	12	13.1±1.0	10.4±0.5*	7.7±0.5*	7.3±0.5*	6.8±0.5*	6.8±0.5*	7.2±0.5*
DMSO(10%)	5	10.4±0.8	11.7±0.3*	8.5±0.7*	8.4±0.4**	8.3±0.4**	7.9±0.5**	7.7±0.2**

* $p < 0.05$, ** $p < 0.01$

Fig. 1. Effects of oxygen radical scavengers and deferoxamine on creatine phosphokinase release in hypoxic-reoxygenated rat heart. Isolated rat hearts were subjects to 90 min of hypoxic, substrate-free perfusion followed by 20 min of oxygen-repleted perfusion. Superoxide dismutase(SOD, 10,000 U), catalase(25,000 U) or deferoxamine(0.5 mM) were infused for 50 min, from 30 min before reoxygenation to the end of perfusion. Dimethylsulfoxide (DMSO, 10%) dissolved in perfusion medium was administered from 5 min before reoxygenation and throughout the reoxygenation period.

Fig. 2. Effects of oxygen radical scavengers and deferoxamine on lactic dehydrogenase release in hypoxic-reoxygenated rat heart. Methods of perfusion and scavenger administration were same as in Fig. 1.

treated hearts. At the end of reoxygenation the flows increased by 5-25% compared to corresponding untreated flows. Oxygen raidical scavengers at concentrations used did not change the coronary flow in the oxygenated control hearts(data not shown).

Enzyme release. Release of intracellular enzymes during hypoxia was not so significant. After 90 min of hypoxia, the rate of CPK release into the coronary effluent was 0.08 U/min/g wet wt. However, in the hearts subjected to hypoxia followed by reoxygenation, there was a sudden, massive enzyme release upon oxygen repletion. As in Fig. 1, the rate of CPK release was abruptly increased to a maximum of 4.6 U/min/g wet wt at 2 min of reoxygneation period, which was a 58 fold increase compared to that of hypoxia. This increased rate was suppressed significantly by SOD, catalase or DMSO. In addition to those scavengers, a specific Fe^{+++}-chelator, deferoxamine also caused a sig-
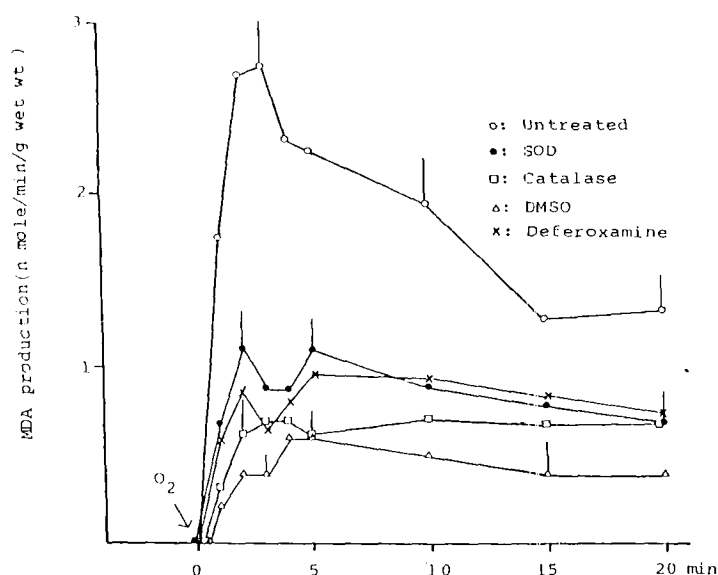
Fig. 3. Effects of oxygen radical scavengers and deferox-
amine on lipid peroxidation in hypoxic-reoxyge-
nated rat heart. Lipid peroxidation of myocardial
tissue was estimated from malondialdehyde re-
lease assayed by thiobarbituric method. Perfusion
conditions and methods of scavenger administra-
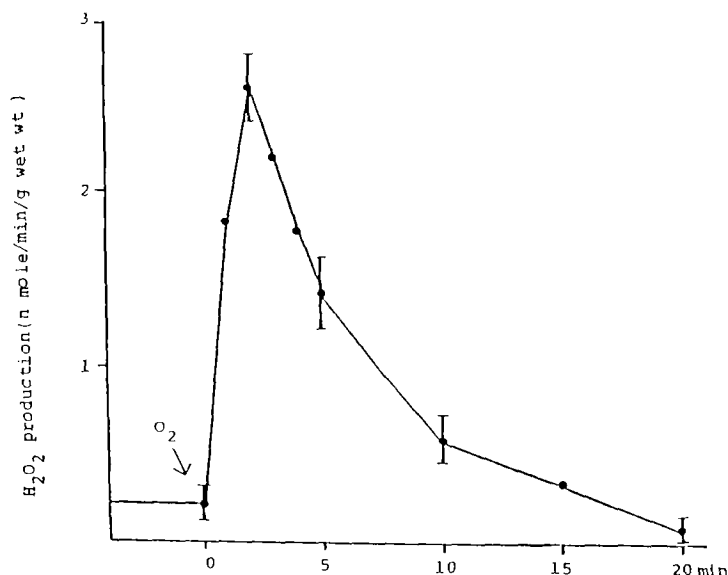tion were same as in Fig. 1.

Fig. 4. H_2O_2 production during reoxygenation in hypoxic
rat heart. Isolated rat hearts were subjected to 90
min of hypoxic, substrate-free perfusion followed
by 20 min of oxygen-repleted perfusion. H_2O_2
released into the coronary effluent during reox-
ygenation period was estimated by fluorescent
dichlorofluorescein assay method.

nificant reduction of CPK release upon reoxygena-
tion.

In LDH(Fig. 2), the rate of release during hypoxia
was 0.1 U/min/g wet wt(at 90 min), and it was
suddenly increased by a maximum of 21-fold at 2
min of reoxygenation. Oxygen radical scavengers or
deferoxamine also suppressed the LDH release sig-
nifcantly during reoxygenation.

The addition of oxygen radical scavengers or de-
feroxamine to the perfusing solutions neither in-
duced nor decreased the intracellular enzyme re-
lease in the control heart preparations perfused
with oxygenated K-H solution for 2 hrs (data not
shown).

Lipid peroxidation. The degree of lipid peroxida-
tion in the myocardial tissue was estimated from
the release of MDA into the coronary effluent. Dur-
ing 90 min of hypoxia, MDA was not detected in
the cornary effluent. However, in the heart prepara-
tions subjected to 90 min of hypoxia followed by 20
min of reoxygenation, MDA was abruptly released.
The maximum rate of release was 2.76 n mole/g
wet wt at 3 min of reoxygenated perfusion(Fig. 3).
This MDA release was prevented significantly in the
presence of oxygen radical scavengers or deferox-
amine. SOD, catalase, DMSO or deferoxamine did
not induce the MDA release in the control hearts
(data not shown).

H_2O_2 production

To confirm the generation of oxygen radical dur-
ing the process of the oxygen paradox, H_2O_2 in the
coronary effluent was measured. H_2O_2 was abrupt-
ly increased with oxygen repletion in the isolated,
hypoxic rat heart preparations. The rate of H_2O_2
production during hypoxia was 0.2 nmole/min/g
wet wt (at 90 min), and it was suddenly increased
to a maximum value of 2.62 nmole/min/g wet wt by
reoxygeation(at 2 min), which was a 13 fold in-
crease compared to hypoxic value(Fig. 4). This
H_2O_2 production was similar in pattern with the
release of intracellular enzymes or MDA. Although
H_2O_2 was never detected in the coronary effluent
in the presence of catalase, it was greatly increased
with the addition of SOD in the perfusing solution.
The rate of H_2O_2 production in the presence of
SOD was 13.25 nmole/min/g wet wt during hypox-
ia(at 90 min) and 24.5 nmole/min/g wet wt upon
oxygen repletion(at 1 min)(Table 2).

DISCUSSION

Although different possible mechanisms which
have been reported as contributing factors for the
myocardial damage during the process of oxygen
paradox include depletion of intracellular high
energy pool(Jennings and Reimer 1981), release
of endogenous catecholamines (Gaudel *et al.*

Table 2. Effects of superoxide dismutase(SOD) and catalase on H_2O_2 production in hypoxic-reoxygenated, isolated rat heart

Conditions	No. of Animal	H_2O_2 production(nmole/min/g wet wt.)								
		Hypoxia(min)	Reoxygenation(min)							
		90	1	2	3	4	5	10	15	20
Untreated	11	0.2 ±0.09	1.82 ±0.38	2.62 ±0.20	2.20 ±0.19	1.78 ±0.35	1.43 ±0.20	0.59 ±0.16	0.59 ±0.10	0.09 ±0.07
SOD (10,000 U)	6	13.25 ±1.31	24.51 ±0.05	19.28 ±1.98	17.08 ±1.32	17.51 ±2.73	14.03 ±2.48	11.60 ±1.51	10.09 ±1.87	6.63 ±2.33
Catalase (25,000 U)	6	0	0	0 ·	0	0	0	0	0	0

1979), excessive intracellular calcium accumulations (Katz and Reuter 1979), activation of phospholipases(Chien et al. 1981) and accumulation of lysophosphatides(Pitts & Okhuysen 1984), there is no known single most probable theory.

Recently, possible invovement of toxic oxygen free radicals has been also suggested to play a crucial role in the development of myocardial damage that occurs during reoxygenation of hypoxic myocardium (Gaudel and Duvelleroy 1984; Guarnieri et al. 1980; Myers et al. 1985). Studies with isolated myocardial tissue in vitro indicate that hypoxia or ischemia causes an increase in intracellular reducing equivalents, a disturbance of mitochondrial electron transport, the conversion of xanthine dehydrogenase to xanthine oxidase, and an accumulation of ATP breakdwn products that act as substrate for oxygen radical production through the action of xanthine oxidase. During reoxygenation of the hypoxic tissue, all of the above conditions favor a burst of oxygen radical production(Fridovich 1978; Hess and Manson 1984; Meerson et al. 1982). In the present study, the release of CPK or LDH into the coronary effluent was abruptly increased by reoxygenation in isolated hypoxic rat hearts, and this increase was suppressed to various degrees by scavengers of O_2^- H_2O_2 or OH· . This result supported the hypothesis that toxic oxygen radicals were involved with the pathogenesis of the oxygen paradox in myocardial tissue.

However, this cadioprotective effect of the scavengers observed in this study and other previous reports(Gaudel and Duvellerory 1984; Myers et al. 1985) provided only indirect evidence of oxygen radical involvement. If the toxic oxygen radicals indeed mediate the phenomenon of oxygen paradox, the radicals should be detected in myocar-

dial tissue during the course of oxygen paradox. Using electron spectroscopy, Rao and Muller(1983) found an increase in the production of a certain kind of free radical in ischemic-reperfused tissue, but oxygen radicals have never been detected directly in myocardial tissue. Because O_2^- and OH· exist transiently in aqueous environment, it is very difficult to measure the production of these radcals in tissues by presently available analytical methods indebted to their oxidation-reduction activities. A relatively stable species, H_2O_2, however, could be measured in biological materials by a sensitive fluorometric method using fluoroscent dichlorofluorescin(Cathcart 1983; Homan-Müller et al. 1975). Homan-Müller and colleagues(1975) quantitated H_2O_2 produced by phagocytizing human granulocytes. In this study, H_2O_2 was detected in the coronary effluent and it was massively increased upon reoxygenation. This H_2O_2 increase was comparable to the enzyme release. Furthermore, the H_2O_2 production was more pronounced with the administration of SOD in the perfusion solution. Considering that SOD catalyzes disumatation reaction converting O_2^- to H_2O_2($O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$), this result seemed to be a reflection of increased production of O_2^-.

By comparison with some of the other oxygen spcies, O_2^- and H_2O_2 are not very reactive in biological system. However, in several biological systems O_2^- and H_2O_2 have been implicated as precursors for a more reactive species, OH· . As a mechanism of production of OH· , iron-catalyzed Haber-Weiss reaction($O_2^- + Fe^{+++} \rightarrow O_2 + Fe^{++}$, $H_2O_2 + Fe^{++} \rightarrow OH· + OH^- + Fe^{+++}$) has been proposed (Fong et al. 1976; Frdovich 1978; Halliwell 1978). The significant reduction of intracellular enzyme and MDA release not only by

SOD, catalase or DMSO, but also by deferoxamine, a highly specific Fe^{+++}-chelator, may therefore be explained in terms of suppression of OH· formation. It was supported by the observations that OH· inhibited calcium binding of cardiac sarcoplasmic reticulumin in *in vitro* reaction conditions containing oxygen radical generating system(Kim *et al.* 1984), and that deferoxamine suppressed inflammation in several animal models and inhibited iorn-catalyzed lipid peroxidation *in vitro*(Blake *et al.* 1983).

The result presented in this paper indicated that the peroxidation of membrane lipids, estimated from MDA production had been triggered by oxygen radicals. This finding agrees with the studies of other investigators(Gaudel and Duvelleroy 1984; Guarnieri *et al.* 1980; Kim and Akera 1987) who reported the prevention of MDA production by oxygen radical scavengers in hypoxic-reoxygenated hearts. The lipid peroxidation and the resultant biochemical changes may alter physical properties of membranes, such as changes in lipid microenvironments of membrane bound enzymes and also increase in membrane permeability (Freeman and Crapo, 1982). This fact was confirmed by the close relation which existed between the rate of intracelular enzymes and MDA releases. In the present study, MDA released into the coronary effluent, instead of its sarcolemmal content were measured, due to the analytical difficulties. The results, therefore, do not specifically show the degree of sarcolemmal lipid peroxidation. However, it should be pointed out that sarcolemmal membranes has been shown to be highly susceptible to oxygen radical-induced lipid peroxidation compared with lipid bilayers of other cellular components(Kramer *et al.* 1984). Therefore, the myocardial damage in hypoxic-reoxygenated cardiac tissue will be largely dependent on hte lipid peroxidation in cell membranes by oxygen radical.

In summary, the present study provides further evidence of the involvement of toxic oxygen radicals, particularly hydroxyl radical (OH·), in the pathogenesis of myocardial damage in the oxygen paradox.

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=국문초록=

저산소 심장의 oxygen paradox시 산소대사물의 역할

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이상동 · 최철희 · 박종완 · 김용식 · 김명석

저산소 심근의 산소재공급에 따른 심근손상(oxygen paradox)에 있어서 유독성 산소대사물인
산소라디칼의 관련성을 확인하고, 심근손상을 이르키는 데 주로 관여하는 라디칼이 무엇인지를
검토하였다. Langendorff 관류장치를 사용한 흰쥐 적출심장에서 실험적 oxygen paradox를 유도
하였으며, 심근손상의 지표로 세포질 효소인 creatine phosphokinase(CPK), lactic
dehydrogenase(LDH), 및 지질과산화 산물의 하나인 malondialdehyde(MDA)의 관상관류액으로
의 유출을 측정하였다. 산소라디칼의 생성을 확인하기 위하여는 역시 관상관류액중의 H_2O_2를
형광성 dichlorofluorescin을 이용한 fluorometry법으로 측정하였다. 저산소관류(90분)에 이은
산소재공급관류(20분)시 CPK, LDH 및 MDA의 관류액으로의 유출이 산소 재공급과 더불어 급
격히 증가하였으며, H_2O_2 또한 산소 재공급시 현저히 증가하였다. O_2^- 제거효소인 superox-
ide dimutase(SOD, 10,000 U), H_2O_2 분해효소인 catalase(25,000 U), OH· 제거물질인
dimethylsulfoxide(DMSO 10%), 철이온 킬레이트제인 deferoxamine(0.5 mM)은 CPK, LDH 및
MDA 유리를 억제하였다. 관상관류액중의 H_2O_2는 catalase 투여시에는 전혀 측정되지 않았으
나, SOD 투여에 의하여는 오히려 더욱 현저한 증가를 보였다. 이상의 실험에서 흰쥐 적출 심장
의 oxygen paradox시 산소라디칼의 생성 증가를 확인하였으며, 산소리디칼 중에서는 OH: 이
심근손상을 유발하는 데 주로 관여할 것으로 결론지었다.