Korean J. of Pharmacology
Vol. 24, No. 1, pp.53-61, 1988

Protective Effect of Antioxidants on the Reoxygenation Injury in Hypoxic Myocardium of Rat

Hyung Ku Yoon, Jung Kyoo Lim and Myung-suk Kim*

Department of Pharmacology, College of Medicine, Seoul National University, Seoul 110-460, Korea

ABSTRACT

The effect of antioxidants on the myocardial cellular damage which occurs during reoxygenation of hypoxic myocardium was examined in isolated rat hearts. The roles of oxygen free radical and lipid peroxidation in reoxygenation injury of myocardium were also investigated. In Langenorff preparation of isolated rat heart, which was made hypoxic by perfusion with the substrate free, hypoxic cardioplegic solution (37℃, 90 min), the release of cytosolic enzymes (creatine phosphokinase, lactic dehydrogenase) and a lipid peroxidation product, malondialdehyde into the coronary effluent were abruptly increased by reoxygenation. The release of enzymes was closely parallel to that of MDA. These increases of enzymes and lipid peroxidation product were suppressed to various degrees in the presence of scavengers of superoxide anion (superoxide dismutase, 10,000 U), hydrogen peroxide (catalase, 25,000 U) and hydroxyl radical (dimethyl sulfoxide, 10%).

A natural antioxidant, α-tocopherol(4.5 uM) and a synthetic one, butylated hydroxytoluene (2 uM) suppressed the release of cytosolic enzymes with the concomittent reduction of lipid peroxidation as measured by malondialdehyde release into the coronary effluent. These effects of antioxidants were dose dependent, and were more pronounced when the antioxidants were administered throughout hypoxic and reoxygenation periods than given during reoxygenation period only.

These results suggest that cytotoxic oxygen free radicals produced in the myocardium during reoxygenation may be responsible for the myocardial cellular injury by enhancing the lipid peroxidation of cellular membranes. Furthermore, the antioxidants may exert protective effect against reoxygenation damage of hypoxic myocardium through the inhibition of lipid peroxidation reaction.

Key Words: Myocardial injury, Hypoxia-reoxygenation, Oxygen radical, Lipid peroxidation, Antioxidant

INTRODUCTION

The myocardial cellular damage which occurs during reoxygenation in isolated hypoxic heart ("Oxygen Paradox") shares simliar biochemical and structural changes with the reperusion injury of ischemic myocardium in vivo(Hearse et al. 1973, 1977; Hess and Manson 1984). One mechanism by which reoxygenation of hypoxic myocardial tissue causes a irreversible cellular damage may be ascribed to the production of reactive oxygen free radicals(superoxide anion, O_2^-; hydrogen peroxide, H_2O_2; hydroxyl radical, $OH\cdot$; singlet oxygen, $^1O_2$) and lipid peroxidation of cellular membranes. This has been supported by the observations that exogenous scavengers of oxygen free radicals prevent functional and biochemical alterations as well as lipid peroxidation reaction in hypoxic and reoxygenated animal tissues(Gaudel and Duvelleroy 1984; Guarnieri et al. 1980; Kim and Akera 1987; Meerson et al. 1982). The oxidative insult on membrane lipid and the resultant damage may cause alterations in activities and functions of membrane-bound enzymes, ion channels and also

*Author for correspondence.

membrane permeability leading to severe disturbance in cellular functions(Freeman and Crapo 1982).

If oxygen free radicals are produced in hypoxic-reoxygenated myocardium indeed, and if they cause membrane damage by inducing lipid peroxidation, then not only the oxygen radical scavengers but also the appropriate preventive measures for the lipid peroxidation should have a protective effect on the myocardial cellular damage during the process of oxygen paradox. In the present study, we examined the protective effects of a natural antioxidant, α-tocopherol and a synthetic one, butylated hydroxytoluene in isolated hypoxic rat heart, and also tried to reinvestigate the role of oxygen free radical and lipid peroxidation in the genesis of reoxygeneation injury of myocardial tissue.

MATERIALS AND METHODS

Adenosine diphosphate (ADP), adenosine monophosphate (AMP), butylated hydroxytoluene (BHT), catalase, creatine phosphate (CP), glucose-6-phosphate dehydrogenase (G-6-PDH), hexokinase, nicotinamide adenine dinucleotide phosphate (NADP), reduced nicotinamide adenine dinucleotide (NADH), sodium pyruvate, superoxide dismutase (SOD), thiobarbituric acid, dl-α-tocopherol acetate were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Dimethylsulfoxide (DMSO) was obtained from E. Merck (Darmstadt, West Germany) and other chemicals were reagent grade.

Induction of oxygen paradox

Sprague-Dawley rats of either sex, weighing 150-200 g were heparizined intraperitoneally. Forty-five minutes after the heparinization, rat was stunned by a blow to the head. The heart was quickly removed and perfused retrogradly through the aorta cannulated with a stainless steel cannula connected to a Langendorff perfusion apparatus. The perfusion solution was Krebs-Henseleit bicarbonate buffer solution(K-H solution, NaCl 118 mM, $NaHCO_3$, 27.2 mM, KCl 4.8 mM, $MgSO_4$, $7H_2O$ 1.2 mM, KH_2PO_4 1 mM, $CaCl_2$ 1.25 mM and glucose 11.1 mM) saturated with a 95% O_2-5 % CO_2 gas mixture yielding a pH value of 7.4 at 37℃. The perfusion pressure was constantly maintained at 100 cmH_2O throughout the whole perfusion period. The heart was kept in a humidified chamber maintained at 37℃ during the perfusion. The pulmonary artery was opened at where it leaves the right ventricule to allow free drainage of the coronary effluent. After 15 min of control perfusion for washing-out the residual blood and equilibration, the heart was subjected to 90 min of hypoxic perfusion and then followed by 20 min of reoxygenated perfusion. The hypoxic and reoxygenated perfusion solution was a cardioplegic solution in which potassium concentration had been increased to 15 mM with a corresponding decrease in sodium concentration. The hypoxic solution was a glucose-free (substituted with equimolar mannitol) solution equilibrtated with a 95 % N_2-5% CO_2 gas mixture. The reoxygenated solution was saturated with a 95% O_2-5% CO_2 gas mixture. Control heart was continuously perfused for 2hrs with oxygenated normal K-H solution. Coronary effluents were collected at indicated time intervals during hypoxic and reoxygenation period for further assay. After completion of the perfusion, the heart was removed from Langendorff apparatus and was weighed after having blotted surface fluid.

The antioxidants studied were α-tocopherol and butylated hydroxytoluene (BHT). They were added to the perfusion solution at a final concentration of 4.5 uM for α-tocopherol and 2 uM for BHT throughout the hypoxic and reoxygenation period. Oxygen radical scavengers used were superoxide dismutase (SOD) which dismutates superoxide anion enzymatically, catalse which degrades hydrogen peroxide, and a hydroxyl radical scavenger, dimethyl sulfoxide (DMSO). Among these, SOD and catalase were administered through the aortic cannula by using an infusion pump. The infusion was continued at a rate of 0.5 ml/min for 50 min starting from 30 min before the end of hypoxic perfusion and thoughout 20 min of the reoxygenation period. Total amounts administered were 10,000 U for SOD and 25,000 U for catalase. DMSO was dissolved in perfusion solution at a concentration of 10% and administered for 25 min from 5 min before oxygen repletion to the end of reoxygenation.

Enzyme assay

Intracellular enzyme, creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) released into the coronary effluent were measured as index of myocardial cellular damage. CPK activity was

assayed spectrophotometrically (Forster et al. 1974). A 0.05 ml aliquot of coronary effluent was added into 2.95 ml of the reaction mixture containing imidazole 100 mM (pH 6.9), glucose 20 mM, $MgCl_2$ 10 mM, ADP 1 mM, AMP 10 mM, CP 20 mM, NADP 0.7 mM, cysteine HCl 10 mM, hexokinase 0.94 U/ml, G-6-PDH 0.48 U/ml. The rate of change of opitcal density was measured at 25℃ and 340 nm with a UV-spectrophotometer (Perkin-Elmer, Model 139). LDH activity was assayed by spectrophotometric method (Bergmeyer and Bernt 1974). The reaction was started by adding 0.5 ml of the coronary effluent into 2.5 ml of the reaction mixture containing 48 mM phosphate buffer (pH 7.5), 0.6 mM pyruvate and 0.18 mM NADH. The rate of change of optical density was recorded at 25℃ and 340 nm with UV-spectrophotometer.

Measurement of lipid peroxidation

The degree of lipid peroxidation in myocardial tissue was estimated from malondialdehyde (MDA) released into the coronary effluent. The concentration of MDA was determined by thiobarbituric acid method (Yagi 1982). A 2.4 ml aliquot of the coronary effluent was transfered to a screw-cap Pyrex tube and mixed with 0.6 ml of 1:1 mixture of 0.67% thiobarbituric acid and glacial acetic acid. The tube was placed on boiling water bath for 60 min and then cooled to room temperature. The absorbance of the reaction mixture was measured at 532 nm with UV-VIS spectrophotometer (Perkin-Elmer, Model 139). The amount of MDA released was expressed as n mole/min/g wet wt using the molar extinction coefficient of 1.56×10^5/M/cm(Placer et al. 1966).

RESULTS

Effects of oxygen radical scavengers

Either in control hearts which were perfused with oxygenated K-H solution for 2 hrs or in hypoxic hearts which were perfused with nitrogen saturated cardioplegic solution for 90 min, there was no significant release of CPK or LDH. However, reoxygenation following hypoxic perfusion caused a massive release of intracellular enzymes. This increased enzyme release was significantly prevented by oxygen radical scavengers. Selected concentrations of SOD, catalase and DMSO reduced the amont of CPK and LDH released during 20 min period of reoxygenation to less than half of those released in untreated hypoxic-reoxygenated hearts. For lipid peroxidation, there was no release of MDA even after oxygenated perfusion of isolated rat heart for 2 hrs.

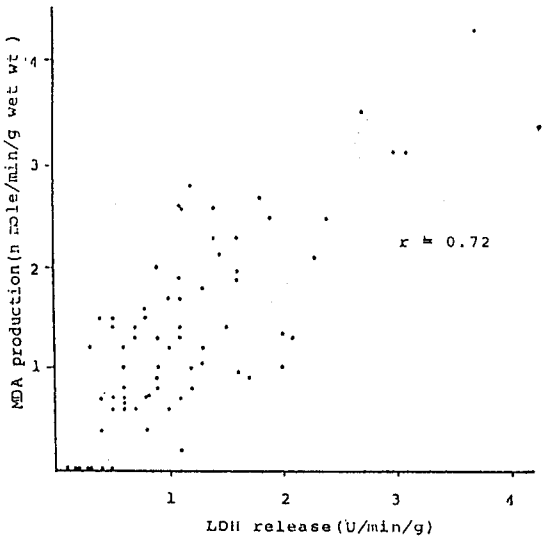

Fig. 1. Relationship between lactic dehydrogenase release and malondialdehyde formation in hypoxic-reoxygenated heart. The plotted points represent MDA and LDH release estimated at 2 min after reoxygenation.

Table 1. Effect of oxygen radical scavengers on CPK, LDH and MDA release in hypoxic-reoxygenated rat hearts

Conditions	No. of animal	CPK (U/g wet. wt)	LDH (U/g wet wt)	MDA (nmole/g wet wt)
Untreated	12	29.11 ± 3.04	15.17 ± 1.51	36.29 ± 3.23
SOD (10,000 i.u.)	6	19.16 ± 2.98*	5.44 ± 0.60**	17.14 ± 3.20**
Catalase (25,000 i.u.)	7	7.42 ± 1.87**	6.16 ± 1.20**	12.88 ± 2.99**
DMSO (10%)	6	9.6 ± 1.91**	6.43 ± 2.19**	8.80 ± 4.0**

Values are total amounts of enzymes and MDA released into the coronary effluent during 20 min of reoxygenation period. * $P < 0.05$, ** $P < 0.01$

Ninety minutes perfusion with hypoxic, cardioplegic solution caused any significant release of MDA neither. Upon reoxygenation of the hypoxic heart, however, the concentraion of MDA in the coronary effluent was markedly increased. This increase of MDA was also significantly prevented by SOD, catalase and DMSO. Among these oxygen radical scavengers, a hydroxyl radical scavenger, DMSO was most effective in reduction of both enzyme release and MDA production (Table 1). A good correlation between the amount of MDA and that of LDH released during reoxygenation was also observed (Fig. 1). The administration of oxygen free radical scavengers neither iduced nor decreased enzyme release or MDA production in the control hearts, and also did not interfere with the analytical methods of enzymes and MDA.

Effects of antioxidant

Coronary flow: In the control heart perfused continuously with oxygenated K-H solution at 37℃ and 100 cmH_2O of perfusion pressure, coronary flow rate was constantly maintatined at about 11 ml/min/g wet wt throughout 120 min perfusion period. Administration of α-tocopherol (4.5 uM) or BHT (2 uM) did not cause a change in control coronary flow rate. After 90 min of hypoxic perfusion, the flow decreased to 60% of the prehypoxic value. Twenty minute reoxygenation following the hypoxia further decreased the flow to about half of prehypoxic value. This decrease in coronary flow was prevented to various degrees by α-tocopherol and BHT. In the hearts treated with these antioxidants, the coronary flows during both hypoxic and reoxygenated periods were considerably greater than those of untreated hearts. After 20 min of reoxygenated perfusion with the antioxidants, the flow was increased 10-50% (Table 2).

Enzyme release and lipid peroxidation: The rate of release of CPK and LDH was abruptly increased in the early minutes of reoxygenation and was maintained high levels thereafter. This increased rate of enzyme was suppressed by α-

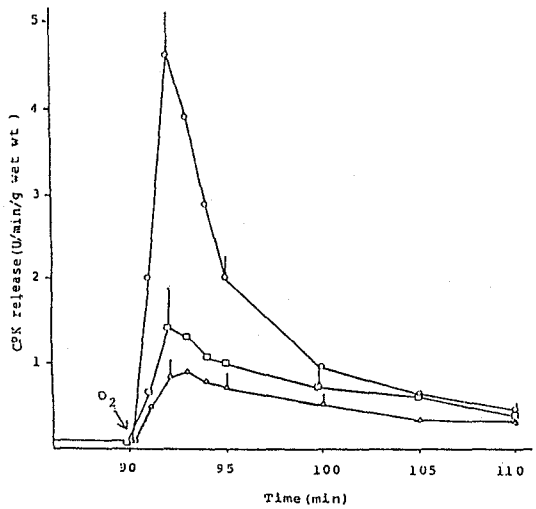

Fig. 2. Effect of antioxidants on CPK release in hypoxic-reoxygenated rat hearts. Rates of CPK release into the coronary effluent from isolated rat hearts subjected to 90 min of hypoxic, substrate-free perfusion followed by 20 min of reoxygenated perfusion were estimated at indicated time. α-tocopherol acetate (4.5 uM, —□—) and butylated hydroxytoluene (2 uM, —△—) dissolved in perfusion medium were administered throughout entire hypoxic and reoxygenation period. Control hypoxic-reoxygenation perfusion (—○—).

Table 2. Effect of antioxidants on coronary flow during hypoxia and reoxygenation in isolated rat hearts

Conditions	No. of animal	Coronary flow (ml/min/g wet wt)							
		Control perfusion	Hypoxia (min)				Reoxygenation (min)			
			2	30	60	90	2	5	10	20
Untreated	12	11.4 ± 0.6	14.4 ± 0.6	10.6 ± 0.5	8.8 ± 0.5	7.2 ± 0.3	6.4 ± 0.2	5.7 ± 0.2	5.6 ± 0.2	5.8 ± 0.2
α-tocopherol, 4.5 uM	6	12.9 ± 0.6	17.2 ± 1.4	12.8* ± 0.5	8.9 ± 0.3	6.6 ± 0.3	6.4 ± 0.2	6.2 ± 0.2	6.2* ± 0.2	6.4 ± 0.4
BHT, 2 uM	8	11.4 ± 1.9	14.9 ± 1.0	13.3** ± 0.8	11.6 ± 0.5	9.6** ± 0.3	9.3** ± 0.5	9.2** ± 0.5	9.3** ± 0.5	8.8** ± 0.3

* $P < 0.05$, ** $P < 0.01$

tocopherol and BHT in a dose-dependent manner (Fig. 2 and 3, Tabl 3). Concentration of MDA in the coronary effluent was massively increased in the first three minutes of reoxygenation and showed elevated levels throughout reoxygenation period. Both α-tocopherol and BHT also significantly prevented this increase in MDA dose dependently(Fig. 4, Table 3). The preventive effects of the antioxidants on LDH release were more pronounced when the agents were administered during both hypoxic and reoxygenated perfusion period than given during either hypoxic or reoxygenation period only (Fig. 5).

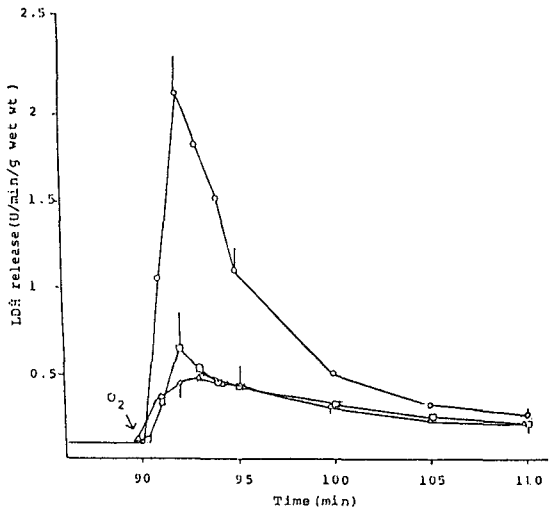

Fig. 3. Effect of antioxidants on LDH release in hypoxic-reoxygenated rat hearts. Perfusion conditions and administration methods of antioxidants are the same as in Fig. 2. Symbols are ; —○—, control hypoxic-reoxygenation perfusion, —□—, 4.5 uM α-tocopherol ;—△—, 2 uM butylated hydroxytoluene.

DISUCSSION

In the present study, ninety minutes of hypoxia

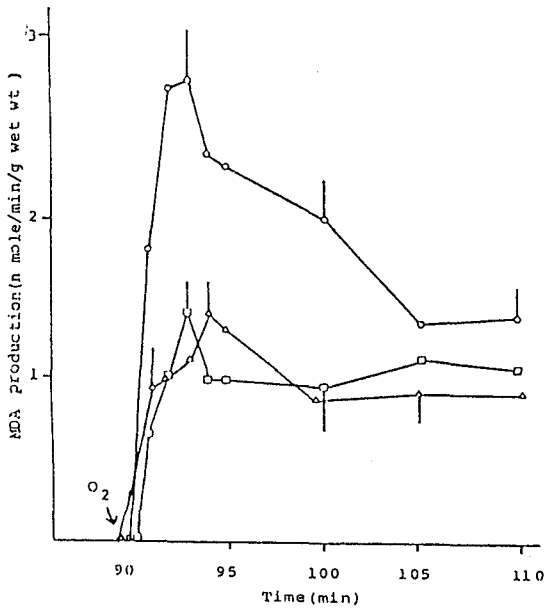

Fig. 4. Effect of antioxidants on MDA formation in hypoxic-reoxygenated rat hearts. Rates of MDA release into the coronary effluent, as estimation of lipid peroxidation, were determined at the indicated time. Perfusion conditions and methods of administration of antioxidant are the same as in Fig. 2. Symbols are ; —○—, control hypoxia-reoxygenation ; —□—, 4.5 uM α-tocopherol ; —△—, 2 uM butylated hydroxytolune.

Table 3. Effect of antioxidants on CPK, LDH and MDA release induced by reoxygenation in isolated hypoxic rat hearts

Conditions	No. of animal	CPK (U/min/g wet wt)	LDH (U/min/g wet wt)	MDA (nmole/min/g wet wt)
Untreated	12	4.63 ± 0.56	2.14 ± 0.21	2.70 ± 0.27
α-tocopherol				
1 um	6	2.83 ± 0.26*	1.02 ± 0.15**	2.99 ± 0.40
4.5 um	6	1.37 ± 0.46**	0.68 ± 0.21**	0.99 ± 0.33**
BHT				
0.5 um	6	1.61 ± 0.3**	0.65 ± 0.09**	1.46 ± 0.30*
2 um	8	1.04 ± 0.12**	0.55 ± 0.08**	0.99 ± 0.20**

The values were estimated in coronary effluent collected at 2 min after reoxygenation. * $P < 0.05$, ** $P < 0.01$

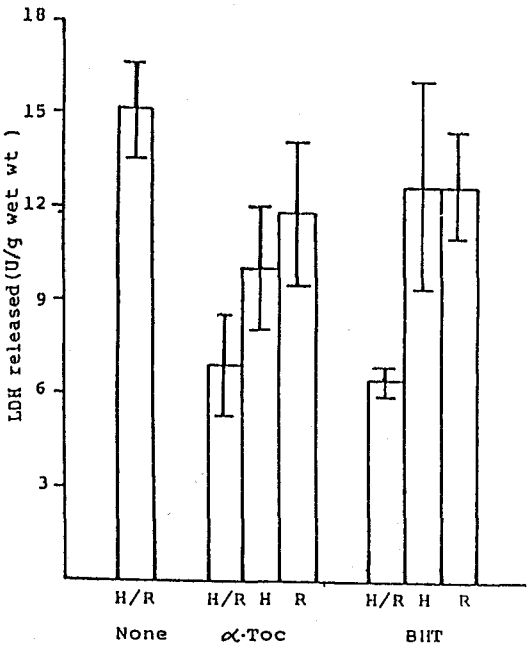

Fig. 5. Effect of antioxidants administered during either hypoxic or reoxygenation periods on LDH release. The antioxidants were administered during either hypoxia (H) or reoxygenation (R) and during both hypoxic and reoxygenation periods (H/R). Concentrations of antioxidants administered are the same as in Fig. 3. Values are total amount of LDH released during reoxygenation (20 min).

followed by twenty minutes of reoxygenation in isolated Langendorff preparations of rat heart caused a marked release of CPK and LDH with a concomittent increase in lipid peroxidation as estimated from the MDA release. This observation of oxygen related toxicity in hypoxic myocardium confirms the original work of Hearse et al. (1973) who has firstly termed the oxygen paradox.

The exact mechanism by which reoxygenation causes cellular damage in hypoxic myocardium has been a subject to controversy. It has been considered that the ability of heart to survive or recover from periods of inadequate oxygenation depends on high energy phosphate reserve available for maintaining cellular functions and membrane integrity. Relatively rapid depletion of cellular high-energy phosphate pool was observed in hypoxic myocardium and this has been suggested to be a main factor in myocardial cellular death (Jennings and Reimer 1981). The cellular depletion of high-energy phosphate, however, can not explain the increase in lipid peroxidation observed in the present study. Moreover, reperfusion of ischemic myocardium with oxygenated soultion restored a tissue ATP pool(Guarnieri et al 1978; Jennings et al 1985) but produced the well-known reperfusion injury in in vivo or isolated animal hearts (Hearse 1977).

Alternatively, changes in cellular calcium metabolism was also thought to play an important role in the genesis of myocardial cellular damage caused by hypoxia and reoxygenation. An excessive intracellular calcium overload was observed in damaged cells of severly ischemic myocardium (Katz and Reuter 1979; Nyler 1981). The intracellular overload of calcium, however, is thought not to be an initial cause for cellular death, but to be a secondary consequence of altered cellular functions or increased membrane permeability due to oxygen deprivation.

Recently, possible involvement of reactive oxygen free radicals has been suggested to play a crucial role in the development of myocardial injury that occurs during reoxygenation of hypoxic myocardium(Gaudel and Duvelleroy 1984; Guarnieri et al. 1980; Kim and Akera 1987). Oxygen free radicals can be produced normally during electron transport in mitochondria(Boveris 1977; Nohl and Hegner 1978). In normal conditions, these oxygen free radicals do not exert any significantly harmful effect, because they are detoxified by endogenously presented quenchers, such a SOD, catalase and glutathione peroxidase. However, in certain pathologic conditions, the production of oxygen free radicals is thought to be increased above the neutralizing capacity of normally existing defensive systems and to contribute to genesis of tissue injuries(Freeman and Crapo 1982). It has been reported that during ischemia or hypoxic condition, there are intracellular accumulations of reducing equivalents (Fridovich 1979), a disturbance of intramitochondrial electron transport (Loschen and AZZI 1976), the conversion of xanthine dehydrogenase to xanthine oxidase (McCord and Roy 1982), and an accumulation of hypoxanthine and xanthine (Jennings and Reimer 1981) which can be used as substrates for oxygen radical production through the action of xanthine oxidase. It is, therefore, likely that all of the above provides favorable conditions for a burst of oxygen radical production upon reoxygenation of hypoxic tissues. The present observations that various scavengers of oxygen radical prevent the release of intracellular enzyme in hypoxic-

reoxygenated rat heart also support the involvement of oxygen radical in the reoxygenation injury of hypoxic myocardium.

Oxygen free radicals are so reactive that can alter most types of cellular macromolecules. The unsaturated fatty acid of membrane lipid is particularly susceptible to oxygen free radical attack. The peroxidation of lipid alters biochemical and physical properties of cellular components, and also the products of lipid peroxidation, such as MDA, interact with proteins to cause cross-linking and polymerization of membrane components. Consequently, the lipid peroxidation induces severe cellular damage including the increase in membrane permeability (Freeman and Crapo 1982; Meerson et al 1982). The ocurrence of lipid peroxidation by oxygen free radical has been documented in liposomes(Pederson and Aust 1973), erythrocytes(Kellog and Fridovich 1977) and cardiac sarcolemmal and microsomal preparations (Kim et al 1985; Kramer et al 1984). In the present study, the increased release of MDA in reoxygenated hypoxic rat heart was significantly reduced by oxygen radical scavengers. This finding agrees with the previous study(Kim and Akera 1987) that has demonstrated the prevention of MDA accumulation by various kinds of oxygen radical quenchers in ischemic-reperfused myocardial tissue. A close correlation observed between MDA production and intracellular enzyme release also confirms the lipid peroxidation-linked cellular damage in isolated, hypoxic-reoxygenated myocardium.

In general, when oxygen radicals react with stable compounds, they generate other radicals leading to chain reactions in which many of stable molecules are oxidized subsequently. Such chain reaction can be terminated by antioxidants which react preferentially with chain propagating radicals to yield nonpropagating products (Aust and Svingen 1982). Therefore, if oxygen free radical initiates an oxidative reaction in myocardial cellular components, antioxidant should limit also the lipid peroxidation and the resultant impairment of myocardial cellular functions. In the previous studies, several investigators (Guarnieri et al. 1978; Kim and Akera 1987) demonstrated that the addition of α-tocopherol to hypoxic and reoxygenated hearts prevented cellular damages. In agreement with this, a natural (α-tocopherol) and a synthetic (BHT) antioxidant examined in this study significantly prevented MDA as well as CPK and LDH release during reoxygenation of isolated hypoxic myocardium of rat. This cardioprotective effect of antioxidant was expressed more markedly when the agent was administered starting from hypoxia and throughtout reoxygenation than given during either hypoxic or reoxygenation period only. It has been demonstrated that myocardial cellular damages caused by reoxygenation is proportional to the preceeding period of hypoxia (Hearse et al. 1973), and also found that a period of hypoxia results in a decrease in defensive enzymes against oxygen radical-induced toxicity (Guarnieri et al. 1980). Thus, considering these facts and existence of oxygen radical production even in the ischemic or hypoxic tissues (Hess and Manson 1984), this differential effect of antioxidant being dependent on time of administration could be explained from the vulnerability of myocardial cells in hypoxic state.

In the present study, the lipid peroxidation was estimated from MDA released into the coronary effluent instead of tissue concentration. The results, therefore, do not show the degree of lipid peroxidation in a specific cellular component. It should be pointed out, however, that sarcolemmal membrane is highly vulnerable to free radical attack, perhaps due to its higher content of cholesterol and unsturated fatty acid in comparison to other cellular mrmbrane systems (Kramer et al. 1984). Thus, a large part of MDA determined in the coronary effluent might be originated from sarcolemma and this would appear to be an estimation of degree of sarcolemmal lipid peroxidation.

REFERENCES

Aust SD and Svingen BA: *The role of iron in enzymatic lipid peroxidation. In Free Radicals in Biology. Vol V, edited by WA Pryor, Academic Press, 1982, pp1-28*

Bergmeyer HU and Bernt E: *UV assay of lactic dehydrogenase activity with pyruvate and NADH. In Methods of Enzymatic Analysis. Vol II, 2nd Ed, edited by HU Bergmeyer, Academic Press, New York, 1974, pp574-579*

Boveris A: *Mitochondrial generation of superoxide and hydrogen peroxide. Adv Exp Biol Med 78:67, 1977*

Forster G, Bernt E, Bergmeyer HU: *Creatine phosphokinase determination with creatine phosphate as substrate. In Methods of Enzymatic Analysis. Vol*

II, 2nd Ed, edited by HU Bergmeyer, Academic Press, New York, 1974, pp789-793

Freeman BA and Crapo JD: *Free radicals and tissue injury. Lab Invest 47:412-426, 1982*

Fridovich I: *Hypoxia and oxygen toxicity. Adv Neurol 26:255-259, 1979*

Gaudel Y and Duvelleroy MA: *Role of oxygen radicals in cardiac injury due to reoxygenation. J Mol Cell Cardiol 16:459-470, 1984*

Guarnieri C, Ferrari R, Visioli O: *Effect of α-tocopherol on hypoxic-perfused and reoxygenated rabbit heart muscle. J Mol Cell Cardiol 10:893-906, 1978*

Guarnieri C, Flamigni F, Caldarea CM: *Role of oxygen in the cellulr damage induced by re-oxygenation of hypoxic heart. J Mol Cell Cardiol 12:797-808, 1980*

Hearse DJ: *Reperfusion of ischemic myocardium. J Mol Cell Cardiol 9:605-616, 1977*

Hearse DJ, Humphrey SM, Chain EB: *Abrupt reoxygenation of the anoxic potassium arrested perfused rat heart; a study of myocardial enzyme release. J Mol Cell Cardiol 5:395-407, 1973*

Hess ML and Manson NH: *Molecular oxygen: Friend and Foe. The role of the oxygen paradox and ischemic/reperfusion injury. J Mol Cell Cardiol 16:969-985, 1984*

Jennings RB and Reimer KA: *Lethal myocardial ischemic injury. Am J Pathol 102:241-255, 1981*

Jennings RB, Schaper J, Hill ML: *Effect of reperfusion late in the phase of reversible ischemic injury. Changes in cell volume, electrolytes, metabolites, and ultrastructure. Cir Res 56:262-278, 1985*

Katz AM and Reuter H: *Cellular calcium and cardiac cell death. Am J Cardiol 181:177-182, 1979*

Kellogg EW and Fridovich I: *Superoxide, hydrogen peroxide and singlet oxygen in lipid peroxidation by xanthine oxidase system. J Biol Chem 250:8812-88, 1975*

Kim HW, Chung MH, Kim MS, Park CW: *A study on the mechanism of calcium binding inhibition of cardiac sarcoplasmic reticulum by oxygen free radicals. Korean J Pharmacol 21:79-89, 1985*

Kim MS and Akera T: *O_2 free radicals; cause of ischemia-reperfusion injury to cardiac Na^+, K^+-ATPase. Am J Physiol. 252:H252-H257, 1987*

Kramer JH, Mak IT, Weglicki WB: *Differential sensitivity of canine cardiac sarcolemmal and microsomal enzymes to inhibition by free radical-induced lipid peroxidation. Cir Res 55:120-124, 1984*

Loschen G and Azzi A: *On the formation of hydrogen peroxide and oxygen radicals in heart mitochondria. In Recent Advances in Studies on Cardiac Structure and Metabolism. Vol 7, edited by Harris P, Bing RJ, Fleckenstein A, University Park Press, Baltimore, 1976, pp3-12*

McCord JM and Roy RS: *The pathophysiology of superoxide: role in inflammation and ischemia. Can J Physiol Pharmacol. 60:1346-1352, 1982*

Meerson FZ, Kagan VE, Kozlov YP: *The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxidant protection of the heart. Basic Res Cardiol 77:465-485, 1982*

Nohl H and Hegner D: *Do mitochondria produce oxygen radicals in vivo? Eur J Biochem 82:563, 1978*

Nyler WG: *The role of caclium in the ischemic myocardium. Am J Pathol 102:262-270, 1981*

Pederson TC and Aust SD: *The role of superoxide and singlet oxygen in lipid peroxdation promoted by xanthine oxidase. Biochem Biophy Res Comm 52:1071-1078, 1973*

Placer ZA, Cushman LL, Johnson BC: *Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical system. Anal Biochem 16:359-364, 1966*

Yagi K: *Assay for serum lipid peroxide level and its clinical significance. In Lipid Peroxides in Biology and Medicine. Academic Press, NY, 1982, pp223-242*

═국문초록═

저산소 심장의 산소 재공급에 따른 심근 손상에 있어서 항산화제의 보호 효과

서울대학교 의과대학 약리학교실

윤 형 구·임 정 규·김 명 석

저산소 심근의 산소 재공급시에 보이는 심근 손상(oxygen paradox) 기전을 규명하고, 이의 예방법을 찾기 위한 연구의 일환으로 유독성 산소 대사물인 산소 라디칼의 관련성과 지질과산화 활성화 및 항산화제의 심근 보호 효과를 검토하였다.

흰쥐 적출 심장을 Langendorff 심장관류법으로 산소 및 glucose 공급을 중단한 cardioplegic 용액으로 관류(37℃, 90분)하여 저산소 상태를 만든 후, 계속해서 산소재공급 관류(20분)를 시행하여 저산소—산소 재공급 심근 손상을 유도 하였다. 심근 손상의 지표로 creatine phosphokinase (CPK), lactic dehydrogenase(LDH)의 관상관류액으로의 유출을, 그리고 지질과산화 척도로는 malondialdehyde(MDA) 생성을 측정하였으며, 이에 대한 산소 라디칼 제거물질과 항산화제 α-tocopherol 및 butylated hydroxytoluene(BHT)의 효과를 검토하여 다음과 같은 성적을 얻었다.

1. 세포질 효소인 CPK 및 LDH의 유출과 지질과산화산물의 하나인 MDA의 생성은 산소 재공급과 더불어 급격히 증가하였다.

2. 산소 재공급시 세포질 효소의 유출과 MDA 생성은 높은 상관관계를 보였다.

3. Superoxide anion(O_2)의 제거 효소인 superoxide dismutase (10,000 U), H_2O_2 제거 효소인 catalase(25,000 U) 그리고 hydroxyl radical(OH) 제거물질인 dimethylsufoxide(10%)는 세포질 효소의 유출 증가와 MDA 생성 증가를 현저히 억제하였다.

4. 생리적 항산화물질인 α-tocopherol(4.5 uM)과 합성 항산화제인 butylated hydroxytoluene(2 uM)은 산소 공급에 따른 MDA 생성 증가와 세포질 효소의 유출 증가를 용량의존적으로 억제하였다.

5. 항산화제들의 심근 보호 효과는 산소 재공급시 투여할 때보다는 저산소 관류시부터 투여한 경우에 더욱 현저하였다.

이상의 결과에서 저산소 심근의 산소 재공급은 유독성 산소 대사물인 산소 라디칼의 생성을 증가시키며, 그에 따른 지질성분의 과산화가 심근 손상을 일으키는데 관여할 것으로 여겨졌으며, 저산소—산소 재공급 심근 손상은 지질과산화 반응을 억제하는 항산화제에 의하여 방지될 것으로 사료되었다.