Korean J. of Pharmacology
Vol.28, No.2, pp.163-170, 1992

Effect of Cyclobuxine on Oxygen Free Radical Production and Cellular Damage Promoted by Arachidonate in Perfused Rat Hearts

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ABSTRACT

The present study was attempted to investigate the effect of cyclobuxine (a steroidal alkaloid) on generation of reactive oxygen metablite and myocardial damage promoted by an exogenous administeration of arachidonate in ischemic-reperfused hearts. Langendorff preparation of the isolated rat heart was made ischemic condition by reducing the flow rate to 0.5 ml/min for 45 min, and then followed by normal reperfusion (7 ml/min) for 5 min. The generation of superoxide anion was estimated by measuring the SOD-inhibitable ferricytochrome C reduction. The degree of lipid peroxidation in myocardial tissue was estimated from the tissue malondialdehyde (MDA) concentration using thiobarbituric acid method. The myocardial cell damage was observed by measuring LDH released into the coronary effluent. Sodium arachidonate (0.1 and 1.0 μg/ml) infused during the period of oxygenated reperfusion stimulated superoxide anion production dose-dependently. The rate of arachidonate-induced superoxide anion generation was markedly inhibited by cyclobuxine (1.0 and 10 μg/ml). The production of malondialdehyde was increased by infusion of arachidonate. This increase was prevented by superoxide dismutase (300 U/ml) and cyclobuxine (1.0 and 10 μg/ml). The release of LDH was increased by sodium arachidonate was also inhibited by superoxide dismutase and cyclobuxine. In conclusion, the present results suggest that cyclobuxine inhibits the production of reactive oxygen metabolite and myocardial damages which were promoted by an administeration of arachidonate during reperfusion of ischemic hearts.

Key Words: Ischemia/reperfusion; Superoxide anion; Arachidonate; Cyclobuxine

INTRODUCTION

The etiology of ischemia is diverse in clinical medicine. It can result from atherosclerosis, thrombolism, or external pressure on vessels (tumor, etc.), or can be iatrogenic during surgery. Ischemia, from whatever source, is accompained by a lack of both oxygen and substrate, and therefore a lack of aerobic energy production. Thus ATP content of the tissue falls rapidly. The lack of ATP initiates a cascade of damaging effects, including inability to maintain membrane ionic balance. There is an influx of sodium into the cells (Bolli *et al.*, 1988; Jennings *et al.*, 1985) which, upon reperfusion, could exchange with calcium (Daly *et al.*, 1982), leading to the activation of injurious enzymes and disruption of the membrane.

The status of intracellular enzymes during ischemia is crucial. First, a potentially detrimental event is the rupture of lysosome (Omar *et al.*, 1991) and the release of protease, which can lead to the degradation of cytosolic proteins. A second

injurious event is the activation of various calcium dependent protease and phospholipase as a result of calcium influx (Weisfeldt, 1987; Murphy *et al.*, 1987). The activation of phospholipase has direct damaging effects on membrane and result in the release of free fatty acid and lysophospholipids. These are noxious by themselves, and can initiate arachidonic acid metabolism (Kontos *et al.*, 1984). The activation of protease can degrade vital enzymes and may induce proteolytic changes which in themselves will increase free radical production on reperfusion (McCord, 1985; Jarasch *et al.*, 1986).

Cyclobuxine extracted from *Buxus microphylla* var. *koreana* Nakai is a steroidal alkaloid. Cyclobuxine inhibited prostaglandin production in vivo and vitro (Lee *et al.*, 1987). It also exerted negative chrono- and inotropic effects in rat hearts (Lee *et al.*, 1989). Therefore, the present study was undertaken to examine the effect of cyclobuxine on oxygen radical production and myocardial damage promoted by arachidonate in ischemic-reperfused rat hearts.

MATERIALS AND METHODS

Perfused heart preparation

Hearts were quickly excised from heparinized (500 U/100 g, i.p.) Sprague-Dawley male rats weighing about 250 g. Isolated hearts were perfused through the aorta in the retrograde manner (Langendorff preparation) with oxygenated Krebs-Henseleit (K-H) solution (mM: NaCl, 120; KCl, 4.8; KHP_2O_4, 12.; $MgSO_4$ 1.2; $CaCl_2$, 1.25; $NaHCO_3$, 25; glucose, 11, pH 7.4 with 95% O_2+5% CO_2) at a constant flow of 7 ml/min with a peristaltic pump. After equilibration for 20 min, ischemia was initiated by reducing flow rate to 0.5 ml/min. After 45 min, perfusion was return to normal flow rate for 5 min to induce reperfusion state.

Cyclobuxine (1.0 and 10 μg/ml) was added to the perfusion fluid throughout ischemia and reperfusion periods. Arachidonic acid (0.1 and 1.0 μg/ml) was administered during the reperfusion period by a peristaltic pump connected to the aortic cannula.

Lipid peroxidation

The degree of lipid peroxidation in myocardial tissue was estimated from the tissue malondialdehyde (MDA) concentration using thiobarbituric acid method (Singal *et al.*, 1983). Homogenates of left ventricular muscle (2~3 mg/ml) were prespared in 0.2 M tris (hydroxymethyl) aminomethane (Tris)-HCl buffer solution (pH 7.4) containing 0.16 M KCl, and incubated for 1 h at 37℃ in a shaking water bath. A 1 ml aliquot was transferred to a Pyrex tube mixed with 0.5 ml of 40% trichloroacetic acid and 0.25 ml of 5 M HCl. Subsequently, 0.25 ml of 2% thiobarbituric acid was added and tubes were placed on boiling water bath for 20 min and then cooled on ice. After cooling, 1 ml of 70% trichloroacetic acid was added, and the mixture was allowed to stand for 20 min at room temperature. The mixture was then centrifuged at 2,500 rpm for 15 min, and the absorbance of the supernatant solution was recorded at 532 nm. The concentration of MDA was expressed as nanomoles per milligram of protein.

Lactic dehydrogenase (LDH) release

LDH released into the coronary perfusate was measured as an index of myocardial cellular damage. Coronary perfusate was collected at the indicated time intervals during first 5 min of repefusion period. LDH activity was assayed by UV-spectrophotometric method (Bergmeyer & Bernt, 1974). A 0.5 ml aliquot of sample was added into a cuvette containing 2.5 ml of reaction mixture consisted of 48 mM phosphate buffer (pH 7.5), 0.6 mM pyruvate and 0.18 mM NADH. The rate of change of absorbance was measured with UV-spectrophotometer (Hitachi 200-20) at 340 nm and 25℃.

Superoxide anion production

Reduction of exogenously administered ferricytochrome C was used to measure superoxide anion production (Salin & McCord, 1974). Starting with reperfusion, ferricytochrome C solution (100 M) either containing SOD (300 U/ml) or not was infused through the aortic cannula at a rate of 0.5 ml/min. The coronary perfusate was collected at an interval of 30 sec. Immediately after determination of volume, optical density was measured at

418 nm with UV-spectrophotometer. Superoxide anion production was estimated from SOD-inhibitable portion of ferricytochrome C reduction. The extent of ferricytochrome C reduction during reperfusion was calculated by using the difference of molar extinction coefficient ($\triangle E_{418}=7.0\times 10^4$/M/cm) between reduced ferricytochrome C and oxidized ferricytochrome C. The total amount of cytochrome C in the perfusate was estimated after reduction of ferricytochrome C by addition of sodium dithionite.

Statistics

Results were expressed as the mean±s.e. Multi-group statistics were calculated by analysis of variance using Duncan's new multiple range test. Differences between groups were considered significant when p was 0.05.

RESULTS

Arachidonate-induced myocardial damage

Activity of LDH was measured in the coronary perfusate as an indicator of cellular injury. LDH released from a nonischemic normal heart was 8.5 U/ml/g wet wt. In ischemic-reperfusion control heart, LDH activity was 41.0 U/ml/g wet wt. In most experimental groups, the peak release of LDH was observed at 1.5 min after starting reperfusion. When arachidonate (0.1 and 1.0 μg/ml) was administered, LDH activities were increased to 52.1 and 63.6 U/ml/g wet wt, respectively. This increase in LDH activity was significantly decreased with SOD treatment to 25.5 U/ml/g wet wt (Fig. 1).

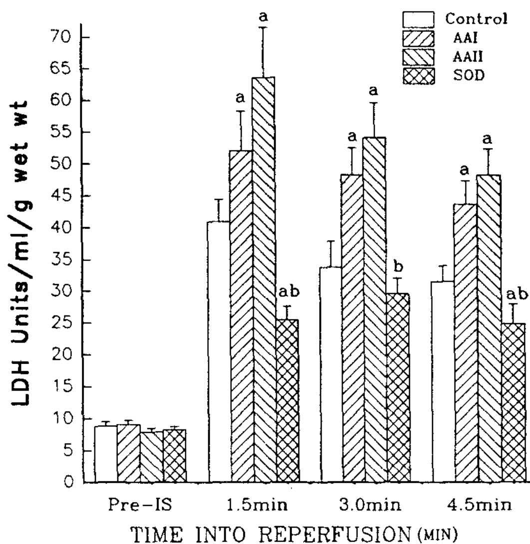

Fig. 1. Effects of arachidonate and superoxide dismutase on LDH release in ischemic-reperfused rat hearts. Arachidonate 0.1 μg/ml (AAI) and 1.0 μg/ml (AAII) were respectively administered with start of reperfusion following 45 min of ischemia. SOD 300 U/ml (SOD) was simultaneously administered with arachidonate (0.1 μg/ml). Results are mean±s.e. of 4~6 experiments. [a]$p<0.05$ vs control, [b]$p<0.05$ vs AAI.

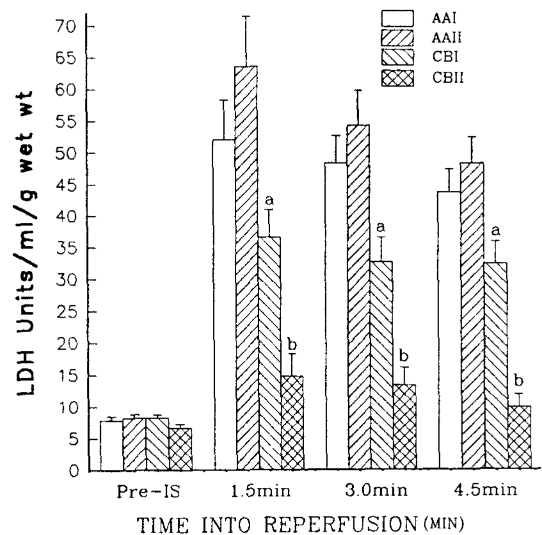

Fig. 2. Effects of cyclobuxine on LDH release induced by an administeration of arachidonate in ischemic-reperfused rat hearts. Arachidonate 0.1 μg/ml (AAI) and 1.0 μg/ml (AAII) were respectively administered with start of reperfusion following 45 min of ischemia. Cyclobuxine 1.0 μg/ml (CBI) and 10 μg/ml (CBII) were respectively pretreated to perfusion fluid prior to ischemia and throughout reperfusion period. Results are mean±s.e. of 4~6 experiments. [a]$p<0.05$ vs AAI, [b]$p<0.05$ vs AAII.

Effect of cyclobuxine on myocardial damage promoted by arachidonate

When cyclobuxine (1.0 and 10 μg/ml) was administered prior to ischemia, it inhibited the increase in LDH release induced by arachidonate (0.1 and 1.0 μg/ml) from 52.1 and 63.6 U/ml/g wet wt to 36.6 and 14.8 U/ml/g wet wt, respectively (Fig. 2). Particularly the inhibitory effect was more significant at a concentration of cyclobuxine (10 μg/ml) which exerted negative chronotropic and inotropic effect in isolated rat hearts.

Arachidonate-induced superoxide anion production and lipid peroxidation

Reduction of ferricytochrome C was increased upon reperfusion in previously ischemic rat hearts. The peak reduction (10.6 nmol/30 sec/g/wet wt) was observed at 1 min after starting reperfusion (Fig. 3). when arachidonate (0.1 and 1.0 μg/ml) was added, the reduction of ferricytochrome C was increased to 18.7 and 21.6 nmol/30 sec/g wet wt, respectively. This arachidonate (0.1 μg/ml) induced ferricytochrome C reduction was significantly suppressed by simutaneous administeration of SOD to 7.9 nmol/30 sec/g wet wt.

The degree of lipid peroxidation in the myocardial tissue was estimated from the production of MDA in left ventricular muscle homogenates. After a continuous perfusion of isolated rat (nonischemic) hearts, the concentration of MDA was 0.55 nmol/mg protein. After ischemia and reperfusion, the average MDA concentration was 0.984 nmol/mg protein. When arachidonate (0.1 and 1.0 μg/ml) was administered, MDA production was increased to 1.153 and 1.537 nmol/mg protein, respectively. This arachidonate (0.1 μg/ml) induced increase was prevented by simultaneous addition of SOD (Fig. 4).

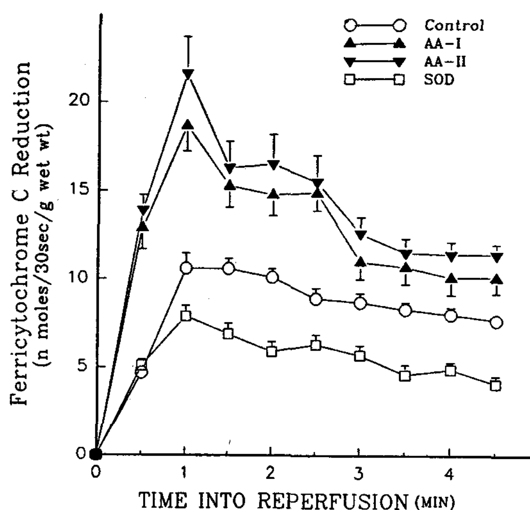

Fig. 3. Effects of arachidonate and superoxide dismutase on ferricytochrome C reduction in ischemic-reperfused rat hearts. Arachidonate 0.1 μg/ml (AA-I) and 1.0 μg/ml (AA-II) were respectively administered with start of reperfusion. Superoxide dismutase 300 U/ml (SOD) was simutaneously added with arachidonate (0.1 μg/ml). Results are mean ± s.e. of 4~6 experiments.

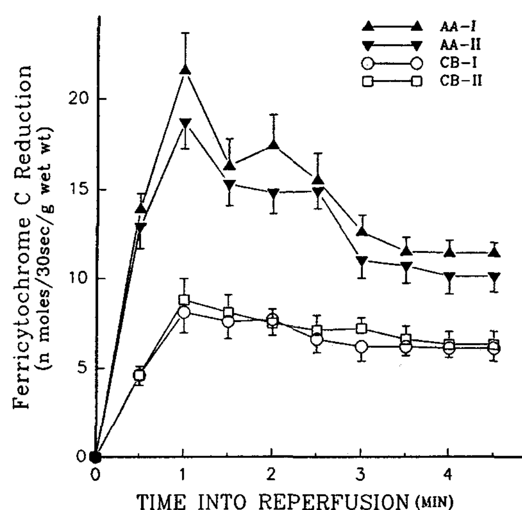

Fig. 4. Effects of cyclobuxine on reduction of ferricytochrome C induced by an administeration arachidonate. Arachidonate 0.1 μg/ml (AA-I) and 1.0 μg/ml (AA-II) were respectively administered with start of reperfusion following 45 min of ischemia. Cyclobuxine 1.0 μg/ml (CB-I) and 10 μg/ml (CB-II) were respectively pretreated to perfusion fluid prior to ischemia and throughout reperfusion period. Results are mean ± s.e. of 4~6 experiments.

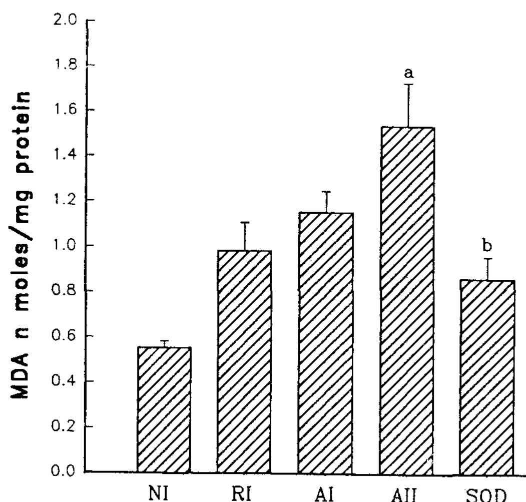

Fig. 5. Effects of arachidonate and superoxide dismutase on lipid peroxidation in sichemic-reperfused rat hearts. Langendorff preparations of rat hearts were subjected to ischemia for 45 min by reducing flow rate and subsequently reperfused for 5 min (IR). Arachidonate 0.1 μg/ml (AI) and 1.0 μg/ml (AII) were respectively administered with start of reperfusion superoxide dismutase 300 U/ml (SOD) was simutaneously added with arachidonate (0.1 μg/ml). Nonischemic hearts (NI) were continuously perfused for comparable time period. Results are mean±s.e. of 4~6 experiments. $^{a}p<0.05$ vs IR, $^{b}p<0.05$ vs AI.

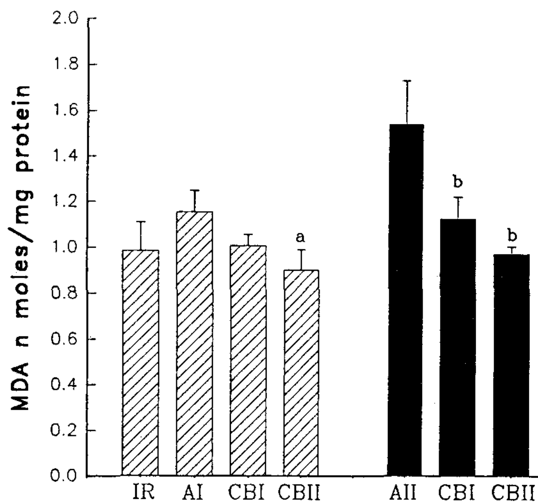

Fig. 6. Effects of cyclobuxine on lipid peroxidation induced by an administeration of arachidonate in ischemic-reperfused rat hearts. Langendorff preparations of rat hearts were subjected to ischemia for 45 min by reducing flow rate and subsequently reperfused for 5 min (IR). Arachidonate 0.1 μg/ml(AI) and 1.0 μg/ml (AII) were respectively administered with start of reperfusion. Cyclobuxine 1.0 μg/ml (CBI) and 10 μg/ml (CBII) were res-pectively pretreated to perfusion fluid prior to ischemia and throughout reperfusion period. Results are mean±s.e. of 4~6 experiments. $^{a}p<0.05$ vs AI, $^{b}p<0.05$ vs AII.

Effect of cyclobuxine on superoxide anion production and lipid peroxidation

Cyclobuxine decreased the arachidonate-induced reduction of ferricytochrome C to the same degree as SOD. The peak reduction (18.7 and 21.6 nmol/30 sec/g wet wt) induced by arachidonate (0.1 and 1.0 μg/ml) was prevented by cyclobuxine (1.0 and 10 μg/ml) to 8.1 and 8.8 nmol/30 sec/g wet wt, respectively (Fig. 5).

The increase in MDA production induced by arachidonate was also prevented by addition of cyclobuxine. When arachidonate (1.0 μg/ml) was administered, MDA production was increased from 0.984 to 1.537 nmol/mg protein. This increase was inhibited with cyclobuxine (1.0 and 10 μg/ml) treatment to 1.124 and 0.971 nmol/mg protein, respectively (Fig. 6).

DISCUSSION

Evidence has accumulated implicating cytotoxic oxygen-derived free radicals as mediators at least of ischemia and reperfusion injury. Reactive oxygen species can be cytotoxic to cells by attacking fatty acids, which lead to lipid peroxidation of membranes, and reacting with proteins, including destruction and oxidation of amino acid, oxidation of sulfhydryl groups, and polypeptide chain scission (Thompson & Hess, 1986). Many intra- and extracellular mechanisms have been suggest-

ed as the sources of oxygen free radical production in ischemic and reperfused hearts. Endothelial xathine oxidase system (Chambers *et al.*, 1983), infiltrated leukocytes in the ischemic region (Simpson & Lucchesi, 1987), electron transport system within mitochondria (McCord, 1988) and pathways of arachidonic acid metabolism (Kontos HA, 1987) were proposed as the sources.

Free fatty acids and lysophospholipids have been reported to be increased in the ischemic and reperfused hearts (Karmazy M, 1986). These are noxious by themselves, and can initiate arachidonate metabolism with the resulting formation of various products and accompanying generation of oxygen free radicals (Yamamoto S, 1983). Many investigators (Eagan *et al.*, 1981; Kontos, 1987; Kukreja *et al.*, 1986) observed that prostaglandin synthetase produced oxygen free radicals. Prostaglandin synthetase is a hemoprotein which has two activities; that is responsible for the oxidation of arachidonate to the hydroperoxide PGG and for the peroxidation of the 15-hydroperoxy group of PGG to a 15-hydroxy group, thus producing PGH. The prostglandin hydroperoxidase is capable of oxidizing a large number of reducing cosubstrates including NADH and NADPH. These oxidations frequently follow chain reaction involving the formation of free radicals (Eagan *et al.*, 1981; Kukreja *et al.*, 1986). The lipoxygenase pathway of metabolism converts arachidonate to hydroperoxides and subsequently to hydroxy acids (Yamamoto S, 1983). The cytochrome p450 oxygenase metabolizes arachidonate to epoxide and is capable of producing superoxide (Kuthan & Ullrich, 1982).

In the present study, when arachidonate was exogenously administered during reperfusion period, superoxide anion production and lipid peroxidation were significantly increased. The release of LDH into cardiac effluents was also increased. Jennings *et al* (1981) observed that reduced pyridine nucleotides (NADH and NADPH) were largely existed in the ischemic myocardial cells. These reduced pyridine neucleotides may promote the production of oxygen radicals during arachidonate metabolism in the ischemic-reperfused heart. Oxygen free radicals exert one of the damaging effects on lipid membrane, the consequences of lipid peroxidation include increased membrane permeability, altered mitochondrial function and formation of cytotoxic metabolites which impair cardiac function (Guarnieri *et al.*, 1980).

Cyclobuxine added prior to ischemia inhibited the increases in superoxide anion production and lipid peroxidation induced by an administeration of arachidonic acid. The release of LDH promoted by arachidonate was also significantly prevented by cyclobuxine. In our previous studies, it was found that cyclobuxine exerted an antiinflammatory effect in carrageenin-induced pleurisy and croton oil-induced granuloma pouch (Lee *et al.*, 1987). It also had negative chrono- and iontropic effects in the isolated rat heart (Lee *et al.*, 1989). Thus it may be concluded that not only inhibition of arachidonate metabolism and but also energy sparing effect are involved in the cardioprotective effect of cyclobuxine against superoxide anion production and myocardial damage induced by an exogenous administeration of arachidonate in the ischemic-reperfused rat heart.

REFERENCES

Bergmeyer HU and Bernt E: *UV assay lactic dehydrogenase activity with pyruvate and NADH. In Methods of Enzymatic Analysis. Vol II. 2nd ed. Edited by Bergmeyer, Academic Press, NY, 574-579, 1974*

Bolli R, Patel BS, Jeroudi MO, Lai EK and McCay PB: *Demonstration of free radical generation in stunned myocardium of intack dogs with the use of the spin trap alpha-phenyl N-butyl nitrone. J Clin Inves 82: 476-485, 1988*

Chambers DE Parks DA and Patterson G: *Xanthine oxidase as a source of free radical damage in the myocardial ischemia. J Mol Cell Cell Cardiol 17: 145-152, 1985*

Daly MJ, Elz JS and Nayler WG: *Sarcolemmmal enzymes and Na-Ca exchange in hypoxic; ischemic and reperfused rat hearts. Am J Physiol 247: H243, 1984*

Eagan RW, Gale P, Baptista EM, Kennicott KL, Vanden-heuvel WJA, Walker RW, Fagerness PE and Kuehl FA: *Oxidation reactions by prostaglandin cyclooxygenase-hydroperoxidase. J Biol Chem 256: 7536, 1981*

Guarnieri C, Flemign F and Caldarera CM: *Role of oxygen in the cellular damage induced by re-oxyge-*

nation of hypoxic heart. J Mol Cell Cardiol 12: 797-806, 1980

Jarasch ED Bruder G and Heid HW: *Significance of xathine oxidase in capillary endotherial cells. Acta Physiol Scand 548 (Suppl): 39-46, 1986*

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

Jennings RB, Schaper J, Hill ML, Steenbergen C Jr and Reimer KA: *Effects of reperfusion late in the phase of reversible ischemic injury. Circ Res 56: 262-278, 1985*

Karmazyn M: *Contribution of prostaglandins to reperfusion-induced ventricular failure in isolated rat hearts. Am J Physiol 251: H133-H140, 1986*

Kontos HA, Wei EP, Povilishock JT and Christman CW: *Oxygen radical mediate the cerebral arteriolar dilation from arachidonate and bradykinin in cats. Circ Res 55: 295-303, 1984*

Kontos KA: *Oxygen radicals from arachidonate metabolism in abnormal vascular responses. Am Rev Respir Dis 136: 474-477, 1987*

Kukreja RC, Kontos HA and Ellis EF: *PGH synthase and lipoxygenase generate superoxide in the presence of NADH or NADPH. Circ Res 59: 612-619, 1986*

Kuthan H and Ullrich V: *Oxidase and oxygen function of the microsomal cytochrome P_{450} monoxygenase system. Eur J Biochem 126: 583-588, 1982*

Lee JH, Moon CK, Kwon JT, Cho BH, Kim YJ, Kim JB, Kim CS, Cha YD and Kim YS: *Effect of cyclobuxine on the derangement induced by hypoxia in the isolated rat heart. New Med J 32 (12): 51-56, 1989*

Lee JH, Park YH, Cho BH, Kim YJ, Kim JB, Kim CM, Kim CS, Cha YD and Kim YS: *Effects of cyclobuxine D on the biosynthesis of prostaglandins in vitro, prostaglandin production and leukocytes migration in vivo. Kor J Pharmcol 23: 51-56, 1987*

Lee JH, Park YH, Cho BH, Kim YJ, Kim JB, Kim CM, Kim CS, Cha YD and Kim YS: *Effects of cyclobuxine D on carrageenin-induced pleurisy and croton oil-induced granuloma pounch in rats. Kor J Pharmacol 23: 151-157, 1987*

McCord JM: *Free radical and myocardial ischemia: Overview and outlook. Free Radical Biol Med 4: 9-14, 1988*

McCord JM: *Oxygen-derived free radicals in postischemic tissue injury. New Engl J Med 312: 159-163, 1985*

Murphy JG, Marsh JD and Smith TW: *The role of calcium in ischemic myocardial injury. Circulation 75 (suppl 5): V15-V24, 1987*

Omar B, McCord J and Downey J: *Ischemia-reperfusion. In Oxidative Stress, edited by Sies H, Academic Press, NY, 1991, pp493-525*

Salin ML and McCord JM: *Superoxide dismutase in polymorphonuclear leukocytes. J Clin Invest 54: 1005-1009, 1974*

Simpson PJ and Lucchesi BR: *Free radicals and myocardial ischemia and reperfusion injury. J Lab Clin Med 110: 13-30, 1987*

Singal PK, Beamish RE and Dhalia NS: *Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. In Myocardial Injury, edited by Spitzer JJ, Plenum, NY, 1983, pp391-401*

Thompson JA and Hess ML: *The oxygen free radical system: A foundmental mechanism in the production of myocardial necrosis. Pro Cardiovasc Dis 28: 449-462, 1986*

Yamamoto S: *Enzymes in the arachidonic acid cascade. In Prostaglandins and Related Substances. edited by Pace-ascieak C and Granstrom E, Elsevier Science Publisher, Amsterdam, 1983, pp171-202*

Weisfeldt ML: *Reperfusion and reperfusion injury. Clin Res 35: 13-20, 1987*

=국문초록=

허혈재-관류 적출심장에서 Arachidonic Acid에 의한 산소래디칼 생성 및 심근손상에 대한 Cyclobuxine의 영향

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이종화 · 권준택 · 조병헌 · 박종안 · 김유재 · 김종배 · 차영덕 · 김창호

흰쥐의 허혈-재관류 적출심장에서 arachidonic acid의 투여에 의해 촉진된 superoxide anion의 생성과 심근손상에 대한 cyclobuxine (스테로이드성 알카로이드)의 영향을 관찰하였다. 적출심장을 Langendorff 관류장치에 현수하고 0.5 ml/min의 저용량으로 45분간 관류한 후 정상관류 (7 ml/min)로 복귀시켜 허혈-재관류 심장으로 사용하였다. 재관류 시 arachidonate (0.1과 1.0 μg/ml)를 투여한 후 superoxide anion의 생성을 관찰하였고, 좌심실 내의 지질 과산화 정도는 MDA의 량으로 측정하였으며, 심근손상의 지표로 lactic dehydrogenase (LDH)유리를 측정하였다. 한편 cyclobuxine (1.0과 10 μg/ml)을 허혈 이전부터 전관류 과정 동안 투여하여 arachidonate에 의해 초래되는 손상에 대한 영향을 관찰하였다.

Arachidonic acid는 용량적으로 superoxide anion의 생성을 증가시켰으며 이 작용은 superoxide dismutase (SOD 300 U/ml)와 cyclobuxine에 의해 현저히 억제되었다. Arachidonate를 투여하였을 때 좌심실 내의 malondialdehyde (MDA)의 생성이 현저히 증가되었으며 cyclobuxine은 MDA의 생성을 용량적으로 억제시켰다. 또한 arachidonate는 LDH의 유리를 증가시켰으며 arachidonate에 의한 LDH의 유리 증가는 SOD와 cyclobuxine에 의해 유의하게 억제되었다.

이상의 결과로 흰쥐의 허혈-재관류 심장에서 스테로이드성 알카로이드인 cyclobuxine이 arachidonate에 의한 반응성 산소대사물의 생성과 심근세포손상을 유의하게 억제하는 것으로 관찰되었다.