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Antioxidant and Pro-oxidant Effects of Epinephrine and Isoprenaline on Peroxidation of LDL and Lipid Liposomes

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Summary

Antioxidant or pro-oxidant properties of epinephrine (EPI) and isoprenaline (ISO) were studied in the absence and presence of Fe^{2+}, Fe^{3+} and Cu^{2+} ions. EPI and ISO (>2 μmol/l) inhibited peroxidation of low density lipoprotein (LDL) induced by 2, 2'-azobis(2-amidino-propane) (AAPH). EPI had a similar inhibitory potency as ISO, but their potency was several times higher than the potency of α-tocopherol (α-TOC). When the LDL peroxidation was induced by 5 μmol/l $CuSO_4$, EPI and ISO enhanced LDL peroxidation at low concentrations (10 μmol/l) and decreased peroxidation at higher concentrations (30 μmol/l). The compounds had a similar tendency to inhibit the peroxidation of phosphatidylcholine liposomes. EPI (3–30 μmol/l) inhibited lipid peroxidation of phosphatidylcholine liposomes induced by 2 mmol/l of AAPH, but it was less effective and even increased the peroxidation, when the samples contained 2 mmol/l AAPH with 50 μmol/l $FeSO_4$ or 2 mmol/l AAPH with 20 μmol/l $FeCl_3$. Inhibition of lipid peroxidation by EPI was also observed when studying decreased oxygen consumption, when the peroxidation of linoleic acid was induced by lipoxidase. In conclusion, EPI and ISO reduced lipid peroxidation, but they exhibit pro-oxidant properties in the presence of Fe^{2+}, Fe^{3+} or Cu^{2+} ions, depending on the catecholamine and ionic concentration.

Key words

Antioxidant – Epinephrine – Isoprenaline – Peroxidation – Low density lipoprotein – Lipoxidase – Iron

Introduction

The results of clinical and experimental animal studies have shown that unusually high levels of catecholamines in the blood cause a variety of morphological and functional changes in the heart (Ferrans *et al.* 1970, Sinhal *et al.* 1981, Wheatley *et al.* 1985). Lipid peroxidation in the myocardium increased in response to isoprenaline treatment of rats and this effect was prevented by pretreatment of the animals with vitamin E (Singal *et al.* 1983). The autooxidation of catecholamines at physiological pH has been proposed to be a source of oxygen radicals leading to damage in myocardial ischaemia-reperfusion injury (Yates *et al.* 1981, Rona 1985, Bindoli *et al.* 1989, 1992, Rump and Klaus 1994). Allen *et al.* (1994) demonstrated that norepinephrine and epinephrine mediated iron release from ferritin under conditions that can occur during ischaemia/reperfusion. These data indicate that catecholamines have negative properties in conditions where free radicals are involved. We reported (Ondriaš *et al.* 1993) that isoprenaline and epinephrine accelerated the decay rate of α-tocopherol radical which could indicate that the compounds reduced the α-tocopherol radical to α-tocopherol. This suggests that catecholamines may have antioxidant properties. We therefore studied the

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effect of epinephrine and isoprenaline on peroxidation of model systems without and with Fe^{2+}, Fe^{3+} or Cu^{2+} ions. The results may contribute to the understanding of the action of catecholamines in free radical-induced toxicity.

Materials and Methods

Chemicals

Epinephrine bitartarate (EPI), isoprenaline hydrochloride (ISO) and α-tocopherol (α-TOC) were from Sigma. 2-thiobarbituric acid (TBA) from Fluka. 2, 2'-azobis(2-amidino-propane) (AAPH) was from Polysciences, Inc. All other chemicals were of analytical grade from commercial sources. Since EPI, ISO and α-TOC are not stable in solution, they were dissolved in the given solution 30–60 s before use.

LDL peroxidation

LDL was prepared from the serum obtained from fresh normal human blood by ultracentrifugation (1.024-1.050 fraction) (Havel *et al.* 1955, Ondriaš *et al.* 1994). Isolated LDL was dialysed for 24 h against a buffer (in mmol/l) NaCl 150, Tris. HCl 5, 7.4 pH. Samples for LDL peroxidation were prepared as follows. Dialysed LDL (20 μl; 18.4 mg protein) in 250 μl of the buffer (in mmol/l: NaCl 150, Tris. HCl 5, 7.4 pH) were incubated at 37 °C for the 3 or 4 h in the dark without and with the studied compounds which were added into the buffer, but α-TOC was added in ethanol (<1 % final ethanol concentration). Peroxidation of the samples was induced by AAPH (1 mmol/l) (Sato *et al.* 1990, Niki *et al.* 1991) or $CuSO_4$ (5 μmol/l) which were added 10 min after the drug. The extent of lipid peroxidation was assessed by measuring the formation of TBA-reactive products (Ondriaš *et al.* 1989). Briefly, after incubation of the sample (250 μl), butylated hydroxytoluene (23 mg in 152 μl ethanol) was added to prevent further peroxidation of the sample. Then 1.5 ml of TBA solution (2.1 g TBA, 84 g trichloroacetic acid, 3.57 ml of 37 % HCl diluted with H_2O to 500 ml) was added and the samples were incubated at 80 °C for 15 min. After that, the samples were cooled at 10 °C in a water-bath and centrifuged at 1600 x *g* for 5 min. The supernatant was analyzed spectroscopically by UV HP-8452A spectrometer, where absorption at 534 nm (TBA-reactive products) was taken as a relative value of lipid peroxidation. Each UV spectrum was accumulated several times by computer, the standard deviation for absorbance being less than 0.0005.

Liposome peroxidation

Phosphatidylcholine (0.2 mg) was hydrated in air with the buffer (in mmol/l: NaCl 150, Tris.HCl 5, 7.4 pH) containing EPI, and the liposomes were prepared by vortexing the samples for one minute. In the case of α-TOC, the control liposomes (phosphatidylcholine hydrated with the buffer) were added to α-TOC and the samples were vortexed for one minute. In order to induce lipid peroxidation, AAPH (2 mmol/l) without and with $FeSO_4$ (50 μmol/l) or $FeCl_3$ (20 μmol/l) were added and the samples were vortexed for 20 s. The liposomes were finally sonicated in a bath for 30 s and incubated for 3 h at 37 °C. The extent of lipid peroxidation was assessed by measuring the formation of TBA-reactive products as described above.

Oxygen consumption

Linoleic acid (1.7 mg) was mixed with 6 ml of the buffer (150 mmol/l NaCl; 5 mmol/l Tris. HCl; 7.4 pH). The suspension (6 ml) was stirred at 37 °C during the measurement. Lipid peroxidation was induced by addition of lipoxidase (4 μg/ml). Epinephrine was added as a powder to the suspension containing linoleic acid 30 s before adding lipoxidase. The oxygen consumption was measured by a Clark-type oxygen electrode and recorded using a chart recorder. The Clark-type oxygen electrode was calibrated using solutions saturated with air (100 %) and bubbled with nitrogen for 10 min (0 %).

Results

The water-soluble AAPH generates free radicals thermally at a constant rate at 37 °C and induces the chain oxidations of polyunsaturated fatty acids or LDL (Sato *et al.* 1990, Niki *et al.* 1991). ISO and EPI inhibited the peroxidation of LDL induced by 1 mmol/l AAPH (Fig. 1, upper panel). Their effect was pronouncedly higher than the effect of α-TOC. Inhibition effect of EPI and ISO was also observed when the peroxidation of LDL was induced by incubation of the samples (at 37 °C for 24 h) without any chemical initiator of the peroxidation (data not shown). We previously reported that α-TOC decreased the peroxidation of LDL induced by 5 μmol/l $CuSO_4$ (Ondriaš *et al.* 1992, 1994). Since EPI and ISO have similar effects on LDL peroxidation probably due to their similar chemical structure, we used only EPI in a further study.

Since catecholamines interact with iron to produce reactive free radicals (Walaas *et al.* 1963, Yagi *et al.* 1992, Allen *et al.* 1994) we next studied the effects of EPI and ISO on peroxidation of LDL induced by 5 μmol/l $CuSO_4$ (Fig. 1, lower panel). The effect of the catecholamines was biphasic, they increased the peroxidation at low concentrations (10 μmol/l), whereas at high concentrations (30 μmol/l) they decreased it.

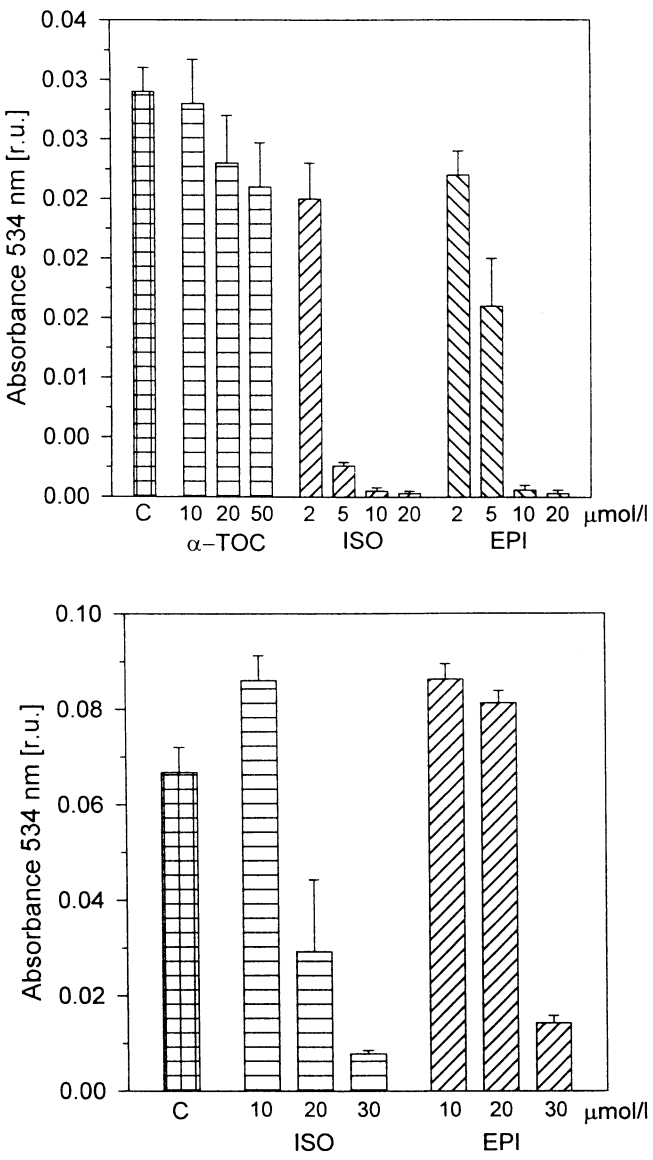

Fig. 1. *Effect of α-TOC, ISO and EPI on the formation of TBA-reactive products in LDL induced by: upper panel, 1 mmol/l AAPH; lower panel, 5 µmol/l $CuSO_4$. Samples were incubated for 4 h at 37 °C. C – control. The data represent means ± S.D. (n=4).*

Effect of EPI on peroxidation of phosphatidylcholine liposomes also depended on the presence or absence of iron in the samples. EPI and α-TOC (3–30 µmol/l) inhibited lipid peroxidation of phosphatidylcholine liposomes induced by 2 mmol/l AAPH (Fig. 2). On the other hand, when the peroxidation was induced by 2 mmol/l AAPH with 50 µmol/l $FeSO_4$ (Fig. 3, upper panel) or by 2 mmol/l AAPH with 20 µmol/l $FeCl_3$ (Fig. 3, lower panel), α-TOC inhibited the peroxidation, but the effect of EPI depended on its concentration in the sample. As is shown in the upper panel of Figure 3, EPI was not effective at concentrations 3–30 µmol/l, it significantly increased the peroxidation at a concentration of 100 µmol/l (Student's t-test, $P<0.001$), and an inhibitory effect on peroxidation was observed only at a high concentration of 300 µmol/l. When peroxidation was induced by 2 mmol/l AAPH with 20 µmol/l $FeCl_3$ (Fig. 3, lower panel), EPI enhanced peroxidation at a concentration of 30 µmol/l and decreased it at lower or higher concentrations.

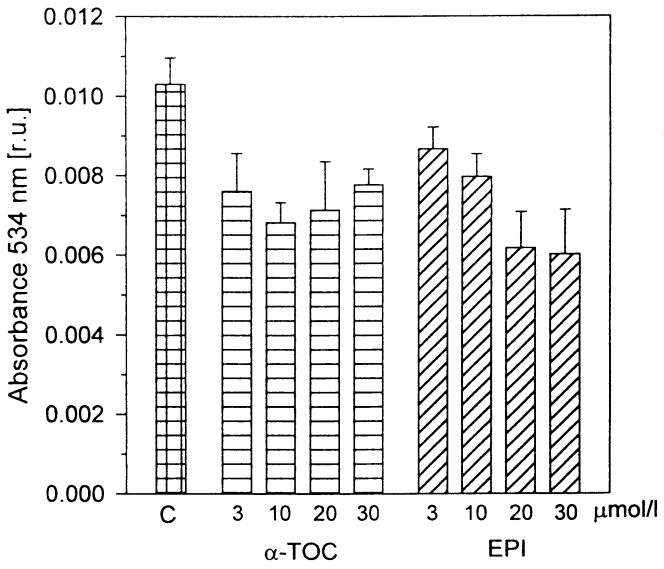

Fig. 2. *Effect of α-TOC and EPI on the formation of TBA-reactive products in phosphatidylcholine liposomes induced by 2 mmol/l AAPH after incubation for 3 h at 37 °C. C – control. The data represent means ± S.D. (n=4).*

In order to confirm the antioxidant properties of EPI in an other system without iron, we studied the effect of EPI on oxygen consumption during peroxidation of linoleic acid, when the peroxidation was induced by lipoxidase (Fig. 4). We used this system since the effect of EPI could be studied immediately and continuously after its dissolution in the sample. In the control samples, oxygen was consumed during peroxidation in 3–4 min after addition of lipoxidase. In the presence of EPI (0.5 and 1 mmol/l) the time lag of oxygen consumption was significantly prolonged (>15 min), indicating its inhibitory effect against peroxidation.

Discussion

It has been proposed that catecholamines undergo autooxidation and redox cycling, giving rise to potentially toxic products such as free radicals, which play a negative role in their toxicity (Yates *et al.* 1981, Rona 1985, Bindoli *et al.* 1989, 1992, Rump and Klaus 1994, Obata and Yamanaka 1997). Free radicals cause lipid peroxidation, e.g. lipid peroxidation in the myocardium increased in response to isoprenaline treatment of rats and this effect was prevented by vitamin E (Singal *et al.* 1983).

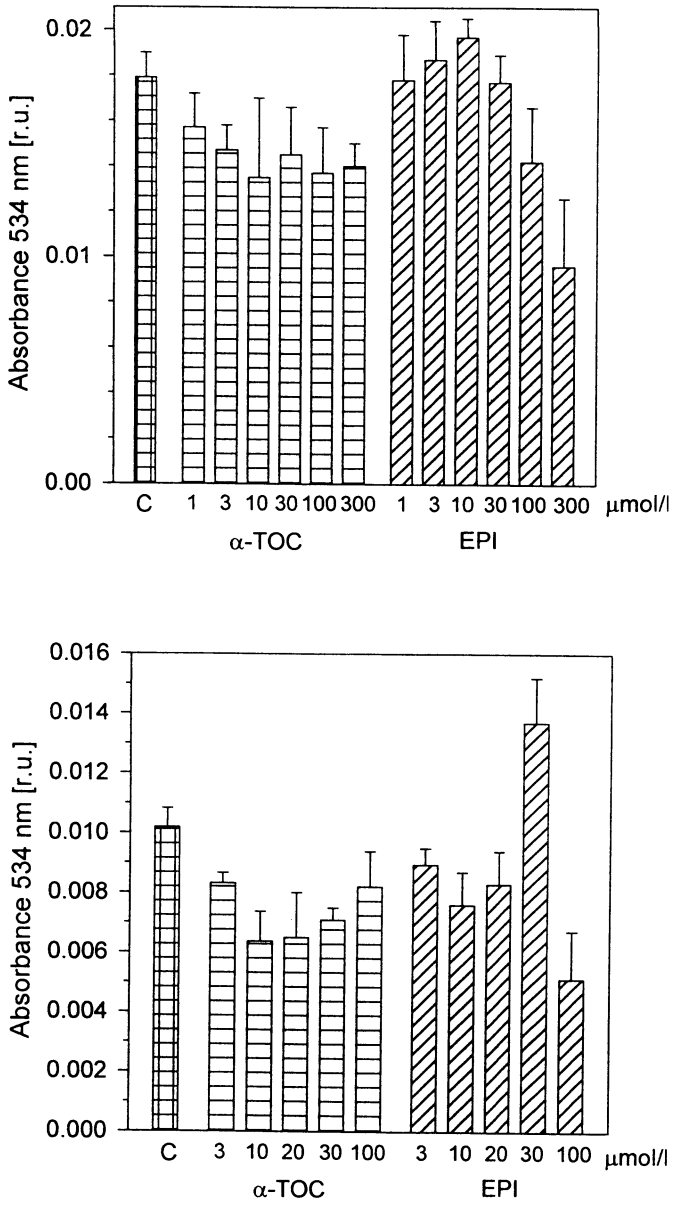

Fig. 3. *Effect of α-TOC and EPI on the formation of TBA-reactive products in phosphatidylcholine liposomes induced by 2 mmol/l AAPH with: upper panel, 50 μmol/l $FeSO_4$; lower panel, 20 μmol/l $FeCl_3$. Samples were incubated for 3 h at 37 °C. C – control. The data represent means ± S.D. (n = 6 for control and EPI, n = 3 for α-TOC).*

However, in the present report we found that the effects of EPI and ISO on lipid peroxidation depended on the presence or absence of metals. EPI and ISO in the absence of metals inhibited lipid peroxidation of LDL and lipid liposomes (Fig. 1, upper panel, Figs 2 and 4). Since AAPH generates free radicals in an aqueous solution (Sato *et al.* 1990, Niki *et al.* 1991), it is probable that higher effect of EPI or ISO in comparison to that of α-TOC (Fig. 1A) in inhibiting LDL peroxidation may be the result of higher solubility of EPI and ISO in aqueous environment in comparison to α-TOC. Esterbauer *et al.* (1987) found that peroxidation of LDL was linked with its α-TOC content. It was suggested that oxidation of LDL is preceded by the destruction of α-TOC. In a previous study, we reported that ISO and EPI may reduce α-tocopherol radical to α-TOC (Ondriaš *et al.* 1993). This may partially explain the observed antioxidant properties of EPI and ISO.

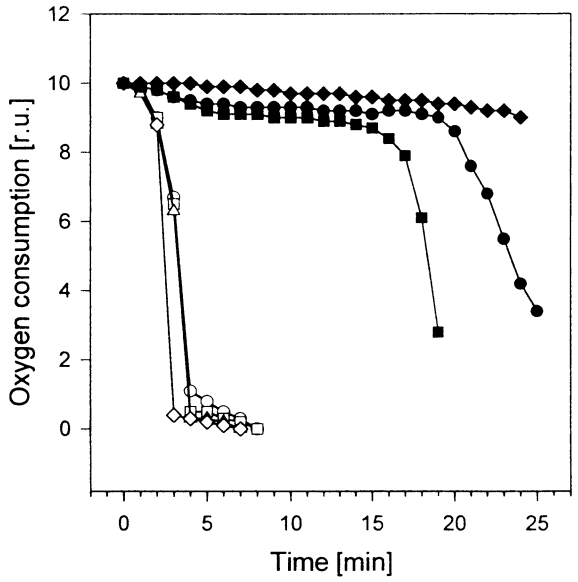

Fig. 4. *Effect of EPI on oxygen consumption during peroxidation of linoleic acid induced by lipoxidase. Open symbols are four experiments for controls. EPI, 0.5 mmol/l (two experiments: full circles and full squares) and 1 mmol/l (full diamonds).*

In the presence of metals, EPI and ISO, depending on the catecholamine and ion concentrations, increased lipid peroxidation (Fig. 1, lower panel and Fig. 3). These results are in agreement with the study of Yagi *et al.* (1992, 1993), who reported that epinephrine-iron complexes generated OH^- radicals from lipid hydroperoxides and from LDL. It was suggested that reactive radicals generated from lipid peroxides by catecholamine-iron complexes and from the metal ion catalyzed oxidation of catecholamine would explain the pro-oxidant effects of EPI and ISO (Walaas *et al.* 1963, Misra and Fridovich 1972, Jewett *et al.* 1989, Bindoli 1992, Yagi *et al.* 1992, 1993). There are several reports that metals play a negative role in catecholamine toxicity. Catecholamines enhanced enzyme inactivation and killed *E. coli* cells in the presence of copper (Aronovitch *et al.* 1991, Correa and Stoppani 1996). The importance of the pro-oxidant effects of EPI and ISO in the presence of iron is emphasized by the finding that norepinephrine and EPI mediate iron release from ferritin under conditions that can occur during ischaemia/reperfusion (Allen *et al.* 1994).

In conclusion, we hypothesize that EPI and ISO have antioxidant properties, they inhibit lipid peroxidation, but in the presence of Fe^{2+}, Fe^{3+} or Cu^{2+} ions, depending on the catecholamine and ion concentrations, they exert pro-oxidant properties which play a role in their toxicity.

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References

ALLEN D.R., WALLIS G.L., McCAY P.B.: Catechol adrenergic agents enhance hydroxyl radical generation in xanthine oxidase systems containing ferritin: implications for ischemia/reperfusion. *Arch. Biochem. Biophys.* **315:** 235–243, 1994.

ARONOVITCH J., GODINGER D., CZAPSKI G.: Bactericidal activity of catecholamine copper complexes. *Free Radic. Res. Commun.* **12–13:** 479–488, 1991.

BINDOLI A., RIGOBELLO M.P., GALZIGNA L.: Toxicity of aminochromes. *Toxicol. Lett.* **48:** 3–20, 1989.

BINDOLI A., RIGOBELLO M.P., DEEBLE D.J.: Biochemical and toxicological properties of the oxidation products of catecholamines. *Free Radic. Biol. Med.* **13:** 391–405, 1992.

CORREA J.G., STOPPANI A.O.: Catecholamines enhance dihydrolipoamide dehydrogenase inactivation by the copper Fenton system. Enzyme protection by copper chelators. *Free Radic. Res.* **24:** 311–322, 1996.

FERRANS V.J., HIBBS R.G.T., WEILY H.S., WEILBAECHER D.G., WALSH J.J., BURCH G.E.: A histochemical and electron microscopic study of epinephrine-induced myocardial necrosis. *J. Mol. Cell. Cardiol.* **1:** 11–22, 1970.

ESTERBAUER H, JÖRGENS G, QUEHENBERGER O., KOLLER E.: Autooxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. *J. Lipid Res.* **28:** 495–509, 1987.

HAVEL R.J., EDER H.A., BRAGDON J.H.: The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353, 1955.

JEWETT S.L., EDDY L.J., HOCHSTEIN P.: Is the autooxidation of catecholamines involved in ischemia-reperfusion injury? *Free Rad. Biol. Med.* **6:** 185–188, 1989.

MISRA H.P., FRIDOVICH I.: The role of superoxide anion in the Autooxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **247:** 3170–3175, 1972.

NIKI E., YAMAMOTO Y., KOMURO E., SATO K.: Membrane damage due to lipid oxidation. *Am. J. Clin. Nutr.* **53:** 201S–205S, 1991.

OBATA T., YAMANAKA Y.: Cardiac microdialysis of salicylic acid. OH generation on nonenzymatic oxidation by norepinephrine in rat heart. *Biochem. Pharmacol.* **53:** 1375–1378, 1997.

ONDRIAŠ K., MIŠÍK V., GERGEĽ D., STAŠKO A.: Lipid peroxidation of phosphatidylcholine liposomes depressed by the calcium channel blockers nifedipine and verapamil and by the antiarrhythmic-antihypoxic drug stobadine. *Biochim. Biophys. Acta* **1003:** 238–245, 1989.

ONDRIAŠ K., STAŠKO A., GERGEĽ D., HROMADOVÁ M., BENEŠ Ľ: Formation of stable free radicals from Kampo medicines TJ-9, TJ-15, TJ-23, TJ-96, TJ-114 and their antioxidant effect on low density lipoproteins. *Free Rad. Res. Commun.* **16:** 227–237, 1992.

ONDRIAŠ K., MIŠÍK V., BREZOVÁ V., STAŠKO A.: Reaction kinetics of α-tocopheroxyl radical with biologically and pharmacologically active substances. *Free Rad. Res. Commun.* **19:** 17–28, 1993.

ONDRIAŠ K., MIŠÍK V., STAŠKO A., GERGEĽ D., HROMADOVÁ M.: Comparison of antioxidant properties of nifedipine and illuminated nifedipine with nitroso spin traps in low density lipoproteins and phosphatidylcholine liposomes. *Biochim. Biophys. Acta* **1211:** 114–119, 1994.

RONA G.: Catecholamine cardiotoxicity. *J. Mol. Cell. Cardiol.* **17:** 291–306, 1985.

RUMP A.F., KLAUS W.: Cardiotoxicity of adrenochrome in isolated rabbit hearts assessed by epicardial NADH fluorescence. *Arch. Toxicol.* **68:** 571–575, 1994.

SATO K, NIKI E., SHIMASAKI H.: Free radical-mediated chain oxidation of low density lipoprotein and its synergistic inhibition by vitamin E and vitamin C. *Arch. Biochem. Biophys.* **279:** 402–405, 1990.

SINGAL P.K., DHILLON K.S., BEAMISH R.E., DHALLA N.S.: Protective effect of zinc against catecholamine-induced myocardial changes: Electrocardiographic and ultrastructural studies. *Lab. Invest.* **44:** 426–433, 1981.

SINGAL P.K., BEAMISH R.E., DHALLA, N.S.: Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. *Adv. Exp. Med. Biol.* **161:** 391–401, 1983.

YAGI K., ISHIDA N., KOMURA S., OHISHI N., KUSAI M., KOHNO M.: Generation of hydroxyl radical from linoleic acid hydroperoxide in the presence of epinephrine and iron. *Biochem. Biophys. Res. Commun.* **183:** 945–951, 1992.

YAGI K., KOMURA S., ISHIDA N., NAGATA N., KOHNO M., OHISHI N.: Generation of hydroxyl radical from lipid hydroperoxides contained in oxidatively modified low-density lipoprotein. *Biochem. Biophys. Res. Commun.* **190:** 386–390, 1993.

YATES J.C., BEAMISH R.E., DHALA N.S.: Ventricular dysfunction and necrosis produced by adrenochrome metabolite of epinephrine: relation to pathogenesis of catecholamine cardiomyopathy. *Am. Heart J.* **102:** 210–221, 1981.

WALAAS E., WALAAS O., HAAVALDSEN S., PEDERSEN B.: Spectrophotometric and electron-spin resonance studies of complexes of catecholamines with Cu(II) ions and the interaction of ceruloplasmin with catecholamines. *Arch. Biochem. Biophys.* **100:** 97–109, 1963.

WHEATLEY A.M., THANDROYEN F.T., OPIE L.H.: Catecholamine-induced myocardial cell damage: catecholamines or adrenochrome. *J. Mol. Cell. Cardiol.* **17:** 349–359, 1985.

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