Effects of Pressor Substances on Low Density Lipoprotein Peroxidation by Cu^{++}

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To investigate whether pressor substances accelerate low density lipoprotein peroxidation by Cu^{++} , low density lipoprotein was dialysed against physiological saline containing 0–5 $\mu\text{mol/l}$ CuCl_2 and/or 1 $\mu\text{mol/l}$ of various pressor substances. Lipid peroxide value of low density lipoprotein changed little with the addition of norepinephrine to the saline, but the addition of norepinephrine to 1 $\mu\text{mol/l}$ CuCl_2 accelerated the peroxidation. When low density lipoprotein was dialysed against saline with norepinephrine, epinephrine, serotonin or dopamine, there were no significant differences in the lipid peroxide values of low density lipoprotein. Although the addition of serotonin into the dialyzate did not accelerate the peroxidation of low density lipoprotein due to Cu^{++} , the addition of norepinephrine, epinephrine and dopamine accelerated the Cu^{++} -peroxidation with a significant increase in the acceleration rate starting at the 36 hour point. Thus, it is speculated that various stresses stimulating the sympathetic nervous system accelerate the peroxidation of low density lipoprotein and produce peroxidized low density lipoprotein in the blood. *J Atheroscler Thromb*, 1997; 4: 85–89.

Key words: Pressor substance, LDL peroxidation, Cathecolamine

Hyperlipidemia (1, 2), hypertension (3, 4), diabetes mellitus (5), obesity (6) and others have been mentioned as risk factors in ischemic heart diseases. Recently, peroxidized low density lipoprotein (pox-LDL) has been reported (7, 8) as one of the important risk factors in the mechanism of atherosclerosis. Although pox-LDL is produced (9) by dialysis of LDL against Cu^{++} *in vitro*, the mechanism of pox-LDL production *in vivo* is unclear. In contrast, many pressor substances are related (10, 11) to the elevation of blood pressure in essential hypertension, and the hypertension complicates various atherosclerotic diseases. Thus, it is very important to clarify how pox-LDL in the plasma is produced and how its production is accelerated. Aronovitch *et al.* (12) reported that copper

ions caused cell damage by forming copper-catecholamine complexes, and subsequently producing radicals. It is unclear, however, whether copper-catecholamine complex accelerates peroxidation of LDL in humans. In the present study, we investigated how Cu^{++} -induced LDL peroxidation is affected by various pressor substances.

Materials and Methods

Preparation of LDL

In tubes containing ethylenediaminetetraacetic acid (EDTA, final 2.5 mmol/l), fasting blood for plasma isolation was collected from healthy individuals. LDL (d. 1.006–1.063 g/ml) was isolated by sequential ultracentrifugation according to Havel's method (13). The LDL fraction was stored at 4°C and dialysed against physiological saline (0.9% NaCl) before use.

Peroxidation of LDL

Three milliliters of LDL (300 μg protein/ml) were

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dialysed against 2000 ml of physiological saline containing 0, 1 or 5 $\mu\text{mol/l}$ CuCl_2 . Aliquots of each LDL were collected at 0, 24 and 48 h after the dialysis and 1/100 vol. of 0.1 mol/l EDTA solution (pH 7.4) was added to stop the peroxidation reaction at each hour point. These methods were carried out at 4°C.

Determination of lipid peroxide (LPO)

LPO was measured with Determiner LPO Kit (Kyowa Medex Co., Ltd., Tokyo, Japan). LDL lipids were extracted according to Bligh-Dyer's method (14) and lipid constituents were separated by thin-layer chromatography (TLC). TLCs were developed with petroleum ether:diethyl ether:acetic acid (75:25:1, v/v) and lipids were stained by spraying an acidic iron-reagent solution (5% acetic anhydrous: 5% sulfuric acid: 0.05% FeCl_3 (v/v/w)) and heated at 110°C for 20 min.

Epinephrine, norepinephrine, dopamine and serotonin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Platelet activating factor (PAF) was purchased from Funakoshi Co., Ltd. (Tokyo, Japan). Angiotensin II and vasopressin were purchased from Sigma Chemical Company (St. Louis, USA).

To investigate the effects of pressor substances on LDL peroxidation by Cu^{++} , 1 $\mu\text{mol/l}$ of each pressor substance was used. Because 1 $\mu\text{mol/l}$ or higher concentration of epinephrine was used to measure platelet aggregability in clinical examination, we needed the comparisons of pressor substances with the same amount for LDL peroxidation.

Statistical analysis

Data are expressed as mean $\pm$ SD. The differences between the groups were evaluated by analysis of variance (ANOVA) and post hoc comparisons of individual groups were calculated with Fisher's PLSD test. The level of statistical significance was $p < 0.05$.

Results

The effects of Cu^{++} and norepinephrine on LDL peroxidation

The LPO value of plasma LDL obtained from blood drawn into the tubes containing 2.5 mmol/l of EDTA was 0.8 ± 0.4 nmol/ml. When LDL was dialysed against physiological saline for 48 h to remove EDTA, the LPO value of LDL increased to 17.8 ± 10.9 nmol/ml (group A, $n=3$). When LDL was stored with EDTA at 4°C for 1 month and then dialysed against physiological saline for 48 h to remove EDTA, the LPO value of LDL increased to 232.7 ± 2.0 nmol/ml (group B, $n=3$). These LDLs at two peroxidation levels were prepared.

By dialysis against the saline for another 48-hour period, the LPO value of LDL in group A changed from 17.8 ± 10.9 nmol/ml to 192.3 ± 19.3 nmol/ml by dialysis against the saline for 48 h in group A (Fig. 1a), and the LPO

Fig. 1. Comparison of Cu^{++} induced-peroxidation in different LDL-LPO concentration levels. (a) LDL with moderate LPO values, (b) LDL with high LPO values.

value in group B changed from 232.7 ± 2.0 nmol/ml to 354.7 ± 9.1 nmol/ml (Fig. 1b). The addition of norepinephrine to saline did not affect the peroxidation of LDL at either level.

Since LDL peroxidation by Cu^{++} has been reported in many papers (15, 16), the dose response of norepinephrine to LDL peroxidation by Cu^{++} was studied. As shown in Fig. 2, peroxidizability of LDL by Cu^{++} increased with norepinephrine concentration in incubation over 24 hours.

Dialysis of LDL against 1 $\mu\text{mol/l}$ CuCl_2 changed the LPO value from 17.8 ± 10.9 nmol/ml to 446.7 ± 37.1 nmol/ml in group A (Fig. 1a) and 232.7 ± 2.0 nmol/ml to 1144.4 ± 36.0 nmol/ml in group B (Fig. 1b). The addition of norepinephrine to 1 $\mu\text{mol/l}$ CuCl_2 significantly accelerated the peroxidation of LDL: from 17.8 ± 10.9 nmol/ml to 1014.9 ± 287.0 nmol/ml in group A (Fig. 1a) and 232.7 ± 2.0 nmol/ml to 2572.3 ± 8.0 nmol/ml in group B (Fig. 1b).

Effects of various pressor substances on LDL peroxidation

Since the degree of peroxidation by Cu^{++} did not differ between group A and group B, subsequent experiments on the effects of pressor substances were carried out

Fig. 2. Dose dependence of norepinephrine on LDL peroxidation by Cu^{++} . LDL was dialysed against $1 \mu\text{mol/l}$ CuCl_2 with each concentration of norepinephrine for 24 hours. LPO value increased with concentration of norepinephrine and clear differences were found at $1,000 \text{ nmol/l}$ norepinephrine.

Fig. 3. Effects of pressor substances on LDL peroxidation by dialysis. LDL was dialysed against $2,000 \text{ ml}$ of (a) saline and (b) $1 \mu\text{M}$ CuCl_2 with each pressor substance.

using LDL at a group B peroxidation level. As shown in Fig. 3a, when LDL was dialysed against saline containing norepinephrine, epinephrine, serotonin or dopamine, no difference was noted in LDL peroxidation. As shown in Fig. 3b, however, the addition of norepinephrine, epinephrine and dopamine to $1 \mu\text{mol/l}$ CuCl_2 significantly accelerated peroxidation of LDL, whereas the addition of serotonin did not. Angiotensin II, PAF and vasopressin did not affect Cu^{++} induced-peroxidation of LDL (data not shown).

Discussion

In various aspects of life-style, i.e., exercise, stress, emotions, cold, etc., pressor substance concentrations increase or decrease in plasma or tissue. When blocking or suppressive agents are administered to patients with essential hypertension, blood pressure is reduced. Therefore, it is reasonable to assume that pressor substance concentrations are closely related to blood pressure regulation. Hypertension is an important risk factor in atherosclerotic diseases and, therefore, it is important to clarify whether these substances influence LDL peroxidation which is strongly related to the pathogenesis of atherosclerosis.

In our experiments, norepinephrine accelerated the peroxidation of LDL by Cu^{++} independently on the peroxidized lipid level of LDL. In addition, epinephrine and dopamine also accelerated the peroxidation of LDL.

In the present experiment, $1 \mu\text{mol/l}$ of catecholamine was used to peroxidize LDL. This concentration of norepinephrine induced higher LPO in the incubation of LDL and Cu^{++} . In addition, this concentration of catecholamine has been used in the measurement of platelet aggregability (17). From this evidence, $1 \mu\text{mol/l}$ of pressor substances were used for LDL peroxidation.

According to Rump *et al.* (18), catecholamine produces oxygen free radicals in isolated rabbit hearts. Wysocki *et al.* (20) reported that hydrogen peroxide was detected in the plasma of patients with coronary heart disease who were undergoing exercise testing. Aronovitch *et al.* (12) published results indicating that copper ions formed complexes with epinephrine or norepinephrine, and that these complexes enhanced cell damage. The cell damage was proportional to the rate of catecholamine oxidation. Furthermore, Baraboi (19) developed a radiation-stress model showing the production of lipid peroxide and the transfer of catecholamine to quinoid. He found that the quinoid intermediated radical reaction. Rosei *et al.* (21) reported that dopamine showed the same catecholamine reaction to oxidation.

The accumulated evidences from these reports support our findings indicating that epinephrine, norepinephrine and dopamine enhance peroxidation of LDL due to Cu^{++} . Although they did not explain clearly the reason for the acceleration of radical oxidation by catecholamines,

Aronovitch *et al.* (12) indicated that the formation of copper-catecholamine complexes was important in radical oxidation.

Generally, the average concentration of pressor substances in serum are 0.5–2 nmol/l. However, it is known that these substances increase more and more under various pathological conditions. Therefore, pressor substances play an important role in the accelerations of LDL peroxidation.

The incubation of various cells and normal LDL induced peroxidized LDL in culture medium with Cu^{++} . Accordingly, our results mentioned above might suggest the enhancement of LDL peroxidation in the vessels.

According to Aronovitch *et al.* (12), the complexes of Cu^{++} and catecholamine enhance cell damage. Therefore, both peroxidized LDL and the damaged cells with catecholamine- Cu^{++} complexes might influence the pathogenesis of the arterial vessels.

In our experiment, serotonin did not cause acceleration of LDL copper oxidation; rather it reduced LPO levels. It is unclear how serotonin reduces LPO levels in Cu^{++} peroxidation.

Sahu *et al.* (22) and Yoneda *et al.* (23) reported that flavonoids or catechin showed strong suppression of radical production. Both these chemical substances have polyphenolic structures in their molecules. According to these reports, polyphenolic structure suppresses the radical oxidation. Serotonin does not have a polyphenolic structure in its molecules. Substances such as angiotensin II, platelet activating substance or vasopressin also had no phenolic or polyphenolic structure. Accordingly, LDL peroxidation by copper ion is not accelerated.

Generally, it has been reported (24, 25) that polyphenol structure suppresses peroxidation. However, catecholamines which have polyphenolic structures accelerated Cu^{++} -peroxidation. Although the reason is unclear, the specificity of the catechol structure making Cu^{++} -complex might be considered as one reason for the acceleration of Cu^{++} -peroxidation. Further experiments are necessary to determine the explanation.

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