Damage to Subcellular Structures Evoked by Lipid Peroxidation

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The influence of lipid peroxidation (LP) on the rate of disruption of rat liver and kidney subcellular structures was studied under two experimental conditions. Damage to cell organelles was found only when peroxidation process carried out into large granule fraction suspensions. Exogenous thiobarbituric acid positive products were noneffective as membrane labilizers. Age, organ and cell organelle-linked differences in the response towards LP produced damage were observed. Rat liver peroxisomes showed higher stability than those of kidney with respect to injury induced by peroxidation process. In addition, in rat kidney and neonatal rat liver samples the lysosomes were found to be more sensitive than mitochondria to the damaging effect of this process. Thiourea, an inhibitor of diene conjugate formation as well as manitol and ethanol known as hydroxyl radical scavengers were tested as terminators of LP and as membrane protectors. Effectiveness was demonstrated only for thiourea.

Peroxidation of membrane lipids plays an important role in cell physiology and pathology. Evidence now favours the belief that the products, derived during lipid peroxidation (LP), can produce unfavourable effects locally as well as far from the site of their formation (Roders *et al.,* 1978). A participation of these products in membrane fusion has been shown (De Duve and Wattiaux, 1966). In addition, a role of nonenzymic LP for prostaglandin synthesis and transformation has also been established (Marnett and Bienkowski,1977; Hornsby and Crivello, 1983). The peroxidative degradation of polyunsaturated fatty acids has been found to produce changes in the fluidity and in other membrane parameters (Archakov, 1975; Ohyashiki and Mohri, 1983; Pradhan *et al.,* 1990). The disorders in lipid organisation of biological membranes result in alterations in the activity of a number of membrane bound enzymes (Hogberg *et al.,* 1973; Baba *et al.,* 1981; De Groot *et al.,* 1986; Ohta *et al.,* 1989).

Some data indicate a relation between LP and reactive oxygen species (Kanofsky, 1986; Nakamura *et al.,* 1987; Engelhardt, 1999). On the basis of this fact attempts have been made to terminate the free radical processes by hydroxyl radical scavengers, by inhibitors of the formation of diene conjugates or by enzymes degrading superoxide anions and H_2O_2 (Halliwell and Grootveld, 1987; Andreyuk and Kiselev, 1988; Puntarulo and Gederbaum, 1988). However, there is no comprehensive information indicating the effectiveness of the free radical scavengers as terminators of the lipid peroxidative process.

Evidence has accumulated that shows the relation of the peroxidation of membrane lipids to some cell functions as well as to many pathological processes (Archakov, 1975; Eduards *et al.,* 1984). Although intensive investigations in this field have been carried out, the molecular mechanisms of tissue damages mediated by LP are not elucidated comprehensively. The objective of the present study was to obtain additional data necessary to answer the question whether LP as a destructive process or toxic products formed during LP are more important for the membrane damage. The effect of some free radical scavengers on membrane injury induced by LP was also examined.

Materials and Methods

Livers from adult male and female and neonatal (3–7 days old) Wistar rats as well as kidneys from adult rats were used . They were taken out immediately after the animals were decapitated and put into an ice-cold 0.25 M sucrose solution. Tissue homogenates were prepared in the same solution by

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a glass-teflon Potter-Elvehjem-type homogeniser. Centrifugal fractionation of liver and kidney homogenates was done as described earlier (Popov *et al.,* 1976; Popov and Yantchev, 1977). Large granule fractions isolated at 15,000×*g* for 20 min were used. They were washed twice with isotonic sucrose solution with subsequent sedimentation at the same centrifugal velocity. Finally granules were resuspended in a small volume of 0.25 M sucrose solution. The concentration of the suspensions was 20–25 mg protein/ml.

Two series of experiments were carried out as follows:

I. Studies, based on the induction of LP into large granule fraction suspensions.

Large granule fractions in isotonic sucrose solutions (concentration of protein about 5 mg/ ml), containing 0.5 mM ascorbate were incubated (usually about 1 h for liver samples and 2 h for kidney samples) with continuous aeration by bubbling until the desired concentration of thiobarbituric acid positive products (TBAPP) was achieved (about 30 nmol malondialdehyde / mg protein of the suspensions). Corresponding suspensions incubated without aeration and addition of ascorbate were used as controls. After incubation, experimental and control suspensions were cooled. Parts of them were used for determination of TBAPP and total enzyme activities. The rest of samples were centrifuged at 20,000×*g* for 20 min. Free activity of marker enzymes for lysosomes, mitochondria and peroxisomes was determined in 20,000 × g supernatants. This activity was expressed as percentages against the total ones. In all cases incubation media, used for determining the enzyme activities, included Triton X-100–0.1% for acid phosphatase (AP) and aspartate aminotransferase (ASAT) and 0.5% for catalase. The activity of AP (EC 3.1.3.2) was determined by the method of Neil and Horner (1964), that of ASAT (EC 2.6.1.1) – according to Reitman and Frankel (Kolb and Kamishnikov, 1982) and that of catalase (EC 1.11.1.6) – according to Cohen *et al.* (1970). The protein content of the samples was estimated by a biuret method (Popov, 1972) and TBAPP according to Popov and Pavlova (1977). A molar extinction coefficient of $1.56 \times 10^5\ cm^2 \times mmol^{-1}$ was used for malondialdehyde (MDA) calculation (Wills, 1969). Manitol, ethanol and thiourea were added to the samples in concentrations of 50 mM.

II. Experiments with large granule fraction suspensions, incubated in the presence of exogenous TBAPP.

Supernatants, separated from rat liver isotonic sucrose homogenates at 15,000×*g* for 20 min, were used to obtain TBAPP. They were incubated with continuous shaking for aeration at 37 °C in the presence of 0.5 mM ascorbate. When the concentration of TBAPP reached the desired levels the flasks with the samples were put into a boiling water bath for 4 min. Corresponding nonaerated controls without ascorbate were heated simultaneously. Precipitated proteins were removed by filtration. Filtrates rich in TBAPP were added to adult rat liver large granule fractions in a concentration, comparable to that of the first series of experiments (about 30 nmol MDA/ mg protein). Then the samples were incubated at 37 °C for 1 h in test tubes, filled with suspensions and closed hermetically to avoid additional accumulation of TBAPP. The subsequent procedures were the same as in the experimental section I.

The results are presented in figures as means ± S. E. or as mean values only. The degrees of significance were determined, using the Student's t-test. $P<0.05$ was interpreted as statistically significant.

Results and Discussion

The results, indicating the changes in the stability of subcellular structures, produced by peroxidation process affecting lipids of granule suspensions, are given in Fig. 1. They show organ and age differences of cell organelles against the damaging process examined. Comparatively high stability of rat liver peroxisomes with respect to LP induced injury was found. The increase of nonsedimentable catalase activity in comparison with corresponding controls was shown to be nonsignificant. Kidney peroxisomes had a higher susceptibility against the labilizing effect of the peroxidation process than liver granules.

LP resulted in liberation of a considerable amount of AP and ASAT from liver and kidney large granule fractions. In rat kidney and neonatal rat liver large granule fractions, lysosomes were shown to be more sensitive than mitochondria to the damage induced by LP. Data given in Fig. 1 indicate a higher degree of nonsedimentable activ-

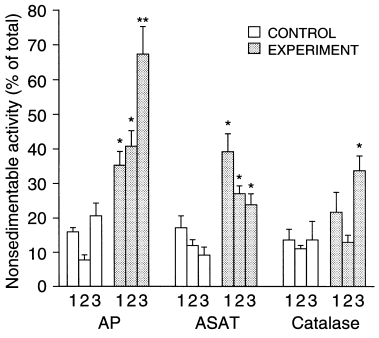

Fig. 1. Release of enzymes (acid phosphatase – AP, aspartate aminotransferase – ASAT and catalase) from subcellular structures evoked by ascorbate-dependent lipid peroxidation (LP). Open columns: controls – preincubation without ascorbate and aeration; dark columns: experimental samples – preincubation in the presence of 0.5 mM ascorbate and continuous aeration. 1 – Adult rat liver large granule fractions (average values from 6 separate experiments with samples from 2 rats each); 2 – Neonatal (3–7 days old) rat liver large granules (from 4 separate experiments with samples from 7–8 neonatal rats each); 3 – Rat kidney large granules (from 5 separate experiments with samples from 2 rats each). Significant difference from the control: *P<0.05; **P<0.001.

ity of AP in comparison with that of ASAT. This difference is well expressed in kidney fractions.

The results from experiments with added exogenous TBAPP to the granule fractions are given in Fig. 2. Lysosomes, mitochondria and peroxisomes, derived from liver and kidney, revealed a similar response. Practically no disruption of subcellular structures was observed when concentrations of exogenous TBAPP (about 30 nmol/ mg protein) comparable with that, produced endogenously by

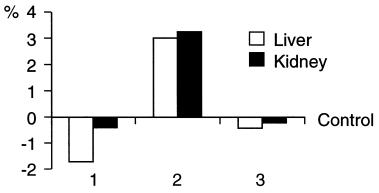

Fig. 2. Free activity of acid phosphatase (AP), aspartate aminotransferase (ASAT) and catalase determined after preincubation of rat liver and kidney large granule fractions (LGF) in the presence of exogenous thiobarbituric acid positive compounds. 1 – AP, 2 – ASAT, 3 – catalase. The values are given in percentages towards the corresponding controls. Mean values from five (liver) and four (kidneys) separate experiments.

LP (first series of experiments) were added to the large granule fraction suspensions prior to incubation. Average values of nonsedimentable activities of AP and catalase were the same as of the controls. In the case of ASAT a slight increase in this activity was observed (Fig. 2). The deviations from the control values were negligible (about + 3%), when compared with those, induced by endogenous TBAPP (+15 ÷ +37%; Fig. 1).

From manitol, ethanol and thiourea, an inhibitory effect on LP was observed for thiourea only (4 nmol MDA/mg protein against 32 nmol MDA/ mg protein for the control suspensions). Probably by this way thiourea retarded the liberation of AP and ASAT from large granule fractions during their incubation under conditions which favour the accumulation of TBAPP (presence of ascorbate and aeration). Under such conditions manitol and ethanol increased slightly the lysosomal labilizing effect of LP. An opposite effect on mitochondria was observed for ethanol (Fig. 3). Manitol and ethanol stabilized liver lysosomes and mitochondria in control granule suspensions (incubation without accumulation of TBAPP). Thiourea could produce such an effect on mitochondria only (Fig. 3). A direct membrane stabilizing influence for polyhydroxyl compounds, such as manitol, has been demonstrated earlier by other authors (Romeo *et al.,* 1967).

As noted above products from degradation of fatty acids can react locally as well as far from the

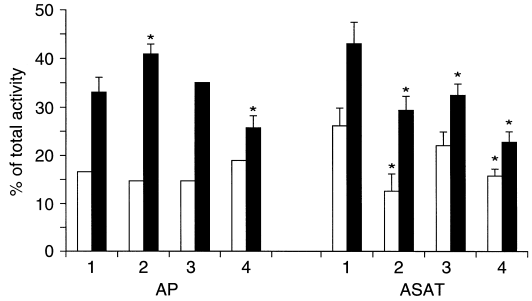

Fig. 3. Effect of manitol, ethanol and thiourea on the rate of release of acid phosphatase (AP) and aspartate aminotransferase (ASAT) from rat liver large granule fractions produced by LP (preincubation in the presence of ascorbate and aeration) –dark columns. Open columns represent corresponding controls (preincubation without ascorbate and aeration). 1 – control; 2 – manitol 50 mM; 3 – ethanol 50 mM; 4 – thiourea 50 mM. Average values from 4 separate experiments.
* Significant difference from the control (P<0.05).

site of their formation (Roders *et al.,* 1978). Damage to cell organelles, produced by LP, has been demonstrated in many investigations. However, there is no firm evidence about the mechanism of action of LP. Does it acts simply as a degradative process, operating within the membranes, or does it induce its unfavourable effect on subcellular structures by toxic products? At present there is no reason to reject the capability of TBAPP to exert pathological action at a distance, but our data supported only local effects of these products and/or LP process on intracellular membrane systems. Powerful damage to subcellular structures has occurred when LP proceeds in the large granule fraction suspensions themselves (Fig. 1). Exogenous TBAPP had a negligible injurious effect on mitochondria (Fig. 2).

Organ, age and subcellular structure-linked differences in response to the LP induced damage were established by the present studies. These differences may be due to unequal overall antioxidant capacity in various tissues and subcellular sites. Different contents and activities of antioxidants and enzymes such as vitamin E, vitamin C, glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase in various tissues have been reported (Lew *et al.,* 1985; Gohil *et al.,* 1986; Di Meo *et al.,* 1996).

Andreyuk G. M. and Kiselev P. A. (1988), Initiation of lipid peroxidation as a result of hemoglobin conversion into hemichrome under effects of free fatty acids. Biokhimiya **53,** 1017–1024 – in Russian.

Archakov A. I. (1975), Microsomal oxidation. Nauka, Moscow – in Russian.

Baba A., Lee E., Ohta A., Tatsuno T. and Iwata H. (1981), Activation of adenylate cyclase of rat brain by lipid peroxidation. J. Biol. Chem. **256,** 3679–3684.

Cohen G., Dembiec D. and Marcus J. (1970), Measurement of catalase activity in tissue extracts. Anal.Biochem. **34**, 30–38.

De Duve C. and Wattiaux R. (1966), Functions of lysosomes. Annu. Rev. Physiol. **28**, 435–492.

De Groot H., Noll T. and Rymsa B. (1986), Alterations of the microsomal glucose-6-phosphatase system evoked by ferrous iron and haloalkane free-radical-mediated lipid peroxidation. Biochim. Biophys. Acta, Gen.Subj. **881 (G122)**, 350–355

Di Meo S., Venditti P. and De Leo T. (1996), Tissue protection against oxidative stress. Experientia **52,** 786–794.

Eduards R. H., Jones D. A. and Jackson M. J. (1984), An approach to treatment trials in muscular dystrophy with particular reference to agents influencing free radical damage. Med.Biol. **62,** 143–147.

Engelhardt J. F. (1999), Redox-mediated gene therapies for environmental injury: Approaches and Concept. Antioxidants and Redox Signaling, Mary Ann Liebert, Inc. **1,** 5–27.

Gohil K., Packer L., Lumen B. de, Brooks G. A. and Terblanche S. E. (1986), Vitamin E deficiency and vitamin C supplements: exercise and mitochondrial oxidation. J. Appl. Physiol. **60,** 1986–1991.

Halliwell B. and Grootveld M. (1987), The measurement of free radical reactions in humans. FEBS Lett. **213,** 9–14.

Hogberg J., Bergstrand A. and Jakobson S. V. (1973), Lipid peroxidation of rat-liver microsomes. Its effect on the microsomal membrane and some membrane-bound microsomal enzymes. Eur. J. Biochem. **37,** 51–59.

Hornsby P. J. and Crivello J. F. (1983), The role of lipid peroxidation and biological antioxidants in the function of the adrenal cortex. Part 1 A. background review. Mol. and Cell. Endocrinol. **30,** 1–20.

Kanofsky J. R. (1986), Singlet oxygen production by bleomycin A. Comparison with heme-containing compounds. J. Biol. Chem. **261,** 13546–13550.

Kolb V. G. and Kamishnikov V. S. (1982), Handbook of Chemical Chemistry. Belorus, Minsk, pp. 111–115 – in Russian.

Lew H., Pyke S. and Quantanilha A. (1985), Changes in the glutathione status of plasma, liver and muscle following exhaustive exercise in rat. FEBS Lett. **185,** 262–266.

Marnett L. J. and Bienkowski M. J. (1977), Nonenzymatic reduction of prostaglandin H by lipoic acid. Biochemistry **16,** 4303–4307.

Nakamura T., Goto M. and Koyama J. (1987), The NADPH oxidase-dependent hemolysis of sheep erythrocytes with membrane fraction of guinea pig peritoneal macrophages. J. Biochem. **101,** 1347–1353.

Neil M. W. and Horner M. W. (1964), Studies on acid hydrolases in adult and foetal tissue. Acid p-nitrophenylphosphate phosphohydrolases of adult guinea-pig liver. Biochem.J. **92,** 217–224.

Ohta A., Mohri T. and Ohyashiki T. (1989), Effect of lipid peroxidation on membrane-bound Ca2+-ATPase activity of the intestinal brush-border membranes. Biochim. Biophys. Acta **984,** 151–157.

Ohyashiki T. and Mohri T. (1983), Effect of ionic strength on the membrane fluidity of rabbit intestinal brushborder membranes. A fluorescence probe study. Biochim. Biophys. Acta **731,** 312–317.

Popov Ch.S. (1972), Effect of chlorpromazine on the stability of rat liver mitochondria, lysosomes and peroxisomes. Compt. rend. Acad. bulg. Sci. **25,**1293–1296.

Popov Ch. S., Yantchev I., Georgiev G. and Geneva L. (1976), Effect of different factors modifying the activity of some enzyme systems of the endoplasmic reticulum on the sensitivity of cell organelles against the damaging action of chemical agents. I. Interrelation between the activity of some enzyme system located in endoplasmic reticulum and hepatotoxicity of carbon tetrachloride. Br. J. Exp. Pathol. **57**, 597–603.

Popov Ch. S. and Pavlova M. (1977), Comparative studies on the sensitivity of rat liver cell organelles to the effect of some vitamins hormones and heavy metal salts. Compt. rend. Acad. bulg. Sci. **30,** 909–912.

Popov Ch. S. and Yantchev I. (1977), Studies on the stability of liver and kidney cell organelles of tumour-bearing rats. Compt. rend. Acad. bulg. Sci. **30,** 1197–1200.

Pradhan D., Weiser M., Lunley-Sapanski K., Frazier D., Kemper S.,

Williamson P. and Schlegel R. A. (1990), Peroxidation-induced perturbations of erythrocyte lipid organization. Biochim. Biophys. Acta, Biomembranes **1023**, 398–404.

Puntarulo S. and Gederbaum A. J. (1988), Increased microsomal interaction with iron and oxygen radical generation after chronic acetone treatment. Biochim. Biophys. Acta **964,** 46–52.

Roders M. K., Glende E. A. and Recknagel R. O. (1978), NADPH- dependent microsomal lipid peroxidation and the problem of pathological action at a distance. New data on induction of red cell damage. Biochem. Pharm. **27,** 437–443.

RomeoD., Stagni N. and Pugliarello M. C. (1967), Protection effect of polyhydroxyl compounds on heart lysosomal structures. Experientia **23,** 247–248.

Wills E. D. (1969), Lipid peroxide formation in microsomes, General Considerations. Biochem. J. **113,** 315–327.