INITIATION OF LIPID PEROXIDATION IN LYSOSOMAL MEMBRANES BY ACTIVATED BLOOD POLYMORPHONUCLEAR LEUKOCYTES

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Interaction between phagocytic cells – polymorphonuclear leukocytes (polymorphs) and macrophages – with soluble and corpuscular agents is accompanied by stimulation of the cells, or "respiratory burst," in the process of which production of various pro-oxidants increases: active forms of oxygen ($O_2^{\cdot}$, $^1O_2$, $OH^{\cdot}$, H_2O_2), cationic proteins [5], and degradation products of unsaturated fatty acids [1]. The appearance of these highly active compounds is linked with the bactericidal and cytotoxic properties of the activated phagocytes [7]. The concrete mechanism of action of the pro-oxidants produced by phagocytes on bacterial and other cells cannot be considered to be finally settled. Data in the literature on this problem are few in number and at times contradictory in nature. One of the targets for the action of activated forms of oxygen is probably the lipids which are components of cell membranes. In model systems, where superoxide radicals are formed by a nonenzymic pathway or in the reaction of xanthine oxidation by xanthine oxidase, initiation of lipid peroxidation (LPO) by hydroxyl radicals has been shown to be possible [10]. However, in such systems it is difficult to rule out completely the possibility of LPO activation by participants in chemical reactions without involving the intervention of activated oxygen.

Accordingly, the present investigation was undertaken with the aim of explaining whether activation of blood polymorphs can initiate LPO in liposomal membranes.

EXPERIMENTAL METHODS

Peripheral blood polymorphs were isolated by the method in [11]. Stratified liposomes were formed from the total fraction of phospholipids from hens' egg yolks by the method in [9]. The final concentration of liposomes in the sample was 5 mg phospholipid to 1 ml of Hanks' solution. The LPO level was determined as the quantity of TBA-active products [8].

To evaluate the role of the superoxide radical and of H_2O_2 in the initiation of LPO in liposomes, solutions of superoxide dismutase (SOD) and catalase were used. SOD was isolated from erythrocytes by the method in [13] and the activity of the enzyme [12] in the sample was 600 U. Catalase (Boehringer, West Germany) was purified before use from thymol, which is added to the preparation as an antiseptic, by gel-filtration on a column with Sephadex G-50. Activity of the enzyme [12] in the sample was 1200 U.

EXPERIMENTAL RESULTS

It was shown previously that active forms of oxygen can pass through a dialysis membrane, so that it was possible to separate the site of generation of oxygen radicals (the xanthine–xanthine oxidase system) from the site of their detection (luminol-dependent chemiluminescence) [3]. A similar method was used in the present investigation. Leukocytes were placed in a dialysis bag, which was immersed in a vessel containing a suspension of liposomes, changes in the LPO level in which were determined. It will be clear from the data that during activation of polymorphs in the inner compartment there was a small (not statistically significant) increase in the content of TBA-active products in the liposomes in the outer compartment (Table 1). This result suggests that superoxide free radicals produced by activated leukocytes, like the H_2O_2 which can be formed as a result of a spontaneous reaction of

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TABLE 1. Effect of Activation of Polymorphs on LPO of Liposomal Membranes

Serial No.	Composition of medium		MDA pmoles/ml
	inside dialysis bag	outside dialysis bag	
1	Hank's solution	Suspension of native liposomes	49
2	Polymorphs+zymosan	PL	60
3	Hank's solution	PL+(Fe-ADP)	50
4	Polymorphs	PL+(Fe-ADP)	57
5	Polymorphs+zymosan	PL+(Fe-ADP)	113
6	Polymorphs+zymosan	PL+(Fe-ADP)+SOD	42
7	Polymorphs+zymosan	PL+(Fe-ADP)+catalase	44
8	Hank's solution	SOL	156
9	Polymorphs+zymosan	SOL	122
10	Polymorphs+zymosan	SOL+(Fe-ADP)	125
11	Polymorphs+(Fe-ADP)+zymosan	SOL	115

Legend. PL) Phospholipids; SOL) suspension of oxidized liposomes. Inside dialysis bag: 2 ml of suspension of polymorphs ($1 \cdot 10^6$ cells in 1 ml) in Hank's solution, not containing glucose, to which opsonized zymosan (2 mg per sample), SOD (600 IU per sample), and catalase (1200 IU per sample) were added. Outside dialysis bag: suspension of liposomes 5 mg/ml, Fe-ADP 10^{-7} M. Incubation time 30 min at 37°C.

disproportioning of superoxide radicals, cannot stimulate LPO in unoxidized phospholipid membranes. We know that hydroxyl radicals, which may be formed from $O_2^{\cdot}$ and H_2O_2 in the presence of ion-complexes of iron, are capable of initiating LPO [6]. In fact, when the Fe–ADP complex was added to a suspension of unoxidized phospholipids, marked activation of LPO was found. Addition of chelated iron to liposomes against the background of unactivated leukocytes (without zymosan) did not lead to intensification of LPO in liposomal membranes. SOD, which removes superoxide radicals, and catalase, which decomposes H_2O_2, prevented LPO stimulation under conditions when they were added to a suspension of liposomes before activation of the leukocytes.

The results obtained in the experiments described above suggest the following order of events in the system consisting of phagocytic cells and phospholipid membranes (Fig. 1).

1. Activation of the cells induces $O_2^{\cdot}$ formation. Some of these radicals react spontaneously with one another, leading to H_2O_2 formation.

2. Active $O_2^{\cdot}$ and H_2O_2 molecules diffuse through the aqueous phase and reach the surface of the phospholipid membrane (in the present experiments diffusion took place through the dialysis membrane). However, these active forms of oxygen are unable to initiate themselves the LPO process in membranes.

3. In the presence of the ion complex of iron LPO still takes place, evidently as a result of the formation of hydroxyl radicals:

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + OH + OH \longrightarrow LPO$$

$$Fe^{2+} + O_2^{\cdot} + 2H^+ \longrightarrow Fe^{3+} + H_2O_2$$

It must be emphasized that the formation of the OH· free radical ought to take place in the immediate proximity of the phospholipid membrane – the oxidation substrate – for if Fe-ATP was added to leukocytes in the inner compartment, and not to the liposomes, no stimulating action on LPO was observed under those circumstances.

In experiments in which activated leukocytes were separated by a semipermeable membrane from pre-oxidized phospholipids, the situation was different. It was found that products produced by the leukocytes did not increase the quantity of lipid peroxides, but instead, on

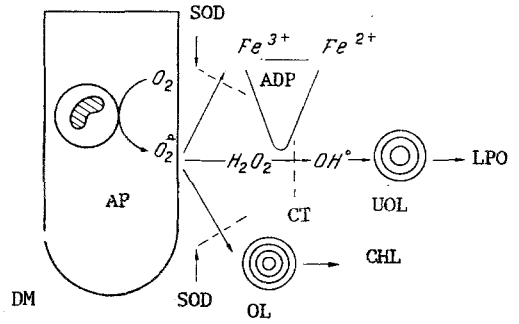

Fig. 1. Diagram of interaction of activated blood polymorphs with liposomal membrane lipids. AP) Activated polymorphs; DM) dialysis membrane; CT) catalase; OL) oxidized liposomes; UOL) unoxidized liposomes; CHL) chemiluminescence.

the contrary, they reduced it somewhat, both in the presence and in the absence of the ion-complex of iron in the liposomal suspension or in the suspension of polymorphs. These results suggest that a certain reaction takes place between lipid peroxides in liposomal membranes and superoxide free radicals, as a result of which the content of LPO products may decrease. The authors of [15], who investigated the reaction between superoxide oxygen radicals and a lipid hydroperoxide in linoleate micelles found that the reaction proceeds with high velocity: the velocityconstant reached a value of $7 \cdot 10^2$ $M^{-1} \cdot sec^{-1}$. It is possible that a similar reaction was responsible for the small decrease in concentration of lipid peroxidation products in a phospholipid suspension also, which was observed in the present experiments.

The results are thus evidence that superoxide radicals and hydrogen peroxide, formed during activation of blood polymorphs, can be displaced for quite a long distance from the sites of their formation, and in the presence of iron ions, they can produce hydroxyl radicals, and this process is accompanied by initiation of LPO in membrane systems. A similar situation is perfectly possible also in vivo, especially in the presence of pathological processes characterized by inflammation, when activation of phagocytes may lead to peroxide damage to the cell membranes and to the plasma lipoproteins.

It was shown previously that with the onset of clinical [4] or experimental [14] myocardial infarction a considerable (more than 10-20-fold) increase in phagocytic activity of the blood neutrophils is observed. Sensitization of leukocytes is accompanied by their chemotaxis, interaction, and infiltration into a zone of necrosis, and a considerable increase in production of pro-oxidants [14]. If it is recalled that this takes place against the background of a decrease in activity of endogenous antioxidant enzymes (SOD, catalase, glutathione peroxidase [2]), then there is every reason to suppose that active forms of oxygen, activation products of lipo- and cyclo-oxygenase of phagocytes, and cationic proteins produced by infiltrated leukocytes actually in the zone of necrosis, may lead through LPO initiation to damage to the cell membranes and to widening of the zone of myocardial necrosis.

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ACTIVATION OF PEROXIDATION OF BRAIN LIPIDS DURING INTRACEREBRAL HEMORRHAGE

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If the impedance of the brain is recorded in conscious cats after intracerebral hemorrhage (ICH) an early decrease is found in the capacity of the brain tissue, preceding the development of edema and swelling of the cells of the cerebral parenchyma [5], reflecting a disturbance of integrity of the membranes. The presence of a phase of acute compression anoxia [6] at the time of hemorrhage suggests that lipid peroxidation (LPO), an important injurious factor for nerve cell membranes during ischemia–anoxia of brain tissue [9], is involved in the pathogenesis of ICH.

The aim of the present investigation was to study the state of LPO in the brain tissue of rats during the first hours and days after ICH.

EXPERIMENTAL METHODS

Experiments were carried out on 58 noninbred male albino rats weighing 250-300 g. To produce ICH in the rats, a hollow needle was inserted stereotaxically under pentobarbital anesthesia (60 mg/kg) into the internal capsule of the right hemisphere. A week after the operation the experimental animals received an injection of 0.15 ml of autologous blood in the course of 2 min. Animals with a needle implanted into the brain but without ICH served as the control. The rats were decapitated 1, 3, and 24 h after ICH, the brain was quickly removed, cooled, weighed, and homogenized. Lipids were extracted with a mixture (2:1) of chloroform and methanol, with tissue and extracting mixture in the ratio of 1:17 (w/v) [17]. Immediately before extraction, nitrogen was bubbled through the mixture to remove oxygen. The concentration of primary products of LPO – diene conjugates (DC) – was determined spectrophotometrically in the lipid phase [11]. Antiradical activity (ARA) of the lipids was investigated with the aid of the stable free radical α-diphenyl-α-picrylhydrazide (DPPH) [8]. The concentration of malonic dialdehyde (MDA), a secondary LPO product, in the brain tissue and the kinetics of its formation in vitro during incubation of brain homogenate with pro-oxidants were investigated as in [10]. Two LPO processes were studied: nonenzymic oxidation, the so-called ascorbate-dependent pathway (ADP), and enzymic, or the NADPH-dependent pathway (NDP) [2]. The incubation medium contained 100 mM KCl, 20 mM Tris-HCl (pH 7.4), 12 μM Mohr's salt, 0.2 mM $Na_2P_4O_7$, and 1 mg/ml of homogenate protein. To study nonenzymic LPO 0.2 mM ascorbate was added to the medium, whereas to investigate enzymic LPO 0.3 mM NADPH was added. Samples, each of 2 ml, of medium were taken after incubation for 5, 15, 30, and 60 min and mixed with 2 ml of 30% TCA. The MDA level in the samples was determined after the reaction with 2-thiobarbituric acid [2]. The MDA concentration was expressed in nanomoles/mg protein. The protein concentration was determined by the biuret method [4]. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney nonparametric test [1].

EXPERIMENTAL RESULTS

ICH led to progressive accumulation of DC and MDA in the rats' brain (Fig. 1). An increase in the DC concentration in the brain was found 3 h (by 21%), and it was even greater

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