Effect of Oxidized Phospholipids on the Chemiluminescence of Zymosan-activated Leukocytes

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Abstract. The effect of liposomes with different degree of oxidation on the zymosan-induced chemiluminescence (CL) of leukocytes was investigated. Non-oxidized liposomes did not influence significantly the CL response of leukocytes. In contrast previously oxidized liposomes increased CL even if liposomes and cells were separated by a dialysis membrane. Based on the observed increase of luminol-activated CL by oxidized liposomes, lipid peroxidation (LPO) products may be suggested to enhance cell activation. Zymosan-activated leukocytes did not affect the amount of malondialdehyde (MDA) in non-oxidized liposomes unless iron salts were added. Fe^{3+} + ADP added to non-oxidized liposomes triggered LPO. Both catalase and superoxide dismutase (SOD) prevented the effect. In experiments with previously oxidized liposomes the activated oxygen species produced by leukocytes did not increase the amount of MDA; on the contrary, they decreased it both in the presence and in the absence of chelated iron in the liposome suspension. The reaction between lipid hydroperoxide and $^{\bullet}O_2^-$ widely accompanied by CL. SOD decreased CL in this system by a factor of 1. 7. On the other hand, peroxidized lipids may "opsonize" initially inactive particles: oxidized liposomes increased CL response of leukocytes similarly as opsonized zymosan routinely used as a phagocyte activator.

Key words: Leukocytes — Chemiluminescence — Lipid peroxidation — Phospholipids — Liposomes

Introduction

There is much current interest in free radical reactions occurring in the living cell. Phagocytosis and lipid peroxidation are two examples of important life processes in which free radicals are somehow implicated (Weiss 1986, Vladi-

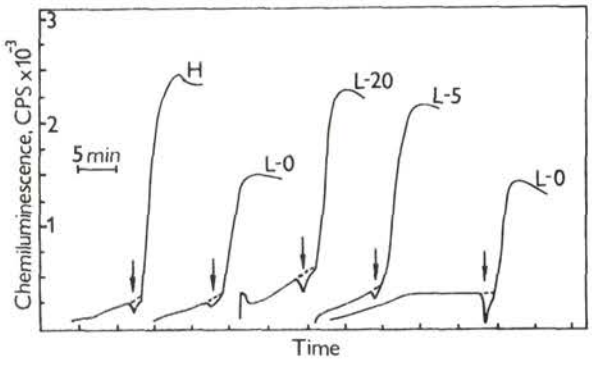

Fig. 2. Activation of CL-responses of leukocytes in the presence of oxidized liposomes. Experiments with a two-chamber cuvette in the absence of luminol. For detailes see "Materials and Methods". Arrows indicate zymosan additions.

leukocytes slightly decreased the zymosan-induced CL response (curve L-0). This decrease may be attributed to the turbidity of the liposome suspension (the optical absorbances of L-0, L-5, and L-20 at 400 nm in 1 cm cuvette were 0.31, 0.35, and 0.39, respectively). Therefore, it may be assumed that the non-oxidized liposomes did not influence significantly the CL response of leukocytes. In contrast, the addition of previously oxidized liposomes to the leukocytes brought about a very pronounced increase of the CL response (cf. curves L-0, L-5 and L-20 in Fig. 1). It should be noted that the CL responses were gradually decreasing within several hours of experiments with a given suspension of neutrophils, both with and without liposomes (cf. the two curves H and the two curves L-0 in Fig. 1). This phenomenon was obviously the result of spontaneous cell injury during their storage after isolation. The difference between L-0, L-5 and L-20 remained approximately constant. The enhanced chemiluminescence of leukocytes in the presence of oxidized liposomes may have several explanations (cf. Gregory et al. 1985). The strong enhancement of CL-response of neutrophils to stimulation in the presence of oxidized liposomes may be the result of direct interaction of the cells with phospholipid vesicles, or may be mediated by small molecules ($^{\bullet}O_2^-$, H_2O_2, ClO^-, water-soluble LPO products) diffusable through the water phase. To investigate the latter possibility, a luminometer with a two-compartment cuvette was used in which the cells and liposomes were separated by a dialysis membrane with average pore radius 2.4 nm. Similar membranes are known to be permeable for small water-soluble molecules and impermeable for large particles such as leukocytes and liposomes. The results obtained in these experiments are presented in Fig. 2. The light emission, in this case, also increased with oxidized liposomes (curves L-5 and

Table I. Accumulation of the lipid peroxidation product malondialdehyde (MDA) outside of the dialysis sack containing leukocyte suspension

Exp. No.	Composition of the suspensions:		MDA nmol/ml
	Inside the dialysis sack	Outside the dialysis sack	
1	Hanks' solution	Phospholipids (PL)	0.049 ± 0.003
2	Leukocytes + zymosan	PL	0.060 ± 0.008
3	Hanks solution	PL + Fe-ATP	0.050 ± 0.001
4	Leukocytes without zymosan	PL + Fe-ATP	0.057 ± 0.002
5	Leukocytes + zymosan	PL + Fe-ATP	0.113 ± 0.005
6	Leukocytes + zymosan	PL + Fe-ATP + SOD	0.042 ± 0.002
7	Leukocytes + zymosan	PL + Fe-ATP + catal.	0.044 ± 0.005

Leukocytes isolated as described under "Materials and Methods" were suspended in standard Hanks' solution in a concentration of 1×10^6 cells/ml. and 2 ml of the suspension were introduced into dialysis sack. The sack was immersed in a suspension of egg-yolk phospholipids in the same solution. The liposome concentration was 5 mg phospholipid per ml (a total of 4 ml). After the addition of 0.1 ml (2 mg) opsonized zymosan to the cells the system was incubated at 37 °C with continuous shaking, and MDA concentration in the outer volume was measured by the method described by Clark and Switzer (1977).

Where indicated, superoxide dismutase SOD) 600 units, and catalase 1200 units per sample were added to the phospholipid suspension.

L-20 as compared with curves L-0). It may be concluded that the enhanced leukocyte chemiluminescence in the presence of oxidized liposomes is due, at least in part, to low—molecular—weight water—soluble compounds which are able to diffuse away from the site of their generation and then interact with oxidized liposomes to produce light quanta. Alternatively, low—molecular—weight LPO products may diffuse through the membrane and make cells more sensitive to the stimulant action. The latter possibility was confirmed in our experiments with water—soluble LPO products which will be described elsewhere.

The aim of the subsequent set of experiments was to establish whether the activated oxygen species produced by the zymosan-activated leukocytes were able to diffuse across dialysis membrane to induce LPO within liposomal membranes.

The level of LPO in these experiments was estimated as the concentration of malondialdehyde-like products (MDA) accumulated in the peroxidized liposomes. Table I summarizes these results. The zymosan-activated leukocytes virtually did not affect the amount of MDA in liposomes not subjected to previous oxidation (experiments 1 and 2). Apparently, the products formed by

LPO products. Luminol can be used for this purpose since it strongly amplifies chemiluminescence of active oxygen species without, or with only very moderate, effect on luminescence associated with LPO. It was found earlier (Sharov et al. 1989) that the addition of luminol to superoxide-producing systems increases the luminescence intensity by several orders of magnitude whereas the increase in systems with LPO is but 2- or 3-fold. Having these facts in mind, we investigated the luminol-dependent CL of activated leukocytes in the presence of non-oxidized or oxidized liposomes.

It should be stressed that the CL intensity of activated leukocyte suspension increased drastically in the presence of luminol. This made it possible to use more diluted cell suspensions: 1×10^4 instead of 1×10^6 cells/ml. (In addition, the luminometer sensitivity was set 2.5 times lower). Under such conditions chemiluminescence produced by lipid peroxide degradation is expected to be negligibly low and variations in the CL responses should reflect only changes in the production of active oxygen species by leukocytes. The results obtained in these experiments are illustrated in Fig. 4. Oxidized liposomes added to the zymosan-activated leukocytes further enhanced the luminol-induced chemiluminescence. The effect of oxidized liposomes resembles in some respects that of zymosan: both LPO and increased zymosan concentration accelerated the CL development and raised the level of CL at certain time intervals (cf. Fig. 4, *A* and *B*). Perhaps, LPO products are able to somehow "opsonize" zymosan in addition to the opsonization produced by the blood serum. The mechanism of this effect remains obscure.

Discussion

The results obtained in this work indicate that the activated leukocytes and phospholipid membranes may influence each other. The activation of leukocytes may result in initiation of LPO if catalytic amounts of iron ions are available in the medium. The enzymes catalase and SOD are able to inhibit significantly this process thus suggesting that the OH radical is involved (see Fig. 3). On the other hand, the effect of $^{\bullet}O_2^-$ produced by activated leukocytes on the oxidized phospholipid membranes is essentially different. The $^{\bullet}O_2^-$ and/or H_2O_2 and ClO^- was shown to decompose the LPO products with concomitant CL burst, the iron being not a participant in this reaction (Table II). It is noteworthy that the decomposition of lipoperoxides by the products formed by stimulated leukocytes is not followed by initiation of new oxidation chains as evidenced by decreased levels of MDA in oxidized liposomes incubated with activated leukocytes but separated from the cells by dialysis membrane (Table II).

The influence of LPO products and/or lipid membranes subjected to

Table II. Lipid peroxidation in previously oxidized liposome suspension (estimated by MDA-like products)

Exp. No.	Composition of the suspensions:		MDA nmol/ml
	Inside the dialysis sack	Outside the dialysis sack	
1	Hanks' solution	Phospholipids (PL)	0.156 ± 0.001
2	Leukocytes + zymosan	PL	0.122 ± 0.002
3	Leukocytes + zymosan	PL + (Fe-ADP)	0.125 ± 0.005
4	(Leuk. + (Fe-ADP) + zymosan	PL	0.125 ± 0.003

For experimental conditions see legend to Table I.

peroxidation on the phagocyting cell activity is a problem of great interest for both immunology and general pathology. The results obtained to date are scanty and contradictory. One possibility is that the peroxidized lipids may "opsonize" initially inactive particles. The results presented in Fig. 4 are in agreement with this concept: oxidized liposomes increased CL response of leukocytes similarly as did additional amounts of opsonized zymosan routinely used as a phagocyte activator did. The biological consequences of this "opsonizing" effect of LPO may appear to be very important. In this way the phagocytes may more efficiently remove foreign cells partially damaged by activated oxygen species produced by the phagocytes. On the other hand, this effect may probably serve as a signal that a cell in the organism is "deadly ill' and should be removed. Further experiments to elucidate this probability are now in progress in our laboratories.

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