5. M. Bessis and R. I. Weed, Adv. Biol. Med. Phys., 14, 35 (1973).
6. M. Bessis, Corpuscles, New York (1974).
7. M. Bessis and N. Mochendas, Blood Cells, 1, 315 (1975).
8. I. A. Clarke and A. I. Salsbury, Nature, 215, 402 (1967).
9. R. P. Garay and P. C. Meyer, Lancet, 1, 349 (1979).
10. R. M. Sutherland, A. Rothstein, and R. I. Weed, J. Cell. Physiol., 69, 185 (1967).

ROLE OF ACTIVATION OF LIPID PEROXIDATION IN THE PATHOGENESIS OF EXPERIMENTAL PERITONITIS

S. G. Konyukhova, A. Yu. Dubikaitis,
L. V. Shabunevich, V. I. Strashnov,
and M. V. Belotserkovskii

UDC 616.381-002-092.9-07:
616.153.915-39

KEY WORDS: lipid peroxidation; peritonitis

The aim of this investigation was to study the role of lipid peroxidation (LPO) in the pathogenesis of development of destructive lesions in the abdomen, accompanied by peritonitis and endotoxicosis. This approach is justified by the fact that these pathological processes are based on damage to membrane structures, whose integrity and functional activity is determined by LPO activity [6].

EXPERIMENTAL METHOD

Experiments were carried out on 38 noninbred dogs weighing 20-25 kg, of which eight formed the control group, undergoing mock operations. The destructive lesion and peritonitis were induced by ligation of the vermiform appendix at its base [7]. Under hexobarbital anesthesia (8-10 mg/kg) a midline laparotomy was performed. During the subsequent days and until death of the animals, the clinical picture of the disease was kept under observation and the following biochemical tests were carried out after 6, 24, 48, and 72 h: 1) biochemical blood analysis (bilirubin, creatinine, urea, sugar, total protein and protein fractions, electrolytes K^+, Na^+, Ca^{++}, alanine-transaminase (ALT) and asparagine-transaminase (AST) activity; 2) determination of the steady-state level of LPO metabolites: diene conjugates (DC) [2], and malonic dialdehyde (MDA), by the reaction with 2-thiobarbituric acid (TBA) in our modification [0.8 ml H_2O and 1.0 ml of 0.6% TBA solution in glacial acetic acid were added to 0.2 ml of plasma; the mixture was boiled for 30 min and, after cooling, 1 ml of 5N KOH and 2 ml isopropanol were added; this was followed by centrifugation at 12,000 rpm for 20 min; the supernatant was subjected to spectrophotometry at 520 nm against a control containing water instead of plasma; the total antioxidant status of the blood plasma was estimated by measuring induced in vitro production of DC (DC_a) and MDA (MDA_a) after activation of blood plasma LPO by Fe^{++} ions (final concentration 0.54 mM) in the presence of $KMnO_4$ (final concentration 0.1 mM), a chaotropic agent]; 3) determination of ceruloplasmin (CP) activity [11], and of superoxide dismutase (SOD) activity [12] and of total blood plasma proteolytic activity (TPPA) [1]; 4) investigation of the level of medium mass molecules (MMM) in the blood plasma [5].

EXPERIMENTAL RESULTS

During the first 6-8 h after the operation the animals were in a state of postanesthetic sleep. After 24 h the general condition of animals of the main group was determined by the presence of the pathological focus in the abdominal cavity, reflected in the typical clinical picture of diffuse peritonitis. After 48 h, 11 animals of the main group had died and the rest were in a serious condition, some showing cerebral coma and decerebrate rigidity. After

Academician I. P. Pavlov First Leningrad Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR F. G. Uglov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 557-559, May, 1989. Original article submitted July 20, 1988.

 © 1989 Plenum Publishing Corporation

TABLE 1. Time Course of Changes in Parameters of LPO and PPA Activity and MMM Level during Development of Peritonitis in Dogs (M ± m)

Parameter	Initially	4–6 h	24 h	48 h	72 h
DC, μmoles/liter	50,9±2,6	56,4±2*	52±3	71,4±1,7**	74,8±2**
	48,3±3,0	51±2	50,1±3	49,3±2,6	47,6±1,9
MDA, μmoles/liter	10,37±0,6	12,65±0,5**	12,60±0,88*	12,63±1,6	15,24±1,9*
	10,5±0,5	11,1±0,5	10,4±0,9	11,6±0,8	11,4±1
DC_a, μmoles/liter	167±13	179±14	234±15**	263±37*	309±31**
	170±18	175±20	181±14	180±32	160±18
MDA_a, μmoles/liter	177±18	209±16	289±16**	378±40**	397±47**
	178±14	200±15	180±20	198±20	201±31
CP, mmoles TP/h/liter	5,42±0,34	6,31±0,17*	5,31±0,34	5,2±0,32	5±0,76
	5,5±0,3	5,72±0,24	5,9±0,28	5,9±0,3	5,6±0,28
SOD, conventional units/ml	15,2±1,3	18,1±3,4	14,2±2	8,1±2**	7,8±1,6**
	15,6±1,8	16,2±1,2	16±0,9	15,8±2	15,3±1,8
TPPA, μmoles arginine/min/100 ml	3,58±0,2	—	3,66±0,26	4,15±0,2*	4,64±0,26**
	3,4±0,18		3,42±0,28	3,32±0,17	3,38±0,21
MMM, conventional units	0,07±0,016	0,074±0,012	0,078±0,02	0,116±0,022*	0,081±0,018
	0,068±0,018	0,073±0,011	0,071±0,024	0,07±0,02	0,072±0,022

Legend. Numerator — parameters of experimental group, denominator — control. *$p \leq 0.05$, **$p \leq 0.01$.

72 h, five animals of the main group were still alive, but they all died during the next day. At autopsy, the peritoneal cavity contained a bloodstained purulent effusion and thrombohemorrhagic and necrobiotic changes were found in the tissues of the internal organs. The general state of the animals of the control group was satisfactory by the end of the 3rd day.

Staggered investigations of traditional biochemical parameters revealed no significant changes in the course of the disease, indicating that their informativeness in this pathological condition is low. The exception was the blood plasma transaminase (ALT and AST) activity, which increased by 1.5-2 times 24 h after the beginning of the disease.

Changes taking place under these conditions in the parameters of LPO and TPPA activity and the level of mean mass molecules (MMM) are shown in Table 1. Clearly 4-6 h after the beginning of the disease activation of LPO took place: the concentration of its most toxic product, namely MDA, in the blood was increased (by 22%). Regulation of activity of LPO processes is based on equilibrium between free-radical processes and the state of the antioxidant system (AOS). In the molecules studied, in the early stages of development of the disease, activation of LPO can be regarded more as the result of accumulation of an excess of acid radicals than as weakening of AOS. The causes of excess formation of oxygen radicals during the development of the pathological process can be taken to include hypoxia of part of the intestine, a marked increase in phagocytosis during inflammation, and hypercatecholaminemia, due to the development of the reactive phase of peritonitis [8]. The presence and combination of these three factors lead to activation of LPO processes as early as 4-6 h after ligation of the vermiform appendix, despite the response of AOS, in the form of increased CP and SOD activity (by 16.4 and 19.1% respectively). After 24 h the process of free-radical oxidation (FRO) of lipids increases in activity, and the system of general antioxidative defense is exhausted, as shown both by lowering of CP and SOD activity and by an increase in induced oxidizability of the lipids. Thus in the early stage a pathogenetic link leading to destabilization of the cell membranes and preparing for the next stage in the destructive process are formed in an early stage. The experimental data obtained agree with clinical observations made by Erokhin and co-workers [3], who found activation of LPO in patients with diffuse peritonitis.

Against this background of exhaustion of the factors of antioxidative defense the process of FRO in the affected tissues is cascade-like in character, which leads to the formation of large quantities of lipid peroxides in biomembranes, including in lysosomal membranes. Destruction of lysosomal membranes as a result of oxidative destruction of phospholipids leads to release of proteolytic enzymes [4], which intensifies hydrolytic processes in the tissues. This is confirmed by an increase both in protease activity and in the MMM concentration in the blood plasma (by 16 and 65% respectively) by 48 h. Accumulation of MDA, which can form

inter- and intramolecular cross-linkages with free amino groups of proteins in the plasma, thereby changing their conformation, can make the proteins more accessible for the action of proteases. The increase in TPAA and accumulation of MDA may be responsible for elevation of the MMM level observed after 48 h.

MMM are now considered to be markers of endotoxicosis, and many investigators [9] have demonstrated their toxicity in vivo. Meanwhile investigations by Tupikova [10] revealed the presence of middle mass oligopeptides with antioxidative activity. The possibility cannot be ruled out that a contribution to this activity may have been made by as yet unidentified low-molecular-weight regulatory peptides, constituting part of the MMM pool. An increase in the MMM concentration may therefore delay to a certain degree the process of hyperactivation of LPO. The fact that the MMM level falls before the animal dies may be the result of their further proteolytic degradation to amino acids, excluding MMM from the system of antioxidative defense. As a result, in the terminal stage of the disease because of critical exhaustion of the factors of AOS, maximal acceleration of LPO processes is observed with accumulation of DC and MDA and with weakening of resistance of the lipids to oxidation.

On the basis of these investigations it can accordingly be postulated that one of the trigger stages in the genesis of peritonitis is activation of LPO, facilitating destruction of lyososomes, and with the outflow of proteolytic structure of proteins, makes them more vulnerable for proteases, which degrade the protein molecules to oligopeptides, and at a certain stage of the disease these may play the role of regulators of LPO activity.

LITERATURE CITED

1. K. N. Veremeenko and L. M. Pogorelova, Lab. Delo, No. 5, 287 (1973).
2. V. B. Gavrilov and M. I. Mishkorudnaya, Lab. Delo, No, 3, 33 (1983).
3. I. A. Eryukhin, V. Ya. Belyi, M. D. Khanevich, et al., Vest. Khir., No. 10, 104 (1987).
4. Yu. P. Kozlov, Bioantioxidants [in Russian], Moscow (1975), pp. 5-14.
5. M. Ya. Malakhova, A. V. Salomennikov, N. A. Belyakov, and A. S. Vladyka, Lab. Delo, No. 3, 224 (1987).
6. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
7. V. A. Popov, Peritonitis [in Russian], Leningrad (1985).
8. K. S. Simonyan, Peritonitis [in Russian], Moscow (1971).
9. Z. A. Tupikova, Vopr. Med. Khimii, No. 1, 2 (1983).
10. Z. A. Tupikova, Vopr. Med. Khimii, No. 3, 108 (1983).
11. R. Fried, Biochimie, 57, 657 (1975).
12. H. Ravin, J. Lab. Clin. Med., 58, 161 (1961).