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EFFECT OF WATER-SOLUBLE ANTIOXIDANTS ON LYSOSOMAL MEMBRANE PERMEABILITY AND ON STRUCTURE OF THE LIVER IN RATS WITH THERMAL BURNS

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The view is held that destructive processes developing in various organs and tissues in pathological states and, in particular, in burns depend on the intensification of lipid peroxidation in these organs and tissues [5, 6]. This dependence is evidently due to the fact that lipid peroxides, formed in excess after burns [3, 11], possess membrane-toxic properties, which are particularly marked in relation to lysosomal membranes [1, 14]. Disturbance of their permeability leads to release of lysosomal hydrolases, stimulation of autolysis, and necrobiosis of the cells. The facts described above served as the basis for the use of inhibitors of peroxidation, i.e., antioxidants, as membrane protectors in various states characterized by intensification of catabolic and destructive processes [7], including in burns [5, 9]. Most membrane-stabilizing natural and synthetic antioxidants (α-tocopherol, ionol) are substances soluble in lipid base. Their effectiveness has been demonstrated experimentally, but it is quite evident that the clinical use of lipid-soluble preparations must be limited to external or peroral administration, and in that case the general membrane-protective action will be dependent on the character of absorption of the substance from the wound surface and mucous membrane of the gastrointestinal tract.

To obtain a preparation suitable for parenteral injection a number of water-soluble antioxidants have been synthesized. Their interaction with the hydrophobic structure of cell membranes has been very closely studied [2], but the action of water-soluble antioxidants in pathological states has been studied extremely inadequately.

The aim of the present investigation was to study water-soluble antioxidants: the water-soluble form of the widely used antioxidant ionol [7] and the antioxidant phenozan, as possible membrane-protectors in relation to lysosomal membranes of liver cells in thermal burns. Ionol in a water-soluble form was generously provided for the experiments by Professor E. G. Nifant'ev (Moscow City Pedagogic Institute), and phenozan by Professor V. V. Ershov (Institute of Chemical Physics, Academy of Sciences of the USSR), to whom the present writers are deeply grateful.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 150-200 g, kept on the ordinary animal house diet. Under superficial ether anesthesia a burn of the IIIB degree was inflicted on the rats (15-20% of the body surface). The investigation began 24 h after burning. De-

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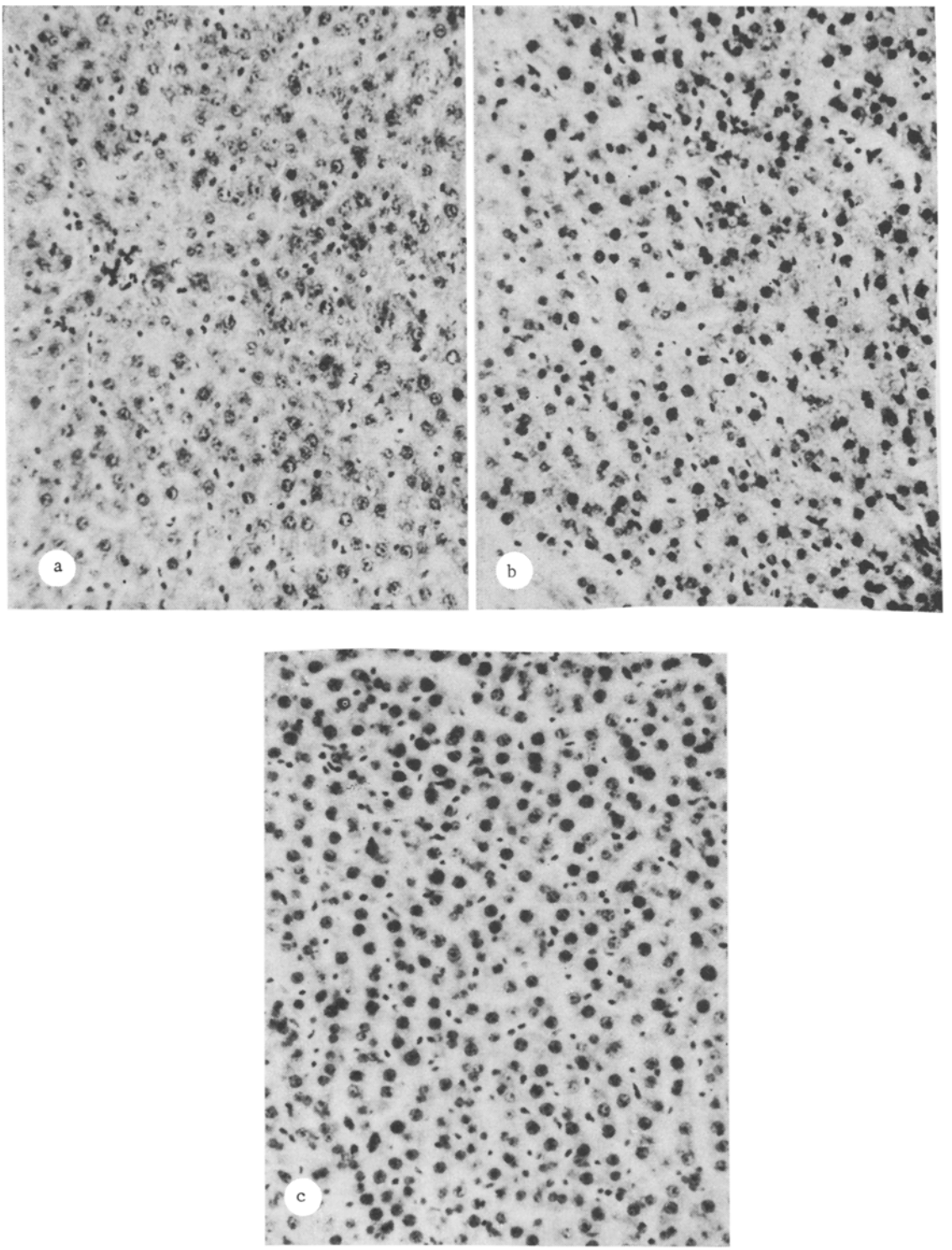

Fig. 1. Morphological picture of rat liver 24 h after burning without injection of antioxidants (a) and when ionol in the water-soluble form (b) and phenozan (c) were injected immediately after burning. a) Marked degenerative changes in liver cells, focal vacuolar degeneration; b) moderate edema of liver tissue, areas of vacuolar degeneration; c) moderate widening of Disse's spaces, no degenerative changes in liver cells, binuclear cells present. Hematoxylin-eosin, 160×.

TABLE 1. Nonsedimented Cathepsin D Activity in Liver of Burned Rats after Injection of Water-Soluble Antioxidants

Experimental conditions	Nonsedimented cathepsin D activity	
	in μg Tyr /mg protein	in % of total activity
Intact control rats (n=12)	5,7±0,5	13,2±1,2
1st day after burning (n=12)	15,0±1,4 (+163%)	36,0±5,9 (+172%)
P	<0,001	<0,001
1st day after burning injection of phenozan (n=10)	6,1±0,7 (—59%)	17,2±3,4 (—52%)
P_1	<0,001	<0,001
1st day after burning injection of ionol in water-soluble form (n=10)	6,6±1,5 (—56%)	16,8±2,4 (—55%)
P_2	<0,001	<0,001

Legend. Significance of differences calculated relative to control (P) and to burns (P_1 and P_2). Change in percent given in parentheses.

pending on the experimental conditions the rats were divided into four groups: 1) control intact rats; 2) burned rats; 3) burned rats receiving ionol in the water-soluble form by intraperitoneal injection in a dose of 75 mg/kg immediately after burning; 4) burned rats receiving phenozan by intraperitoneal injection in a dose of 40 mg/kg immediately after burning.

The dose of the antioxidants was calculated on the basis of their action on antioxidative activity of lipids determined previously, and relative to the antioxidative activity of the lipid-soluble antioxidant dibunol (ionol).

The rats were decapitated 24 h after burning and after injection of the antioxidants and the liver was homogenized at 0-4°C in a glass Potter's homogenizer with teflon pestle in 0.25 M sucrose solution (pH 7.4) containing 0.001 M EDTA for 90 sec at 1200 rpm. The final dilution of the homogenate was 1:19 (w/v). Stability of the lysosomal membranes of the hepatocytes was judged by the ratio of activity of cathepsin D, an enzyme of the lysosomal matrix, unsedimented under the conditions specified to the total activity of the enzyme in the liver homogenate. The liver homogenate was centrifuged initially at 1000g for 10 min to remove tissue fragments and nuclei. Total cathepsin D activity was determined in part of the supernatant after destruction of the lysosomes by Triton X-100 in a final concentration of 0.2%. Nonsedimented activity was determined in the supernatant after centrifugation at 26,000g [5, 12]. Activity of cathepsin D was determined by the method in [10] and expressed in μg tyrosine/mg protein. Histological investigations of liver sections accompanied each experiment.

EXPERIMENTAL RESULTS

Data on the action of water-soluble antioxidants on permeability of lysosomal membranes of the hepatocytes are given in Table 1.

It will be clear from Table 1 that 24 h after burning nonsedimented cathepsin D activity was considerably increased both in absolute values and as a percentage of total activity.

This is evidence of increased passage of the lysosomal matrix enzyme cathepsin D into the cytoplasm as a result of increased permeability of the lysosomal membrane, one result of which could be intensification of autolysis, which the present writers and others have observed in previous studies [4, 13]. Water-soluble antioxidants had a marked membrane-protective action. Both substances (phenozan and ionol in the water-soluble form) considerably reduced nonsedimented cathepsin D activity in absolute values and as a percentage of total activity. Stabilization of the lysosomal membrane thus took place, with a considerable decrease

in the passage of the lysosomal matrix enzyme into the cytosol. No significant differences were observed between the two antioxidants with regard to the parameters studied.

It can be postulated that there are fewer grounds for intracellular destruction of proteins and, possibly, of death of the cells after stabilization of the lysosomal membranes than in animals with burns and with disturbed integrity of the lysosomal membranes in the hepatocytes. Morphological investigations provided evidence in support of such conclusions.

Even in the early period after burning (24 h) substantial disturbances of the usual morphological picture could be observed in the liver (Fig. 1a): The liver cells were separated by edema and the Disse's spaces were moderately widened. Evidence of cloudy swelling degeneration could be seen in the hepatocytes, with necrobiotic changes in individual cells. In the liver of some animals focal degenerative changes succeeded in developing after 24 h, and they were particularly marked in the central areas of the hepatic lobules. Areas of necrosis consisting of fragments of hepatocytes and amorphous eosinophilic bodies, with extensive hemorrhages, could be seen in those regions. In the hepatocytes which still remained intact at the periphery of the lobules, marked granular vacuolar degeneration was present. Considerable deformation of the hepatocyte nuclei could be seen. In some parts of the liver tissue signs of fatty degeneration were present.

In most cases 24 h after burning and injection of ionol, in the water-soluble form, into the burned animals the architectonics of the liver tissue showed a mild degree of vacuolar degeneration. In a few cases islands of fatty infiltration could be seen (Fig. 1b).

Degenerative changes in liver tissue 24 h after burning and injection of phenozan into the animals were either very mild in degree of absent (Fig. 1c). The liver cells contained glycogen and they showed only very slight evidence of fatty degeneration.

Injection of water-soluble antioxidants into burned animals thus proved highly effective: It not only stabilized the lysosomal membrane, limiting release of lysosomal hydrolases into the cytoplasm and reducing the potential possibility of their action, but also evidently led to real inhibition of autolysis, which was reflected in preservation of the structure of the liver.

The mechanism of action of the water-soluble antioxidants phenozan and ionol in the water-soluble form is undoubtedly linked with their interaction with the lipid components of biological membranes. The solubility of these compounds in an aqueous medium must facilitate their more rapid transport to the cells of the organs and also, perhaps, their penetration into the cell. The effectiveness of water-soluble antioxidants in these experiments opens up prospects for their clinical trial and possible use in the combined treatment of burns.

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