Fe^{++}-DEPENDENT SERUM CHEMILUMINESCENCE DURING PROLONGED ASSISTED CIRCULATION

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Considerable attention has been paid to the problem of a prolonged assisted circulation (AC) during open heart operations [1, 2, 6]. It is said that reduction of the postoperative mortality depends not only on the correctly chosen conditions and technique of AC [9], but also on the use of soundly based methods correcting the disturbances of homoeostasis. Thus, according to certain data, administration of antioxidant before perfusion promotes a reduction of hemolysis [12]. Products of lipid peroxidation (LPO) are known to have a harmful action on membranes [3] and, in particular, on erythrocyte membranes, with the probability of their subsequent destruction under the influence of various physicochemical and mechanical factors. However, the state of LPO during AC has not hitherto been studied. Investigation of the intensity of free-radical processes may be of general theoretical and practical importance for the understanding of the molecular mechanisms of the damaging action of prolonged AC on the body.

The aim of this investigation was to study the time course of LPO in the blood serum during AC by the chemiluminescence (ChL) method in the presence of Fe^{++}.

EXPERIMENTAL METHOD

Experiments were carried out on 20 mongrel dogs of both sexes weighing 12-16 kg. A full AC was carried out with the aid of a foam-film counter-current oxygenator by the method described previously [14] for 3 h. Blood samples were taken from the femoral vein: after thoracotomy and before connection to the AC apparatus (ACA, initial level), at the beginning of AC, after 10, 20, 30, 60, 90, 120, 150, and 180 min of the perfusion period, and 10, 20, 60, and 120 min after disconnection of the apparatus. Serum was obtained from the blood by centrifugation for 15 min at 3600g.

Serum ChL was measured on a chemiluminometer by the method suggested previously [4]. The light sum of the slow flash (S_7), determined as the area beneath the ChL curve during the first 7 min of its development (in relative units), was recorded.

Before measurement of ChL and after initiation of LPO, samples were taken from the cuvette for determination of malonic dialdehyde (MDA) accumulation. The test solution, in a volume of 1 ml, was mixed with 1 ml of 30% TCA and centrifuged for 30 min at 6000g. To determine MDA, 2 ml of a 0.5% solution of 2-thiobarbituric acid was added to 1 ml of supernatant and the mixture was incubated for 45 min in a waterbath at 60°C. To convert optical density at 535 nm to MDA concentration, the molar coefficient of absorption of the colored product was taken to be 156,000 $cm^{-1} \cdot M^{-1}$ [13].

The level of hemolysis was determined spectrophotometrically from absorbance at 578 nm. The free hemoglobin concentration was determined by means of a calibration curve.

EXPERIMENTAL RESULTS

The light sum of ChL increased after the beginning of AC to reach a maximum at 10 min (Fig. 1). It then fell, to regain its initial level at about 60 min. The fall continued until 90 min, when the light sum was lower than initially. Toward the end of AC there was

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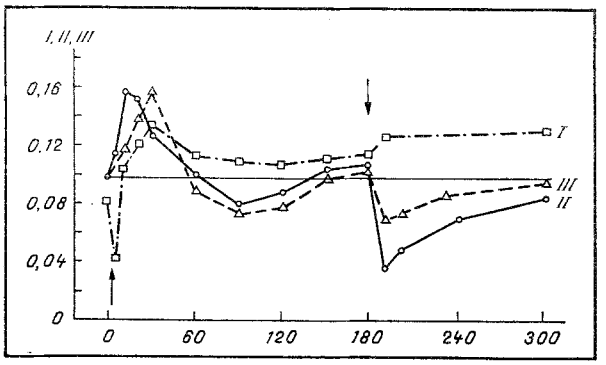

Fig. 1. Intensity of LPO in blood serum during AC. Abscissa, time (in min); ordinate: I) free hemoglobin concentration (in mM); II) amplitude of slow flash of ChL (in % of initial); III) increase in MDA concentration (in % of initial). Arrow pointing upward indicates beginning, arrow pointing downward indicates end of perfusion.

a small increase in the values of this parameter. After disconnection of the ACA a sharp fall in the intensity of ChL was observed, followed, however, by a rise. Thus the changes in ChL were in three phases.

During the first 30 min of AC the difference in MDA concentrations before and after addition of Fe^{++} was maximal (Fig. 1). This difference then decreased, and starting with the 60th minute it became smaller than initially.

During AC hemolysis increased (Fig. 1). Comparison of the trend of the serum ChL parameters and the free serum hemoglobin level showed that the changes in their values were almost parallel.

The first phase of the change in ChL during AC was connected with an increase in the ability of the serum lipoproteins to be oxidized. At the beginning of AC, the animals received blood with high pO_2, and this may have been the primary factor initiating LPO [3]. Indirect evidence in support of this view is given also by increased MDA accumulation and increased hemolysis. In the second stage, in response to the increased ability of the lipoproteins to undergo oxidation, compensatory mechanisms regulating peroxide homeostasis were evidently activated, so that ChL fell by the 30th-60th minutes of AC, and MDA accumulation decreased after the addition of Fe^{++}. We know, for example, that in hyperoxia 11-hydroxycorticosteroids are released into the blood stream [10] and, as has been shown [5], steroid hormones possess antioxidant properties. Very probably the antioxidative enzymes also were activated. Meanwhile there was a small decrease in the free serum hemoglobin concentration. In the third phase, during reaction of antioxidants with free radicals in LPO reactions, exhaustion of the protective antioxidative system evidently took place, so that it could no longer inhibit free-radical reactions to the required degree. Starting from the 90th minute of AC the intensity of ChL increased again, indicating increased ability of the serum lipoproteins to undergo oxidation.

There is no doubt that AC is accompanied by the development of hypoxia of the organs and tissues. The sharp decline in the intensity of ChL after disconnection of the ACA may be the result of mobilization of products of ischemia, which evidently possess antioxidative properties [7, 8, 11].

The results are thus evidence of the important role of LPO in the pathogenesis of injuries caused by AC, and they indicate that antioxidants may be used for the prevention of these injuries. The method of recording the serum ChL in the presence of Fe^{++} can be used for the rapid estimation of the state of peroxide homeostasis during the prolonged use of AC.

LITERATURE CITED

1. V. V. Antipov, Yu. M. Gal'perin, N. M. Ermakova, et al., Grud. Khir., No. 4, 108 (1960).
2. V. N. Bukharin and V. P. Podzolkov, Thoracic Surgery of Childhood [in Russian], Moscow (1975), pp. 342-450.
3. Yu. A. Vladimirov, and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).

4. Yu. A. Vladimirov, R. R. Farkhutdinov, and M. N. Molodenkov, Vopr. Med. Khim., No. 2, 216 (1976).
5. V. M. Gukasov and V. K. Fedorov, The Role of Changes in the Structure of Membranes in Cell Pathology [in Russian], Moscow (1977), pp. 8-52.
6. G. D. Ioseliani, S. M. Chilaya, V. M. Gurchiani, et al., Grud. Khir., No. 1, 15 (1979).
7. Yu. M. Lopukhin, Yu. A. Vladimirov, M. N. Molodenkov, et al., Vopr. Med. Khim., No. 1, 116 (1983).

PERMEABILITY OF BIOLOGICAL BARRIERS OF THE ENDOCRINE ORGANS FOR SERUM PROTEINS IN GUINEA PIGS

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Transport of macromolecules both into and out of the parenchyma of a gland is interesting in connection with regulation of the functions of these organs by the aid of peptide hormones, and also in connection with transport of the actual hormones produced by these glands. Penetration of proteins is also important for the development of pathological processes in organs of the endocrine system. However, the blood-tissue barriers in the endocrine glands have received little study.

The aim of this investigation was to study the permeability of barriers of the thyroid and parathyroid, thymus, and adrenal glands, the pancreas, the testes, and ovaries for autologous native and heterologous serum proteins.

EXPERIMENTAL METHOD

Experiments were carried out on healthy guinea pigs. Penetration of autologous serum proteins of the animals was studied in tissues of the endocrine glands with the aid of fluorescent antibodies by the standard method [2], using antisera against albumins, globulins, and whole serum proteins of guinea pigs with an antibody titer of up to 1:20,000 according to dilution of the antigen in Ouchterlony's test. Penetration of heterologous serum proteins was investigated with the aid of horse serum labeled with fluorescein isothiocyanate (FITC), 1 and 10 h after intravenous injection of a 3% solution of serum proteins in a dose of 1 ml/100 g body weight. After luminescence investigation, the sections were stained along with parallel sections with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Autologous and heterologous serum proteins penetrated through the vessels of the thyroid and parathyroid glands into the interstices and were detected in the fibers and cytoplasm of the connective tissue cells. Serum antigenic proteins were distributed in the parathyroid gland among the interstitial tissues between secretory cells, surrounding them on all sides. In the thyroid gland, autologous and heterologous serum proteins penetrated to make contact with the interstitial surface of the follicular epithelium. These proteins were detected in the cytoplasm of the follicular epithelium, particularly in the low epithelium, and also in the colloid of some follicles. The number of follicles into whose lumen autologous and heterologous serum proteins penetrated varied in different animals from a few to half of the total number. The arrangement of follicles containing and not containing serum proteins in a section through tissue of the thyroid gland was not uniform. In the thymus, autologous and heterologous serum proteins penetrated from the vessels into the interlobular connective tissue, and spread through it to make contact with the surface epitheliocytes of the cortical

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