VERY WEAK LUMINESCENCE OF BLOOD PLASMA IN EXPERIMENTAL EMBOLISM OF THE MAIN LIMB ARTERIES

V. A. Shestakov, N. P. Istomin, O. B. Nutsubidze, and M. P. Sherstnev

UDC 616.13-031.37/.38-005.7-092.9-07:612.117.5

Changes in the level of chemiluminescence of the blood plasma, initiated by hydrogen peroxide and bivalent iron, were studied in 15 dogs with experimental embolism of the bifurcation of the aorta. After occlusion of the artery the level of chemiluminescence initiated by iron fell. Meanwhile, the level of chemiluminescence initiated by hydrogen peroxide rose to reach a maximum 6 h after the beginning of occlusion, followed by a tendency to fall. A statistically significant increase in luminescence initiated by hydrogen peroxide can be used to judge the duration of the disease. After restoration of the blood flow to the limb there was a decrease in chemiluminescence initiated by hydrogen peroxide together with a further decrease in chemiluminescence initiated by iron.

KEY WORDS: chemiluminescence of blood; aortic embolism; blood flow to the limb.

Diagnostic methods in vascular surgery are constantly being improved and augmented. The state of patients can consequently be evaluated more completely and the tactics of treatment correctly chosen. An urgent problem in emergency vascular surgery is the diagnosis of acute occlusion of the trunk arteries [10]. There are as yet no sufficiently simple but accurate methods which can give a complete picture within a short time of the degree of ischemia, the duration of the disease, the collateral blood flow, and the extent of thrombosis accompanying embolism of the trunk arteries. Hence the importance of seeking express methods of diagnosis of acute disturbances of arterial patency.

The method of measuring chemiluminescence initiated by bivalent iron in blood plasma and serum, the intensity of which depends mainly on the concentration of β-lipoproteins [3], has been adequately developed [2] and is used for the diagnosis of various surgical diseases: acute appendicitis [12] and various forms of cholecystitis [9]. It has also been shown that during the development of experimental ischemia of organs the level of chemiluminescence initiated by bivalent iron in the blood serum falls, reflecting the severity of the condition of the experimental animals [8].

Addition of hydrogen peroxide to an aqueous solution of protein is accompanied by the development of a flash of chemiluminescence [6]. Chemiluminescence initiated by hydrogen peroxide has been used to study normal and cancer cells during the development of an experimental tumor of the thymus in mice [4]. This method evidently provides more complete over-all information on the state of proteins undergoing oxidation by products of free-radical chain oxidation of lipids.

The object of the present investigation was to study changes in chemiluminescence of blood plasma initiated by hydrogen peroxide and bivalent iron in experimental embolism of the main trunk arteries and after restoration of the circulation to the ischemized limb in order to discover the diagnostic potential of these modifications of the chemiluminescence method.

EXPERIMENTAL METHOD

Experiments were carried out on 15 mongrel dogs of both sexes weighing from 17 to 22 kg. The animals were given an injection of 1% morphine solution in a dose of 1 ml/kg body weight, after which the bifurcation

Professorial Surgical Clinic, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Savel'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 9, pp. 304-306, September, 1979. Original article submitted July 14, 1978.

0007-4888/79/8809-1011$07.50

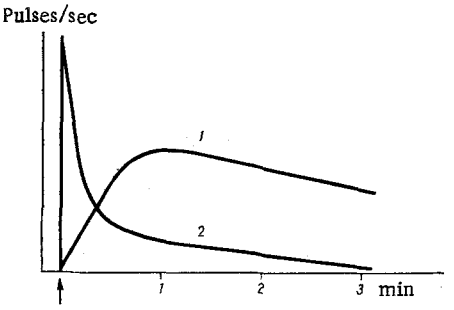

Fig. 1. Chemiluminescence of dog blood plasma. 1) Chemiluminescence initiated by hydrogen peroxide; 2) chemiluminescence initiated by bivalent iron. Arrow indicates time of addition of initiator. Abscissa, time (in min); ordinate, intensity of chemiluminescence (in pulses/sec).

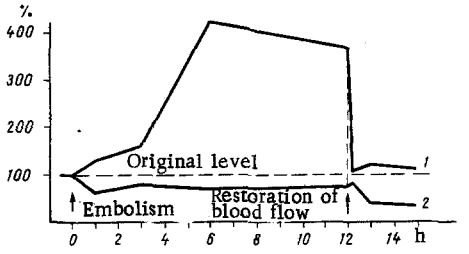

Fig. 2. Changes in chemiluminescence during embolism of main arteries of limbs. 1) Chemiluminescence initiated by hydrogen peroxide; 2) chemiluminescence initiated by bivalent iron. Abscissa, time (in h); ordinate, intensity of chemiluminescence (in % of original level).

of the aorta was artifically occluded by introducing and dilating a balloon catheter in the region of the bifurcation by the method described in [7]. Occlusion of the arteries was judged from disappearance of pulsation in the limb vessels.

Blood samples from the general circulation of the animals were taken before the experiment, and 15 min and 1, 3, 6, and 12 h after embolism. The balloon was removed 12 h after embolism and blood samples taken 15 min and 1 and 3 h after restoration of the circulation to the ischemized limbs.

To obtain plasma, 1 ml of 3.8% sodium citrate solution was added to 4 ml blood and the mixture was centrifuged for 10 min at 1500 rpm.

Chemiluminescence initiated by hydrogen peroxide or by bivalent iron was measured by a system incorporating an FÉU-35 photomultiplier. Blood plasma in a volume of 1 ml was poured into a cuvette kept in a dark chamber. The volume in the cuvette was made up to 4 ml by addition of 134 mM phosphate buffer, pH 7.4. The apparatus was switched on and 2 ml 15 mM hydrogen peroxide or 2 ml 15 mM bivalent iron solution ($FeSO_4 \cdot 7H_2O$) was introduced into the cuvette through tubes. In both cases a flash of chemiluminescence appeared, although it differed in shape (Fig. 1). Chemiluminescence was evaluated from the total light flux, determined as the area beneath the luminescence curve over a period of 5 min. The results were compared as percentages of the initial value of the chemiluminescence flash. One investigation took about 25 min.

EXPERIMENTAL RESULTS

Analysis of the results shows that the intensity of chemiluminescence initiated by bivalent iron fell statistically significantly during the development of ischemia relative to the initial level, in agreement with observations by other workers [1, 8]. In particular, 1 h after the development of occlusion the chemiluminescence was 59 ± 8.4% ($P < 0.05$) of its original level, after 3 h it was 86 ± 13.6% ($P > 0.05$), after 6 h 69 ± 8.6% ($P < 0.05$), and after 12 h 75 ± 12.3% ($P < 0.05$) (Fig. 2). Restoration of the circulation to the ischemized limb was accompanied by a further fall in chemiluminescence, which reached 41 ± 2.3% of its initial level 1 h after restoration of the blood flow ($P < 0.01$).

When the chemiluminescence of samples taken 1, 3, 6, and 12 h after embolism was compared, no statistically significant differences were found between them. From the results obtained by this method it is thus impossible to judge the time elapsing after the moment of acute occlusion of an artery.

Chemiluminescence initiated by iron is a sensitive test for recording quenching agents, for during embolism it always stays below the original level.

Analysis of the results of chemiluminescence initiated by hydrogen peroxide showed that luminescence increased during experimental embolism to 134 ± 33% 1 h after occlusion, and to 161 ± 60% ($P > 0.05$) of the original level after 3 h (Fig. 2). After 6 h the chemiluminescence was statistically significantly higher than the original level and reached its maximum at 422 ± 130% ($P < 0.05$). Later, while remaining at a high level, chemiluminescence gradually fell to 370 ± 131% toward 12 h ($P < 0.05$).

Elevation of the level of luminescence initiated by hydrogen peroxide can be explained as the result of a stress reaction to the acute disturbance of arterial patency. Zhuravlev and Zhuravleva [5] made a detailed examination of the mechanisms of the increase in intensity of spontaneous chemiluminescence as a result of stress. The same mechanisms evidently lie at the basis of the increased intensity of chemiluminescence initiated by hydrogen peroxide.

At the beginning of development of the stress reaction catecholamines are released and this is accompanied by liberation of lipids into the systemic blood flow (although this does not happen at once), but not until a short time after discharge of the catecholamines. The rise in the lipid concentration evidently explains the gradual increase in the intensity of luminescence toward 6 h after the beginning of embolism. In this context it is interesting to note a tendency for chemiluminescence initiated by iron to increase toward 3 h after the beginning of embolism.

Because of the increase in the level of chemiluminescence initiated by hydrogen peroxide toward 6 h after the beginning of embolism, this method can be used as an additional diagnostic method for determining the duration of the disease.

Restoration of the blood flow to the limb leads to a sharp fall in luminescence initiated by hydrogen peroxide, so that 15 min after removal of the balloon it was 107 ± 27%. The level of chemiluminescence 3 h after restoration of the circulation was 117%, not statistically significantly different from the level of luminescence before the development of embolism.

The fall in chemiluminescence initiated by hydrogen peroxide after restoration of the blood flow, the decrease in luminescence initiated by iron throughout the period of experimental embolism, and the even greater decrease after restoration of the blood flow, took place on account of the liberation of low-molecular-weight compounds which, as investigations by Malyugin et al. [8] have shown, have a quenching effect, into the general blood stream through the cell membrane damaged as a result of ischemia. It may be that the quenchers are kinins, whose molecular weight is about 1000. Kinins are formed during tissue hypoxia, and after restoration of the blood flow in the ischemized limb they are discharged in large quantities into the general blood flow [11]; the decrease in chemiluminescence is evidently connected with this phenomenon. Both the tendency for the level of chemiluminescence initiated by hydrogen peroxide to fall from 422 to 370% and the rapid fall in luminescence initiated by iron are evidently due to the liberation of kinins into the blood flow on account of the small but constantly functioning collateral blood flow.

The authors are grateful to Professor Yu. A. Vladimirov for technical help.

LITERATURE CITED

1. N. A. Belyakov, R. R. Farkhutdinov, and N. I. Teterev, Éksp. Khir., No. 6, 79 (1975).
2. Yu. A. Vladimirov, V. I. Olenev, and T. B. Suslova, in: Very Weak Luminescence of Blood Plasma in Clinical Diagnosis [in Russian], Moscow (1974), pp. 6-33.

3. Yu. A. Vladimirov, R. R. Farkhutdinov, and M. M. Molodenkov, Vopr. Med. Khim., No. 2, 216 (1976).
4. A. K. Govorun, V. I. Levin, A. I. Svirnovskii, et al., Biofizika, No. 1, 100 (1974).
5. A. I. Zhuravlev and A. L. Zhuravleva, Very Weak Luminescence of Blood Serum and Its Role in Combined Diagnosis [in Russian], Moscow (1975).
6. A. E. Zakaryan and Yu. V. Babok, in: Very Weak Luminescence in Medicine and Agriculture [in Russian], Moscow (1974), pp. 83-88.
7. I. I. Zatevakhin, V. M. Koshkin, N. P. Istomin, et al., Éksp. Khir., No. 3, 16 (1976).
8. É. F. Malyugin, Yu. A. Vladimirov, N. A. Belyakov, et al., in: Experimental Basis of the Treatment of Hepatic Failure [in Russian], Moscow (1975), pp. 19-24.
9. M. N. Molodenkov and V. N. Shilov, in: Very Weak Luminescence of Blood Plasma in Clinical Diagnosis [in Russian], Moscow (1974), pp. 57-62.
10. V. S. Savel'ev, Yu. S. Petrosyan, L. S. Zingerman, et al., Angiographic Diagnosis of Diseases of the Aorta and Its Branches [in Russian], Moscow (1975).
11. L. D. Chirkova, "The kallikrein–kinin system of blood plasma in patients with acute arterial occlusion of the main limb vessels," Candidate's Dissertation, Moscow (1975).
12. A. P. Sharov and I. M. Artyukhov, in: Very Weak Luminescence of Blood Plasma in Clinical Diagnosis [in Russian], Moscow (1974), pp. 55-56.