Comparative analysis of oxidative metabolism alterations in blood at acute cerebral hemorrhagic insult in clinic and experiment

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Summary

The present work was aimed to study oxidative metabolism alterations in blood at acute cerebral hemorrhagic insult in clinic and experiment. Total of 52 patients suffering from acute cerebral hemorrhagic insult (with the age range 31-76 years. 32- man and 20 - woman), and 48 laboratory rats (with body weight range 250- 300 g) have been investigated. Electronic paramagnetic centers of the blood of investigated patients and laboratory rats were studied with the use of electronic paramagnetic resonance (EPR) method. EPR signals were measured on the Radiospectrometer РЭ - 1307 (Russia). Acute hemorrhagic insult of the brain in rats was induced with the use of experimental noninvasive photochemical method. Obtained data were analyzed by Student's t criterion. According to the results obtained after investigations has been concluded that at acute cerebral dishemia both, in the blood of patients and experimental rats develops pro-oxidants (Mn^{2+} and Fe^{2+} ions) and antioxidants (ceruloplasmin, Fe^{3+}-transferrin) ratio disbalance, increases synthesis of nitric oxide and formation of nitrosil complexes of heme iron (HbNO) and nitrosil complexes of nonheme iron (FeSNO).

Has been suggested that identity of alterations of oxidative metabolism in blood at acute cerebral hemorrhagic insult detected in clinical trials and experimental rats indicates that revealed phenomenon represents general biologic reaction, and one of the main circles in the pathogenesis of hemocirculatory alterations in the brain.

Key words: Hemorrhagic insult, oxidative metabolism, EPR method.

Introduction

Nowadays, among cerebro-vascular diseases acute cerebral hemorrhagic insult has been considered as very topical. That's why, interest to study, and clarify mechanisms of pathogenesis of the mentioned disease increases worldwide.

The present work was aimed to investigate oxidative metabolism alterations in blood at acute cerebral hemorrhagic insult in clinic and experiment, and provide comparative analysis of data.

Material and methods:

Neurology department at Tbilisi State Medical University and I. Beritashvili Institute of physiology supplied us with clinical materials for investigation.

Experiments have been carried out on 52 patients suffering from acute cerebral hemorrhagic insult. They applied clinic within 2 to 24 hours after onset of cerebral insult. Patients age range was from 31 to 76 years (32 – man, and 20 woman).

Investigations also involved 48 laboratory white rats (with body weight range 250- 300 g). Experimental animals were subdivided into two major groups: the control- (12 rats), and experimental (36 rats) groups.

Acute hemorrhagic insult of the brain in rats was induced using experimental noninvasive photochemical method.

Laboratory white rats were anesthetized with chlorhydrate narcosis (1ml of 4% solution per 100g body weight), during 2-3 minutes at 37^0 C. Photosensitive dye - Bengale rose solution (0,13 ml of 0,75% solution per 100 g body weight) was administered in animals femoral vein. Thereafter rats were kept in stereotactic equipment followed by baring of the cranium. Duration of exposure to illumination was 0,5, 1 and 1,5 hours.

From the halogen lamp source (243, 250 w), owing to optical ray conductive fiber (with diameter 2,5 mm), the final capacity for lighting (upon the cranium) was 0,64 w/cm^2.

Has been studied area of the brain's hemorrhagic insult directly exposed to illumination and adjacent area as well, in which pathological process was extended with diminishing intensity.

Electronic paramagnetic centers of the blood of investigated patients and laboratory rats were studied with the use of electronic paramagnetic resonance (EPR) method at TSMU central scientific laboratory. Free nitric oxide (NO) content in the blood was determined using diethyldithiocarbamate trap. Diethyldithiocarbamate was added to blood with the dose of 0,2 ml/1ml. EPR specters of cerebral cortex tissue were registered using the Radiospectrometer РЭ - 1307 (Russia). Materials for investigation were placed in polyethylene tubes and kept in liquid nitrogen at -196°C temperature.

Results and discussion

Alterations of electronic paramagnetic centers of the blood of investigated patients suffering from acute cerebral hemorrhagic insult are presented on table N1.

Studied have shown that at cerebral dishemia intensity of EPR signal of oxidized ceruloplasmin increases extremely (by 45% compared to control group data), while intensity of EPR signal of Fe^{3+}

-transferrin – decreases and amounts to about 74% of control data. At the same time intense signals of Met-Hb, Fe^{2+}, and Mn^{2+} ions display, increases intensity of EPR signal of nitric oxide (NO) by 12% compared to control data, the intense signal of nitrosil complexes of heme iron (HbNO) and low intensity signals of nitrosil complexes of nonheme iron (FeSNO) appear.

The above-mentioned alterations of oxidative metabolism have potential for further initiation of free radical oxidation processes triggering exaggerated lipid peroxidation leading to cell- and tissue destruction respectively.

Alterations of electronic paramagnetic centers of the blood of rats at experimental acute cerebral hemorrhagic insult are presented on table N2.

As we can see, after 0,5 hour exposure to illumination, intensity of EPR signal of oxidized ceruloplasmin increases by 26%. Intensity of EPR signal progresses in parallel with increase in duration of exposure to illumination, and after 1 hour exposure - amounts to 150% of control data indicating elevation of oxygen reactive forms in the blood.

At the same time of observation intensity of EPR signal of Fe^{3+} -transferrin decreases by 13% compared to control data, and correlates with appearance of Fe^{2+} ions at the end of observation (after 1,5 hours exposure to illumination).

Intensity of EPR signal of NO increases by 28% after 1 hour exposure to illumination, and than decreases due to formation of nitrosil complexes of heme iron (HbNO) and nitrosil complexes of nonheme iron (FeSNO).

Conclusion

Thus, according to the results of our study can be concluded that:

1. At acute cerebral dishemia both, in the blood of patients and experimental rats develops pro-oxidants (Mn^{2+} and Fe^{2+} ions) and antioxidants (ceruloplasmin, Fe^{3+}-transferrin) ratio disbalance.
2. At acute cerebral hemorrhagic insult increases synthesis of nitric oxide and formation of nitrosil complexes of heme iron (HbNO) and nitrosil complexes of nonheme iron (FeSNO).
3. Identity of alterations of oxidative metabolism in blood at acute cerebral hemorrhagic insult detected in clinical trials and experimental rats indicates that revealed phenomenon represents general biologic reaction, and one of the main circles in the pathogenesis of hemocirculatory alterations in the brain.

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სისხლში ჟანგვითი მეტაბოლიზმის ცვლილებების შედარებითი ანალიზი მწვავე ცერებრული ჰემორაგიული ინსულტის დროს კლინიკასა და ექსპერიმენტში

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Table N1. Changes of paramagnetic centers intensity (1mm/mg) of the blood in patients at acute cerebral hemorrhagic insult

	N	NO	HbNO	FeSNO	Cerulo-	Fe^{3+}-	Met-Hb g=6,0	Mn^{2+}	Fe^{2+}

		g=2,01	g=2,01	g=2,03	plasmin g=2,056	transferrin g=4,2		g=2,14	g=2,25
Control 1	20	14,0±1,02	-	-	20,0±1,0	30,0±1,5	-	-	-
Hemor-rhagic insult 2	52	16,97±1,05 $P_{1.2}$<0,05	10,06±2,0	1,7±0,5	29,06±1,6 $P_{1.2}$<0,001	22,2±1,2 $P_{1.2}$<0,01	9,87±1,6	15,26±0,9	20,1±3,8

Table N2. Changes of paramagnetic centers intensity (1mm/mg) of the blood in rats at experimental acute cerebral hemorrhagic insult.

	N	NO g=2,01	HbNO g=2,01	FeSNO g=2,03	Cerulo-plasmin g=2,056	Fe^{3+}-transferrin g=4,2	Met-Hb g=6,0	Mn^{2+} g=2,14	Fe^{2+} g=2,25
Control 1	6	12,0±0,5	-	-	12,0±0,8	19,0±0,5	-	-	-
0,5 hour 2	6	12,2±2,2 $P_{1.2}$<0,1	-	-	15,2±1,0 $P_{1.2}$<0,05	16,7±0,9 $P_{1.2}$<0,01	9,3±0,4	10,0±0,4	-
1 hour 3	6	25,3±0,6 $P_{1.2}$<0,02	-	-	18,0±1,0 $P_{1.3}$<0,02	15,7±0,5 $P_{1.3}$<0,02	11,0±0,6	10,3±0,4	-
1,5 hour 4	6	10,3±0,4 $P_{1.2}$<0,05	7,5±0,5	6,0±0,5	19,6±0,5 $P_{1.4}$<0,01	14,9±1,5 $P_{1.4}$<0,05	11,2±2,3	10,6±0,5	50,0±2,4