CHEMICALLY MODIFIED HEMOGLOBIN AS AN ARTIFICIAL OXYGEN CARRIER IN DOGS WITH EXPERIMENTAL HEMORRHAGIC SHOCK

E. A. Selivanov, N. I. Kochetygov, K. A. Gerbut, M. A. Azhigirova, E. P. Vyazova, A. M. Zeinalov, I. M. Bystrova, and E. I. Molokovskaya

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Solutions of chemically modified hemoglobin (PH-Pp) differ in the molecular-weight distribution and functional characteristics of the hemoglobin. The side effects of PH-Pp solutions are associated, in particular, with the high-molecular-weight fraction in its composition [2]. It has been shown to be best to use PH-Pp compounds which do not contain fractions above 300,000 daltons.

The aim of this investigation was to study the gas-transport properties of a PH-Pp solution, with none of these high-molecular-weight fractions in its composition, on a model of hemorrhagic shock in dogs.

EXPERIMENTAL METHOD

PH-Pp was obtained with the aid of glutaraldehyde as cross-linking agent and pyridoxal-5-phosphate as regulator of affinity for oxygen [1]. Its degree of polymerization was 20-25% and its molecular weight did not exceed 140,000 daltons. The compound had a narrow molecular-weight distribution. An investigation in vitro of the affinity of PH-Pp for oxygen showed that P_{50} of the polyhemoglobins was 26-29 mm Hg. This is identical with the affinity of hemoglobin of freshly prepared donated blood. Values of Hill's coefficient, namely 2.0-2.1, confirmed that structural changes in the protein in the region of the environment of heme as a result of chemical modification were not significant (Hill's coefficient for native hemoglobin is 2.6-2.8). Thus the mechanisms of cooperativeness of the reversible oxygenation process were undisturbed in this PH-Pp. It was used as the basis to prepare a 7.5% blood-substituting solution.

The hemodynamic and gas-transport properties of this solution were studied on a model of hemorrhagic shock. The cardiac output (CO, $ml \cdot min^{-1} \cdot kg^{-1}$) was recorded in the dogs by the thermodilution method, and the arterial blood pressure (BP, in mm Hg) and the electrocardiogram were recorded on a "Mingograf-7" polygraph (Siemens-Elema). The heart rate (HR) was determined from the ECG. The stroke volume (SV, $ml \cdot kg^{-1}$) and total peripheral resistance ($TPR \cdot 10^4$, $dyn \cdot sec \cdot cm^{-5}$) were calculated. A catheter was introduced through the left femoral vein in the right atrium, from which a sample of mixed venous blood was obtained. Samples of arterial blood were taken as bleeding carried out from the left femoral artery. The hemoglobin concentration (Hb, $g \cdot dl^{-1}$), partial pressure of oxygen (pO_2, mm Hg), and the degree of oxygenation (HbO_2, %) in arterial (a) and venous (v) blood and plasma, were determined on the ABL-3 instrument (Radiometer). The oxygen capacity (CaO_2, CvO_2, $ml \cdot dl^{-1}$), arteriovenous difference in oxygen concentration in the blood (CaO_2—CvO_2, $ml \cdot dl^{-1}$), systemic oxygen transport (QO_2, $ml \cdot min^{-1} \cdot kg^{-1}$), and oxygen consumption (VO_2, $ml \cdot min^{-1} \cdot kg^{-1}$ were calculated. Massive blood loss (45-50 $ml \cdot kg^{-1}$) was carried out fractionally. Hypotension was maintained for 60 min. There were two series of experiments on 12 mongrel dogs. In series I the lost blood was replaced by

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TABLE 1. Systemic Hemodynamics during Hemorrhagic Shock and Its Treatment by Infusion of Polyglucin (Series I) and PH-Pp Solution (Series II)

Parameter	Series	Initial	Before treatment	After treatment			
				10 min	1 h	2 h	24 h
BP	I	171±5	26±3	143±5	134±5	119±4	120±7
	II	175±8	33±5	179±8	188±7	150±8	127±13
SV	I	1,1±0,1	0,2±0,04	2,2±0,5	1,1±0,2	0,7±0,1	0,7±0,1
	II	1,2±0,1	0,2±0,05	1,9±0,1	1,6±0,1	1,2±0,2	0,9±0,1
CO	I	192±19	38±8	424±60	202±21	141±15	149±19
	II	209±41	40±4	315±28	181±12	130±5	115±5
HR	I	162±6	179±17	194±17	200±25	192±23	206±16
	II	128±12	174±13	167±17	114±13	120±17	149±14
TPR	I	7,5±0,9	5,9±0,8	2,8±0,3	5,3±0,3	6,9±0,8	6,7±0,7
	II	8,8±0,6	6,7±1,1	5,1±0,6	7,6±0,8	9,2±0,8	8,8±0,9

Legend. Here and in Table 2, * indicates significance of differences compared with series I $p < 0.05$.

TABLE 2. Oxygen Balance during Hemorrhagic Shock and Its Treatment by Infusions of Polyglucin (Series I) and a Solution of Modified Hemoglobin (Series II)

Parameter	Series	Initial	Before treatment	After treatment			
				10 min	1 h	2 h	24 h
Hb_a	B I	19,8±1,0	16,0±0,9	6,5±0,6	7,8±0,6	8,5±0,5	7,6±0,9
	B II	21,3±0,9	17,8±0,9	10,2±0,5	12,0±0,9	12,4±0,6	10,9±0,4
	P II			3,8±0,2	4,3±0,2	4,3±0,1	2,1±0,1
	E II			6,4±1,0	7,6±1,1	8,1±1,0	8,8±1,1
CaO_2	B I	26,6±1,3	21,4±1,2	8,6±0,7	10,4±0,9	11,3±0,6	10,4±1,3
	B II	28,6±1,3	23,9±1,2	13,7±0,6	16,1±0,9	16,6±0,9	14,5±0,7*
	P II			5,1±0,3	5,8±0,3	5,7±0,2	2,5±0,2
	E II			8,6±0,5	10,2±0,6	10,9±0,6	11,7±0,7
Q_{O_2}	B I	51,5±6,2	8,0±1,5	36,3±5,9	21,1±2,9	16,2±2,5	17,1±2,4
	B II	47,7±4,3	10,1±2,1	42,3±3,4	26,0±2,7	19,1±1,3	15,9±1,2
	P II			12,9±1,3	7,8±1,6	5,2±0,5	2,4±0,1
	E II			29,2±2,5	18,7±1,9	14,2±1,0	14,0±1,2
CaO_2	B I	5,1±0,3	18,1±1,3	2,6±0,3	4,7±0,7	6,2±0,4	5,4±1,3
	B II	6,9±1,0	21,4±1,4	4,1±0,3	7,8±0,8	9,4±1,3*	9,2±0,2*
CaO_2-CvO_2	P II			1,1±0,2	3,3±0,3	3,2±0,2	1,4±0,1
	E II			3,0±0,4	4,5±0,9	6,1±0,9	7,8±0,2
V_{O_2}	B I	9,7±0,6	7,2±1,1	11,7±1,3	9,3±0,4	8,7±0,9	8,3±2,1
	B II	11,7±1,7	8,8±1,7	12,8±2,0	12,4±1,3	11,3±1,0	10,5±0,6
	P II			2,8±0,3	4,4±0,7	3,4±0,4	1,2±0,2
	E II			10,2±2,4	8,3±0,9	7,9±0,8	9,2±0,6
$HbVO_2$	B I	69,9±7,0	10,4±1,3	65,4±3,8	50,5±5,6	43,5±6,2	41,6±4,5
	B II	74,1±2,4	13,6±2,7	67,5±3,4	48,3±4,8	42,6±5,1	36,8±4,3
	P II			73,9±2,5	49,4±6,2	45,5±5,4	52,6±2,8
P_{VO_2}	B I	47,5±1,7	22,3±5,6	48,7±3,3	36,4±4,6	29,6±2,6	29,5±1,6
	B II	46,1±2,7	17,7±2,5	46,6±1,4	36,6±5,4	28,7±1,9	25,1±1,2
	P II			52,4±4,4	32,9±2,7	29,8±2,0	32,3±2,5

Legend. B) blood, P) plasma, E) erythrocytes.

injection of polyglucin, in series II by the 7.5% PH-Pp solution. Parameters of the central hemodynamics and oxygen balance of the body were recorded before bleeding (initial data) after 60 min of hypotension, and again 10, 60, and 120 min and 24 h after transfusion of the blood substitutes. The numerical results were subjected to statistical analysis by a nonparametric method.

EXPERIMENTAL RESULTS

In the experiments of series I (control) transfusion of polyglucin into animals in a state of hemorrhagic shock led, to judge from the hematocrit, to the development of marked hemodilution, connected with an increase in the plasma volume of the blood, arterial blood pressure, CO, and SV, which were close to the original values or even a little higher

during 1 h after infusion. After 2 and 24 h they were significantly lower. TPR immediately after infusion was depressed, but later it rose to its original level (Table 1).

The oxygen capacity of the blood fell on account of hemodilution, but because of the increase in CO, QO_2 increased, although it remained 1.5-2 times lower than initially. The arteriovenous difference in oxygen saturation and concentration in the blood was reduced. VO_2 fell after blood loss, and as a result of infusion it rose and did not differ statistically significantly from the initial level. Reduction of the oxygen deficiency was indicated also by a rise of pVO_2, from 22.3 ± 5.6 to 48.7 ± 3.3 mm Hg (Table 2).

In series II hemodilution also developed after transfusion of the PH-Pp solution. BP at all times of observation was higher, but CO somewhat lower (but not statistically significantly so) than in series I. HR also was lower. SV 10 min after infusion was the same as in series I, but after 1 and 2 h it was actually higher. After 24 h no difference could be found in the value of SV between the two series. It will be clear from Table 1 that a higher BP was maintained in the experiments of series II by a higher TPR than in series I.

During infusion of the polyhemoglobin solution, although CaO_2 did not regain its initial values, it was nevertheless about 50% greater than after infusion of polyglucin (Table 2). Its increase took place entirely on account of the plasma hemoglobin, i.e., of the PH-Pp introduced into the blood stream. The value of QO_2 was correspondingly higher and, what is most important, the increase in the quantity of oxygen extracted by the tissues from unit volume of blood in the experiments of series II took place on account of oxygen transported by the injected PH-Pp. The fraction of this oxygen was particularly great 1 and 2 h after infusion. It was due to extraction of this oxygen from the blood that an increase took place in the net consumption of oxygen by the animal at the times mentioned. This was achieved because deoxygenation of the PH-Pp of the plasma was almost the same as that of the hemoglobin in the erythrocytes (Table 2).

However, the partial pressure of oxygen in mixed venous blood after transfusion of the hemoglobin solution was no higher than in series I. The reason may perhaps be that in the series with injection of PH-Pp solution the animals' body temperature was 1.0-1.5°C higher than in series I and, consequently, the oxygen consumption was higher. This increase in the body temperature of the animals was evidently connected with the pyrogenic properties of the hemoglobin solution.

Thus the blood substitute solution based on chemically modified hemoglobin is able to restore the normal systemic circulation, to increase the oxygen capacity of the blood, and to provide an additional quantity of oxygen for the tissues per unit volume of the blood, as a result of the prolonged circulation of hemoglobin in the plasma and its deoxygenation.

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