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EXPERIMENTAL EVALUATION OF MODIFIED POLYHEMOGLOBIN ON A MODEL OF LIMITING HEMODILUTION

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KEY WORDS: blood substitute; artificial oxygen carrier; hemoglobin; hemodilution; hemodynamics; oxygen supply

Definite progress has been made in the creation of an artificial oxygen carrier (AOC) on the basis of hemoglobin (Hb). Solutions of modified polymerized Hb, freed from stroma, have high oxygen capacity and can remain for quite a long time in the blood stream [5, 7, 8, 11]. How effectively an AOC can carry out the gas-transporting function of blood is currently under discussion in the scientific press [4, 9, 12]. The fullest answer to this question, in our opinion, can be given by the use of an experimental model of limiting hemodilution. In such a case, only 5-10% of the experimental animal's own blood remains in the blood stream, and the remaining volume is made up of the test preparation, so that the oxygen supply to the body is virtually entirely dependent on the properties of the OAC.

The aim of this investigation was to evaluate the efficacy of modified polyhemoglobin, proposed as an AOC, on a model of limiting hemodilution in dogs.

EXPERIMENTAL METHOD

Experiments were carried out on 14 mongrel dogs, male and female, weighing 11.3 ± 1.9 kg and anesthetized with pentobarbital sodium in a dose of 30 mg/kg. A blood substitute based on a modified Hb polymer, containing pyridoxal-5'-phosphate (PH-PP) as regulator of reversible oxygenation, was used as the AOC (the preparation was developed at the All-Union Research Center for Hematology and its physicochemical properties have been described previously [1]). The limiting hemodilution model was obtained by replacement transfusion with a 10% solution of PH-PP (8 dogs) or rheopolyglucin (6 dogs — control). Salts were added to both preparations before use in accordance with the electrolyte formula of Ringer—Locke solution [2]. Blood was removed from the femoral artery and the blood substitute was injected simultaneously into the femoral vein at the rate of 1.4-2.0 ml/kg/min until the hematocrit (Ht) index fell to 5% or below. The following parameters of the systemic hemodynamics were determined in all animals: blood pressure (BP), cardiac output (CO), and circulating blood volume (CBV), by the dye (cardiogreen) dilution method on a "Cardiac Output Computer" (USA). Other parameters studied included Ht, the blood Ht concentration, and the partial pressure of oxygen (pO_2) separately in plasma from arterial and mixed venous blood, by the methemoglobin-cyanide method on an AVL gas microanalyzer (Switzerland). The oxygen concentration in the blood and plasma was calculated from the data thus obtained by the equation

$$O_2 \text{ concentration} = 1.34Hb[pO_2/p^{50}]^n/1 + [pO_2/p^{50}]^n,$$

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TABLE 1. Basic Parameters of Hemodynamics during Hemodilution with 10% PH-PP (experiment) and Rheopolyglucin (control) Solution (M ± m)

Stage of observation	BP, mm Hg		CO, ml/kg·min		CBV, ml/kg	
	experiment	control	experiment	control	experiment	control
Initial	140±6,4	156±3,5	157±11,3	180±16,9	67±3,3	72±3,4
Hemodilution:						
To Ht 15 %	148±7,7	147±4,5	144±19,5	373±41,6**	79±5,5	113±8,6
To Ht 10 %	136±14,3	143±7,2	182±53,6	334±18,7***	91±7,8	137±18,2
To Ht 7 %	150±13,7	125±7,5**	242±38,9	310±7,7***	128±13,9***	150±15,2***
To Ht 5 %	136±8,9	91±13,4	229±40,4	216±31,8	134±12,8***	163±19,1***
10 min after hemodilution	139±7,7	Died	212±36,6	Died	142±14,2***	Died
1 h after	136±6,6		174±21,2		120±11,7***	
2 h after	133±8,8		152±9,0		100±16,0	
3 h after	126±9,3		133±6,6		92±4,7**	
4 h after	121±9,1		117±4,9		91±5,3*	

Legend. Here and in Table 2: *p < 0.05, **p < 0.01, ***p < 0.001 (values differing significantly from initial).

TABLE 2. Total O_2 Consumption by Animal and Percent O_2 Extraction from Arterial Blood during Hemodilution with 10% Solution of PH-PP (experiment) and Rheopolyglucin (control) (M ± m)

Stage of observation	O_2 consumption, ml/kg·min		Per cent of total O_2 consumption due to PH-PP	Per cent O_2 extraction	
	experiment	control		experiment	control
Initial	5,7±1,23	3,7±0,44	—	12,3±1,95	11,7±1,11
Hemodilution:					
To Ht 15 %	2,4±0,63*	3,8±0,74	45	11,8±4,41	21,7±3,55*
To Ht 10 %	2,1±0,28*	5,0±1,14	58	14,3±1,15	20,4±4,07
To Ht 7 %	3,0±0,32	5,8±1,25	68	32,3±4,28**	28,5±4,96*
To Ht 5 %	2,4±0,37*	5,3±0,88	77	39,9±2,01***	32,6±4,87**
10 min after hemodilution	Died	6,5±1,99*	80	Died	34,0±4,07***
1 h after		6,2±1,19	83		41,0±6,05***
2 h after		5,9±0,83	82		45,6±6,92***
3 h after		4,8±0,88	79		45,3±7,41***
4 h after		4,8±0,71	75		49,8±8,20***

where Hb denotes the Hb concentration (in per cent), and p^{50} and n are coefficients characterizing the ability of Hb and its derivatives to bind and give up oxygen [3]; the total oxygen consumption was determined as the product of CO and the difference between the oxygen concentrations in arterial and venous blood; oxygen extraction from arterial blood was determined by the equation:

$$\text{per cent extraction } O_2 = 100 \times (\text{conc. } O_2 \text{ art.} - \text{conc. } O_2 \text{ ven})/\text{conc. } O_2 \text{ art.}$$

The parameters studied were measured in the initial state, during exchange transfusion with the blood substitute at stages corresponding to Ht values of 15, 10, 7, and 5%, and again 10 min and 1, 2, 3, and 4 h after the end of exchange transfusion. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Exchange transfusion of PH-PP solution or rheopolyglucin occupied on average 1.5-2.0 h and amounted to 191 ± 18 ml/kg body weight in both experimental and control groups.

BP in animals of the experimental group did not transgress physiologically normal limits at any time during the experiment. In animals of the control group BP did not begin to fall until Ht had reached 5% (Table 1).

During exchange transfusion with rheopolyglucin, CO increased more than during exchange with PH-PP solution. In the control group, for instance, when Ht had fallen only to 15% CO was twice as high as initially, and in the animals of the experimental group the maximal value of CO was 1.5 times higher than initially when Ht was 7% (Table 1).

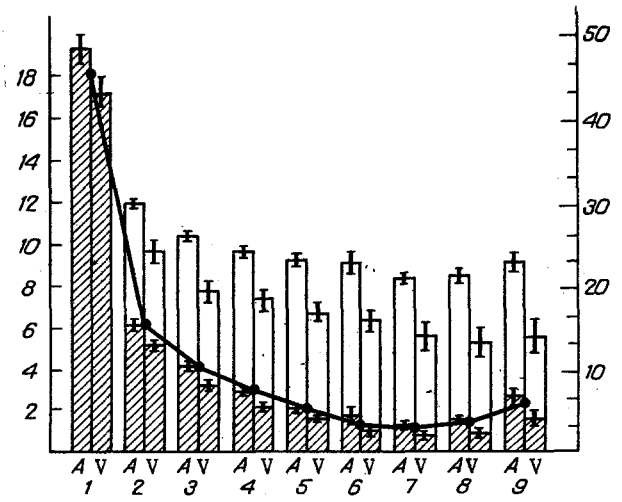

Fig. 1. O_2 concentration in arterial (A) and mixed venous (V) blood during hemodilution with 10% PH-PP solution. Ordinate, O_2 concentration (in vols. %) and Ht (in %); abscissa, stage of observation: 1) initial; 2, 3, 4, 5) during replacement of blood by 10% PH-PP solution to Ht values of 15, 10, 7, and 5% respectively; 6, 7, 8, 9) 10 min and 1, 2, and 4 h respectively after end of blood replacement by 10% PH-PP solution; shaded part of columns represents amount of O_2 bound with Hb of erythrocytes; unshaded part — amount of O_2 bound with Hb of PH-PP present in blood plasma. Curve shows value of Ht during experiment.

A more marked increase in the blood flow rate in animals of the control group compared with the experimental can be explained by the known reduction in blood viscosity taking place in response to injection of rheopolyglucin [6, 10]. This increase in CO may also be compensatory in response to the progressive decline in the oxygen capacity of the blood during hemodilution by the plasma expander.

During gradual dilution of the blood with rheopolyglucin, the percentage oxygen extraction from arterial blood also increased; and by the end of exchange transfusion it was 3.5 times higher than initially (Table 2).

However, despite the considerable increase in CO and in the percentage oxygen extraction, a decrease in the total oxygen supply of the body was observed in animals of the control group (Table 2). During hemodilution this value amounted to 45-55% of the initial level, and further replacement of blood by rheopolyglucin to Ht = 5% or below, led to a critical reduction of the oxygen capacity of the blood and to the development of acute anemic hypoxia, culminating in death of 100% of the animals.

Replacement of blood by 10% PH-PP solution caused the oxygen concentration to fall in both arterial and venous blood by half compared with the initial values, and it was maintained at this level throughout the experiment (Fig. 1). Despite this, however, the animal's oxygen consumption did not decrease with deepening of the hemodilution. A smaller proportion of the oxygen consumed by the body was transported in this case by erythrocytes which remained in the blood stream, and a considerably greater part (75-80%) by Hb present in the blood plasma in the composition of PH-PP (Table 2).

After the end of exchange transfusion with PH-PP, the total oxygen consumption remained unchange for 4 h of observation. This was possible because the increased percentage of oxygen extraction from arterial blood and the hemodynamic parameters of the circulation were maintained at a sufficiently physiological level. For instance, fluctuations of CO were not significant throughout the experiment except during the last stage 4 h after the end of exchange transfusion. CBV, which rose progressively during hemodilution, returned to its normal values gradually during the next 4 h (Table 1).

Thus all the animals of the control group died after total replacement of blood by rheopolyglucin, whereas all the animals of the experimental group survived during 4 h of observation after total replacement of blood by a 10% solution of PH-PP. This result can be explained on the grounds that the AOC under investigation can maintain the oxygen supply of the body at an adequate level, and at the same time it possesses a marked hemodynamic action.

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MICRODIALYSIS STUDY OF EFFECTS OF ATYPICAL NEUROLEPTICS AND ANXIOLYTICS ON STRIATAL DOPAMINE RELEASE AND METABOLISM IN CONSCIOUS RATS

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Cerebral microdialysis in conscious animals is a method of investigating dynamic changes in neurotransmitter release and metabolism in vivo [9]. The method is based on stereotactic implantation of a microdialysis catheter into brain tissue, followed by perfusion and analysis of the dialysate. The concentration of neurotransmitters and their metabolites in the dialysate is determined by their concentration in the extracellular space and it reflects activity of the concrete neurotransmitter system [11]. The basic feature of the method of brain microdialysis which distinguishes it from in vitro methods is that neurochemical processes are studied in the whole brain, with its regulatory mechanisms preserved.

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