RUSSIAN ACADEMY OF SCIENCES

"PERFTORAN" SCIENTIFIC-PRODUCTION COMPANY

PERFTORAN

BLOOD SUBSTITUTE WITH GAS-TRANSPORTING FUNCTION

Professor Felix F. Beloyartsev
(1941–1985)
He was a scientific leader of
the topic from 1979 to 1985

THE CHRONOLOGY OF "ARTIFICIAL BLOOD" BASED ON PERFLUOROCARBON EMULSION IN RUSSIA

The basic directions of the scientific investigations on creating the Perfluorocarbon blood substitute – PERFTORAN – were developed in 1979 and divided into the following stages:

1979 Study of composition:
– method of obtaining, control methods, source of raw materials;

1981 Pre-clinical study:
– pharmacodynamics, parmacokinetics, specific activity, acute and chronic toxicity, carcinogenic, mutagenic, teratogenic and immunotrophic effects, etc.;

1984 Pharmacological Committee:
– consideration of pre-clinical study results in order to receive a permit to clinical trials;

1984 Clinical investigations:
study of effectiveness, tolerance, safety, specific activity;

1986 Preparation of the normative documents:
– instructions, Provisional Pharmacopoeia Article, Experimental Industrial Regulations;

1994 Pharmacological Committee:
– consideration of three phases of clinical trials, receiving a permit to clinical use;

1994 Pharmacological Committee:
– consideration and approval of instructions, Provisional Pharmacopoeia Article, Experimental Industrial Regulations;

1995 Pharmacopoeia Committee:
– consideration and approval of instructions, Provisional Pharmacopoeia Article, Experimental Industrial Regulations;

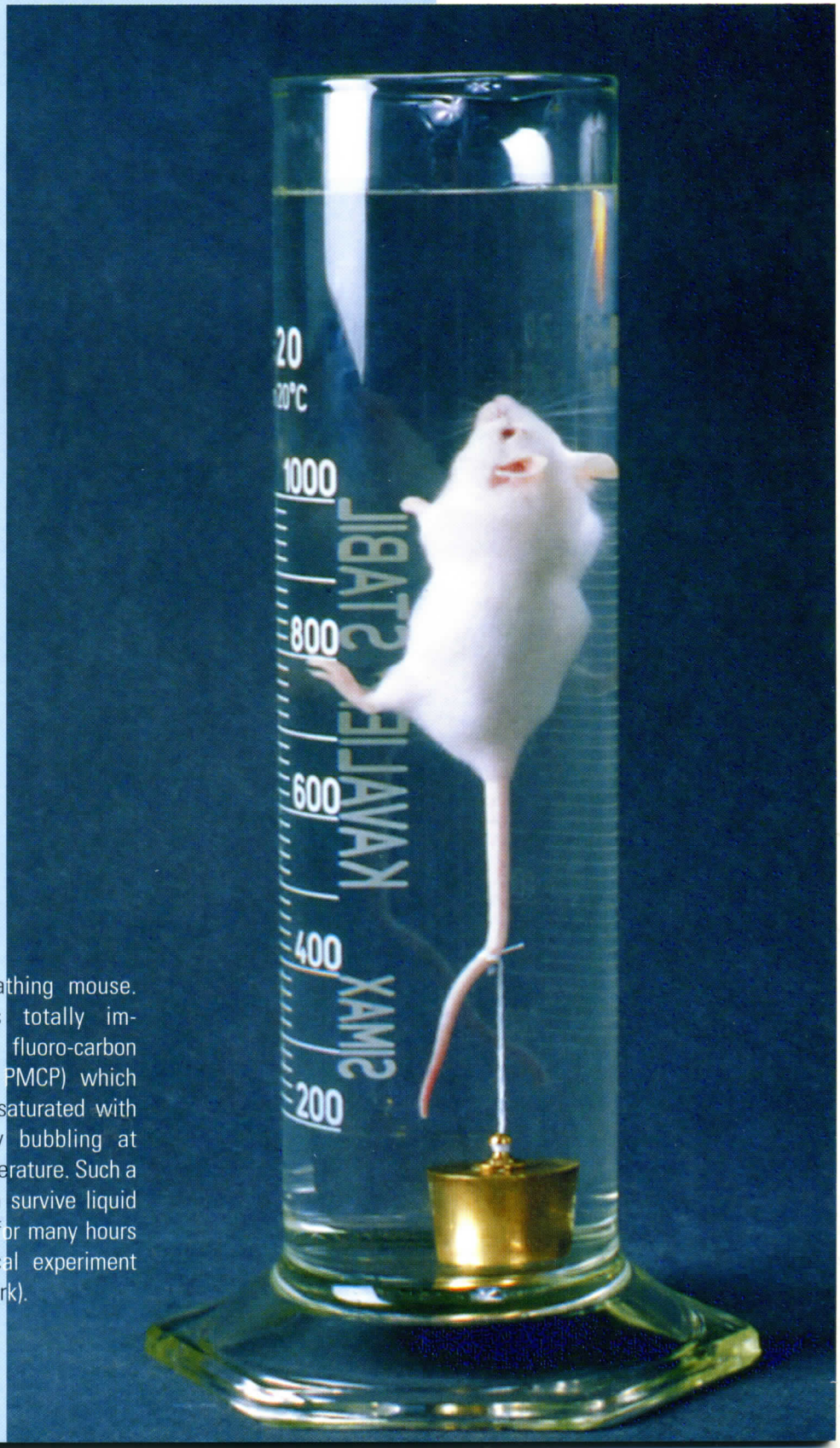
1996 State control inspection:
– physicochemical and biological control;

1996 Ministry of Public Health:
– the order to clinical use and Registration Certificate for medical use and industrial output;

1997 Ministry of Public Health:
– Licence for sale and industrial output.

Prof. Genrikh Ivanitsky, D.Sc.
Associat-member RAS
Institute of Theoretical
and Experimental
Biophysics of Russian
Academy of Sciences,
President of the
"Perftoran" Comp.

Serguei Vorobyev, D.Sc.
Institute of Theoretical
and Experimental
Biophysics of Russian
Academy of Sciences,
Vice-president of the
"Perftoran" Comp.



Liquid-breathing mouse. Mouse is totally immersed in fluoro-carbon (PFD and PMCP) which has been saturated with oxygen by bubbling at room temperature. Such a mouse can survive liquid breathing for many hours (by classical experiment of L. C. Clark).

Main Information

Drug characteristics

Perftoran is 10 vol. % compound emulsion of submicronic fluoroorganic particles.
Perftoran composition consists of ten reagents.
Perftoran is a gas-carrier.
Perftoran improves gas exchange and metabolism in tissues;
Perftoran increases O_2-carrying capacity of blood;
Perftoran stabilizes the membranes;
Perftoran improves blood flow and peripheral microcirculation;
Perftoran restores the central hemodynamics;
Perftoran apparently protects myocardium;
Perftoran acts as a diuretic;
Perftoran is a calcium slow channel blocking agent.

Qualitative and quantitative composition

White emulsion with slightly blue tint.

Reagents:

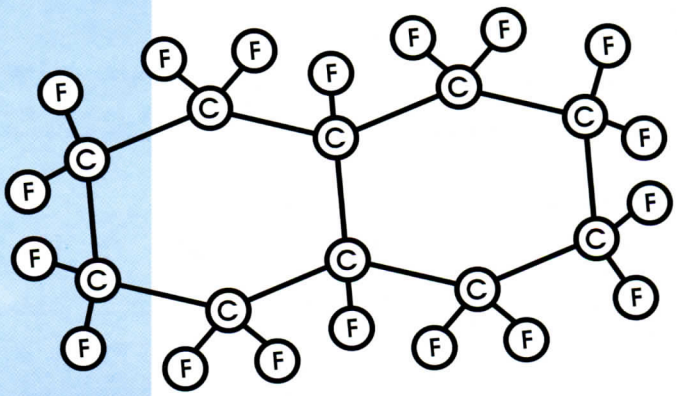
$C_{10}F_{18}$ – perfluorodecalin (Mw=462 D)	13 g
$C_{12}F_{23}N$ – perfluoromethylcyclohexylpiperidin (Mw=595 D)	6.5 g
Proxanol – 268 (Mw ≈ 8000 D)	4.0 g
NaCl – sodium chloride	0.6 g
KCl – potassium chloride	0.039 g
$MgCl_2$ – magnesium chloride	0.019 g
$NaHCO_3$ – sodium hydrocarbonate	0.065 g
NaH_2PO_4 – sodium hydrophosphate	0.02 g
$C_6H_{12}O_6$ – glucose	0.2 g
H_2O – water for injection	100 ml

Physical properties:

F^- – content of free fluorine ions	$<10^{-5}$ M
Average particle size	0.03 ÷ 0.15 μm
Osmolality	280 ÷ 340 mOsm
Viscosity	2.5 sP
pH	7.2 ÷ 7.8
vol. % O_2 – O_2 solubility (pO_2=760 mm Hg., t=20°C)	~ 7.0 vol. %
vol. % CO_2 – CO_2 solubility (pCO_2=760 mm Hg., t=20°C)	~ 60 vol. %

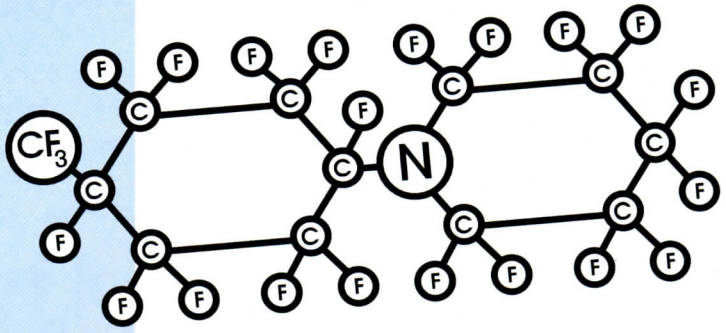
Chemical and structural characteristics of Perftoran's main compounds:

Perfluorodecalin (PFD) $C_{10}F_{18}$



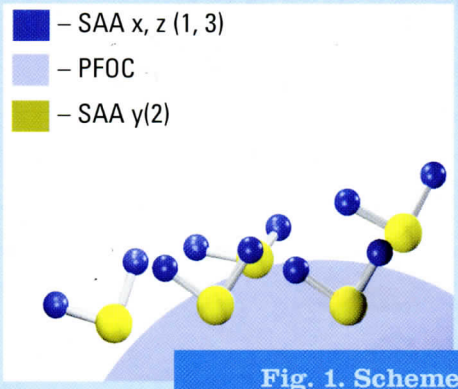
Molecular weight 462 D
Trans-isomer – 50-55%
Cis-isomer – 45-50%
Main ingredient – 93%
(perfluoride admixture 7%)

Perfluoromethylcyclohexylpiperidin (PFMCP) $C_{12}F_{23}N$



Molecular weight 595 D.
It contains 3 isomers:
$C_{12}F_{23}N$ Mw = 595 D 62-71%
$C_{12}F_{23}N$ Mw = 595 D 20-39%
$C_{12}F_{23}N$ Mw = 633 D 4-9%

Main ingredient – 97%
(perfluoride admixtures 0.5-3%)



Proxanol is a surface-active agent (SAA)

It consists of two compounds: oxyethylene and oxypropylene:
$HO-(C_2H_4O)x-(C_3H_6O)y-(C_2H_4O)z-H$
y(2) – number of oxypropylene chains, x, z (1,3) – number of oxyethylene chains.

Fig. 1. Scheme of Perfotran's particle.

Indications for application

Perftoran is recommended to be used as a plasma substitute with O_2 – and CO_2 – carrying function.

It is used in case of:

acute and chronic hypovolemia (traumatic, hemorrhagic shock from burns and septic shock, cranial trauma, during or after operations);

alteration of microcirculation and peripheral blood flow (insufficient perfusion of tissues, septic shock, infections, fat embolism);

anti-ischemic protection of transplants;

heart operations (in heart-lung apparatus);

local use (local perfusion, bronchopulmonary lavage).

Contraindications

Perftoran is contraindicated in patients with hemophilia and allergic diseases. It should not be used during pregnancy.

Adverse effects

Sometimes the injection of a test-dose can cause allergic reactions, such as: hyperaemia, tachicardia, decrease in blood pressure, hypertermia, headache, chest pain and lumbal pain, difficulty in breathing, neutropenia, and anaphylactic reactions. The adverse reactions occur rarely and they disappear in 10-15 minutes without special treatment.

When adverse reactions occur, the infusion of Perftoran should be stopped and corticosteroids should be used immediately.

Drug interactions

Perftoran can be administered together with albumin, saline solutions, glucose, and antibiotics.

In contrast to colloid solutions saline solutions do not alter the biological, physical and chemical features of Perftoran.

Colloid solutions cause a significant increase in particle size and change the biological and chemical characteristics of Perftoran. Therefore, colloid infusions should be used after administration of Perftoran or through other peripheral venous access.

Dosage and administration

Perftoran is a broad-spectrum emulsion. The dose depends on the severity and character of disease.

Perfluorocarbon emulsion is administered as bolus or continuos infusion. Before use it is recommended to provide a biological test after the first 30 drops. The infusion should be started slowly.

Treatment of acute and chronic hypovolemia.

The dosage of Perftoran is 5 to 30 ml/kg. It is administered intravenous as short or continuous infusion. To achieve best results the patient should receive adequate oxygenation with 40% to 60% inspired O_2 during 24 hours after the infusion of Perftoran.

Treatment of microcirculation and metabolism disorders.

The dosage of Perftoran is 4 to 8 ml/kg (max. single dose is 30 ml/kg). It is used as continuous infusion with an interval between administrations of 1-4 days. The total dose is 100 ml/kg. To achieve best results the patient should receive adequate oxygenation with 40% to 60% inspired O_2 during 24 hours after the infusion of Perftoran.

Anti-ischemic protection of transplants.
Perftoran is administered to donor and recipient at a dose of 20 ml/kg as short or continuous infusion 2 hours before operation.
Heart operations.
Perftoran is used as the main solution for artificial blood flow at a dose of 10 to 40 ml/kg.
Regional usage.
Perftoran is used as a solution for standard oxygenation apparatus for regional perfusion at a dose of 40 ml/kg.
Local usage.
(Bronchopulmonal lavage, etc.).
The administration of Perftoran is similar to administration of traditional treatment.

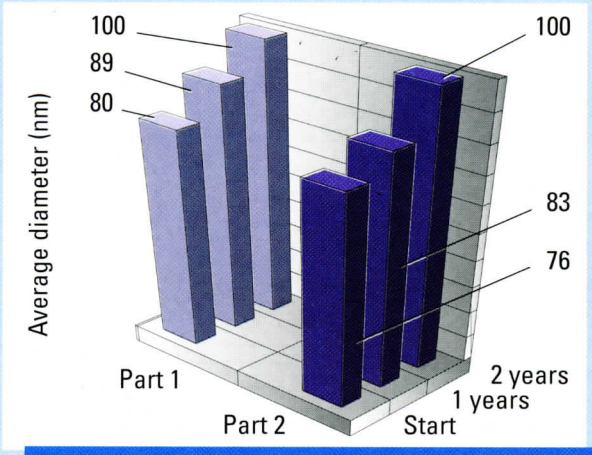


Fig. 2.0. Change of fluorocarbon emulsion's average diametre (nm) after storage at -18°C for 2 years.

Precautions

Follow the storage and thawing instructions. Proper storage and thawing avoid an increase in particle size and appearance of sediment in the emulsion (free perfluorocarbon phase). If sediment precipitates emulsion should not be used.

Perftoran should not be mixed in one syringe or system with the dextranes – polyglukin, reopolyglukin or oxyethylstarch with molecular weight < 100000. Use this solutions after the administration of Perftoran or through other peripheral venous access.

Shelf life and storage

Perftoran should be stored at the temperature, -5°C to -18°C.

The shelf life is 2 years (Fig. 2.0). After thawing the emulsion can be stored at the temperature, +4°C for 2 weeks (Fig. 2.1).
Inappropriate storage or thawing leads to an increase in particle size. Maximal average size should be 0,15 μm (or 150 nm).

Perftoran is recommended to be thawed at room temperature. Perftoran may be thawed and refrozen 5 times. After thawing the emulsion should be thoroughly shaked.
The emulsion should not be used in case of:
stratification of emulsion (even after shaking);
opacity of emulsion (milky colour);
sediment in emulsion (transparent oily drops on the vial wall).
The perfluorocarbon emulsion should not be stored at temperatures below –18°C or thawed at temperatures above +30°C.

Package

Glass vials, containing 100, 200, and 400 ml of perfluorocarbon emulsion.

Company and address

Russia, SPC "Perftoran", production.
142292 Moscow region, Pushchino,
ITEB RAS, "artificial blood" building.
FAX/TEL.: (0967) 79-0546.

Russia, SPC "Perftoran",
office in Moscow.
113035, Moscow, 34 "B",
Sophiyskaya nab., off. 419.
FAX: (095) 953-4500
TEL.: (095) 953-5468, 234-4906.

http://www.perftoran.ru
E-mail: perftoran@perftoran.ru

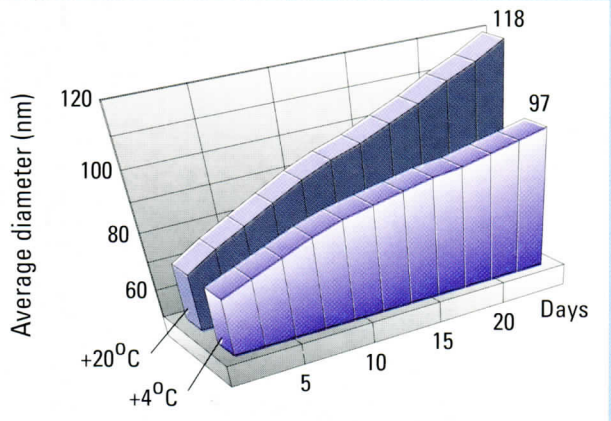


Fig. 2.1. Change of fluorocarbon emulsion's average diameter (nm) after storage at 4°C and at 20°C for 1 month.

Pharmacology (Efficacy)

Gas-carrying capacity

Perfluorocarbon emulsions are used because of their gas-carrying capacity. In perfluorocarbon emulsion physical solubility of gases is observed in contrast to chemical solubility of gases in hemoglobin molecules. In hemoglobin oxygen is bound to iron and the consumption curve has a S-form.

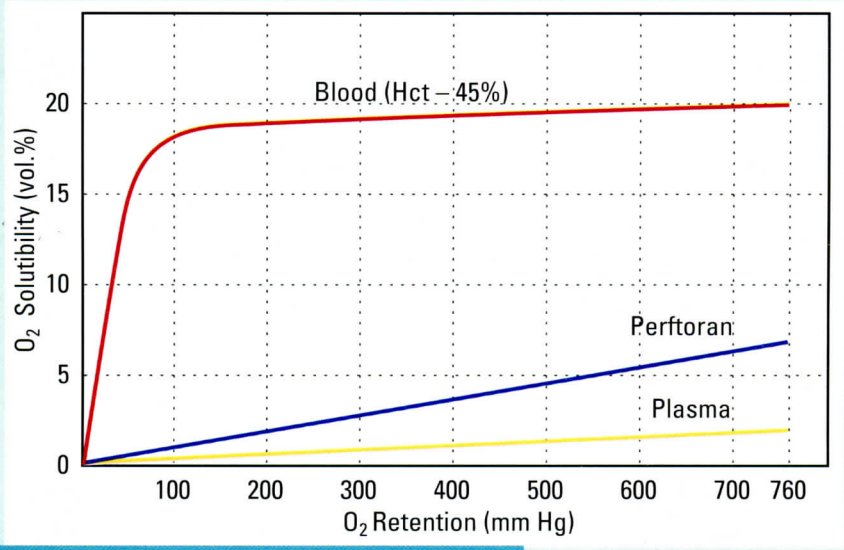


Fig.3. O_2 concentration in different fluids.

Plasma – 2,3 vol. %
Perftoran – 7 vol. %
Blood – 20 vol. %

In contrast, in perfluorocarbon emulsion a linear increase of O_2 solubility according to Henry's law is observed. (Fig.3). The O_2 content in arterial blood is about 20 vol. % . After O_2 is dissolved in tissues its content in venous blood decreases up to 15 vol. % Consequently, the O_2-carrying capacity of blood is used incompletely. The 15 vol. % of oxygen becomes a reserve.

The removal of O_2 from perfluorocarbon emulsions to tissues reaches more than 90%. Comparing the solubility of gases in plasma, which carries 2.3 vol. % of oxygen, with solubility in PFOC* shows that the same values of O_2 retention in perfluorocarbon emulsions allow to dissolve about 40 vol. % of oxygen. (Fig. 4). The gas-carrying capacity of Perftoran is possible due to perfluorodecalin and perfluoromethylcyclohexylpiperidin contained in the proportion 2 to 1, respectively. It is important that perfluorocarbon remains dispersed and it is rapidly excreted from the body.

*PFOC – perfluoroorganic compounds

Table 1. Some values of gas-exchange and oxygen solubility during the infusion of Perftoran at the dose of 10 ml/kg.

Compounds	O_2 solubility (%)	Grog's constanta of diffusion for O_2	Oxygenation time (μs)	Respiratory surface area (m^2)
Erythrocytes	98,21	–	200-250	3500
Plasma	1,29	$5,3x10^{-5}$	–	–
PFOC	0,50*	$4,4x10^{-4}$**	14-26***	45000****

* increase in physically dissolved O_2 by 1/3
** increase in O_2 transportation due to increased diffusion
*** increase in saturation rate of O_2
**** increase in O_2 transportation due to larger respiratory surface area

The respiratory function of blood is based on the correlation between the diffusion rate and gas exchange rate. When pO_2 decreases the only way to maintain the total diffusion rate of O_2 is to increase the surface area of diffusion. The research data show that the respiratory surface area of lungs is 120 m^2 and the respiratory surface area of erythrocytes is about 3-4 thousands m^2.

The surface area of capillaries of the whole body is 10-15 thousands m^2. Decreased pO_2 leads to an increase in the surface area of diffusion.

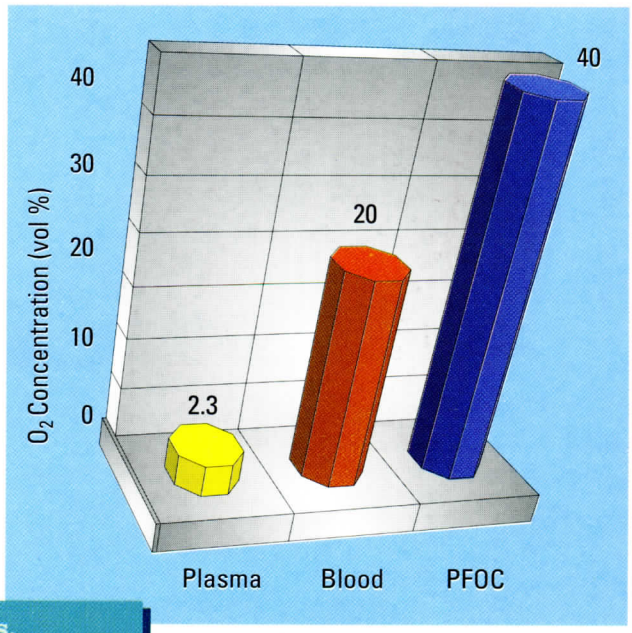


Fig.4. O_2 concentration in different fluids.

The total respiratory surface area of the perfluorocarbon emulsion's particles at the dose of 10 ml/kg is 45000 m^2, which is approximately 10-times higher than the respiratory surface area of erythrocytes at the same pO_2 – 100 mm Hg (Table 1).

Thus, the total gas exchange rate is significantly higher in perfluorocarbon emulsion than in erythrocytes due to the larger respiratory surface area.

The submicronic particles of perfluorocarbon emulsion is another factor that plays a great role in O_2 delivery. The small particle size (the average particle size is 0.07 μm that is 100 -times smaller than the size of erythrocyte) allows to deliver O_2 to every part of the organism, even to the hypertrophied tissues (Fig. 5).

The small particle size provides adequate O_2 supply to tissues with poor blood flow. The relationship between the emulsion's particle size and the size of capillaries provides laminar blood flow and low vessel resistance. Proxanol (a stabiliser in the emulsion) avoids the aggregation of blood cells.

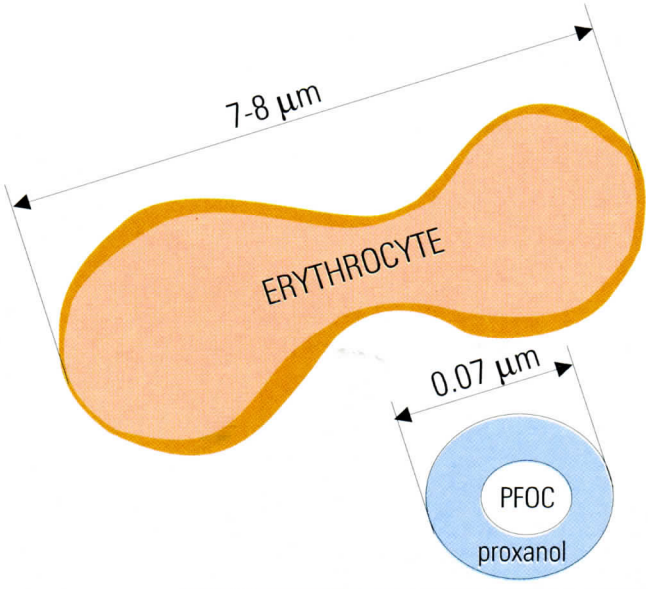


Fig.5. Average particle size and erythrocyte's size.

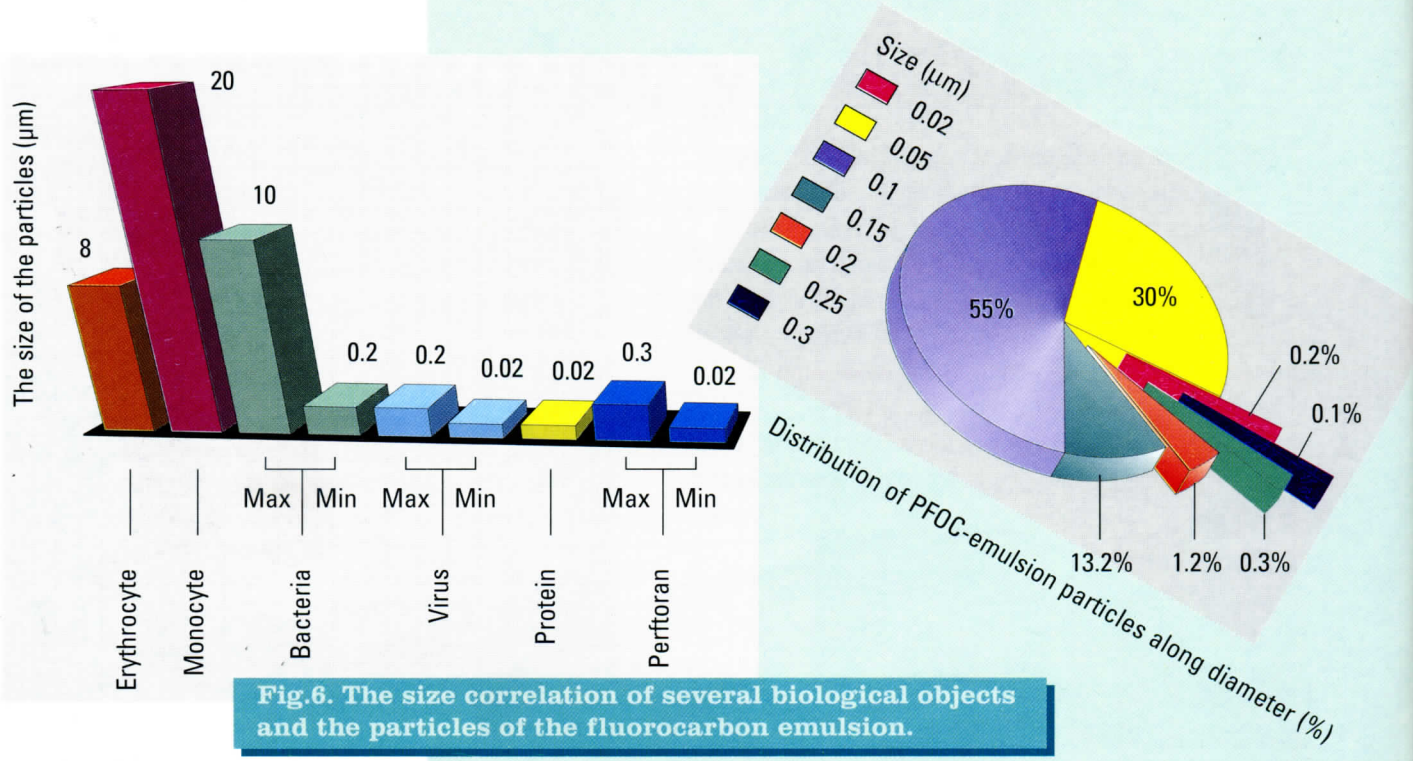


Fig.6. The size correlation of several biological objects and the particles of the fluorocarbon emulsion.

Specific activity of perftoran determined in rats

Specific animal studies have been carried out. They have evaluated the survival rate after massive volume replacement with Perftoran in rats (Table 2). Studies showed that after massive blood loss (Hb 5-8 g%) and subsequent transfusion of Perftoran emulsion (65-85 ml/kg) or colloid-saline solutions (65-90 ml/kg) containing saline composition and 3% albumin, the survival rate was better in the Perftoran group. Additional blood samples evaluating Hb level were obtained in both groups. During and after the infusion animals were oxygenated ($pO_2 > 500$ mm Hg). In the experimental group 9 of 30 animals survived.

All animals in the control group (20 animals) died during the first 3 hours.

Table 2. Survival rate after massive blood loss treated either with Perftoran or with colloid-saline solution (CSS) in rats.

Animal group	Number of animals	Hb level (%)		Elapsed time after transfusion (h)				The number of living animals
		baseline	after transfusion	1-3	4-6	7-9	10-12	
				The number of dead animals				
control (CSS)	20	14±0.05	1.5±0.01	20	–	–	–	0
experiment (Perftoran)	30	14±0.06	1.4±0.02	–	–	10	11	9

Eight rats died at the end of transfusion from significant anaemia and hypoxemia. In the experimental group 21 rats died after 8-12 hours. All the deaths were caused by acute massive blood loss and significant post-transfusion hypovolemia occurring 5-6 hours after the Perftoran transfusion. Apparently, the latter occurred as the result of increased diuresis.
Thus, massive blood loss in rats can be treated by Perftoran emulsion.

Tabl. Comparision of emultion physical properties (Riess J., 1995)

Properties	Perftoran (Russia)	Fluosol (Japan)	Oxygent (USA)		
			AF0104	AF0143	AF0144
pH	7,4	7,3	7,0	7,0	7,1
Osmolality (mOsm/kg)	288	201	305	317	307
Free fluoride (μmol/L					
presterile	1,0	21	n.a.	1,0	1,2
poststerile			16	18	10
Viscosity at 1 Hz (mPas)	2,6	3,8	31	55	4
Particle size					
median diameter (μm)	0,07	0,13	0,27	0,15	0,16
% particles>0,5 μm	0,0	1,0	9,5	0,2	1,4

Total O_2 concentration in arterial and venous blood, chemically bound and physically dissolved oxygen in arterial and venous blood, arterial venous difference (AVD) O_2 were determined by calculations.
The O_2 capacity of blood was decreased in both groups. However, the level of physically dissolved O_2 in the Perftoran group was 3.5 time higher (2.94±0.31) than in the control group (0.88±0.02) (Table 3). That compensated for the decrease in AVD by O_2 and provided equal to baseline (66.50±0.85) the real value of O_2-carrying capacity, which was 70±0.4 ml/min·m^2. In the control group equal blood loss, volume replacement and level of cardiac index (2.2±0.1; 2.1±0.1) caused a decrease in O_2-carrying capacity by 37% and it became 44±0.3 ($p<0.05$). This showed insufficient O_2 delivery to tissues (Fig. 7).

THE STUDY OF O_2-CARRYING CAPACITY OF PERFTORAN IN DOGS

Seventeen dogs were involved in the study. The experimental group consisted of 9 animals and the control group consisted of 8 animals. In all animals transfusions were made through the cannula in a.femoralis. Exchange transfusion of 50 ml of blood per kg of body weight was performed in isovolemic regimen under the control of blood pressure in aorta and vena cava superior and ECG. The pH, pO_2 and pCO_2 in arterial and venous blood, cardiac output, heart rate, Hb level, and Perftoran concentration were measured during transfusion and during the first 2 hours after transfusion.

Thus, the results obtained from this study showed the advantages of Perftoran over polyglukin, traditionally used as a plasma substitute. Substituting 60–70% of circulating blood volume Perftoran successfully provides gas-carrying function and maintains the normal rate of O_2 delivery.

Table 3. The values of blood gases after replacement either with polyglukin or with Perftoran in dogs.

Values	Before replacement	2 hours after replacement	
		Polyglukin	Perftoran
Hb level (%)	12±0.95	4.7±1.2	3.6±0.34
pO_2 a (mm Hg)	234±13.6	259±58	360±42
pO_2 v (mm Hg)	52±8	53±8.4	45±3.4
pCO_2 a (mm Hg)	53±3.1	40.5±3	57±7
pCO_2 v (mm Hg)	59±2.5	47.7±4.1	61±4.5
pH a	7.35±0.06	7.33±0.10	7.34±0.08
pH v	7.23±0.02	7.26±0.10	7.24±0.16
Level of O_2 conjugated with Hb (vol. %)	16.1±1.2	6.30±0.7	5.06±0.6
AVD by O_2 conjugated with Hb (vol. %)	3.96±0.9	1.41±0.1	0.96±0.05
Level of O_2 in arterial blood, physically dissolved (vol. %)	0.86±0.01	0.88±0.02	2.94±0.31
AVD by O_2, physically dissolved (vol. %)	0.67±0.01	0.69±0.01	2.40±0.30
Total AVD by O_2 (vol. %)	4.85±0.9	2.01±0.1	3.35±0.30
Real transportation of O_2 (ml/min·m^2)	66.5±0.35	44±0.3	70±0.4

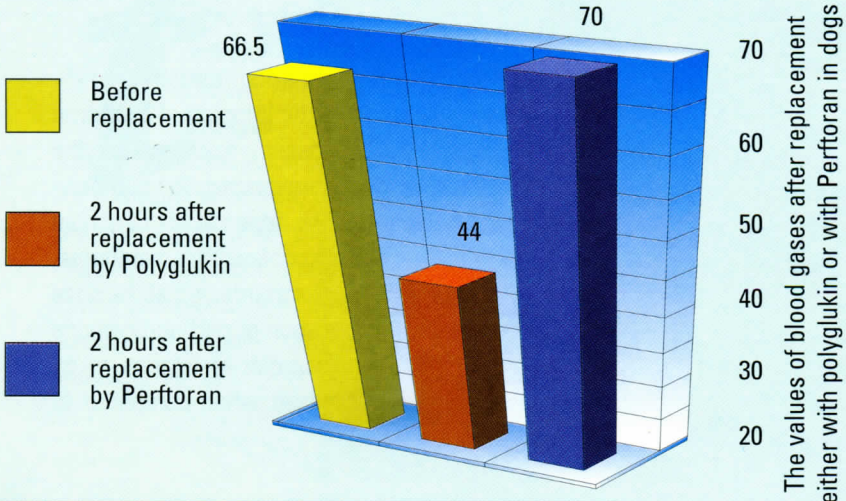


Fig.7. Real transportation of O_2 (ml/min/m^2).

RHEOLOGICAL CHARACTERISTICS OF PERFTORAN

Acute ischemia of the organs is often assosiated with a number of rheological disorders. The rheological disorders (impairment of blood viscosity, etc.) lead to impaired microcirculation and progressive reduction of organ perfusion. It is evident that the improvement of rheological characteristics of blood will increase perfusion in organs.

Several studies have demonstrated that one of the compounds of perfluorocarbon emulsion – proxanol – improves the rheology of spherocytes (Smith C. et al., 1987), inhibits the effects of vasoactive products (Saeeb M. et al., 1979), and reduces death rates due to haemorrhagic shock (Hymes A. et al., 1971).

The effect of proxanol on myocardial ischemia caused by coronary occlusion was studied and performed in dogs. An equal decrease in myocardial contractility was observed in experimental and control groups 60 minutes after occlusion. The cardiac index was reduced to 80% ($p<0.05$); coronary blood flow was reduced to 54% ($p<0.05$); coronary resistance increased to 82% ($p<0.05$).

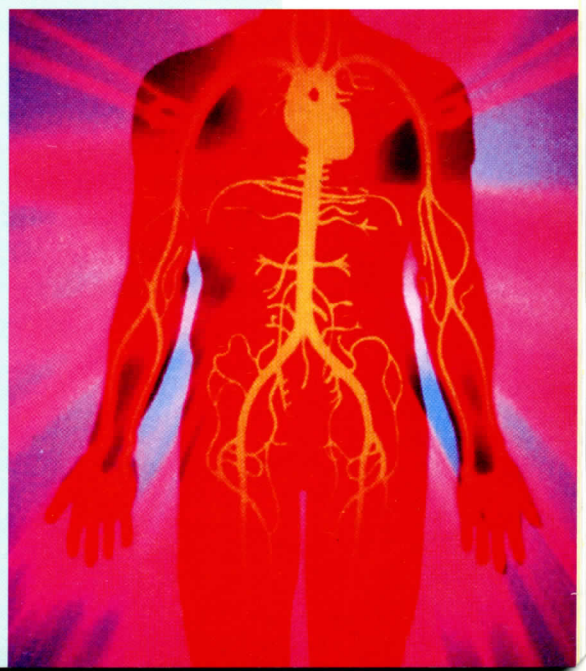
The infusion of saline solutions in the control group did not influence the cause of myocardial ischemia. Hemodynamics and rheology were impaired.

The infusion of 4% proxanol emulsion in the experimental group led to partial correction of coronary blood flow (to 81%) and coronary resistance (to 135%).

In addition, the infusion of proxanol considerably increased the cardiac index (to 89%) and decreased relative blood viscosity (by 12%).

The increase in coronary blood flow was related with the reduction in blood viscosity. The latter was significantly reduced after the infusion of proxanol.

Proxanol improves microcirculation due to increased blood flow. C. Smith et al. (1987) showed that pluronic (an analogue of proxanol) corrects the endothelial adherence of sedimented spherocytes, improves the passage of spherocytes through micropores, reduces rigidity of membranes, and provides a "greasing effect" on the cell surface.



A.I. Miroshnikov et al. (1984) have shown that perfluorocarbon emulsion with 4% proxanol enhances electrophoretic mobility of erythrocytes possibly due to an increase in the superficial charge of erythrocytes. A.M. Treshinski et al. (1984) have shown that superficial charge plays a great role in the prevention of erythrocyte aggregation, which leads to better microcirculation.

The increase in myocardial resistance to ischemia by prophylactic infusion of Perftoran emulsion can be possibly explained by the presence of perfluorocarbons in cardiomyocytes' membranes. This process depends on threshold concentration of PFOC in the sarcoplasmatic membrane. For example, one hour after Perftoran infusion at the dose of 20 ml/kg at minimal concentration of perfluorocarbons in membranes (2.1 μg/kg) myocardial resistance to ischemia was significantly increased when compared to the control infusion.

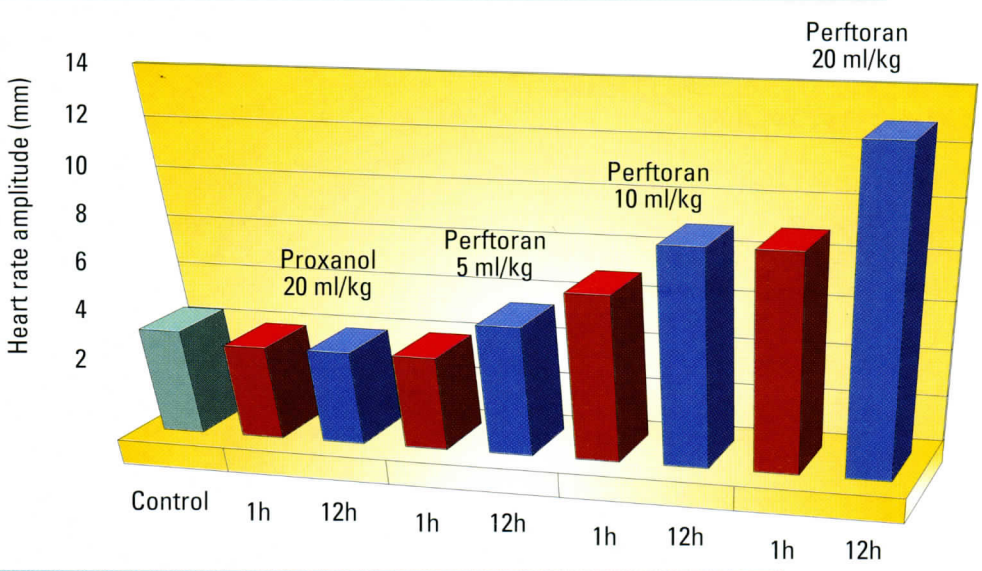


Fig.8. Recovery of heart rate amplitude in post-ischemic period after prophylactic infusion of Perftoran.

AFFINITY TO MEMBRANES

Prophylactic infusion of Perftoran emulsion enhances myocardial stability against ischemia, which can be explained by the presence of small amounts of PFOC in cardiomyocytes. This increase is dose-time dependent (Fig. 8). The increase in cell membrane permeability during ischemia is the main factor that damages cells.

After 12 and 24 hours following the infusion myocardial resistance did not change significantly, although the concentration of perfluorocarbons in the membrane increased. An increase in the time of circulation of small amounts of emulsion (10 ml/kg) in the blood from one to 12 hours before ischemia as well as the increased dose of emulsion (to 20 ml/kg) infused one hour before ischemia enhance myocardial resistance to damages.

Thus, an increase in myocardial resistance to ischemia depends on dose and time.

Transient accumulation of perfluorocarbons in sarcoplasmatic membranes of cardiomyocytes after the prophylactic infusion of emulsion leads to modification of cell membranes and diminishes myocardial damage due to ischemia and reperfusion.

Protection is caused by the direct influence of perfluorocarbon on membranous phospholipids as well as by interaction with hydrophobic parts of membranous proteins which can lead to restorage of homeostazis in the damaged cell.

Immunotropic characteristics

Perftoran stimulates the humoral immune system by activation of macrophages and increasing T-helpers activity.

Anti-ischemic features

The best way to interrupt the pathophysiological changes caused by myocardial ischemia is to restore coronary blood flow. The infusion of Perftoran at the dose of 10 ml/kg to animals has restored coronary blood flow (occlusion of 70-80%), improved oxygen delivery and acid-alkaline balance of myocardium and consequently improved myocardial contractility and cardiac output.

Myocardial improvement can be explained by the ability of proxanol to change blood viscosity. After the infusion of emulsion relative blood viscosity was decreased by 12% accompanied by an increase in the superficial charge of erythrocytes which prevented their aggregation and improved microcirculation.

Pharmacology (Safety)

Acute toxicity studies

Determination of median lethal dose – LD_{50}

Perftoran was infused at the rate of 0,7 ml/min at the dose of 120, 140, and 160 ml/kg. During 5 days the number of deaths was registered and corresponding probit values were calculated from the table (Table 4).

LD_{50} was determined from the curve of correlation between dead animals and experimental doses (in probits). It was 130 ml/kg. In grams the LD_{50} is 35.3 g/kg. The LD_{50} for polyglukin is 15.3 g/kg.

Table 4. The results of toxicity studies of Perftoran.

Infused dose (ml/kg)	Number of mice		Dead mice (%)	Probit
	baseline	after 5 days		
120	10	2	20	4.16
140	10	8	80	5.84
160	10	10	100	6.64

Chronic toxicity studies

Chronic toxicity studies of Perftoran were performed in rats and rabbits. Perftoran was infused every day in the course of 10 days at the dose of 10 ml/kg. In the control group either polyglukin or saline solution with 3% albumin were administered.

Several parameters were evaluated: appearance of animals, motor activity, digestive function, weight, and survival rate. Rabbits and rats were butchered by air embolism decapitation, respectively, for gross and histologic postmortem examination.

The following results were obtained:

1. Plethoric hepatic capillaries;
2. Significant vacuolization of hepatocytes in all animals which received polyglukin and Perftoran and in some animals which received saline solution with 3% albumin;
3. Haemorrhage in alveoli in animals which received either Perftoran or polyglukin;
4. Small perivascular infiltrates in lungs in animals which received either Perftoran or polyglukin;
5. In liver, spleen and lymphnodes of all animals which received Perftoran vacuolized macrophages containing PFOC and small granulome were observed. However, the structure of these organs did not differ from the control group.

Thus, pathological changes were not observed after multiple administrations of Perftoran at the dose of 10 ml/kg body weight to rabbits. Registered vacuolization of hepatic tissue was observed after administration of Perftoran and polyglukin. Vacuolization of hepatocytes is a physiological reaction which developed after massive transfusion of blood substitutes. Other registered changes seems to be the results of butcher procedure.

Carcinogenic studies

Carcinogenic studies were carried out in mice and rats. Six hundred and eighty rats (7 groups) and 890 mice (9 groups) were involved in the study. Median follow-up was 150 weeks for rats and 132 weeks for mice. The drug was administered subcutaneously (sc.), intraperitoneally (pi) and intravenously (iv) (including blood substitution).

Possible carcinogenic effects were estimated by the following parameters: effective count; latency period; increased incidence of tumour types occurring in the experimental group compared to control; occurrence of tumour earlier than in the controls; increased multiplicity of tumours (coefficient of multiplicity).

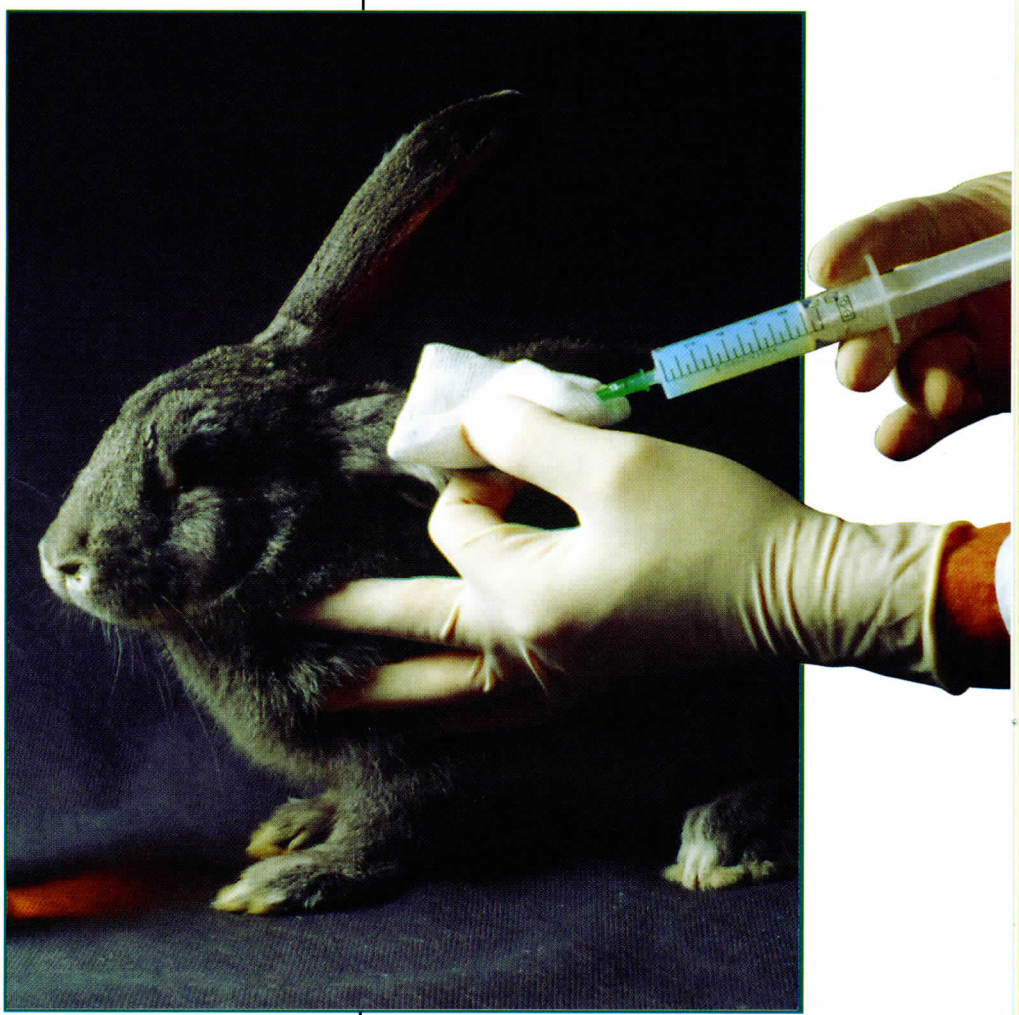
The difference in incidence of induced tumours in control and experimental mice was not statistically significant. In contrast to inactive control solution multiple infusion of saline solution causes a reduction of the latency period ($p<0.001$). A tendency to reduction of the latency period was registered in the experimental group at the maximal doses of Perftoran (20 ml/kg).

Morphological evaluation in mice did not show differences in localization, structure and malignancy of tumours in the two groups. The incidence of breast adenocarcinoma prevailed in this study. Differences in incidence of tumour and structure in control and experimental groups were not statistically significant.

The difference in incidence of tumours in rats in control and experimental groups was not statistically significant. A tendency to an increase in coefficient of multiplicity was registered in the control group receiving saline solution of Perftoran. Malignant (nondifferentiated cancer) lung tumours prevailed in this group (11.6% from all tumours) and in the group receiving blood substitution (15.7%).

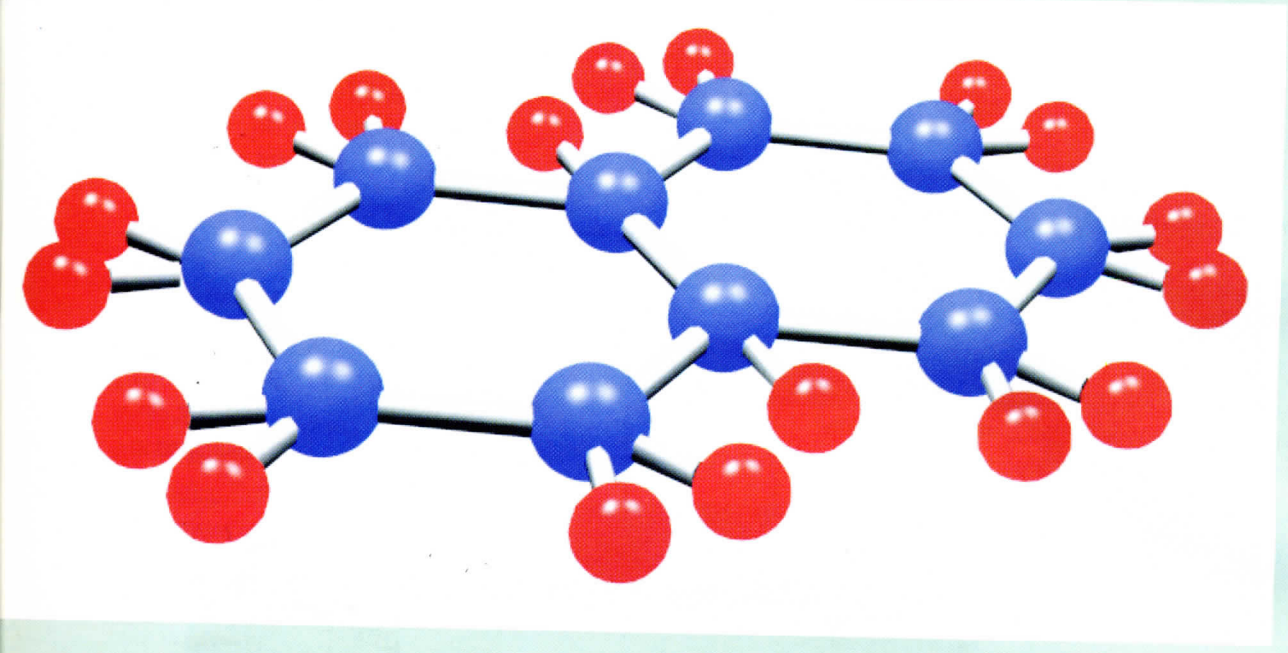
The difference in incidence of tumours in the two groups was not statistically significant. The incidence of breast tumours prevailed in all groups.

Thus, the absence of statistically significant differences in control and experimental groups in mice and rats shows that Perftoran has no carcinogenic activity. All tumours developed in animals were spontaneous.



Teratogenic studies

Teratogenic studies showed that several Perftoran regimens (25 ml/kg every day during 5-10 days, total dose 125-250 ml/kg) provided teratogenic effect in non-lineage rats.



Teratogenic effect of a single dose of Perftoran is observed at a dose above maximum therapeutic dose (25 ml/kg). However, the dose that can cause teratogenic effect (5-6 times higher than 25 ml/kg) is unlikely to be used in practice (i.e., the single dose causing teratogenic effect would be 125-250 ml/kg).

In pregnant women Perftoran should be used only if mother has vital indications. The use of Perftoran during pregnancy can cause the impairments of fetus.

Mutagenic studies

Mutagenic studies were conducted according to the recommendations of Pharmacological Committee of the Russian Federation on evaluating potential mutagenic effects.

The ability of Perftoran to induce gene mutation in bacteria under condition of metabolic activation in vitro and in vivo, chromosome aberration in bone marrow cells in mice and in human lymphocytes and dominant lethal mutation in germinal cells in mice were evaluated. The emulsion did not show mutagenic activity.

Immunology

Studies showed that blood substitute based on PFOC – Perftoran – stimulates the humoral immune system. Production of antibodies in immunised and non-immunized animals is stimulated by the proliferation of progenitor B-cells and the activation of macrophages. Migration of cells from bone marrow to the sites of antibody production is confirmed by alteration of the number of nuclear cells in parenchymatous organs while using of Perftoran. Perftoran does not appear to stimulate the cellular immune system.

Pharmacokinetics

Perftoran, an emulsion with submicron structure, is phagocyted by the cells of reticulo-endothelial system and accumulates in the liver, spleen and bone marrow. Phagocyted particles lead to the development of multiple phagosomes. Duration of the accumulation of perfluorocarbon compounds depends on their physicochemical features.

Perfluorodecalin (PFD) is cyclic perfluorocarbon without nitrogen and oxygen heteroatoms. Elimination time is one month.

Perfluoromethylcyclohexylpiperidin (PFMCP) consists of two cyclic structures, CF_3 group and nitrogen heteroatom. Elimination time is 18-24 months.

Perfluorocarbon compounds in Perftoran emulsion are chemically inactive compounds which are not metabolised in the body.

PFOC are eliminated via lungs in expired air and skin.

The time of Perftoran circulation in the blood depends on dosage of emulsion and species of animal. Half-period of elimination from the body after administration of 20 ml/kg to rabbits is 24-26 hours and to rats is 8-10 hours.

Proxanol, the surface active agent, is eliminated via kidney in 24 hours.

Excretion

Successful usage of perfluorocarbon emulsions depends on their elimination rate. Particles should not be eliminated too fast from blood and they should not be accumulated too long in organs. The main part of perfluorocarbon compounds is eliminated via the lungs in expired air (Fig. 9) and via the skin.

Intensive phagocytosis of Perftoran particles is conducted by cells of reticulo-endothelial system in the lungs (predominantly by alveolar macrophages) and in the liver, spleen, and bone marrow (A.M. Golubev et al., 1993). Phagocyted particles lead to the development of multiply phagosomes, which move to the apical part of the alveolar macrophages and then are eliminated by exocytosis due to the destruction of the apical parts of cells. (N.B. Shibaev et al., 1983). Perfluorocarbon compounds are chemically inactive and they are not metabolized in tissues. The detection of drug concentration in tissues and in blood is exact.

The duration of accumulation of the drug in the body depends on physicochemical features. For example, perfluorodecalin (PFD), cyclic perfluorocarbon without nitrogen and oxygen heteroatoms, is eliminated from the body for one month. Another perfluorocarbon, perfluoromethylcyclohexylpiperidin (PFMCP) consists of two cyclic structures and CF3 group with nitrogen heteroatom and is eliminated from the rat's body for 18-24 months without any damage of tissues and organs (Table 5, Fig. 10).

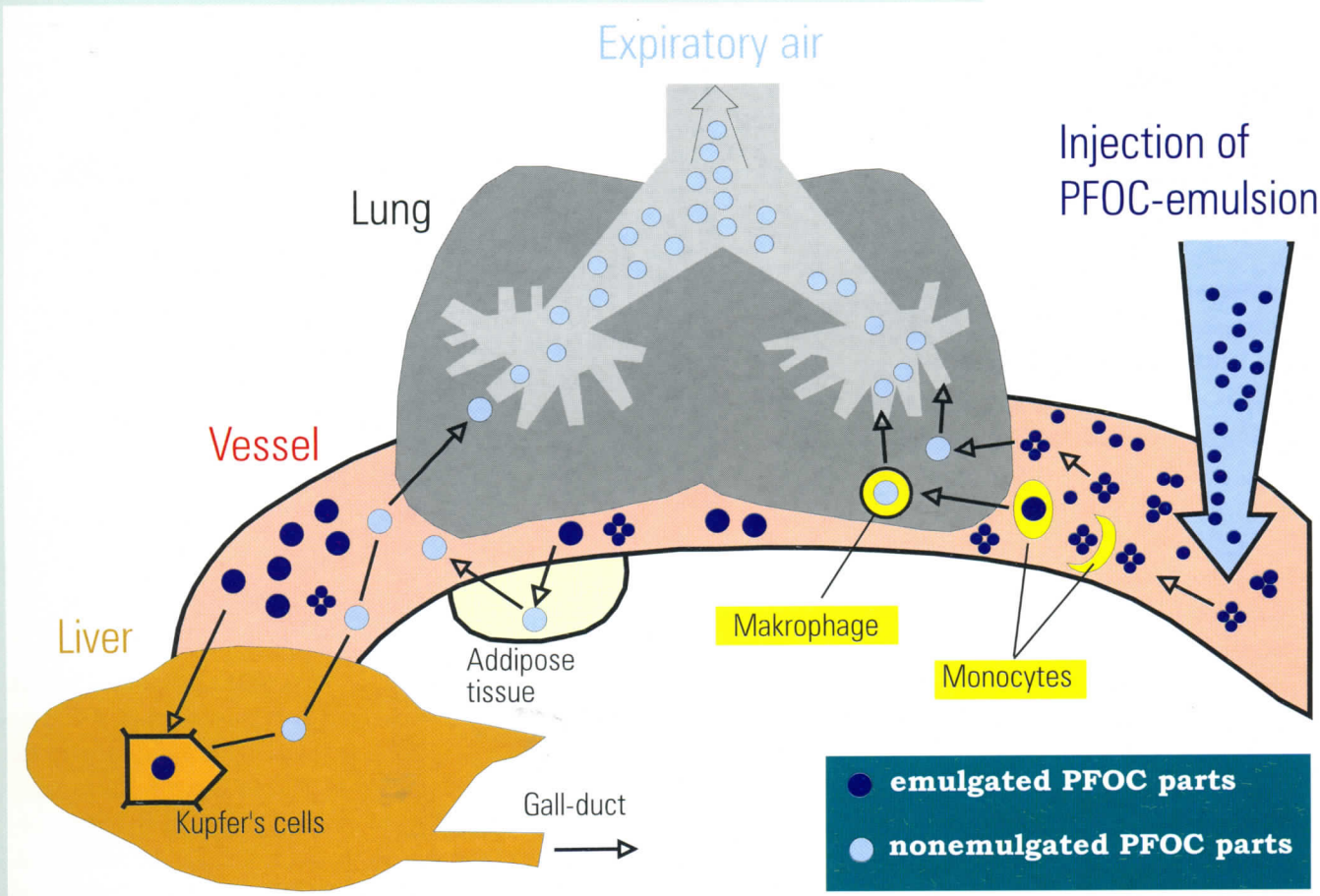


Fig.9. Ways of PFOC-emulsional excretion from an organism.

Table 5. Elimination of PFMCP from rat's body.

Regimen of infusion	Time after infusion	PFMCP level in organs (%) from total dose of infused PFMCP
Replacement of 50-60% with Perftoran	3 days	33.2±1.92
	14 days	18.1±1.99
	1 month	11.5±1.74
	2 months	9.5±0.32
	6 months	2.9±0.21
	8 months	2.1
	13 months	1.25
	18 months	0.5
	24 months	flakes*
Plethoric infusion of Perftoran (10 ml/kg)	7 days	18.8
	1 month	10.0
	3 moths	5.4
	6 months	2.4
	12 moths	1.1
	18 months	0.85
	24 months	flakes*

* – levels below 0.05 mg/g of tissue

Chromatographic analysis showed flakes of PFMCP in the body by the end of the 24th month after the administration of Perftoran emulsion. Perfluorodecalin is eliminated earlier.

The addition of cyclic perfluorocarbon with nitrogen heteroatom (PFMCP) to the mixture with perfluorodecalin is necessary to create a more stable emulsion.

PFD, the real cyclic perfluorocarbon, is rapidly eliminated from the body but it is not stable. PFMCP is stable but it is eliminated slowly from the body. That is why a mixture of these two compounds was created in the proportion of 2 parts of PFD to one part of PFMCP.

This mixture is the basis for gas transportation in Perftoran emulsion.The time of curculation of perfluorocarbon emulsion in the blood depends on average particle size and the type of surfactants. Standard Perftoran emulsion with average particle size of 0.07 μm, emulgated by block-copolymer of oxyetylene and oxypropylene (proxanol), with Mw = 8000 D, where the hydrophobic block makes up 20% of the whole molecule, administered at the dose of 20 ml/kg has a half-period of elimination in rabbits of 24 hours. In contrast to PFOC, proxanol is eliminated via the kidney during 24 hours.

Within 2 hours after administration of Perftoran (proxanol concentration is 4%) the content of proxanol in the blood does not exceed 1.5% of the total administered dose. Further the level remains 0.5-0.3% during 24 hours.

Morphological evaluation did not show pathological alterations of tissues and organs after the use of perfluorocarbon emulsion.

Pharmacodynamics

Perftoran consists of chemically inactive perfluorocarbon compounds which are not metabolised in the body of humans or animals.

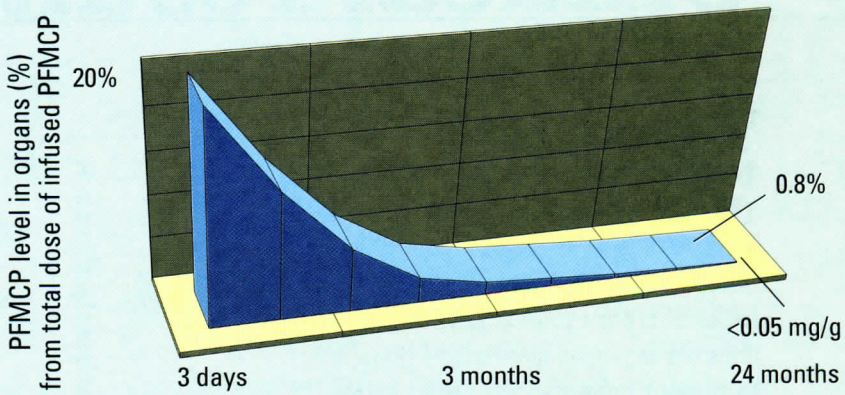


Fig.10. Elimination of PFMCP from rat's body (plethoric infusion of Perftoran (10 ml/kg)).

Induction of cytochrome P-450-dependent monoxygenase system of liver.

Perfluorodecalin, the main compound of the emulsion, is a biologically active agent. It increases the activity of enzymes of the monoxygenase system. The level of cytochrome P-450 by the third day after Perftoran infusion is 1.8 nmol/mg of protein, which is 2.8 times higher than control values.

Perfluorodecalin develops a complex with cytochrome P-450 in experiments in vivo. The absorbtion spectrum looks like a butterfly. Maximum absorption is 390nm and minimum is 420 nm, well known for type I substrates hydroxylated in monoxygenase system.

The quantitative increase in microsomal cytochrome P-450 was accompanied by an increase in the free NADPH oxidation rate by microsomes, selected from the liver of animals receiving Perftoran. The maximum NADPH oxidation rate was determined by the second day after Perftoran infusion and it was 225% in comparison to the control.

The NADPH-dependent oxidation of lipids by the second day after Perftoran infusion decreased by 10 times; by the tenth day it was similar to the control.

A number of investigations (method of double diffusion, method of inhibition of specific substrates metabolism by antibodies against individual forms of cytochrome) showed the presence of isoform of cytochrome P-450 immunologicaly equivalent to cytochrome P-450 in the liver of rats and the absence of isoform equivalent to cytochrome P-448, induced in the liver after injection of polycyclic aromatic carbohydrates, benzapyren, some mutagens and cancerogens.

The method of hexenal sleep was used to determine the changes in liver function in vivo. The duration of hexenal sleep by the third day after infusion of Perftoran was reduced to 3 min compared to control (18-19 min). Perftoran stimulates the activity of the monooxygenase system against some barbiturates due to induction of Phenobarbital form of cytochrome P-450 in the liver of animals.

The duration of induction is determined by the dose of infused emulsion. Perftoran has the same induction ability as Phenobarbital.

Clinical results

THE USE OF PERFTORAN EMULSION IN NEUROSURGERY

Seventy-nine patients with severe forms of cranial trauma have received different regimens of intensive therapy to prevent brain hypoxia. The characteristics of the adaptation process reducing neurological disorders in the acute phase of cranial trauma and improving the survival rate were determined.

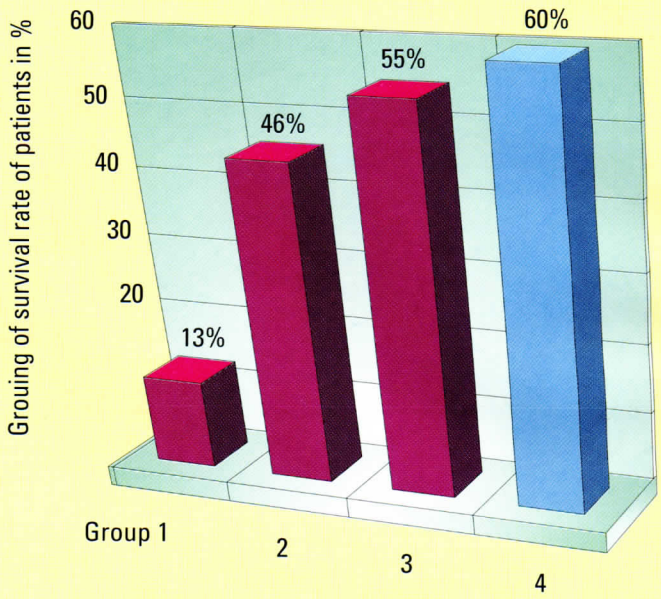


Fig.11. Survival rate of patients after cranial trauma (II coma) by different pharmacological protection:
Group 1 (coma 6.5 days): GABA+B (gamma-aminobutiric acid + barbiturates)
Group 2 (coma 5.7 days): GABA+B+HBO (hyperbaric oxygenation)
Group 3 (coma 5.4 days): GABA+B+HBO+"Antihypoxin" food supplement
Group 4 (coma 10 days): GABA+B+HBO+Perftoran emulsion

Among 38 patients with coma II (according to Glasgow scale) receiving combination of gamma-amino butyrate acid (GABA) and barbiturates (group 1) only 5, patients are alive (13%). Mean duration of coma was 6.5 days.

Among 20 patients with coma II receiving combination of gamma-amino butyrate acid (GABA), barbiturates and hyperbaric oxygenation (HBO) (group 2) 8 patients are alive (40%). Mean duration of coma was 5.7 days.

In group 3 patients received GABA, barbiturates, HBO and "Antihypoxin" food supplement. Mean duration of coma was 5.4 days. 5 patients of 9 are alive (55%).

The patients in group 4 received the same therapy as the patients in group 3 plus Perftoran emulsion at the dose of 3-5 ml/kg. Although coma lasted longes in this group (more then 10 days), 7 patients of 12 are alive. This survival rate is higher than in previous 3 groups (60%) (Fig 11).

Other studies showed that Perftoran infusion to 20 patients with cranial trauma and coma II increased the survival rate to 38.5% versus 7.6% in the control group. Thus, Perftoran emulsion has a wholesome effect on patients with cranial trauma. The prevention of brain hypoxia in both groups was provided with sodium tyopental and GABA. The duration of coma was 6-10 days. 5-7 ml/kg of Perftoran were additionally administered to patients in the experimental group the first day after injury.

Thus, therapy where Perftoran is used to prevent brain hypoxia improves survival rates.

THE USE OF PERFTORAN IN TRANSPLANTOLOGY

Transplants are damaged due to different conditions. For example the results of kidney transplantation in patients with chronic renal failure are still unsatisfactory.

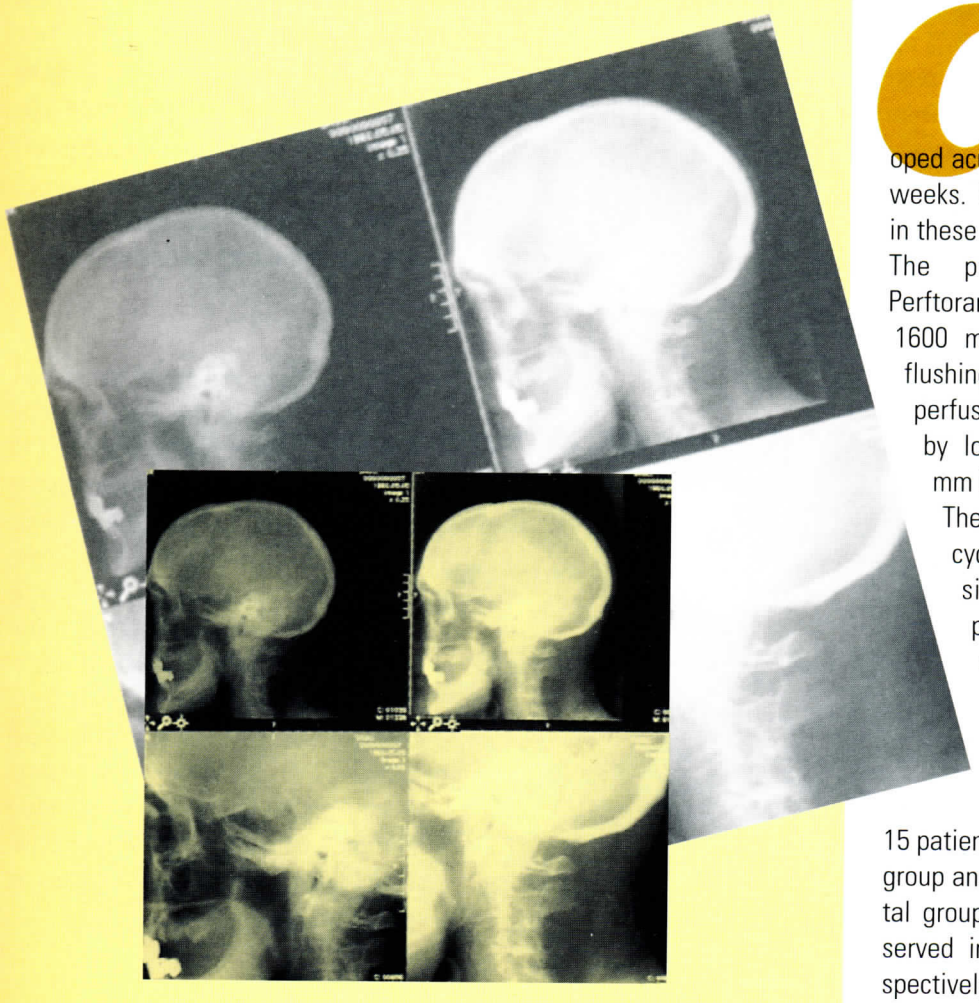
Perftoran have been used to prevent the ischemia of transplant and to improve the results of kidney transplantation. The following goals were determined:

- evaluation of transplant function while infusion of Perftoran to dying donor;
- evaluation of transplanted kidney function after the preparation of transplant in donor's body.

Evaluation has been made in 76 cadavers; in 50 cases in dying donors the standard preservation regimen were used (control group) and in 26 cases the preservation regimen included Perftoran (experimental group).

In the control group 26 kidneys were used for kidney transplantation (2 kidneys were not used due to technical difficulties). In the experimental group kidneys were transplanted to 44 recipients (8 kidneys were not used due to technical difficulties). All donors were patients who died from cranial trauma. Perftoran was infused intravenous (1.2-1.6 l) to cadavers before kidney were removed.

The results of the standard preservation regimen in the control group showed that oligoanuria (30-50 ml/h) remained even after the arterial blood pressure had recovered. Volume perfusion rate of removed kidneys with preserving solution was 60-80 ml/min by perfusion pressure of 60-80 mm Hg.



Duration of transplant's flushing was 5-8 min. In post-transplant period 50% of recipients developed acute renal failure lasting for 2-4 weeks. Hemodyalysis was performed in these patients.

The preservation regimen with Perftoran enhanced diuresis to 1300-1600 ml/h. Duration of transplant's flushing was 2-4 min. The volume perfusion rate was 120-150 ml/min by low perfusion pressure (40-60 mm Hg.).

The results in recipients receiving cyclosporin A (immunnosuppressive agent) with or without preservation with Perftoran showed that acute renal failure in early post-transplant period developed rarely in the group with Perftoran in contrast to controls. Among 15 patients receiving CsA in the control group and 15 patient in the experimental group acute renal failure was observed in eight and one patient, respectively.

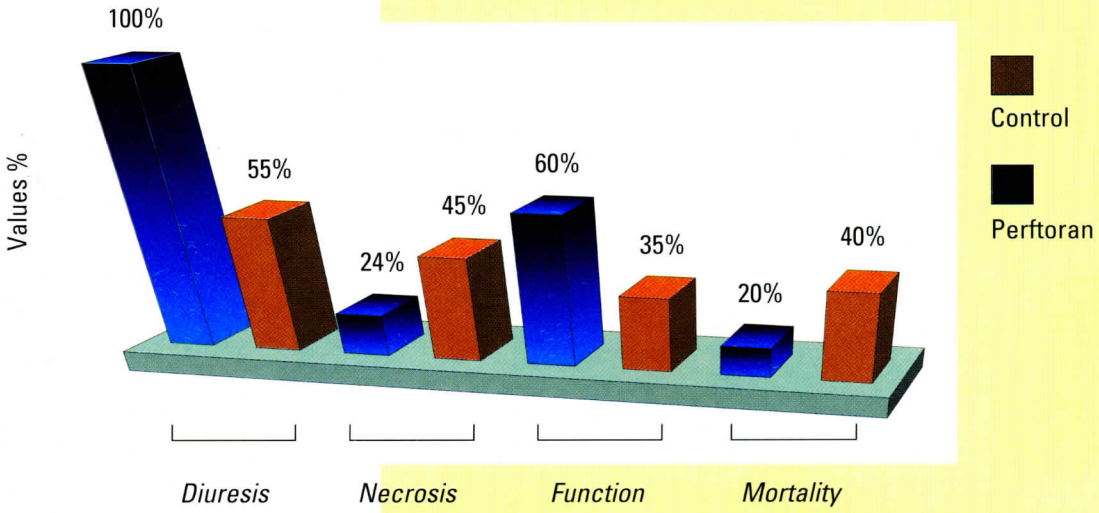


Fig. 12. Clinical values of kidney transplant in recipients with different anti-ischemic protection.

Within one hour after transplantation normal diuresis was observed in 100% patients in the experimental group and in 55% patients in the control group; the rate of acute calcium necrosis was 24% and 45% respectively; the rate of transplants with normal function (after discharge from hospital) was 60% and 35%, respectively; mortality was 20% and 40%, respectively (Fig. 12).

Thus, the use of Perftoran allows to improve the results of kidney transplantation approximately 2 times.

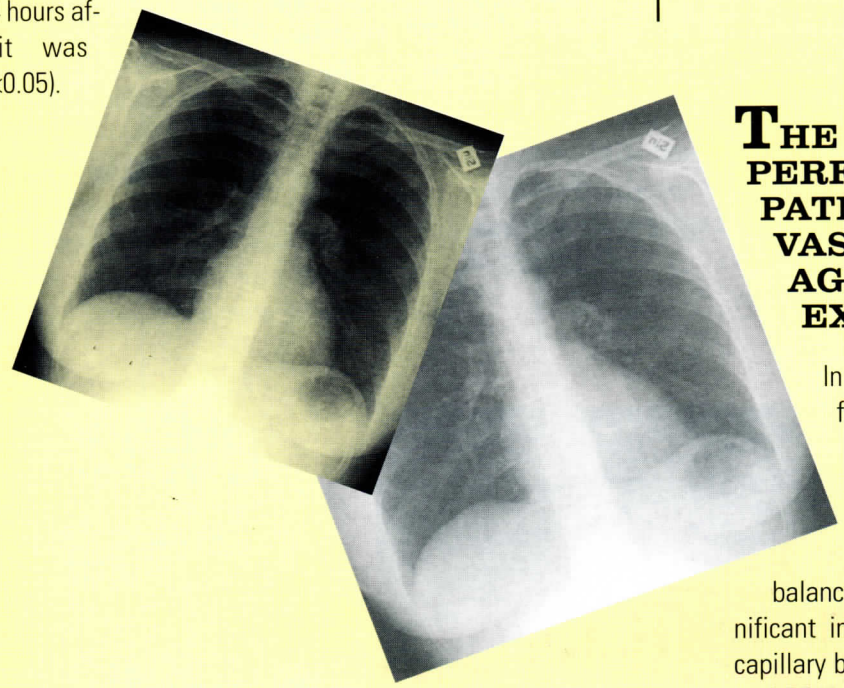
INFLUENCE OF PERFTORAN EMULSION ON GAS-EXCHANGE RATE IN PATIENTS WITH SEVERE COMBINED TRAUMA

Perftoran has been used in 25 patients with traumatic shock grades II-III accompanied by acute blood loss. Perftoran was infused to each patient iv as continuous infusion at a dose of 800 ml within 8-10 hours after injury.

The volume of transfusion during the first day after injury was 0.37±0/0.1 L of erythroconcentrate. During the same period in the Perftoran group transfusion of blood and its components was not performed.

The use of Perftoran in intensive therapy leads to the improvement in microcirculation in the lungs, which is documented by a reduction in alveolar dead space (ADS). Significant improvement in microcirculation in lungs after Perftoran infusion was observed in patients with very severe disorders in gas-exchange.

In 16 patients receiving Perftoran within 8 hours after injury pO_2 was 117.6±0.1 ml/min·m^2, ADS before infusion was 30.9±8.0%; 20 min after infusion it was 15.5±7.8%; 24 hours after injury it was 15.6±5.3% ($p<0.05$).



The usage of Perftoran as a plasma substitute with gas-carrying function in patients in posttraumatic shock leads to the improvement in tissue perfusion (increase in AVD) (Table 6). Thus, the use of Perftoran in patients with severe combined trauma improves pulmonary functions due to an increase in microcirculation in lungs.

THE USE OF PERFTORAN IN PATIENTS WITH VASCULAR DAMAGE OF LOWER EXTREMITIES

In 20 patients Perftoran was infused at a dose of 5-8 ml/kg. In all patients, except one, the injured extremities were warm. The measurement of the acid-alkaline balance in the blood showed a significant increase in pO_2 by 20.3% in capillary blood. The values of pCO_2, pH and BE were not changed. In the control group (polyglukin) pO_2 was not increased.

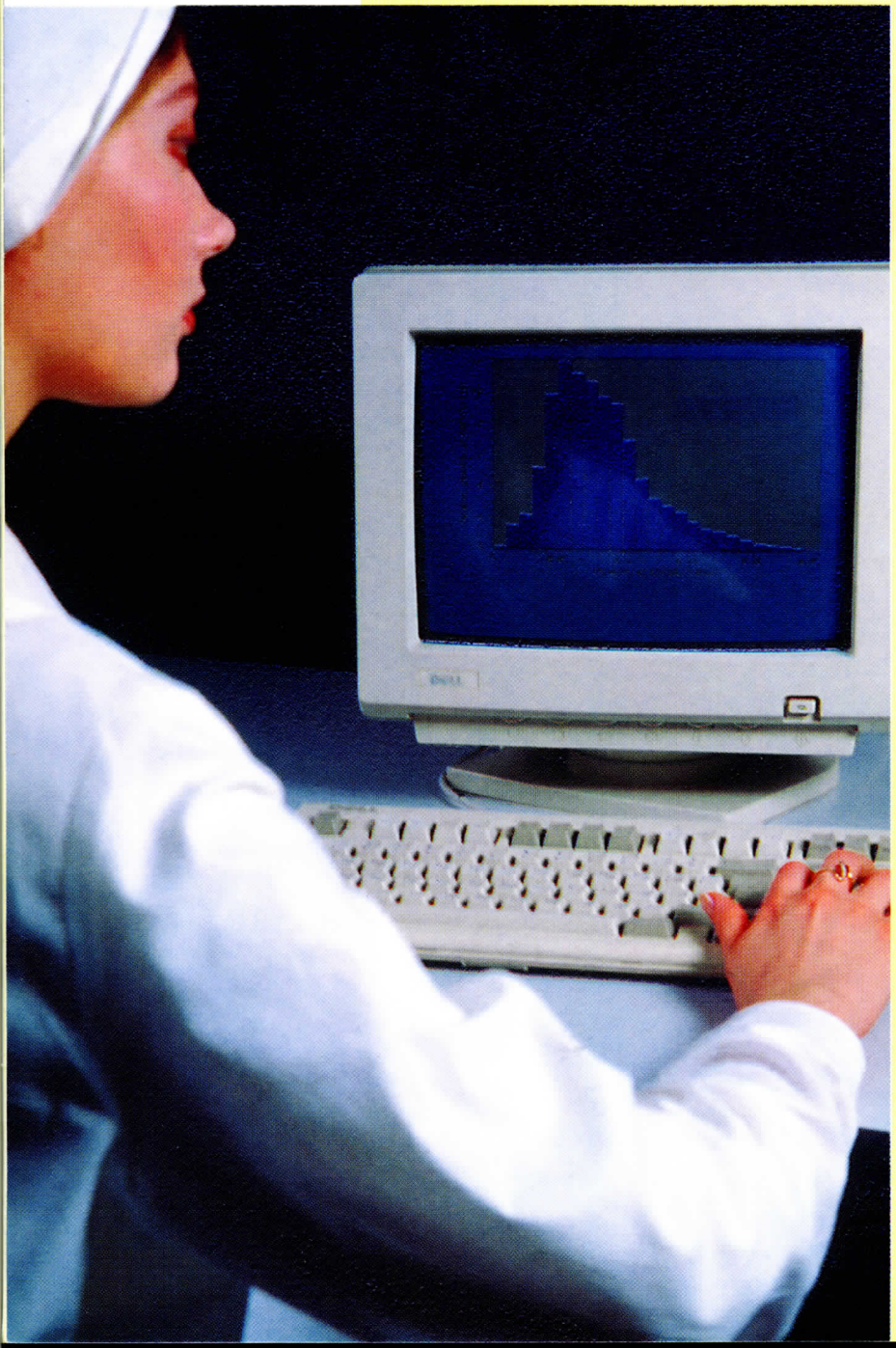
Table 6. The influence of Perftoran on arterial-venous difference (ml/l) by oxygen.

	Time	Experimental group, Perftoran(n=25)	Control group, optimized IT*(n=64)	Control group, routine IT* (n=32)
1	8 hour after injury	35.8±4.4	25.7±2.1	25.2±3.0
2	After infusion of Perftoran	34.0±4.6	–	–
3	24 hours after injury	48.7±4.8	34.8±2.4	28.6±4.0

* IT – intensive therapy

The level of milk acid (MA) in the blood was significantly decreased after the infusion of Perftoran (from 13.71±0.94 to 7.28±1.06 mg %), i. e. aerobiotic oxidation was increased due to enhanced transportation of O_2 to damaged tissues. In the control group the decrease in MA level was not evident (from 15.37±1.62 to 13.25±1.92 mg%). In the Perftoran group muscular blood flow was increased by 43.6% at rest and by 136.7% after exercise. In the control group muscular blood flow was increased by 25.8% at rest and by 57.8% after exercise.

In comparison to controls the infusion of Perftoran increased blood flow approximately 2 times. Angiography showed the increased rheographic index in the Perftoran group (in feet – 15.2%; in shins – 30.7%).
In the control group in blood flow was less increased: in ankles – 25.7%, in feet – 3.8%. In patients with ischemia, grade IV, after using polyglukin blood flow remained unchanged. In the experimental group an increase in blood flow in ischemic area was evident.

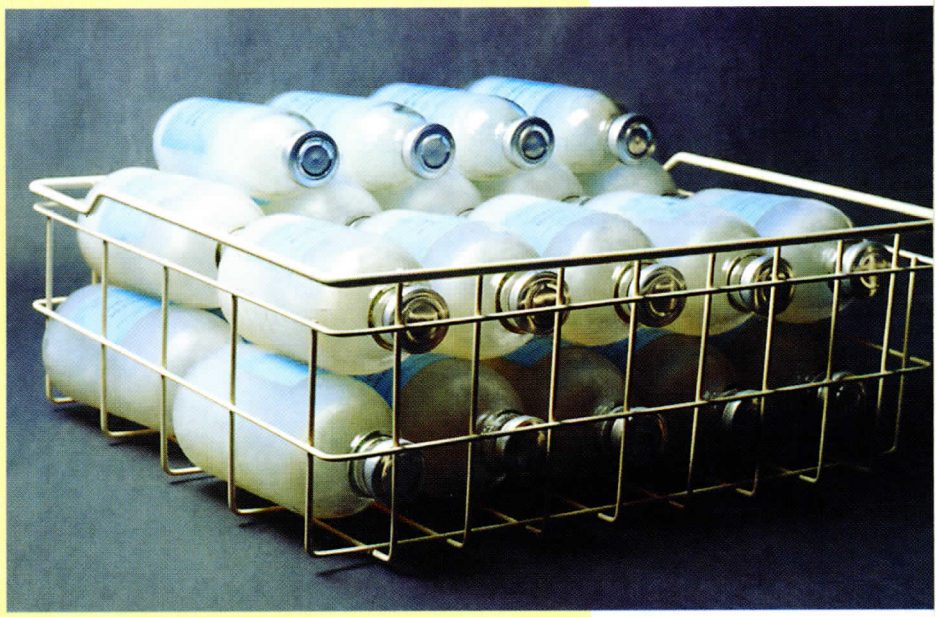


In the control group an increase in microcirculation was less: at rest, by 24.1%, after exercise, by 43.3%. In the experimental group an increase was significant: by 61.2% at rest and by 50.7% after exercise.
Thus, a single infusion of Perftoran at a dose of 5-8 ml/kg considerably increases oxygenation of ischemic area and it can be used in patients with severe ischemia in the lower extremities.

THE USE OF PERFTORAN EMULSION IN PATIENTS WITH DISORDERS IN MICROCIRCULATION, TISSUE PERFUSION AND METABOLISM

Perftoran was used at a dose of 6-8 ml/kg as single or multiple infusion in patients with disorders in microcirculation, tissue perfusion and metabolism caused by sepsis, peritonitis, anaerobic infections, toxic hepatitis, cranial trauma, fat embolism, vascular disorders or malignant tumours. Perftoran was used with other drugs and by oxygenation with 40 to 60% inspired O_2.

Transient increase in the following values was observed: central venous pressure – by 15-30% – with the following decrease caused by an increase in effective surface area of vessels and in vascular volume; cardiac stroke volume – by 16-22%; blood osmolality – by 6-10%; volume of circulating blood – by 12-118% – due to volume of plasma. Levels of Hb, Htc and trombocytes did not change due to hemodilution.



Homeostasis was not significantly changed. Steady significant reduction of creatinin and BUN levels indicated an increase in microcirculation in the kidneys; steady significant reduction of ALT and AST indicated a decrease in tissue hypoxia. The total O_2-capacity of blood was increased insignificantly by 1.0-1.5 vol. % after the use of Perftoran. However, O_2 retention in tissues was increased by 20-40%.

Evident improvement in tissue perfusion may be explained by activation of hepatic functions: infusion of 400 ml of Perftoran increased the rate of antipyrin hydrohylation 2 times. (Antipyrin was used as a test-drug.)

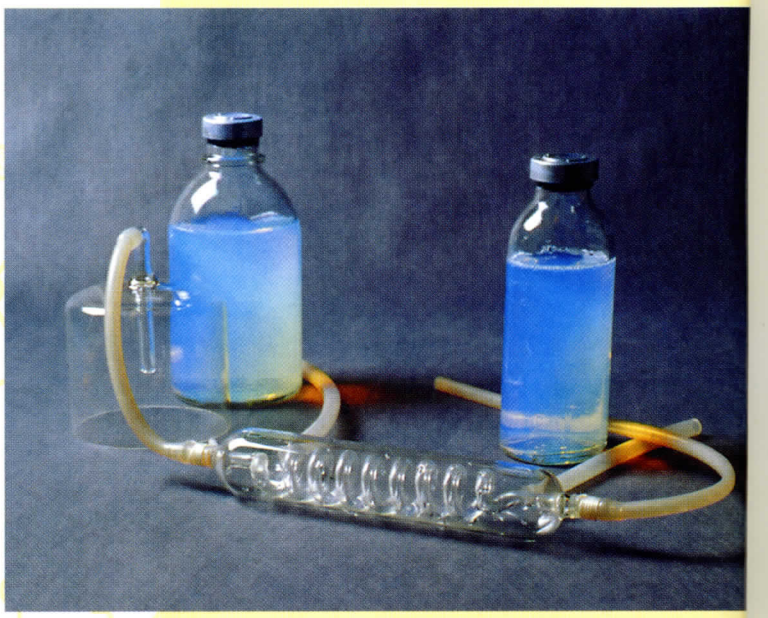
Indirect signs of activation of hepatic function were: less susceptibility to narcotics and tranquilizers in patients receiving Perftoran possibly due to transient induction of cytochrome P-450 in liver.

Infusion of Perftoran at a dose 400 ml bid or tid for 1 or 2 days led to reduction of symptoms of hepatitis of different aetiology: reduction of jaundice, decrease in bilirubin levels, ALT, AST, increase in total protein levels, improvement of homeostatic functions.

Infusion of Perftoran at the dose of 400-600 ml for 1-2 days, 6-8 times in patients with occlusive arterial diseases of the lower extremities and traumatic edema leads to reduction of pain, recovering skin colour, reduction of edema, increase in skin temperature, by 2,3±0.35°C ($p<0.05$), decrease in milk acid level in venous blood returned out of damaged extremity, by 51.3±1,3% ($p<0.05$), increase in reographic index in ankles by 22-32%, healing of trophic ulcers.

This improvement is caused by Perftoran's O_2-carrying function and by an increase in diffusion of physically dissolved oxygen contained in perfluorocarbon in tissues and better O_2 transportation and CO_2 removal from the area with damaged microcirculation due to submicronic size of particles. In patients with cranial trauma, cerebrovascular disorders and encephalopathy of various aetiology the infusion of Perftoran decreased (according to clinical data, CT, MRI and EEG data) the signs of cerebral edema and encephalopathy; infusion of Perftoran at the dose of 400 ml, 1-3 times by extracranial anostomosis led to uncomplicated post-operative period. Thrombosis and anastomosis were not observed.

Patients who were conscious, told about a feeling of "freshness and lightness" in the head after the infusion of Perftoran possibly caused by better oxygenation in the brains and enhanced removal of CO_2. EEG showed a significant increase in rapid electrical activity especially in the frontal lobes. This condition was explained by patients as a feeling of "freshness and lightness" in the head.



Perftoran prevents the development of fat embolism and treats its clinical symptoms. This was confirmed by the experiment.

The possible explanation is that Perftoran is a liquid sorbent with an active surface area of 600 m^2 per 100 ml of emulsion. Each one ml of Perftoran binds 10 mg of lipids; 400-3000 ml can destroy by sorption 4-30 g of lipids, the basis for fat emboles. 2 times decreased viscosity, hyperosmolality and the presence of proxanol, lead to reduction of edema, increased diuretic effect, increased perfusion in tissues and removal of CO_2.

Perftoran improves lung drainage due to an increase in mucosal secretion in tracheobronchial tree. The use of Perftoran in patients with respiratory distress syndrome, pneumonia, heart failure leads to easier explosion of sputum, more rapid disappearance of X-ray signs of interstitial and alveolar edema, recovery of spontaneous breathing, the improvement of lung functions, reduction of symptoms of hypoxia.

Infusion of Perftoran at the dose of 400 ml, 2-3 times in patients with gastric or duodenal ulcers resistant to traditional treatment leads to better and more rapid healing. The results of electrogastrogramma, phonoenterogramma and motor activity of intestine in these patients showed signs of enhanced peristaltic caused by the improvement of blood circulation and oxygenation of gastrointestinal system.

The use of Perftoran in patients with infectious disorders after adequate drainage of the site of infection leads to resolution of fever during 24 hours. In patients with chronic infectious (osteomyelitis, chronic pyelonephritis) or in patients with inadequate drainage of site of infection (peritonitis, infection of soft tissues) the infusion of Perftoran led to fever (increase of temperature by $0.6\pm0.11^{o}C$) within 1 to 3 hours.

Usually these patients received hemosorbtion before the administration of Perftoran. Patients become febrile because of the "appearance of toxin" from the sites of infection due to improvement of blood rheology.

Infusion of Perftoran to oncological patients led to better tolerance of chemo- and radiotherapy (infusion of 400-600 ml of Perftoran with following administration of radiotherapy, 6-8 times). When compared to the control group significant changes in peripheral blood parameters and biochemical parameters were not observed in the experimental group; more evident tumor regression was registered in the experimental group.

Since it is well known that a hypoxic tumor is resistant to chemo- and radiotherapy, better blood circulation and better oxygenation of the tumor make it more sensible to chemo- and radiotherapy.
Thus, the use of Perftoran in patients with disorders of microcirculation, tissue perfusion and metabolism of various etiology showed the following symptoms improving. They are: a gas-exchange in tissues, decrease in acidosis, the improvement of capillary pulse, an increase in local blood flow, the reduction of total peripheral resistance, increase in skin temperature, an increased diuresis, increased peristalsis, the reduction of signs of organ ischemia.

References

Beloyartsev F.F. Perfluorocarbons in biology and medicine.// Perfluorocarbons in biology and medicine: Col. – Pushchino, 1980 – p.5-21.

Beloyartsev F.F., Ivanitsky G.R., Maevsky E.I., Obraztsov V.V., Shechtman D.G. Chemically inactive fluorocarbons – inducers enzyme of hepatic monooxigenase system.// DAS USSR – 1986 – v.286 – № 3. – p.729-732

Beloyartsev F.F., Kaidash A.N., Islamov B.I., Maevsky E.I., Tverskoi A.L., Shibaev N.V., Luchitsky V.N., Kobrinsky E.M. Cardioplegia with perfluorocarbon emulsion for the evention of myocardium during heart operations.// Medico-biological aspects of the use of perfluorocarbons: Col. – Pushchino, 1983. – p.116-127.

Beloyartsev F.F., Kaidash A.N., Islamov B.I., Maevsky E.I., Kokoz Y.M., Freydin A.A., Obraztsov V.V. Evaluation of possibilities of the use of perfluorocarbon cardioplegia as an anti-ischemic protection of myocardium.// Annual annals of Acad. Med. Sci. USSR. – 1986. – M 6. – p.37-43.

Brustovetsky N.N., Maevsky E.I. The influence of perfluorocarbon compounds on mitochondria.// Perfluorocarbons in biology and medicine: Col. – Pushchino, 1980. – p.81-85.

Cligunenko E.N., Cotlyarov V.S., Doronin A.G. Morphological changes in the cells of brain while treating clinical cranial trauma with the use of "Perftoran" preparation.// The Physiological Activity of Fluorine-Containing Compounds, Pushchino, 1995, p.p. 153-163.

Cligunenko E.N. The preparation "Perftoran" and its efficiency in the prevention of brain hypoxia (by neurological deficiency in catamnesis).// The Physiological Activity of Fluorine- Containing Compounds, Pushchino, 1995, p.p. 176-183.

Golubev A.M., Beloyartsev F.F., Vasiliev A.E., Pokrovsky Y.E. The reaction of biological system during blood replacement with the perfluorocarbon emulsions.// Moscow – TEIS – 1993.

Hymes A.S., Safavian M.I., Gunter T.// J.Surg.Res. – 1971. – vol.11. – pp-191-197.

Ivanitsky G.R., Beloyartsev F.F. Re. the development of fundamental and applied research on Perfluorocarbons in biology and medicine. – USSR Acad.Sci. Preprint – 1983.

Ivanitsky G.R., Vorobyev S.I. Engineering of artificial blood substitutes with gas-carrying function based on the perfluorocarbon emulsion.// Active perfluorocarbon medium for medicine and biology (new aspects): Col. – Pushchino. – 1993. – p.5-33.

Ivanitsky G.R., Obraztsov V.V., Sklifas A.N., Sologub G.R. Perfluorochemicals and biological membranes: interaction and related problems// 11 th Intern Symp. Chem. – Berlin, 1985. – D-29.

Ivanitsky G.R., Vorobyev S.I. Anti-ischemic perfluorocarbon emulsions.// Intern. Congress of Artif. Blood. Canada. – 1991. – p.1132.

Ivanitsky G.R., Vorobyev S.I. Organisation of motile structures in blood flow – the basis of functioning perfluorocarbon "Artificial Blood" – Biophysics, v.41, № 1, 1996 pp. 189-201.

Ivanitsky G.R., Vorobyev S.I. The Physiological Activity of Fluorine-Containing Compounds, Pushchino, 1995, pp. 246.

Islamov B.I. Anti-ischemic myocardial protection by the perfluorocarbon emulsion: Theses. Doc. Med. S. – M., 1987.

Ivanov K.P. State of the art issues on respiratory blood function and pulmonary gas-exchange.// Physiological journal. – 1992. – № 78. – v.11 – p.11-26.

Kobrinsky E.M., Freydin A.A., Maevsky E.I., Lazarev A.V., Illarionov E.F., Chernokhvostov V.V., Gelfer C.M., Ovsyannikova S.V., Kokoz Y.M. Influence of proxanol on sodium and potassium channels in myocardium.// Perfluorocarbon gas-carrying media: Col. – Pushchino, 1984. – p.79-85.

Koval E.A., Maevsky E.I. Potassium permeability and stability of erythrocyte membranes during interaction with perfluorocarbons.// Perfluorocarbon in biology and medicine: Col. – Pushchino, 1980. – p.85-90.

Kokoz Y.M., Kobrinsky E.M., Freydin A.A., Islamov B.I., Maevsky E.I., Beloyartsev F.F., Ivanitsky G.R. The ef-

fect of gas-carrying perfluorocarbon emulsion (ionic transport, contractility and sensibility to mediators).// Dagestan Acad.Sci. USSR. – 1983. – v.270. – № 2 – p.459-461.

Kuznetsova I.N., Bezrukova A.G. The determination of particle size in the perfluorocarbon emulsion.// Chemical-pharmaceutical journal. – 1982. – № 11. – p.122-126.

Kuznetsova I.N., Tohman N.S. The spectrophotometrical method of determination of proxanol in water solutions and biological liquids.// Chemical-pharmaceutical journal. – 1982. – № 9. – p.287-292.

Kuznetsova I.N., Herbut K.A. Correction of values of physical-chemical homeostasis by infusion of the perfluorocarbon emulsion while treatment of hemorrhagic shock in dogs.// Hematology and transfusiology. – 1987. – № 7. – p.36-40.

Ladilov Y.V., Islamov B.I., Vorobyev S.I., Ivanitsky G.R. Anti-ischemic effect of the perfluorocarbon emulsion (FCE) on myocardium in dogs.// Bulletin of exper. biology and medicine. – 1991. – № 2 – p.139-142.

Ladilov Y.V., Islamov B.I., Vorobiev S.I. Influence of proxanol, surfactant, on ischemic myocardium.// Experimental and clinical pharmacology. – 1992. – vol.55. – p.23-25.

Ladilov Y.V., Islamov B.I., Vorobyev S.I., Ivanitsky G.R. Influence of various perfluorocarbon emulsion's doses on hemodynamics and contractility of ischemic myocardium.// Bulletin of exper. biology and medicine. – 1992. – № 6 – p.593-595.

Lutz J., Bauml M., Schulze H. Comparison of Fluosol-DA 35% with Fluosol-DA 20% in the circulation of the liver.// Proc. of the IVth Intern. Symp. of perfluorochemical blood substitute – Kyoto, 1978 – pp. 123-135.

Maevsky E.I. Biological effects of perfluorocarbons and proxanols. // Perfluorocarbons in biology and in medicine. – Col. – Pushchino, 1980. – p.76-81.

Maevsky E.I., Shibaev N.V., Illarionov E.F., Arkhipov V.V., Obraztsov V.V., Islamov B.I., Brustovetsky N.N., Sklifas A.N., Ponomarchuk V.V. Creation and medico-biological approbval of perfluorocarbon emulsions (PCE) – O_2 carrier. // Transplantology of organs and tissues: Col. – Tbilisi, 1982. – p.262-263.

Makarov K.N., Mirsabekian N.S., Snegirev V.F., Melnichenko B.A., Prokudin I.P., Maximov B.N., Yakhlakova O.M., Knunianz I.L. Synthesis and physico-chemical features of perfluoroalkyl– and 1,4-dialkylreplaced cyclohexans.// Perfluorocarbons in biology and medicine. Col. – Pushchino, 1980. – p.21-30

Miroshnikov A.I., Temnov A.V., Islamov B.I. et. al.// Perfluorocarbon gas-carrying media – Pushchino, 1984. p.152-156.

Moroz V.V., Krylov N.A., Ivanitsky G.R., Kaidash A.N., Onichshenko N.A., Simanov V.A., Vorobyev S.I. The use of Perftoran in clinical medicine.// Anesthesiology and Intensive Medicine – 1995, 6., p.12-17.

Obraztsov V.V., Shekhtman D.G., Sologub G.R., Beloyartsev F.F. Induction of microsomal cytochromes in rat's liver after intravenous infusion of perfluorocarbon emulsion.// Biochemistry. – 1985. – v.50. – № 7. – p.1220-1227.

Onishchenko N.A., Serniak P.S., Kalinin D.I., Kovalenko N.V., Chernobryvzev P.A., Luneva A.G., Vorobyev S.I. Treatment of allograft nephrectomy.// Authorized Certificate № 1827253 of 13.10.92.

Saeed M., Hartmann A., Bing R. Inhibition of vasoactive agents by the perfluorochemical emulsion.// Life Science. – 1987. – vol.40. – pp.1971-1979.

Shibaev N.V., Popov V.I., Allakhverdov B.L. Ultrastructural analysis of intracellular accumulation and elimination of PFOC.// Medical-biological aspects of the use of PFOC emulsions. – Pushchino. – 1983. – p.175-186.

Sklifas A.N., Shibaev N.V., Brustovetsky N.N., Maevsky E.I. Content of perfluorocarbons in organs of animals after blood replacement with PFOC emulsions.// Medico-biological aspects of the use of perfluorocarbons: Col. – Pushchino. – 1983. – p.90-95.

Smith C.M., Hebbel R.R., Tukey D.P., Glawson C.C., White J.G., Versellotti G.M. Pluronic F-68 reduced endothelial

adherence and improves the theology of liganded sickle erythrocytes.// Blood. – 1987, 69, 6, pp.1631-1636.

Treshchinsky A.M., Mishchuk I.I.// Anesthesiology and Intensive medicine. 1984, № 4, p.17-21.

Usenko L.V., Cligunenko E.N., Vorobyev S.I., Kolomiizev L.F., Stebelsky A.S. Several methods to prevent brain hypoxia and their role in therapy of neurological disorders in patients with severe cranial trauma.// Clinical aspects of posthypoxic encephalopathy: Col.– 1992. – p.66-68.

Usenko L.V. To the background of creation and use of gas-carrying transfusion media.// The Physiological Activity of Fluorine-Containing Compounds, Pushchino, 1995, p.p. 167-173.

Usenko L.V., Cligunenko E.N. Outlooks and possibilities for the use of "Perftoran" preparation in a complex treatment of critical states.// The physiological Activity of Fluorine-Containing Compounds, Pushchino, 1995, p.p. 173-176.

Usenko L.V., Cligunenko E.N. Doronin A.G. Bacrground of the use of "Perftoran" preparation in treatment of severe cranial trauma.// Active perfluorocarbon media for medicine and biology (new aspects), Pushchino, 1993, p.p. 47-63.

Usenko L.V., Petrenko V.G. "Perftoran" preparation in intensive therapy of a syndrome of erythrocyte membranepathia in patients with acute severe cranial trauma. // Physical chemical and clinical investigations of perfluorocarbon compounds, Pushchino, 1994, p.p. 76-95.

Usenko L.V., Cligunenko E.N. Mechanisms of adaptation while Perftoran treatment severe cranial trauma. // Physical chemical and clinical investigations of perfluorocarbon compounds, Pushchino, 1994, p.p. 95-116.

Varushenko R.M., Bulgakova L.L., Druzhinina A.I., Mirsabekiants N.S., Makarov K.N. Steam pressure and entalpia evaporation of several perfluorocarbons and their nitrogen– and oxygen derivatives.// Perfluorocarbons in biology and medicine: Col. – Pushchino, 1980. – p.30-34.

Vorobyev S.I., Ivanitsky G.R., Ladilov Y.V., Obraztscov V.V., Sklifas A.N., Ponomarchuk V.V., Onishchenko N.A. Modification of cell membranes by perfluorocarbons as the possible mechanism of reduction of ischemia in myocardium.// DAN USSR. – 1988. – 299. – 1. – p.228-230.

Vorobyev S.I., Ladilov Y.V., Obraztsov V.V., Ivanitsky G.R. Prophylactic use of the emulsion – a new method of anti-ischemic protection of myocardium.// Bulletin of exper. biology and medicine. – 1990. – № 7. – p.19-21.

Vorobyev S.I., Vasiliev A.E., Islamov B.I., Kravchenko V.A. Evaluation of possible use of the perfluorocarbon emulsion for normotermal perfusion of removed heart.// Pathophysiology and exper. therapy. – 1991. № 6. – p.34-36.

Vorobyev S.I., Ladilov Y.V., Popov L.A., Baum O.V., Kulikova L.A., Sidorenko V.G., Shkvorchenko G.O, Ivanitsky G.R. Influence of chemically inactive compounds (contained in emulsion) on amplitude of cardiac contractility.// Annual annals of Acad. Med. Sci. USSR. – 1991. – № 6. – p.61-64.

Vorobyev S.I., Ivanitsky G.R., Makarov K.N., Moroz V.V., Kutyshenko V.P. The perfluorocarbon emulsions stabilized by non-ionic block-co-polymers.// Active perfluorocarbon media for medicine and biology (new aspects): Col. – Pushchino. – 1993. – p.33-47.

Vorobyev S.I., Kuznetsov A.I., Obraztsov V.V., Ponomarchuk V.V., Moiseeva A.A., Kulikova L.A., Goryushkina V.N., Kravchenko V.V., Simanov V.A., Moroz V.V., Ivanitsky G.R. The effect of non-ionic superficial active agent (proxanol) on erythrocyte resistance.// Active perfluorocarbon media for medicine and biology (new aspects): Col. – Pushchino. – 1993. – p.225-230.

Vorobyev S.I., Starovoitova L.N., Senina R.Y., Moiseeva A.A., Kulakova G.M., Maevsky E.I., Yuryev N.V. To the discussion of technology of submicronic emulsions.// Physicalchemical and clinical investigations of perfluorocarbon compounds: Col. – Pushchino. – 1994. – p.24-33.

Vorobyev S.I., Islamov B.I., Ladilov Y.V. The possible use of the perfluorocarbon emulsions for long-duration storage of donor's heart.// Chest and cardiac surgery. – 1990. № 4. – p.38-41.

RUSSIAN ACADEMY OF SCIENCES

"PERFTORAN" SCIENTIFIC-PRODUCTION COMPANY

PERFTORAN

BLOOD SUBSTITUTE WITH GAS-TRANSPORTING FUNCTION