Oxygen-Dependent and Oxygen-Independent Effects of Perftoran

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Summary. A brief review of the main properties of the low-concentrated emulsion Perftoran which contains perfluorodecalin and perfluoromethylcyclohexylpiperidine rapidly and slowly eliminated from the organism respectively. Perftoran carries an expected small volume of oxygen, but improves the oxygen regime in tissues and organisms due to kinetic acceleration of the oxygen flow and facilitates functions of the remaining erythrocytes. Activation of Adenosine triphosphate (ATP) synthesis in rat liver mitochondria after massive blood replacement and an improvement of the energy state and the survival of kidney grafts isolated during a severe hemorrhagic shock served as good indexes of the sufficient oxygen delivery by Perftoran. It has relatively low reactogenicity of Perftoran due to a small average particle size of emulsion of about 0.07 μm. The allowed threshold in particles size is smaller than 0.14 μm for Perftoran. The main oxygen-independent properties of Perftoran are the following: a decrease in rigidity of heart muscle during cardioplegia due to a reversible inhibition of Ca-current and a decrease in its sensitivity to epinephrine; immunosuppressive effect is connected with the inhibition of hyperactivated macrophages and primed neutrophils; de novo synthesis of the phenobarbital isoforms of P450 in liver is induced by perfluorodecalin.

Key words. Perfluorochemicals, Energy metabolism, Cardioplegia, Cytochrome P450

It was a great honor to present a paper devoted to Perftoran in Japan, where the pioneer studies in creating the first commercial standard perfluoro-

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chemial (PFC) emulsions Fluosol 43 and Fluosol DA were done. This brief review elucidates some oxygen-dependent and oxygen-independent properties of Perftoran that is a low-concentrated emulsion of two types of PFCs: rapidly and slowly eliminated from the organism perfluorodecalin (PFD) and perfluoro-methylcyclohexylpiperidine (PFMCP). Perftoran contains 10 volume percent of PFCs emulsion stabilized by surfactant Proxanol which is similar to Pluronic F68. Water-salt solution in Perftoran is biocompatible and does not contain any plasma expander. An important peculiarity of Perftoran emulsion is a small average particles size of about 0.07 μm.

Perftoran capability to transfer oxygen is a trivial broadly described characteristics of PFC emulsion [1] but the oxygen-independent properties are due to unexpected biomedical effects connected with a modification of the cells and tissues functions after their biophysical relationships with PFC emulsion [2].

Oxygen-Dependent Properties of Perftoran

The main question arises as to whether the low-concentrated PFC emulsion can improve oxygen delivery. The biomedical and clinical studies such as the experiments on perfusion of the isolated heart, heart-lung complex and kidneys, partial and subtotal blood replacement in rats; substitution of a massive blood loss, treatment of hemorrhagic and traumatic shocks and heart infarcts have shown that Perftoran can maintain an oxygen regime in the organism and isolated organs on the satisfactory level, better than traditional protein-salt or polymer solutions [3–6].

Perftoran has all the well-known features of PFC emulsion to carry oxygen described in detail recently by J.G. Riess [1], among them: additional oxygen capacity of PFC emulsion, accelerated diffusion rate of oxygen and carbon dioxide in PFCs, enlarged diffusion surface for gas transfer, increased oxygen and carbon dioxide gradients between erythrocytes and tissues, and finally PFC particles can go through the occlusive and spastic vessels impermeable for erythrocytes because their size is 70 times larger than that of emulsion particles. In direct experiments [7] it was shown that the rate of oxygenation and deoxygenation in a mixture of blood with PFC emulsion was significantly higher than in the mixture of blood and water-salt solution. It is also necessary to take into account that PFD, one of the components of Perftoran, dissolves in the phospholipids of erythrocyte membranes [8]. This phenomenon is accompanied with an increase in lateral mobility of lipids in membranes and makes erythrocytes more elastic, improves their mechanical and chemical resistance.

Here we would like to emphasize that in most cases after a massive blood loss and hemodilution, the oxygen capacity of the remaining red blood cells

(RBC) is sufficient to transfer the necessary amount of oxygen. The problem lies not in the complete replacement of the lost RBC but in facilitating oxygen delivery to tissues together with the remaining RBC. Under these conditions oxygen delivery suffers from damaged kinetic characteristics and high affinity of Hb to O_2 in RBC, their increased rigidity and fusion, and also because of vasoconstriction which blockades the RBC penetration into the capillary blood stream. Due to its kinetics, diffusion and particle size advantages, Perftoran improved oxygen delivery from lungs to peripheric tissues together with the remaining RBCs. Apparently PFC droplets and red blood cells form the rapid and reversible gas-carrying conveyer.

To estimate oxygen-carrying efficiency of Perftoran we determined as the indexes of the sufficient oxygen delivery the functions of rat liver mitochondria (RLM) after massive blood replacement and the survival of kidney grafts isolated during severe hemorrhagic shock in dogs.

Saving of Oxidative Phosphorylation in RLM is Good Evidence of Perftoran Efficacy at Massive Blood Replacement

Changes in mitochondrial functions during hypoxia and ischemia play a crucial role in cell survival and in maintaining of tissue functions. After ischemia mitochondria lose respiratory control and decline adenosine triphosphate (ATP) synthesis [9] while after hypoxia they have an increased oxidative phosphorylation rate [10]. In our experiments [11] under promedol and ether anesthesia, the isovolemic blood replacement was carried out on male Wistar rats with Perftoran (supplemented by albumin to 3%) and with mixture of 3% albumin and salt solution, or with autoblood in control animals. During and after operation animals were placed in the chamber with high PO_2 of about 550–600 mm Hg. The hemoglobin content in rat blood decreased from 16.0 ± 1.4 to 5.0 ± 0.6, arterial PO_2 after 2 and 6 h reached 500 ± 12 and 480 ± 10 respectively. Venous pH before blood replacement (during anesthesia) was 7.22 ± 0.04 and after blood replacement with Perftoran it was 7.20 ± 0.04 and 7.15 ± 0.04 within 2 and 6 h respectively, while salt-albumin solution did not maintain venous pH higher than 7.10–7.12. RLM were isolated 6 h later after blood replacement and respiratory parameters were measured in thermostatic 1 ml cell with a Clark-type oxygen electrode [9,11].

Most animals survived in both groups almost identically (in the parallel experiments): 19 from 20 in the Perftoran group and 18 from 20 in the albumin group. But the price of life was different: 6 h after the blood replacement with salt-albumin solution the isolated RLM could not phosphorylate ATP. It was a symptom of deep ischemia. In contrast, after the blood replacement with Perftoran RLM phosphorylated ATP more actively than organelles

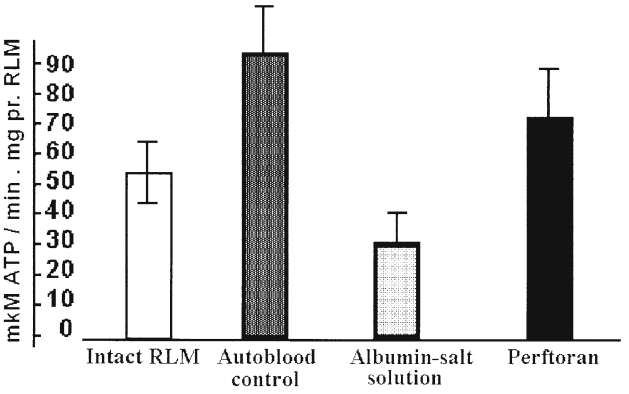

FIG. 1. Changes in the phosphorylation rate of ATP in rat liver mitochondria (RLM) isolated 6 h later after 70% blood replacement with autoblood or albumin-salt solution, or Perftoran together with 3 % albumin

from the intact animals (Fig. 1). Such activation of oxidative phosphorylation in RLM is a good evidence of Perftoran efficiency and of the fact that the organ has undergone only acute hypoxia but not ischemia.

Survival of Dog Kidney Grafts Transplanted After Hemorrhagic Shock Is Evidence of Perftoran Efficacy [12]

Hemorrhagic shock was induced by acute blood loss of about 35 ml/kg until the arterial blood pressure decreased from 150/70 to 50/30 mm Hg, the kidney blood flow dropped from 2000 ± 120 ml/min kg to 800 ± 60 ml/min kg. One hour later the bleeding dogs were treated with infusions of dextran 60 or Perftoran in the volume of about 40 ml/kg together with breathing of oxygen-air mixture and injections of furosemid (to 1.5–2 mg/kg), droperidol (0.03–0.05 mg/kg), heparin (25000 U). After 2 h hemorrhagic shock kidneys were isolated. Survival of kidney grafts isolated from the "hemorrhagic" animals treated with Perftoran and transplanted into the dogs that were previously undergone nephtoectomy was much better than that with dextran. Perftoran in contrast to dextran enabled to keep ATP/ADP and lactate/pyruvate ratios in the dog kidney tissue (Fig. 2), prevented an increase in creatine and urea levels in the recipient blood, and prolonged kidney grafts life spans four- to five-fold.

Oxygen Delivery Is Impossible if There Is Anaphylactic Side Reaction

The obtained improvement of oxygen transfer would be impossible if Perftoran were an anaphylactic drug because of the anaphylactic reactions are accompanied by damages in microcirculation, by a drop of arterial pressure

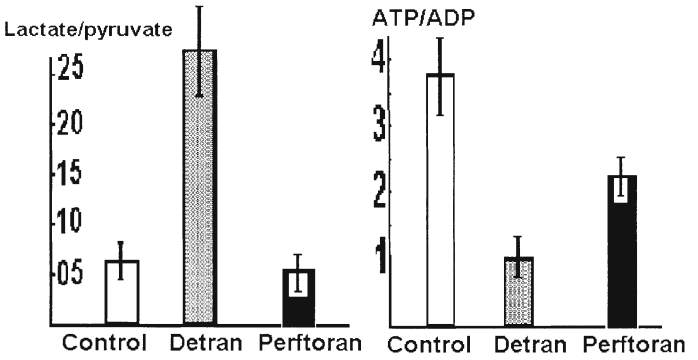

FIG. 2. Maintenance of ATP/ADP and lactate/pyruvate ratios in the dog kidney tissue isolated from the "hemorrhagic" dogs treated with Dextran 60 or Perftoran. *Control*, before blood losses. *Detran* and *Perftoran*, 2 h after blood losses and 1 h after infusions of Detran 60 or Perftoran

and by decrease in oxygen tension. A conventional point of view attributed these reactions of PFC emulsion to the presence of surfactant Pluronic F68 [13]. If the probability of anaphylactic side reactions is high, there is no other way but to exclude the use of such PFC emulsions. But the problem proves to be more complicated. Pyatovskaya [14] found that the neutropenic index which reflected reactogenicity was as a rule low after injection of Perftoran if the latter had particles with the size smaller than 0.1 μm after a short period of storage. Enlargement of the particle size owing to deviation in technology, temperature regime and storage time, conditions of transportation, and defrosting enhanced the danger of anaphylactic reactions development. The allowable threshold in particles size is of about 0.14 μm for Perftoran. Therefore the actual technology accepted in 1996 is focused on the average size of particles to be in the range of 0.07–0.08 μm, and it is prohibited to have particles with the size more than 0.16–0.18 μm. Thanks to that the average frequency of reactions decreased from 7% before 1996 (revealed on 912 patients) to 2 % (on 1824 patients).

Some Oxygen-Independent Properties of Perftoran

Relaxation and Preventing of Ca Overload Within Cardioplegia

Perftoran significantly increased the relaxation of heart muscle during cardioplegia [15–18]: ischemic contracture was much lower in the presence of PFC emulsion or proxanol solution in comparison with the usual hyperpotassium solution (Fig. 3). This phenomenon is due to the reversible inhibition of Ca-current through the cell membrane and a decrease in sensitivity

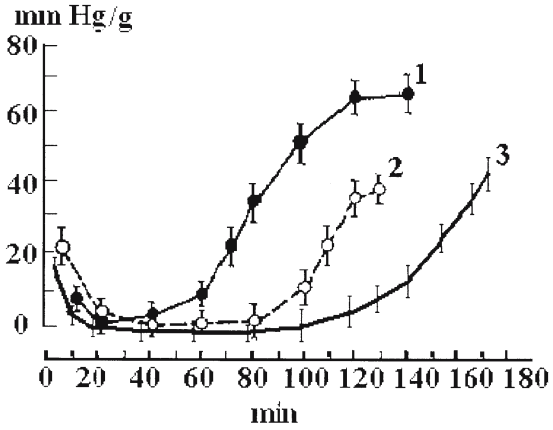

FIG. 3. Changes in the rest tensions of rat heart during cardioplegia at 17°C. *1*, hyperpotassium solution (71.8 mM NaCl, 29 mM KCl, 2.1 mM $MgCl_2$, 15 mM $NaHCO_3$, 1.4 mM NaH_2PO_4, 44 mM Mannitol, 28 mM Glucose, 1.2 mM Ca gluconate); *2*, hyperpotassium solution with 3% proxanol (surfactant from Perftoran); *3*, hyperpotassium solution with Perftoran. Rest tension in mm Hg per g heart weight

of Ca-channels to epinephrine caused by Perftoran independently on its saturation by oxygen. These mechanisms may be also responsible for diminishing of ischemic and reperfusion damages and for alleviating of resuscitation of heart electric activity and contractility after cardioplegia.

Immunosuppressive Effect of Perftoran

The immunosuppressive effect of Perftoran is apparently connected with the inhibition of hyperactivated macrophages and primed neutrophils after sorption and phagocytosis of emulsion droplets by these cells. In vitro, Obraszov et al. [19] showed that Perftoran inhibited the exaggerated production of reactive oxygen metabolites in neutrophils in responce to activating stimuli. In vivo we tested Perftoran inhibition of hyperactivated macrophages on the model of mice infected by retrovirus of Rausher erythroblast leukosis (study was performed by V. Buhman). The hyperactivation macrophages and production of virus particles by macrophages are accompanied by splenomegaly and formation of a great number of fused erythroblasts colonies in the spleen of infected mice. Intravenous or intraabdominal Perftoran infusions decreased the splenomegaly half and the formation of fusion colonies three- to five-fold. These results may bring us closer to the understanding why Perftoran can also have an antiinflammatory effect and decrease the frequency of graft rejections after organ transplantations.

Perftoran Infusion Induces De Novo Synthesis of Cytochrome P450 in Liver

Perftoran infusion induces de novo synthesis of cytochrome P450 in liver because of the substrate-enzyme complex formation between perfluorodecalin and cytochrome P450 [20,21]. Immune electrophoresis and substrate affinity evidenced that Perftoran induced noncancerogenic phenobarbital isoforms of P450. That induction resulted in accelerating xenobiotics hydroxy-

lation, shortening phenobarbital sleep, inhibition of lipid peroxydation, and stimulation of the second phase of detoxycation. The duration of P450 induction is almost equal to the half-retention time of perfluorodecalin in liver.

Influence of Long Retention of PFMCP on the Tissues

A great number of investigators did not find any pathologic damage in the organs that accumulated PFMCP, which has the half-retention time of 90 days. Vasil'ev and Golubev [22] observed evaluation of histological picture of tissues which accumulated PFMCP. They found specific granulomas formed by macrophages containing PFCs. The granulomas went through several stages of development from macrophages' to epithelioids' and then to lymphatics' granulomas and after that disappeared without any trace. But parenchymal cells surrounding granulomas acquired an increased regeneration potential. Perhaps thanks to that Perftoran inhibited the connective tissue growth during hepatitis and cirrhosis [23].

Summing up, it would be necessary to emphasize that Perftoran carried the expected small volume of oxygen, but significantly improved the oxygen regime due to kinetic acceleration of the oxygen flow facilitating erythrocytes functions. Modification of some enzymes and membranes in the presence of Perftoran in the blood and PFCs in the tissues endows Perftoran with the unexpected properties which can determine its new field of applications in experimental and clinical practice.

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