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PERFLUOROCARBONS: CHEMICALLY INERT BUT BIOLOGICALLY ACTIVE?

U. GROSS and St. RÜDIGER

Central Institute of Inorganic Chemistry,
O-1199 Berlin, Rudower Chaussee 5, (F.R.G.)

H. REICHELT

Academy of Military Medicine, Institute of Clinical Chemistry,
O-1242 Bad Saarow, (F.R.G.)

SUMMARY

One of the most striking properties of perfluorocarbons is their chemical inertness. Due to this, these compounds are said to be physiobiologically compatible. Therefore, they are suggested for a variety of medical uses including the one as component of blood substitutes. However, it was the blood substitute research, which revealed biological activities of perfluorocarbons. Some of these biological effects are described shortly.

INTRODUCTION

The early development of perfluoroorganic compounds ('perfluorocarbons, PFCs') was due to the urgent need for chemically highly inert liquids [1]. Perfluorocarbons, i.e. compounds composed only from C and F and some organic perfluorides containing also N and/or O atoms, and without having multiple bonds, proved to be very stable under different conditions at a variety of applications. This is effected by the high stability of the C-F bond and the shielding of the

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molecular skeleton by the closely packed F atoms. The chemical stability of PFCs and the fact that mammals do not have enzymes to split the C-F bond should likewise result in a high physiological inertness. This was first proved by Clark and Gollan [2] with their impressive experiment of 'mouse as fish', i.e. a mouse breathing oxygen-saturated pure liquid PFC. As a consequence of their physiological compatibility and high gas solubility, numerous biological and medical applications of PFCs have been developed, with the use of perfluorocarbon emulsions for blood substitution being the most exciting one [3]. Since blood substitutes would probably be used in quantities up to several litres per patient, the whole composition and all of its constituents have to be tested thoroughly in high concentration.

EXPERIMENTAL

Perfluorocarbon emulsions were prepared on the basis of F-dibutylmethylamine and perfluorodecalin (PFD), F-cyclohexylmethylmorpholine and PFD (E 47), F-cyclohexyloxyethylmorpholine and PFD, F-benzodioxan ethyl ether, and bis(F-butyl)-ethene (F-44 E). Additionally, the Russian perftoran, an emulsion from F-methylcyclohexylpiperidine and PFD, was tested. The emulsions were obtained from a mixture of the fluorocarbons, EO-PO-block copolymer emulsifier and distilled water by a homogenization process at high pressure up to 700 atm or sonication at an energy density up to 300 W/cm^2 . The particle diameter were in the range of 150 - 250 nm.
Animal experiments and the resulting physiological and biochemical parameters (Table 1) are only presented for the 20 % w/v E 47 (14 % PFD, 6 % F-cyclohexylmethylmorpholine, 2.5 % emulsifier). The blood substitute contained an additive solution with human albumin as the oncotic agent, electrolytes, buffer and glucose in the well-known physiological concentration.
The other above mentioned fluorocarbon emulsions gave in animal blood substitution experiments not identical but in general comparable results to that of E 47.

TABLE 1

Changes of selected biochemical parameters after exchanging blood for the blood substitute BS 47 in rat (n = 12)

Time of taking blood samples	Initial	Post-perfusion	2 h after perfusion	6 h after perfusion	8 h after perfusion
Haematocrit (%)	40.5±1.0	19.5±2.5	21.0±1.2	21.5±1.3	22.6±1.3
Fluorocrit (%)	0	8	8	7	6
colloid osmotic pressure at 20°C (kPa)	3.03±0.06	3.56±0.42	2.51±0.25	2.99±0.53	3.01±0.28
viscosity of blood plasma (mPa s)	2.3±0.06	1.09±0.07	1.11±0.07	1.08±0.07	1.04±0.06
Lactate (mmol/l)	2.64±0.13	2.45±0.76	2.18±0.25	8.49±0.28	9.84±0.72
ATP (mmol/l)	0.352±0.018	0.278±0.026	0.384±0.017	0.215±0.056	0.143±0.094
Phosphate (mmol/l)	2.45±0.21	2.51±0.26	2.81±0.36	3.85±0.92	5.98±0.95
Na^+ (mmol/l)	140.5±1.3	144±1.4	144.4±0.7	145.4±3.2	144.6±3.6
K^+ (mmol/l)	3.47±0.27	3.45±0.29	3.93±0.28	4.16±0.3	6.95±1.76
Ca^{++} (mmol/l)	2.35±0.24	2.38±0.2	2.42±0.2	2.41±0.2	2.43±0.2
pH	7.44±0.0	7.46±0.22	7.56±0.07	7.22±0.03	7.10 ±0.11
PO_2 (kPa)	12.2±0.6	55.6±5.8	53.9±3.3	49.1±2.1	51.1±4.6
pCO_2 (kPa)	3.73±0.21	3.97±0.73	3.37±0.06	3.20±0.14	2.94±0.68
HCO_3^- (mmol/l)	25.4±2.4	24.3±2.8	25.6±1.6	21.6±2.8	15.4±3.9
Base excess	0.4	-0.5	0.5	-3.5	-10.1

Animal experiments

Isovolaemic blood exchange was performed on nonanaesthetized conscious rats of 230 - 260 g body weight. The exchange rate was 2 ml/5 minutes carried out via jugular venous and right carotial artery catheters. Animals were catheterized under hexobarbital narcosis the day before. From the beginning of blood exchange animals breathed oxygen-enriched air in a box (FiO_2 = 0.8). In the tests reported here the blood exchange was stopped at a haematocrit of 0.24 - 0.20, but in principle it can be performed to 0.01 - 0.02 ('bloodless' rat).

RESULTS AND DISCUSSION

In course of our research programme for the development of storage stable perfluorocarbon (PFC) emulsion for medical use, an emulsion based on perfluorodecalin and perfluorocyclohexylmethylmorpholine, emulsified by ultrasound with emulgator Prohalyt P 24 (home made EO-PO-block polymer like Pluronic F 68) proved to be very stable at room temperature.
A thorough biological-medical investigation in rats of a blood substitute (BS) based on the mentioned PFCs (E 47) revealed an unfortunate phenomenon: Several hours after massive exchange of blood against the blood substitute in conscious rats, at increased oxygen partial pressure (due to breathing oxygen enriched gas) in the intravasal BS-blood mixture, we observed a derangement of oxygen utilization in the tissue (hypoxia), an accumulation of substrate hydrogen from the metabolism, and a decrease of energy formation. These results can be discussed in terms of a disturbation of the mitochondrial respiratory chain in which the hydrogen oxidation is catalyzed by the cytochromes.
This phenomenon does not depend on specific structural types of PFCs, since it was similarly observed with emulsions of perfluorocyclohexyloxyethylmorpholine and F-decalin, of F-44 E (kindly supplied by J.G. Riess), of perfluoromethylcyclohexylpiperidine and F-decalin, and of others too, respectively. It does not result from certain impurities in the PFCs, as investigations with F-cyclohexylmethylmorpholine of graduated purity

showed, neither from the Prohalyt P 24, as proved by separate experiments with rats.

Finally, it was tested, whether the disturbance of energy generation was reversible or not. Therefore, the blood substitute was reexchanged for autologous or homologous erythrocytes after characteristic biochemical changes had occured. However, the pathological process which got started once, continued and couldn't be influenced by this procedure. This seems to be an evident indication of the fact that the pathological process has no intravascular origin.
Histological findings were almost the same in all blood substitute experiments. Besides the well-known findings of PFC accumulation mainly in liver, spleen and lung tissue, signs of tissue hypoxia (tissue ischaemia) are most important. They are regarded as cause of death from the pathological aspect.
The observed, PFC-induced obstruction of the formation of energy can be discussed in terms of the following mechanism: The normal, undisturbed process of mitochondric energy (ATP) production results in the formation of protons, due to the redox-activity of the cytochromes, which are released into the cytosol of the cells. As a consequence, a proton gradient is built up at the membrane, the electrochemical potential of which yields the energy needed by the proton transporting ATPase for the synthesis of ATP. This process, known as chemical-osmotic coupling, depends on the impermeability of the membrane for H^+ and OH^- ions.
It can be taken for sure that PFCs, especially the most lipophilic perfluorodecalin and perfluorooctylbromide, can be intercalated or dissolved into mitochondrial membranes [4], where the hydrophobic part of the ATPase is located. This way interactions with ATPase are very likely. ATPase is responsible for the formation of proton channels. It is known [5] that interactions with dissociated ionophores as well as with lipophilic nondissociated decouplers and inhibitors cause a disordered permeability of the mitochondrial membranes for protons. Then the electrochemical potential decreases. Mitochondrial respiration and oxydative phosphorylation become decoupled. Biochemically, this process was characterized by

decreasing ATP, increasing ADP, phosphate, potassium and substrate protons, shifting of the NAD/NADH balance towards NADH, and increasing of lactate concentration in spite of normal oxygen concentrations in blood and tissue.
According to this mechanism, PFCs which differ in their solubility into mitochondric membranes should cause different biological effects. There are already some reports referring to adverse biological effects of perfluorocarbon emulsions. Lowe, who has summarized most of them, has emphasized the possible responsibility of the surfactant used (Pluronic F 68) or of its impurities [6]. On the contrary, it was shown by Obraztsov et al. [7] and independently by others [8] that neat perfluorocarbons can induce cytochrome P-450 formation, with perfluorodecalin (PFD) having great inductory activity whereas perfluorotributylamine has none. Likewise, PFD induces the formation of NADPH-cytochrome reductase [9]. These authors have found further on that PFD emulsions in concentrations above 1.3 g PFD per kg are lethal to rabbits. However, if a perfluoroamine emulsion (e.g. perfluoromethylcyclohexylpiperidine) is administered immediately after the PFD emulsion, the rabbits survive [10]. Obviously, the differing lipid solubilities of PFCs, as indicated by their CST-values in n-hexane, cause a state of dissolution, where the highly soluble PFD is re-extracted from the membrane by the lipid insoluble F-amines

Besides these effects, which can be traced back to PFC lipid solubility, there are other possible interactions to be taken into considerations explaining biological changes:

- tissue damage due to hyperbaric oxygen
- corpuscular interactions of PFC emulsion droplets with phagocytes and cells of RES
- PFC-surface interaction with proteins, lipids etc. causing conformational modifications and adsorption.

The consequences of all these findings and of the open questions for future applications of perfluorocarbons in medicine are: Firstly, to extend these studies and thereby look for perfluorocarbons which do not show such effects. Secondly, to limit the maximum dose of perfluorocarbon administered. And

thirdly, to make use of special biological effects, e.g. of the cytochrome P-450 induction in case of intoxication.

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