Effect of Perfluorodecalin on Viability of Ehrlich Ascites Tumor Cells under Conditions of Hypoxia

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Perfluorodecalin increased survival rate of Ehrlich ascites tumor cells under pathological conditions of hypoxia in combination with hyperkalemia. High potassium medium increased the content of lysophospholipids in samples, while in the presence of perfluorodecalin, phosphatidylethanolamine level decreased.

Key Words: *perfluorodecalin; Ehrlich ascites carcinoma; hypoxia*

Perfluorinated organic compounds (POC) are extremely chemically and metabolically stable [8] and are widely used in biology and medicine due to their high ability to dissolve different gases, *e.g.* CO_2 and O_2 [1]. Russian biophysicists showed that POC have biological activity independent of their gas transport properties [1]. In particular, lipophilic POC dissolved in cell plasma membranes and interacting with hydrophobic parts of polyenzyme complexes, modifying their functional activity. This manifested in modification of erythrocyte membranes, changes in activity of the first complex of the mitochondrial respiratory chain, phenobarbital-type induction of cytochrome P-450, and inhibition of the production of reactive oxygen species by activated leukocytes. In addition, POC and their emulsions have strong sorption properties [5]. However, until recently the physiological effects of POC were associated primarily with their gas-transporting properties and all other types of activity were considered secondary and unimportant.

Membrane damage during hypoxia accompanying many diseases of chemical etiology occurs due to energy and ATP deficit. This leads to changes in the energy-dependent ion transport. The overall ionic permeability of membranes is also modified because of changes in membrane surface charge and hydrophobicity degree of the membrane lipid phase. Both of these factors operate simultaneously, although their relative contribution to the resultant change in membrane permeability varies in different cases. These same factors determine, ultimately, nonspecific effect of various compounds such as steroids, proteins, *etc.* on membrane permeability.

Direct influence POC on biological systems can not be explained by only their gas-transporting ability.

Based on preclinical and clinical studies, perftoran not as blood substitute, but as a medicine, which has antihypoxic and antiischemic action, found increasing application in the wide range of pathological conditions [3]. Shift in the ratio of pro- and antioxidant systems determining the development of cell apoptosis and necrosis is a key elements of hypoxic and ischemic states. Controversial views exist regarding the impact of plethoric perftoran administration on the state of pro- and antioxidant systems of the organism.

POC exhibit affinity to phospholipids, essential components of cell membranes. This suggests the possibility of hydrophobic interaction of POC with the membrane followed by its conformational changes. This is confirmed by the effect of perfluorocarbons on bilayer lipid membranes, isolated mitochondria of erythrocytes, and microsomes. It is known that greater resistance of cells to transforming factors correlates

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with higher degree of hydrophobicity of their lipid spectrum. Therefore, we studied the possibility of increasing hydrophobicity of membrane lipids by incubation with perfluorocarbons and the effect of these changes on cell viability under stress conditions, particularly during hypoxia. Such an objective is relevant regarding manifold functions of perfluorocarbons.

Perfluorodecalin (PFD) was selected as a substance increasing hydrophobicity of membranes [4]. Fluorinated hydrocarbons can stabilize cells due to not only their gas-transporting properties [6], but also integration into the membrane [7] and compensation for excess of detergent lipid components, produced by cell at some pathologies including cancer. It is known that tumor cells contain higher amount of lipid hydrolysis products. Normally, lysoforms are also formed during cell activation, but these changes are compensated by re-acylation. Lysophospholipids are biomarkers of cancer [13], so membrane lysoform concentration may serve as a criterion of cell malignization. Accumulation of lipid hydrolysis products leads to depolarization of the cell membrane and increases the risk of metastases. Physicochemical properties of perfluorocarbons are opposite to those of detergent lipid derivatives, such as lysophospholipids, fatty acids, and lipoperoxide fragments. Highly hydrophobic perfluorocarbon molecules may act as agents compensating for excessive hydrophilicity of lipid derivatives in membranes of cancer cells. In these cases, membrane potential increases and the excitability of the cell declines.

It was assumed that under conditions of hypoxia cell death occurs due to accumulation of excess fatty acids and lysoforms. Combination of cell overexcitation with hypoxia leads to more pronounced accumulation of hydrophilic lipid products, causing an increase in membrane permeability. We studied PFD used in medicine and biology as a gas transporting medium, to improve the viability of cells under hypoxia.

MATERIALS AND METHODS

Ehrlich ascites tumor cells (EAC) were derived by [2]. Medium for cell isolation and incubation was Hanks' solution+20 mM HEPES, pH 7.2 at 2°C and pH 7.4 at 37°C, respectively. Exocytosis was induced by ionomycin, component 48/80, A23187, and ATP. Changes in cell shape and size were recorded by right-angle light scattering in the suspension (λ=620 nm). The efflux of potassium from the cells upon ATP stimulation was recorded using ion-selective potassium electrode with lower limit of sensitivity of 10^{-6} M (Nico-Analyte) placed in thermostatic fluoroplastic mixing chamber.

To simulate hypoxia, the incubation medium was purged with argon for 1 h and treated with sodium dithionite ($Na_2O_4S_2$) and sodium sulfite (NaS), which are efficient oxygen absorbers [10] often used to create hypoxia [9]. Due to rapid loss of oxygen-absorbing capacity in contact with water, solutions of dithionite and sodium sulfite (40 mg/ml) were prepared immediately prior to the introduction of an aliquot into the working cell. Oxygen content in the incubation medium was assessed using an oxygen electrode.

For thin-layer chromatography, 20 μl of 10% EDTA and 30% sodium metabisulfite were added to samples and shaken 50 times. Samples were centrifuged in the cold at 1500 rpm. An aliquot of isopropanol 2 ml in volume was added to the precipitate and shaken 50 times. Isopropanol extract was separated from the precipitate by centrifugation at 1500 rpm. Isopropanol in a volume of 2 ml was added again to the precipitate, and the procedure was repeated. EDTA, metabisulfite, and sodium sulfate were added to the resulting supernatant to remove the remaining water. The resulting samples were dried. The composition of phospholipids was determined by two-dimensional thin layer chromatography. Separation of lipids was carried out in the following solvent systems: chloroform:hexane 75:25 (v/v); chloroform:methanol:methyl acetate:water 53:34:9:4 (v/v), and carbon tetrachloride.

The dried plates were kept in concentrated hydrochloric acid for 40 min, and then in the thermostat at 70°C for 1 h, and stained with iodine. The plates were processed using TSH Manager soft. Statistical data processing was performed using Student's *t* test.

RESULTS

We studied possible stabilizing effect of PFD on tumor cells plasma membranes under hypoxic conditions. A suspension of EAC cells was placed in oxygen-free environment for 3 h. Membrane resistance to the action of the damaging agent was assessed by the ratio of cells permeable for trypan blue dye and the total number of cells. Oxygen concentration in the incubation medium during argon purging decreased, but cell death did not increase. The same result was obtained by placing the cells in the hyper-potassium medium with 25 mM and 50 mM KCl under low O_2. Deterioration of the cell functional state was recorded in the absence of glucose at three higher KCl concentrations in the medium: 73, 99, and 143 mM (total concentrations of Na^+ and K^+ remained constant). Cell death did not exceed 20%. These three concentrations combined with hypoxia were used to estimate PFD influence of on cell survival.

The percentage of survived cells increased in the presence of PFD under combination of hypoxia and high potassium medium (Table 1). The maximum effect was observed at a concentration of 143 mM KCl; cell death rate decreased by 3.4 times.

TABLE 1. Percentage of Cell Death Estimated by Trypan Blue Inclusion (incubation under hypoxic conditions at 37°C)

KCl concentration in cell incubation medium, mM	Incubation for 15 min, 37°C	Incubation for 3 h, 37°C	
	under argon	under argon	under argon in the presence of PFD
143	4±1	17±2	5±2
99	10±4	8±2	4±2
73	3±1	11±2	5±1

We analyzed the total lipid membrane composition of cells incubated for 3 h in media with 50, 99, and 143 mM KCl with a low content of O_2, in the presence and absence of PFD. It was shown that high potassium medium induces accumulation of lysophospholipids. In samples with PFD, the level of phosphatidylethanolamine decreased. The addition of PFD to the cell suspension did not lead to significant changes in the lysoform content.

The cell responds to different types of activation with lipid hydrolysis. We hypothesized that PFD added to the cells overexcited by high potassium medium will increase their viability.

Under severe hypoxia, *i.e.* in the presence of sodium dithionite causing the formation of peroxides, the time course of K^+ concentrations in the extracellular environment was recorded to test the excitation of EAC cells under the influence of ATP.

We investigated 4 variants of the effect of ATP (5 mM) on extracellular K^+ concentrations in EAC cell suspension:

- interaction of cell suspension (10^8 cells/ml) with ATP (control);
- interaction of cell suspension (10^8 cells/ml) with ATP 50 sec after pretreatment with 40 mg/ml sodium dithionite;
- interaction of cell suspension (10^8 cells/ml) with ATP after its pre-incubation with PFD (1.3% of the cuvette volume);
- ATP 50 sec after addition of 40 mg/ml sodium dithionite to the cell suspension against the background of pre-incubation with PFD (1.3% of the cuvette volume).

After incubation of the suspension in the presence of PFD, the increase in extracellular K^+ levels in response to ATP was not followed by its normalization within 8 minutes (Fig. 1). In the control, normalization of potassium levels occurred within 3 min (Fig. 1, *1*).

In response to ATP, return to normal extracellular potassium levels was suppressed after treatment with both sodium dithionite and PFD. However, pre-incubation of cell suspension with PFD abolished this effect (Fig. 1, *2*).

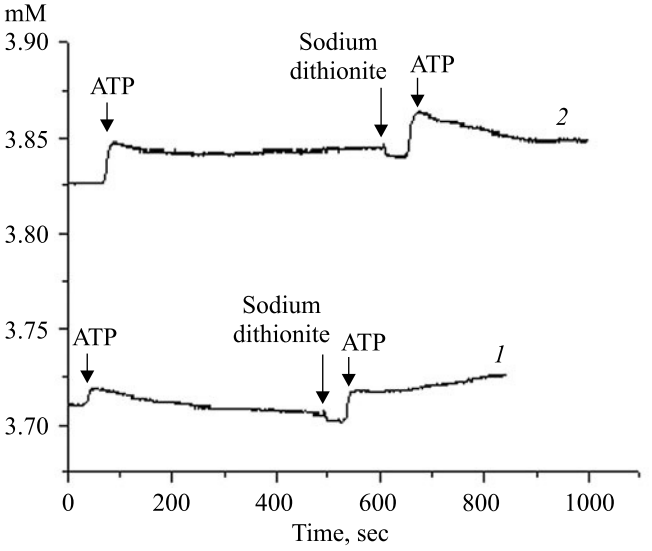

Fig. 1. Changes in K^+ concentration in the incubation medium during incubation with ATP and sodium dithionite in the control sample (*1*) and after PFD pretreatment (*2*).

Oxidative stress intensifies peroxidation of unsaturated fatty acids in the membrane. The capacity of sodium dithionite to induce oxidative stress in cells suggests that the effects of PFD and sodium dithionite might probably compensate each other. Sodium dithionite apparently simulates combination of hypoxia with oxidative stress typical of many pathologies. In this regard, PFD and other perfluorocarbons can be considered as promising preventive and therapeutic agents, which are able to compensate excessive cell membrane hydrophilization.

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