Blood-Protecting Effect of Perfluorodecalin

V. I. Skorik, A. V. Sudus, V. V. Shilov, E. B. Zhiburt, V. I. Vashchenko, and E. N. Rozhdestvenskaya

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The ability of perfluorodecalin to improve the properties of blood and protect it from harmful agents was assessed by evaluating the functional state of donor blood treated with this compound and then exposed to O_2 in a bubble (contact) oxygenator. The results of determining the acidic resistance of erythrocytes, serum levels of free hemoglobin, the amount of malonic dialdehyde in lipid extracts of erythrocyte membranes, erythrocyte deformability, and blood viscosity support the notion that organoperfluoric compounds are chemically and biologically inert. Pretreatment of blood with perfluorodecalin protects it from the adverse effects of contact oxygenation and improves its rheological properties.

Key Words: *perfluorodecalin; blood; oxygenation; blood protection*

The unique gas-transporting capacities of organoperfluoric (OPF) compounds are well known (they are capable of dissolving up to 50% of O_2 and up to 190% of CO_2 by volume [2]) and are exploited when attempts are made to produce artificial blood or to develop devices for extrapulmonary membrane gas exchange or other purposes [2-6,8,10,12,13].

Other properties of OPF compounds are less well known. Thus, there are just a few reports of the their ability to render erythrocytes more resistant to injurious chemical or physical agents [2,5,9,10,12], although erythrocyte stability is a problem of much concern in modern transfusion services [2,6,9,10,12,13].

The purpose of the present study was to evaluate the blood-protecting potential of one OPF compound, perfluorodecalin (PFD), by determining the functional state of blood after its treatment with this compound and subsequent exposure to O_2 in a bubble (contact) oxygenator.

MATERIALS AND METHODS

Samples of donor blood, either fresh or stored (with the preservative glugicir added in a 1:4 ratio to the blood), were used, divided into groups of nine samples in each: intact (control) blood (group 1); blood treated with PFD for 60 min in two chambers connected in tandem, one of which served to mix up the liquids on disks of a rotor and the other, to effect complete and reliable separation of the blood from PFD (group 2); blood through which O_2 was bubbled for 4 to 12 h in a contact oxygenator at a rate of 100 ml/min per 100 ml blood (group 3); and blood treated with PFD and then oxygenated as above (group 4).

The ability of PFD to improve the properties of blood and protect it from harmful agents was evaluated by measuring the acidic resistance of erythrocytes [7], the serum level of free hemoglobin (Hb) (in the reaction with azopyram using the conventional procedure), the amount of malonic dialdehyde (MDA) in lipid extracts of erythrocyte membranes as an index of lipid peroxidation [1], and erythrocyte deformability and blood viscosity [11]. The results were statistically analyzed by Student's t test using Statgraphics software.

RESULTS

The acidic resistance of erythrocytes reflects their ability to withstand the action of hydrochloric acid,

P. A. Kuprianov Clinic of Cardiovascular Surgery, Academy of Military Medicine, St. Petersburg

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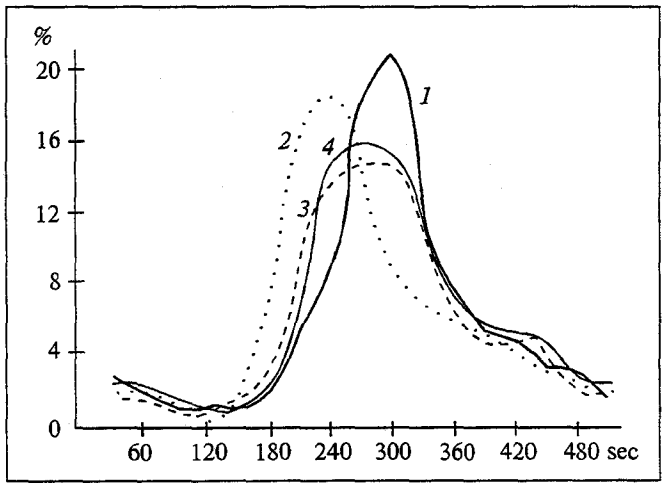

Fig. 1. Acidic erythrocyte resistance in intact blood (*1*), oxygenated blood (*2*), and perfluorodecalin-treated blood before (*3*) and after (*4*) its oxygenation.

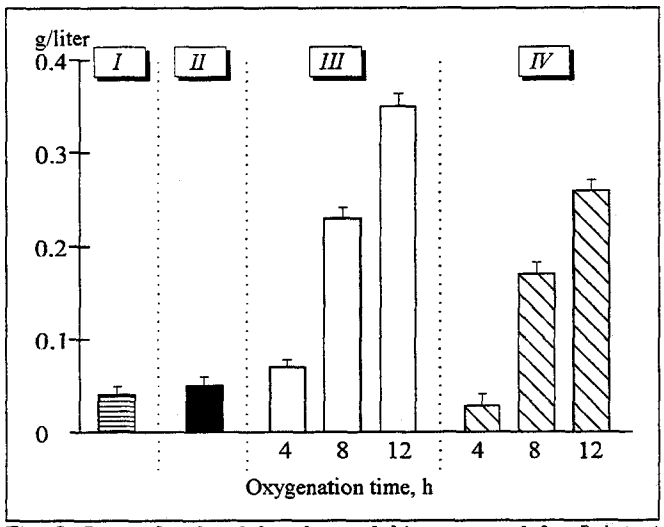

Fig. 2. Serum levels of free hemoglobin measured for *I*) intact blood; *II*) perfluorodecalin(PFD)–treated blood; *III*) oxygenated blood; *IV*) blood oxygenated after PFD treatment.

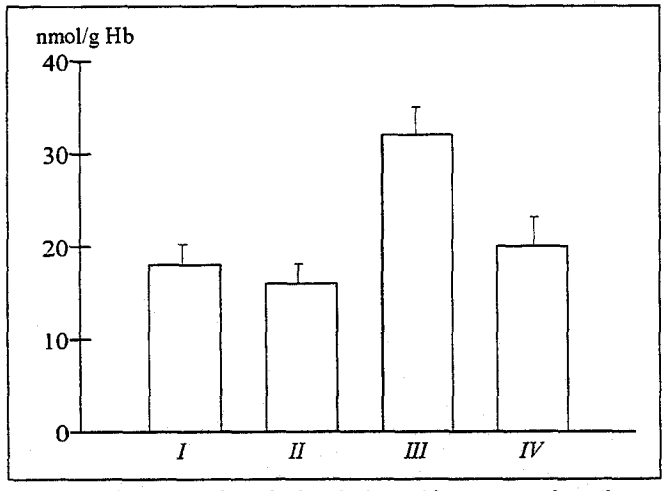

Fig. 3. Malonic dialdehyde levels in lipid extracts of erythrocyte membranes prepared from intact blood (*I*), perfluorodecalin(PFD)–treated blood (*II*), oxygenated blood (*III*), and blood oxygenated after PFD treatment.

which in a concentration of 0.004 M causes complete hemolysis over a period of time (normally for 8-10 min) rather than abruptly. The duration and peak of hemolysis together with the distribution of destroyed erythrocytes over particular time intervals characterize their functional state and degree of maturity.

In our study, erythrocytes in the intact and PFD-treated blood showed similar degrees of acidic resistance, the proportion of cells with medium resistance being significantly higher (60.1% and 55.7%, respectively) than those of weakly resistant (18.4% and 22.8%) and highly resistant (20.5% and 21.5%) cells. The damaging action of contact oxygenation was manifested in a significantly increased proportion (43.5%) of weakly resistant erythrocytes at the expense of a drop in the number of cells with medium resistance to 37.8%. The blood samples treated with PFD before oxygenation were found to contain weakly and medium-resistant erythrocytes in proportions found in intact blood (20% and 59.8%, respectively). The proportions of highly resistant erythrocytes in all blood samples (intact, oxygenated, and PFD-treated and then oxygenated) were similar (20.5%, 18.7%, and 20.2%, respectively).

Inspection of the hemolysis curves obtained in testing acidic erythrocyte resistance showed that the hemolysis of oxygenated blood peaked somewhat earlier (by the 240th second) than did the hemolysis of intact blood (by the 300th second), which is an indication of impaired blood stability after contact oxygenation. However, the rates at which the hemolysis of intact or oxygenated blood peaked and then declined were higher than in the case of PFD-treated blood or of blood oxygenated after its treatment with PFD. In the latter two cases, as shown in Fig. 1 (curves *3* and *4*), the percentage of maximum hemolysis is lower and the hemolysis curves are dome-shaped rather than peaky.

The treatment of intact blood with PFD for 60 min did not alter the plasma concentration of free Hb, which supports the notion that this compound is inert both chemically and biologically. However, the blood samples thus treated and then exposed to O_2 in the bubble oxygenator for different periods were found to have undergone significantly less hemolysis (by 53.4%, 20%, and 26.8% after 4, 8, and 12 h of oxygenation, respectively) than the PFD-untreated samples subjected to oxygenation (Fig. 2).

Measurements of erythrocyte deformability and blood viscosity demonstrated (Table 1) that the oxygenation had adversely affected the rheological properties of the blood (the erythrocytes became less deformable and the blood more viscous). PFD treatment was found to improve its rheological status. Thus, as shown in Table 1, erythrocyte deformabili-

TABLE 1. Rheological Properties of Intact (Group 1), PFD-Treated (Group 2), Oxygenated (Group 3), and PFD-Pretreated Oxygenated (Group 4) Blood Samples ($M\pm m$, n=9)

Rheological properties	Group of assayed blood			
	1	2	3	4
Erythrocyte deformability, rel. units	1.59±0.20	2.37±0.58	1.37±0.29	1.52±0.35
Blood viscosity, rel. units	1.97±0.08	1.64±0.25	2.05±0.14	1.66±0.18

ty was much higher in the PFD-treated blood samples (group 2) than in the samples of intact blood (group 1) and similar in the latter group and group 4 (PFD-pretreated oxygenated samples).

Very revealing results were yielded by measuring MDA levels in lipid extracts of erythrocyte membranes prepared from blood samples of the different groups (Fig. 3). The oxygenation increased lipid peroxidation through the direct action of O_2 on blood cells (the MDA concentration being 32.8±4.1 nmol/g Hb *vs.* 18.3±3.6 nmol/g Hb in the lipid extracts prepared from intact blood). PFD treatment not only lowered the MDA level to 16±2 nmol/g Hb ($p<0.01$) in the intact blood samples, but also inhibited its rise in the samples subjected to contact oxygenation (20.2± 3 nmol/g Hb *vs.* 32.8±4.1 nmol/g Hg recorded for the PFD-untreated oxygenated samples). These results show that PFD prevents the blood in direct contact with oxygen from the latter's prooxidant action. That this compound has a stabilizing effect on erythrocyte membranes has also been reported by other workers [5,6,9,12], although the precise mode of its protective action on the blood remains to be elucidated.

When the blood is exposed to an injurious agent, such stabilization can prevent the activation of a mechanism whereby intensive lipid peroxidation is initiated and erythrocyte membrane phospholipids undergo degradation.

In our view, the results of the present study and the evidence reported in the literature prove that PFD does protect the blood from damage. Considering its well-known chemical and biological inertness, this substance can be used to advantage as a means of protecting blood from the adverse effects of artificial circulation in open heart surgery, during long-lasting extrapulmonary blood oxygenation, and in other situations involving prolonged blood transfusion.

A sine qua non for the use of PFD to these ends is the assurance that this hemoprotector has been completely separated from the blood after its treatment. Previous attempts to achieve such separation all failed. The complete and reliable separation of the hemoprotector from blood accomplished in our studies has cleared the way for its clinical application. A description of how the separating device works is beyond the scope of this article and will be provided in our forthcoming publications.

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