Activation of Plasma Complement by Perfluorocarbon Artificial Blood: Probable Mechanism of Adverse Pulmonary Reactions in Treated Patients and Rationale for Corticosteroid Prophylaxis

By Gregory M. Vercellotti, Dale E. Hammerschmidt, Philip R. Craddock, and Harry S. Jacob

Perfluorocarbons have shown promise as clinical blood substitutes. Although early experience in Japan with one such product—Fluosol-DA—has been uncomplicated, we observed an adverse pulmonary reaction in the first American patient to receive it and know of similar reactions in two other Americans so treated. Postulating that activation of plasma complement (C) by the perfluorocarbon emulsion might have caused the reaction, we tested the product to determine if it is an activator of complement. Incubation of Fluosol with plasma led to C3 conversion, decrement in CH50, and generation of C5a-related PMN aggregating activity; EDTA prevented such activation, while EGTA did not, suggesting that it proceeded via the alternative C pathway. Infusion of Fluosol into rabbits produced hypoxemia, neutropenia, thrombocytopenia, and pulmonary leukostasis, mimicking abnormalities previously demonstrated in rabbits receiving infusions of zymosan-activated plasma C. These deleterious responses to Fluosol were diminished by predemediating rabbits with corticosteroids (which had seemed of benefit when used empirically in our patient). In vitro and in vivo, Fluosol’s effects were reproduced by Pluronic F-68, the nonionic detergent used to maintain the emulsion stability of Fluosol-DA. We conclude that adverse reactions to Fluosol are probably mediated by C activation and that steroid premedication may prevent them in susceptible patients.

There are many indications for a synthetic erythrocyte substitute that can both carry oxygen and expand plasma volume (A) in patients who refuse blood transfusions on religious grounds; (B) in those with acute hypovolemic shock in nonhospital environments; (C) in isolated organ perfusion; (D) in patients with rare blood types or other crossmatching difficulties. To date, the most promising erythrocyte substitutes are the perfluorochemicals; one such compound, Fluosol-DA (Green Cross Corp., Osaka, Japan), an emulsion of perfluorodecalin and perfluorotripropylamine (Table 1), has been found clinically safe and efficacious in Japan.2-4

We recently had the opportunity to observe the first patient treated in the United States with Fluosol-DA. In contrast to its previous uncomplicated use, our patient had an adverse pulmonary reaction when he received Fluosol; no reaction occurred with a subsequent infusion after premedication with corticosteroids in high dose. His pulmonary reaction was like those occurring with complement (C) activation; we therefore postulated that Fluosol might activate plasma C, stimulating granulocytes and leading in turn to pulmonary dysfunction, as we have reported in several other contexts.5-9 This hypothesis was then tested both in vitro and in experimental animals.

CASE REPORT

A 67-yr-old man with severe peripheral vascular disease underwent an arterial bypass graft for relief of claudication. He refused blood on religious grounds, but bled little perioperatively. However, he later returned to the hospital for treatment of cellulitis of his surgical wound. He fell, profuse bleeding from his wound ensued, and occasioned surgical intervention. His hemoglobin fell to 3.8 g/dl, and he became lethargic and disoriented. Because he refused transfusion, an attempt was made to deliver oxygen with Fluosol-DA (supplied by Dr. E. Levine, Alpha Therapeutics, Pasadena, Calif.) after consent of the family and of the Food and Drug Administration had been obtained. Thirty milliliters of Fluosol were given over 15 min while the patient breathed 100% O2; the infusion was stopped because of chest tightness, shortness of breath, and tachycardia. His pulse rate rose from 90 to 140/per min, while mean arterial pressure fell from 108 to 84 mm Hg. The mean pulmonary artery pressure rose from 28 to 43 mm Hg, while the pulmonary artery wedge pressure remained 18 mm Hg. The arterial PO2 fell from 195 mm Hg to 92 mm Hg (FIO2-100%). Fifty milligrams diphenhydramine and 2 g methylprednisolone were administered intravenously, with subsequent stabilization of vital signs. Because the patient became even more anemic and stuporous, another infusion of Fluosol was attempted the next day, but after empiric i.v. medication with 2 g methylprednisolone and 50 mg diphenhydramine. He tolerated this infusion without shortness of breath, chest pain, or change in hemodynamic indices or PaO2. Two liters of Fluosol were infused over 9 hr, which resulted in a 4 vol% concentration of Fluosol in the blood. Hypoxic symptoms abated following the infusion, the patient became alert, and was able to remember his age and the date, as well as recognize friends and relatives. His infection cleared with antibiotic therapy; his hemoglobin rose to 9 g/dl, no further bleeding occurred, and he was discharged.

MATERIALS AND METHODS

Fluosol-DA and its detergent component, Pluronic F-68, were supplied by Alpha Therapeutics. Fluosol-DA was added to mini-

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mally heparinized (2 U/ml) normal human plasma in a 1:30 (v/v) ratio to duplicate approximately the level achieved in the patient's plasma. After incubation for 1 hr (37°C), Fluosol was removed by centrifugation (12,000 g for 10 min at 4°C). As a positive control for complement activation, zymosan (5 mg/ml) was incubated in parallel with plasma and removed by centrifugation. The following evidence of complement activation was compared among Fluosol-incubated, zymosan-incubated, and saline-incubated (control) plasmas: (A) presence of C5a, as detected by granulocyte (PMN) aggregometry; (B) electroaphoretically demonstrable C3 conversion; (C) decrements in CH50.

To test the C dependence of PMN aggregating activity developing in Fluosol-incubated plasma. Fluosol was also incubated with plasma that had been decomplemented by heat (56°C for 30 min) or EDTA (10 mM) or with plasma in which the classical C pathway had been selectively blocked by 10 mM Mg-EGTA. In addition, Fluosol was incubated with plasma obtained from a patient with a severe congenital deficiency of C5.

To determine, if possible, which component of Fluosol-DA activated complement, fresh normal plasma was also incubated with each of the major components of the emulsion (Table 1): perfluorodecalin, perfluorotripropylamine, Pluronic F-68, yolk phospholipid, glycerol, and hydroxyethylstarch (Alpha Therapeutics); each was added to plasma in an amount equal to that provided in a 1:30 mix of Fluosol and plasma and was incubated as described above.

Fluosol-DA was infused intravenously into 3–4 kg New Zealand white rabbits at a rate of 1 ml/min for 30 min. Arterial blood was sampled for measurement of blood gases and blood counts (by standard techniques) immediately prior to infusion and at intervals for 1 hr. Control rabbits (4) received like volumes of physiologic saline. Three rabbits received infusions of Pluronic F-68 alone (2.7 vol% in saline), and 3 rabbits received 60 mg methylprednisolone (sodium succinate; Upjohn Company, Kalamazoo, Mich.) per kg body mass i.v. 15 min prior to Fluosol infusion. Lungs were removed from the rabbits near the end of the infusions, were prepared in routine fashion for light microscopy, and were examined for leukostasis.

Statistical significance of differences among groups was assessed by Student’s t test and by the Mann-Whitney U test.

**RESULTS**

Fluosol-incubated plasma, when added to a stirred suspension of granulocytes, provoked aggregation (Fig. 1) similar to that produced on addition of zymosan-activated plasma, a known source of C5a. Such aggregating activity failed to develop when Fluosol was incubated with plasma that had been decomple-

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**Table 1. Components of Fluosol-DA (T.M.)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Wt/v%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorodecalin</td>
<td>14.0</td>
</tr>
<tr>
<td>Perfluorotripropylamine</td>
<td>6.0</td>
</tr>
<tr>
<td>Pluronic F-68</td>
<td>2.7</td>
</tr>
<tr>
<td>Yolk phospholipids</td>
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</tr>
<tr>
<td>Glycerol</td>
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<tr>
<td>NaCl</td>
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</tr>
<tr>
<td>KCl</td>
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</tr>
<tr>
<td>MgCl2</td>
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</tr>
<tr>
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<tr>
<td>NaHCO3</td>
<td>0.210</td>
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<tr>
<td>Glucose</td>
<td>0.180</td>
</tr>
<tr>
<td>Hydroxyethylstarch</td>
<td>3.0</td>
</tr>
</tbody>
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**Figure 1. Generation of granulocyte aggregating activity in plasma by Fluosol.**

Fluosol-DA incubated plasma evidenced a 24% ± 5% (SEM) decrement in CH50 as well as immunoelectrophoretic evidence of C3 conversion. C3 conversion was not demonstrable in EDTA-treated plasma, but continued unabated in EGTA-treated plasma.

The component of Fluosol-DA responsible for C activation appears to be Pluronic F-68, the nonionic detergent used to maintain emulsion stability. On incubation with plasma, Pluronic—but no other component—engendered PMN aggregating activity, as well as C3 conversion and a >20% decrement in CH50 (not shown).
When Fluosol was infused into rabbits (Fig. 2), granulocyte counts fell by 65% ± 7% at 10 min, with a return toward baseline thereafter; this fall in granulocyte count was accompanied by a 38% ± 11.5% fall in platelet count and a 20% ± 8.6% decrement in PaO₂. As with in vitro C activation, this effect was mimicked by infusion of Pluronic alone, while saline infusion was without significant effect (not shown).

When lungs were removed from Fluosol-infused rabbits at the time of the nadir of the granulocyte count, small vessels were observed to be filled with granulocytes (Fig. 3, lower panel). Similar findings were present in the lungs of rabbits receiving Pluronic alone (not shown), while rabbits receiving saline had only occasional granulocytes in the pulmonary vessels (Fig. 3, upper panel).

Because methylprednisolone is known to block C5a-induced PMN aggregation, and because our patient seemed to benefit from empiric premedication with high-dose methylprednisolone, we studied the effect of treatment of rabbits with this agent. Indeed, intravenous administration of methylprednisolone (60 mg/kg) to rabbits prior to Fluosol infusion significantly (p < 0.001) blunted the decrement in circulating granulocytes and ablated hypoxemia (p < 0.05) (Fig. 4).

**DISCUSSION**

A synthetic erythrocyte substitute that could expand plasma volume and carry oxygen is greatly needed, and the most promising agents to date have been the perfluorochemicals. One such agent, Fluosol-DA, has been given to over 100 Japanese patients with apparent safety and efficacy. This agent is an emulsion of perfluorodecalin and perfluorotripropylamine, maintained by a nonionic detergent (Pluronic F-68) in a balanced salt solution containing glycerol, yolk phospholipid, and hydroxyethylstarch (Table 1). It has a
blood half-life of 13 hr and a tissue half-life of 8.9 days (as measured in rats). Fluosol is capable of reversibly binding \( O_2 \) with a nearly linear \( O_2 \) dissociation curve; it binds a third as much \( O_2 \) as does normal blood (Hct, 45%) at \( P_{O_2} \) 760 mm Hg. While this carrying capacity is inferior to that of blood, it is great enough that benefit might be expected in severe anemia. Such benefit is suggested by our patient, whose cerebral function improved dramatically after Fluosol.

During developmental trials of other perfluorochemicals, adverse pulmonary reactions have occasionally been noted. These have been attributed to a variety of physical properties of the chemicals, including particle size, vapor pressure, and emulsion stability. For example, pulmonary hypertension and thrombocytopenia were reported in rabbits that received perfluorobutyltetrahydrofuran. At autopsy, these animals have mottled hemorrhagic lungs with dilated pulmonary vessels and right heart chambers. Coagulation defects were noted in some rabbits, leading to the hypothesis that clots or platelet thrombi may have caused the pulmonary dysfunction. The possibility that the emulsifier might be a determinant of toxicity has also previously been suspected. Specifically, no complications occurred in dogs receiving perfluorochemicals emulsified with yolk phospholipid, while pulmonary dysfunction attended the administration of products containing Pluronic F-68. When studied, Pluronic F-68 was found to precipitate plasma proteins, activate plasminogen, and generate fibrin split-products in vitro.

Our patient’s pulmonary symptoms were like those associated with transfusion reactions or endotoxemia, situations in which complement and PMNs are activated. Other work from our laboratory has suggested that the stimulated PMN is important in adverse pulmonary reactions associated with C activation. We therefore postulated that Fluosol might have caused our patient’s difficulties by directly stimulating granulocytes or by activating C with the generation of the PMN-stimulatory fragment \( C_5a_{span} \). In agreement with this latter hypothesis, we found that Fluosol did not directly activate PMNs; however, it did activate plasma C via the alternative pathway, leading thereby to PMN activation. In other situations, C-dependent PMN aggregating activity has been due to the generation of \( C_5a \) or of its desarginine derivative. That this is also the case when Fluosol is incubated with plasma is suggested by the inability to generate aggregating activity in \( C_5 \)-deficient plasma (Fig. 1, tracing 5). The component of Fluosol responsible for C activation seems to be the nonionic detergent Pluronic F-68, since neither any other individual component mimicked the effects of while Fluosol.

Fluosol infusion into rabbits caused transient granulocytopenia, hypoxemia, and pulmonary leukostasis, similar to the effects of infusion of other complement activators or of plasma in which C has been deliberately activated ex vivo. Thrombocytopenia was also evident during Fluosol infusion. This decrease in platelet count could also be due to C activation, since lapine platelets are sensitive to damage when C is activated; however, a direct effect of Fluosol on platelets has not been excluded. This observation agrees with earlier studies; however, thrombocytopenia has been observed with fluorocarbon infusions into some experimental animals, although the mechanism has not been clearly elucidated. Regardless of whether it is C-mediated, coincident platelet activation may have accentuated the pulmonary dysfunction in our experimental animals and perhaps even in our patient. Recent studies in our laboratory suggest this possibility, in that platelets increase the endothelial injury wrought by C-stimulated PMNs in vitro.
pulmonary dysfunction by activating complement, which leads to leukoembolization and pulmonary leukostasis. The activated granulocytes—in concert with platelets—may injure pulmonary vascular endothelium and release vasoactive substances, leading to capillary leak, perivascular edema, V/Q mismatching, and resultant hypoxemia.

Glucocorticoids, which we have shown to decrease the PMN response to activated C,20,31 blunted the granulocytopenia and hypoxemia in rabbits given Fluosol and prevented pulmonary compromise on rechallenge of our patient. The exact mechanism of this protection is uncertain, but it may well be related to inhibition of PMN and platelet function. Very high doses of steroids affect the fluidity of cell membranes32 and interfere with PMN receptor function.33 This results in decreased PMN aggregability, as well as decreased ability of stimulated granulocytes to injure endothelium;34 platelet responses to diverse stimuli are blunted as well.

It is of interest that no untoward reactions have been reported in over 100 Japanese patients treated with Fluosol-DA. Our patient's pulmonary dysfunction and that of at least two other Americans of whom we are aware with similar adverse reactions suggests the possibility of racial differences in susceptibility to adverse effects of fluorocarbons; the reason for this dichotomy is not at all clear. However, lipoprotein profiles between Japanese and Americans differ, and similar adverse reactions suggest the hypothesis that one could predict which patients will respond adversely to Fluosol by quantitating C5a generation in individual patients' plasmas incubated with Fluosol prior to therapy.

The identification of the detergent as the probably culprit raises the additional possibility that alternative means of maintaining emulsion stability might be devised to improve tolerance of the product. Until that goal is achieved, our studies suggest that adverse pulmonary reactions to perfluorochemical emulsions may be prevented by steroid pretreatment of susceptible patients. Further study, with careful hemodynamic, pulmonary, and hematologic monitoring of future patients receiving Fluosol, will help further delineate this problem.

ACKNOWLEDGMENT

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