EFFECTS OF PERFLUOROCARBON EMULSION IN RHEOLOGY (Abstract): Aim: To study effects of a perfluorocarbon emulsion on plasma and whole human blood viscosity in the presence of albumin or modified fluid gelatin. Material and methods: We investigated the effects of several PFC concentrations on plasma and whole blood viscosity in the presence of human albumin solution (HAS) or modified fluid gelatine (MFG; Gelofusine) to obtain three PFC emulsion concentrations (4, 8 and 15 g/dL). Three hematocrit levels (Hct) were investigated: 30, 20 and 13%, corresponding to different clinical situations. Plasma and whole blood viscosity was measured at 37°C, using a Couette viscometer for shear rates ranging from 0.2 to 128 s⁻¹. Results and discussion: All PFC concentrations increased plasma and whole blood viscosity for the same Hct. Viscosity values similar to physiological ones were observed at Hct 13%, with MFG – PFC 4, 8 g/dL and HAS – PFC 15g/dL; at Hct 20%, with MFG – PFC 4g/dL and HAS - PFC 15g/dL; at Hct 30%, and HAS – PFC 4, 8 g/dL. Conclusions: We conclude that this PFC emulsion increases plasma and blood viscosity and that among the three studied volume expanders, the interaction with MFG can result in viscosity values above the physiological one even at low Hct values. Our results suggest that such increased blood viscosity could decrease skeletal muscle oxygen pressure. Keywords: PFC EMULSION, WHOLE HUMAN BLOOD, VISCOSITY, HEMATOCRIT.

Severe acute blood loss resulting in hemorrhagic shock (HS) can lead to severe tissue damage responsible for high morbidity and mortality (1). The recovery of physiological macrohemodynamics parameters such as arterial pressure and cardiac output, as well as the fast restoration of microvascular perfusion and oxygen supply to vital organs, are the major aims of primary therapy of HS. Intravenous volume replacement with an acellular fluid may restore cardiac output but fails to promote microvascular perfusion (2). Severe hemodilution, due to massive infusions of volume expanders, may further impair tissue oxygenation because of decreased oxygen transport. Survival from shock is directly dependent on the restitution of adequate blood oxygen carrying capacity once the hematocrit (Hct) falls below a critical range of 20-25% (3).

It has been shown that stored as opposed to fresh RBCs have a decreased capacity to deliver oxygen in the microcirculation.

Artificial oxygen carriers based on PFC emulsions have been developed because they can increase oxygen transport in the initial stages of resuscitation (4). Indeed, in the case of acute severe anemia, or acute...
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Normovolemic hemodilution (ANH), these artificial oxygen carriers can increase the oxygen carrying capacity of hemodiluted fluid. PFCs are chemically and biologically inert synthetic compounds (perfluorinated hydrocarbon compounds). PFCs have high oxygen solubility through weak van der Waals forces (4).

The rheological properties of blood regulate many physiological and pathophysiological parameters of the cardiovascular system, such as arterial blood pressure, blood flow in the microcirculation, hemostasis, inflammation, etc. Viscosity contributes to endothelial shear stress (5, 6).

The interactions among PFC and the different volume expanders used in clinical practice have not been studied.

We made the hypotheses that the PFC emulsion interacts with the three above-cited types of colloids and that these interactions modify plasma and blood viscosity; the interactions depend on the value of Hct.

MATERIAL AND METHODS

Human whole blood was diluted with the PFC emulsion mixed with different volume expanders. Three PFC concentrations were tested: 4, 8 and 15 g/dL each combined with Hct values of 13, 20 and 30%. Controls used were human whole blood at 40% Hct and diluted with autologous plasma or the volume expanders at Hct 13, 20 and 30%.

The volume expanders used were: human albumin solution 20% (HAS) diluted in Ringer solution at 50 g/L; gelofusine 4%: a modified fluid gelatin (MFG).

The viscosity of plasma, PFC and volume expanders was measured at 37°C with an automatic capillary viscometer, and expressed in mPa.s⁻¹. The viscosity of the different compounds: plasma, volume expanders and whole blood at different Hct levels and of mixtures of each compound with PFC at different concentrations, was determined at 37°C, using a Couette type coaxial viscometer at shear rates from 0.2 to 128 sec⁻¹ and expressed in mPa.s⁻¹.

RESULTS

The intrinsic viscosities of volume expanders alone or mixed with plasma, in the absence or presence of increasing concentrations of PFC are presented in table I. Volume expanders alone (or in the presence of plasma) exhibit an inverse linear viscosity as a function of shear rate (lower shear rate values are associated with higher viscosity). When PFC emulsion was added, the viscosity increased as a function of the PFC concentration. The intrinsic viscosity was similar between plasma and HAS, but increased strongly with MFG (tab. I).

In preliminary experiments we verified that the presence of the PFC emulsion, even at high concentrations, did not interfere with the measurements of viscosity.

A total of 45 situations, according to Hct values (40, 30, 20 and 13%), volume expanders (plasma, MFG, HAS) and PFC concentrations (0, 4, 8, and 15 g/dL) have been studied. The control situations of whole blood at Hct values of 40%, and blood diluted with autologous plasma and with volume expanders, at Hct values of 30, 20 and 13% respectively were represented in figure 1.

When blood was mixed only with volume expanders (fig. 1), whatever the final Hct, the hemodiluted blood presented always viscosities lower than whole blood at 40% Hct. In the situation where Hct value was 13%, whatever the volume expander used, the viscosity values were similar with those observed upon dilution with autologous...
gous plasma.

When PFC was added to blood hemodiluted with the volume expanders, the viscosity was statistically higher. In fact, the presence of the PFC emulsion, independently of the Hct values, always resulted in highly increased blood viscosity (fig. 2, 3).

**TABLE I**

**Intrinsic viscosity of the human plasma, volume expanders and mixtures of volume expanders with different PFC emulsion concentrations**

<table>
<thead>
<tr>
<th>Solutions</th>
<th>PFC emulsion (g/dL)</th>
<th>Intrinsic viscosity (mPa.s⁻¹) at shear rates 20.4 s⁻¹ (left), 128 s⁻¹ (right)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Alone</strong></td>
</tr>
<tr>
<td>Human plasma</td>
<td>0</td>
<td>1.4</td>
</tr>
<tr>
<td>HAS</td>
<td>0</td>
<td>1.0/1.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.1/1.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.7/1.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.0/2.8</td>
</tr>
<tr>
<td>MFG</td>
<td>0</td>
<td>1.7/1.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.5/2.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.9/2.9</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>58/off limit</td>
</tr>
</tbody>
</table>

**Fig. 1.** The effects of shear rates on the viscosity of red blood cells diluted with autologous plasma at Hct 40, 30, 20, and 13% and with different volume expanders at Hct 30, 20, and 13% (n = 6 for each group)
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Fig. 2. The effects of shear rates on the viscosity of red blood cells mixed with volume expanders plus different PFC emulsion concentrations at Hct 13%

When hemodilution reached a final Hct 13%, and was made with HAS, a strong decrease of blood viscosity was observed. When 15 g/dL of PFC emulsion was added, this restored the physiological viscosity (NS vs control at Hct 40%) (fig. 2). A similar viscosity as control 40% Hct was observed in the presence of MFG and PFC 4 and 8 g/dL. In contrast to what was observed with HAS, when all the other volume expanders studied were mixed with 15 g/dL PFC to obtain identical hemodilution, the viscosity was always higher than the physiological value (Hct of 40%). However, at these same Hct values, HAS with lower PFC emulsion concentrations (4 and 8 g/dL) led always to viscosity values below those observed at Hct 40%.

When hemodilution reached a final Hct of 20% (fig. 3), blood viscosity was significantly higher than the physiological viscosity with MFG and PFC of 8 and 15 g/dL respectively. Viscosity values were similar to physiological values for MFG PFC 4 g/dL and HAS PFC 15 g/dL. All other combinations of volume expanders and PFC at different concentrations resulted in viscosity values below the physiological ones (fig. 3).

When hemodilution reached a final Hct of 30% (fig. 4), blood viscosity was significantly higher than the physiological viscosity with MFG and PFC of 4 and 8g/dL respectively. Viscosity values were similar to physiological values for HAS PFC 4 and 8g/dL. All other combinations of volume expanders and PFC at different concentrations resulted in viscosity values below the physiological ones (fig. 4).

DISCUSSION

The effects of PFC on blood rheology are more difficult to analyze because in clinical practice, the final results of resuscitation with volume expanders and oxygen carriers is a complex mixture of native red
blood cells, plasma, volume expanders and eventually PFC. These complex mixtures could have unpredictable effects on blood rheology.

**Fig. 3.** The effects of shear rates on the viscosity of red blood cells mixed with volume expanders plus different PFC emulsion concentrations at Hct 20%

**Fig. 4.** The effects of shear rates on the viscosity of red blood cells mixed with volume expanders plus different PFC emulsion concentrations at Hct 30%

We measured *in vitro* the viscosity of human whole blood diluted with the PFC emulsion mixed with different volume expanders. Three PFC concentrations were tested: 4, 8 and 15 g/dL each combined with Hct of 13, 20 and 30%. Controls used were human whole blood at 40% Hct (physiological blood viscosity values) and
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diluted with autologous plasma or the respective volume expanders at Hct 13, 20 and 30%.

For this study, we have chosen different PFC concentrations: 4 g/dL, 8 g/dL and 15 g/dL. The higher doses, although not used in clinical practice could allow to magnify potential effects and to better resolve trends in the rheological behavior of mixtures. Different shear rates (2.8 to 128 s\(^{-1}\)) which represent the different blood flow inside post capillary venules and large vessels (7). Different Hct values to explore the different cases susceptible to be meeting in acute clinical situation such as HS (Hct values of 10-15%) or cardiopulmonary bypass (Hct values of 20-30%).

Freyburger et al have shown significant alterations on the blood rheological properties in vivo hemodilution situation (8). MFG has been shown to facilitate red blood cell aggregation in vitro by reducing the electrostatic repulsive forces after adsorption on the cell membrane. MFG has been shown to interact specifically with plasma proteins such as fibronectin, and also may enhance the formation of bridges between red blood cells (9).

The effect of PFC on the rheological parameters of blood in a living organism at the Hct twice lower than the normal values was studied by Kuznetsova et al. (10). These authors showed that an increase in emulsion concentration leads to an increase in blood asymptotic viscosity and the aggregation of erythrocytes. It was concluded that only a small volume of PFC emulsion, as compared with the total amount of erythrocytes, can improve the blood fluidity upon disturbed circulation. The results suggest that PFC and MFG interact to increase viscosity and even at low doses of PFC and low Hct values, the presence of MFG results in viscosity values above those considered to be physiological.

CONCLUSIONS

The main findings of this study are that: addition of PFC, even at low clinically used concentrations, to different volume expanders, in the absence or presence of RBC, always resulted in increased viscosity values. At several concentrations, PFC increased viscosity values above the physiological values (that measured for 40% Hct in plasma). For instance, at an extreme Hct value of 13%, the addition of MFG, 4 g/dL of PFC restored the physiological viscosity values. For higher PFC concentrations, viscosity is dramatically higher than physiological values even at such low Hct values. These observed viscosities were more important when Hct was increased.

If the concentrations of PFC must be increased, the albumin solution has the smallest effect on blood rheology.

REFERENCES

NEW TARGET FOR THE OLD ASPIRIN

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, a classical and authentic cyclooxygenase (COX) inhibitor, reduces the risk of developing several types of cancer. On site of inflammation, induction of COX-2 quickly results in the biosynthesis of prostaglandins leading to inflammatory response. Overexpression of inducible COX-2 and an increased prostaglandin biosynthesis play a significant role in carcinogenesis. Ogawa and coworkers investigated the effect of aspirin on regional lymph node metastasis during the development of lung cancer in mice. The carcinoma model was obtained by injecting a solution containing Lewis lung carcinoma (LLC) cells overexpressing green fluorescent protein (GFP) and BD Matrigel in the male mice lungs. Operative mortality was 10%. Single pulmonary nodules developed at the implanted site in 95% of animals, and regional mediastinal lymph node metastasis were observed 14 days after LLC-GFP cell injection in mice. Aspirin was orally administered (100 mg/kg, twice a day) after LLC-GFP cell injection. The reducing of percentage metastasis to regional lymph nodes was observed beginning with day 12 (63%), with no significant suppression of primary tumor growth in the lungs. Aspirin treatment led to a significant reduction in mortality (survival rate 70%; P < 0.0001). The authors suggested that COX inhibitors have potential to prevent lymph node metastasis (Ogawa F, Amano H, Ito Y, Matsui Y et al. Aspirin reduces lung cancer metastasis to regional lymph nodes. Biomed Pharmacother 2014; 68:79–86).