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Influence of parenteral fat emulsion Intralipos® and citric acid on blood viscosity and erythrocyte morphology *in vitro*

Yiyao Liu [a,*], Hong Yang [a], Quansheng Su [b], Akio Sakanishi [c]

[a] *School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, Sichuan Province, PR China*
[b] *Lab of Sport Physiology and Biochemistry, Department of Sport Medicine, Chengdu Sport University, Chengdu 610041, Sichuan Province, PR China*
[c] *Department of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, Japan*

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Abstract

In most studies fat emulsion had been administered by enteral route. Recently a parenteral soybean-oil emulsion has been developed. In this study we assessed the effects of a parenteral soybean-oil emulsion (Intralipos®) and citric acid on blood rheology and erythrocyte morphology *in vitro*. Porcine blood was incubated *in vitro* with increasing concentrations of fat emulsion Intralipos® and citric acid at 37 °C for 1 h. Viscosity of plasma and whole blood was measured using a FASCO-2050 digital viscometer. Red blood cell morphology was examined by light microscopy. The viscosity of whole blood represented an ascending dose-dependence for different Intralipos® concentrations at high shear rate of 90 and 225 s^{-1}, however, it decreased with citric acid concentrations. On the other hand, the whole blood viscosity also declined with citric acid concentrations in presence of 30% Intralipos® (v/v), and there is a minimal viscosity at 0.67% citric acid (v/v). There are some thorns on the blood membrane at 40% Intralipos® as compared with control (no Intralipos® addition), which indicates the Intralipos® compound may affect blood cell membranes, and resulted in whole blood viscosity increase. We concluded that the intravenous soybean-oil preparation Intralipos® interacts with the erythrocyte membrane, and citric acid could alleviate the whole blood viscosity.

Keywords: Fat emulsion; Intralipos®; Citric acid; Blood viscosity; Erythrocyte morphology; Intravenous injection

1. Introduction

Total parenteral nutrition (TPN), which is also known as parenteral hyperalimentation, provides complete nutritional therapy by the infusion of nutrients directly into the systemic circulation. TPN is widely used to provide complete nutritional support in a variety of pathophysiologic settings in which patients are unable to eat, for instance, critical ill infants [1,2]. Fat emulsions are also one of the energy resources for animals or humans. Intravenous fat emulsions, especially soybean oil, are now available as resource of essential fatty acids in patients receiving TPN, and has been found to be clinically safe, sable and economical [3].

During the recent years, the pharmacology and pharmacokinetics of fat emulsions have been well documented [4,5]. Hepatic dysfunction and steatosis are the most common complication associated with TPN, but infants, it is said to express itself as cholestasis [1]. Hepatoprotective management, including the provision of an efficient energy source in an appropriate dosage, is necessary during TPN to promote normal growth in infants. The liver is the main source of serum albumin. In protein-energy malnutrition, not only the serum albumin level but also the hepatic albumin mRNA level is known to be markedly decreased [6].

However, deleterious microcirculatory effects of fat emulsion infusion may be caused by hemorheological or vascular effects. Few reports describing their rheology aspects during TPN treatment have appeared in the literatures. In the present study, we focused on investigating effect of parenteral fat emulsion Intralipos® and citric acid on erythrocyte morphology and blood viscosity *in vitro*. In this report, we also discuss how to alleviate the side-effects of fat emulsion Intralipos® by citric acid addition.

* Corresponding author at: Lab of Biomechanics and Biorheology, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, Sichuan Province, PR China.
Tel.: +86 28 8320 6124; fax: +86 28 8320 6124.
E-mail address: liuyiyao@hotmail.com (Y. Liu).

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2. Materials and methods

Fresh swine blood was anti-coagulated with K2EDTA (1 mg/mL). Plasma and red blood cells (RBCs) were isolated from the swine blood by 3000 rpm centrifugation for 10 min. Soybean-oil emulsion (20% Intralipos®) was purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Citric acid was obtained from Wako (Osaka, Japan). A 2 ml RBCs were mixed with 2 ml different prepared soybean-oil emulsion Intralipos® and citric acid. The final concentrations of Intralipos® are $\Phi = 1$, 2.5, 5, 7.5, 10, 20, 30 and 40%; final concentrations of citric acid are $\Phi' = 0.33$, 0.67, 1.66, 3.33 and 6.67%. For whole blood, its hematocrit H is 40%, very near to the mean value of physiological level. Preparation with no Intralipos® and citric acid is the control in these experiments. After all the samples are kept in incubator for 1 h at 37 °C, we used FASCO-2050 digital viscometer (Veiduo, Chongqing, China) to measure their viscosity values. Morphology of red blood cells was examined by light microscopy.

3. Results

Fig. 1 shows the dependence of viscosity on the shear rate for whole blood and plasma. The viscosity of both whole blood and plasma declines as exponential function with increase of shear rate (Fig. 1a and b). For whole blood, the viscosity increases with the Intralipos® concentration ($\Phi = 10$, 20, 30 and 40%) at a given shear rate (Fig. 1a). However, this phenomena have not appeared for plasma, and for various there are almost no obvious difference at a given shear rate (Fig. 1b).

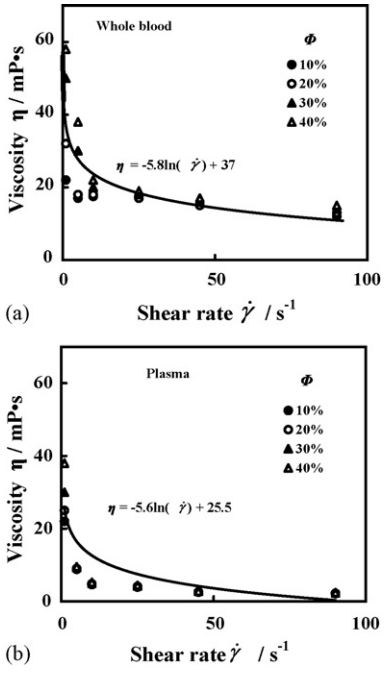

Fig. 1. Viscosity (η) dependence of swine whole blood (Hematocrit $H = 40\%$) (a) and plasma (b) at different Intralipos® concentrations Φ of 10, 20, 30 and 40% at 37 °C. Their decrease tendency as exponential functions of $\eta = -5.8 \ln \dot{\gamma} + 37$ and $\eta = -5.6 \ln \dot{\gamma} + 25.5$ for whole blood and plasma, respectively. Data points are represented as the means of three separate experiments.

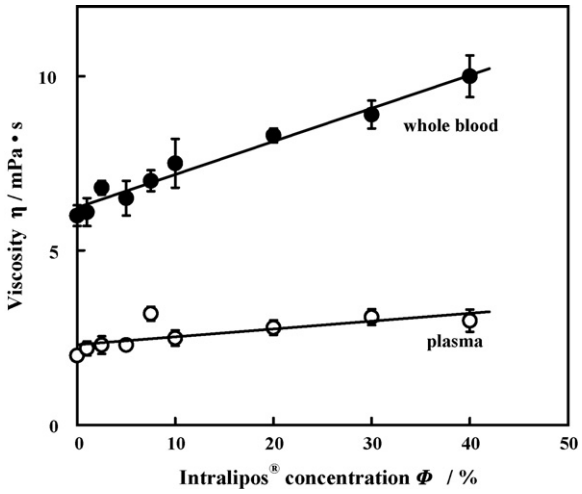

Fig. 2. Viscosity of whole blood and plasma against the Intralipos® concentrations Φ of 1, 2.5, 5, 7.5, 10, 20, 30 and 40% at 90 s^{-1} shear rate. Data points are represented as mean ± S.D.

At a high shear rate of 90 s^{-1}, viscosity of whole blood and plasma presents a linear dependence against the Intralipos® concentration Φ as shown in Fig. 2. It is also found that the whole blood viscosity increases with Φ, but there is no significant difference for plasma at shear rate of 90 s^{-1}. It is very well coincided with Fig. 1. In order to explore the mechanisms of high viscosity induced by Intralipos®, the morphology of RBCs is observed by light microscope. At $\Phi = 40\%$, the RBCs morphology of the Intralipos® addition sample and the control (no Intralipos®) is compared (Fig. 3). There are some thorns on the blood cell membrane at 40% Intralipos® (Fig. 3b), but it is not observed in the control (Fig. 3a).

At very high shear rate of 225 s^{-1}, the dependence of viscosity for whole blood and plasma on the citric acid concentration Φ' is shown in Fig. 4. The viscosity of whole blood without Intralipos® decreases with Φ', but viscosity tendency is very near a flat line and almost no change with Φ' for plasma. For the samples of whole blood with 30% Intralipos®, the viscosity firstly decreases, and then increases with Φ'. In other words, there is optimal citric acid concentration Φ' that alleviate the blood viscosity at $\Phi' = 0.67\%$ (v/v) *in vitro* (Fig. 4).

4. Discussion

The long-term impact of nutrition on health and disease is well documented, and the amount and type of lipid intake has been implicated in the pathogenesis of arteriosclerosis and ischemic heart disease [7–9]. Recent reports have shown an influence of enteral nutrition on morbidity and mortality of intensive care patients. In a multicenter study on the adult respiratory distress syndrome, tailored nutrition with eicosapentaenoic acid (EPA), [3]γ-linoleic acid, and antioxidants improved oxygenation, reduced the length of mechanical ventilation, decreased the incidence of new organ failure, and shortened the length of stay in the intensive care unit [10]. In another multicenter study of septic patients, a customized diet including arginine, nucleotides,