Effects of Bolus Injection of Soybean-Based Fat Emulsion and Fatty Acids on Pulmonary Gas Exchange Function

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To determine whether or not a “bolus injection” of soybean-based fat emulsion (SFE), which contains oleic acid (OA), a potent lung-toxic unsaturated C-18 fatty acid, can induce pulmonary dysfunction, we examined the effect of SFE injection on the partial oxygen pressure of arterial blood (Pao2) and pulmonary vascular permeability. In addition, we compared the effect of an injection of SFE with that of OA, soybean oil (a source of SFE), emulsified OA and C-18 fatty acids. Bolus injection of SFE (0.3–4.8 ml/kg) had little effect on Pao2 and pulmonary vascular permeability. Injection of an equivalent amount of OA, on the other hand, significantly decreased Pao2 and increased pulmonary vascular hyper-permeability. This decrease in Pao2 was attenuated by emulsification. Unemulsified soybean oil also induced a decrease in Pao2, although the effect was weaker than that of OA. Other unsaturated C-18 fatty acids (linoleic and linolenic acid) induced a decrease in Pao2 as potent as OA while stearic acid, a C-18 saturated fatty acid, had little effect. Although we did not observe pulmonary toxicity as a result of “bolus injection” of SFE, the chemical form, for example, emulsification and the degree of saturability of the carbon chain, seems to influence the pulmonary toxicities of lipids and fatty acids. Furthermore, the potent pulmonary toxicity of OA seems to depend not only on pulmonary vascular embolization but also pharmacological and/or inflammation-inducing properties.

Key words soybean-based fat emulsion; lung injury; oleic acid; hypoxemia; pulmonary vascular hyper-permeability

The unsaturated C-18 fatty acid oleic acid (OA) is known as a potent lung toxicant.1–3) Intravenous injection of OA to animals produces acute lung injury with hypoxemia and an increase in pulmonary vascular permeability, and has been used as a model of clinical acute lung injury including acute respiratory distress syndrome (ARDS).1–4)

Soybean-based fat emulsions (SFEs) are clinically used worldwide for nutritional support. SFEs contain a high level of OA (e.g., 22–25% of the fatty acid in Intralipid®4, a formulation of SFE),2) yet despite this, do not induce lung injury when used properly. The reason for the low pulmonary toxicity of SFEs remains unclear, although it has been postulated that “bolus injection” may induce an acute load of OA to the lungs and subsequent lung injury. However, since SFEs are clinically administered by continuous injection, little has been reported on the effects of “bolus injection” of SFE on pulmonary function.

This study was conducted to examine whether a “bolus injection” of SFE induces acute lung injury accompanied by pulmonary gas exchange and/or vascular hyper-permeability as is the case with OA. In addition, we also compared the effect of an injection of SFE with that of OA, soybean oil (a source of SFE), emulsified OA and C-18 fatty acids, namely, stearic, linoleic and linolenic acid, on pulmonary gas exchange and evaluated the possibility of lung injury induced by these lipids.

MATERIALS AND METHODS

Materials Intralipid®, a representative SFE for ethical use, was used as the SFE throughout this study. Intralipid® was purchased from Otsuka Pharmaceutical Co. (Tokyo, Japan). OA, soybean oil, Evans blue, formamide, stearic acid, linoleic acid and linolenic acid were purchased from SIGMA Chemical Co. (St. Louis, MO, U.S.A.). Other reagents and solvents were of reagent grade. Emulsified OA was prepared by adding 1 ml of OA to 4 ml of cold water using a microsyringe with vibration. This mixture was then sonicated for 5 min. The average particle diameter of the OA emulsion particles was less than 1 μm. The OA emulsion was prepared just before use. Deionized distilled water was used throughout.

Animal Care and Handling This study was approved by the Animal Care and Use Committee of Kumamoto University. The care and handling of animals were performed in accordance with the National Institutes of Health guidelines for the care and handling of animals. All operations and measurements mentioned below were performed as described previously.3,4)

Measurement of the Partial Oxygen Pressure of Arterial Blood (Pao2) To examine the effect of bolus injection of SFE and other lipids on pulmonary gas exchange (Table 1), we measured Pao2. Briefly, guinea pigs were randomly divided into 6 groups and treated with the following: 1) 0.3—4.8 ml/kg SFE; 2) 4.8 ml/kg saline (as a control group); 3) 15—60 μl/kg OA; 4) 65—260 μl/kg soy bean oil; 5) 75—300 μl/kg emulsified OA; or 6) 15 μl/kg C-18 fatty acids (stearic, linoleic or linolenic acid). Reagents were adminis-

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blood gas analyzer (ABL 300, RADIOMETER Ltd., Copenhagen, Denmark).

**Determination of Pulmonary Vascular Permeability**

To examine the effect of bolus injection of SFE on pulmonary vascular permeability, we measured extravasated Evans blue dye in the lungs. Briefly, 30 mg/kg of dye was administered 1 min before the intravenous injection of 0.3—4.8 ml/kg SFE, 15 μl/kg OA or 4.8 ml/kg saline. Ninety minutes after the reagent injection, the chest cavity was opened. The pulmonary intravascular space was then washed out by perfusion with saline. The lungs were cut into sections and Evans blue was extracted with 20 ml of 100% formamide solution. The concentration was then determined by light absorbance at 620 nm and interpolation of the data was performed using a standard curve. The amount of Evans blue is expressed as ng/mg of the wet weight of tissue.

**Statistical Analysis** Multiple comparisons were made to examine the statistical significance of the data. When uniform variance was identified by Bartlett’s analysis ($p<0.05$), one-way analysis of variance (ANOVA) or repeated measures ANOVA was used to test for statistical significance. When significant differences ($p<0.05$) were identified, the data were further analyzed by Dunnnett’s or Tukey’s multiple range test.

**RESULTS**

**Effects of a Bolus Injection of SFE on Pao$_2$** A bolus injection of 0.3—4.8 ml/kg SFE, which contains approximately 15—240 μl/kg OA, had little effect on Pao$_2$ after 75 min (Fig. 1A). There were no statistical differences between values at all times and all dosages compared with the saline group. On the other hand, injection of OA (15, 30, 60 μl/kg) induced a decrease in Pao$_2$ in a dose-dependent manner (Fig. 1B).

**Effects of Bolus Injections of SFE and OA on Pulmonary Vascular Permeability** Bolus injection of SFE had little effect on pulmonary vascular permeability 90 min after the injection. There were no statistical differences in pulmonary vascular permeability at all dosages compared with the saline-treated group. OA (15 μl/kg) injection, on the other hand, increased the pulmonary vascular permeability (Fig. 2).

**Effects of Bolus Injections of Soybean Oil, Emulsified OA and C-18 Fatty Acids on Pao$_2$** Although intravenous injection of soybean oil at doses of 65 and 130 μl/kg did not have any effect on Pao$_2$, 260 μl/kg caused a significant decrease in Pao$_2$ compared with the initial value (Fig. 3A). The maximum decrease was approximately 65% of the initial value. Intravenous injection of emulsified OA seemed to have little effect on the decrease in Pao$_2$ compared with naked OA injection. Although a significant difference was observed between the 6 min value and initial value in the 75 μl/kg-treated group, there were no statistical differences in Pao$_2$ between the initial value and those at all other times in the 150 and 300 μl/kg-treated groups (Fig. 3B). As shown in Fig. 3C, injection of 15 μl/kg of linoleic or linolenic acid induced a significant decrease in Pao$_2$ compared with the initial value, while stearic acid did not.

**Comparative Effects of the Four Lipids on Pao$_2$** A significant difference was observed between treatment with SFE and soybean oil, both of which included 60 μl/kg of OA. There were also significant differences between the OA and emulsified OA groups at all doses. The OA-induced decrease in Pao$_2$ was attenuated by emulsification. In addition, the decrease in Pao$_2$ induced by soybean oil was weaker than that by OA, and there was a significant difference between the soybean oil and OA groups.

**DISCUSSION**

The results indicate that a bolus injection of SFE did not induce acute lung injury in guinea pigs in our system. Generally, when the diameter exceeds about 15 μm, emulsion
sified OA had a lesser effect on Pao2. These results suggest an acute load of OA. Compared with "unemulsified" OA, emulsified OA particles, on the other hand, do not seem to cause inflammation and injury of the lungs. Accordingly, when intravenously administered, OA may form emboli and achieve a sufficient concentration to cause inflammation and injury of the lungs. Our results suggest that even n-3/n-6 fatty acids induce lung injury when administered intravenously as free fatty acid form. The potential effect of linolenic acid on the development of lung injury was more powerful than that of linoleic acid. In addition, stearic acid, a saturated C-18 fatty acid, did not induce lung injury that was distinct from the effect of unsaturated C-18 fatty acids. The reason for this remains unexplained at present; however, further elucidation will be important in determining the pathophysiology of lung injury induced by fatty acids such as OA.

Although some clinical studies suggest that the "rate of infusion" may be a cause of SFE-induced lung injury, this is not the sole factor, but rather may act as an additional or supportive factor. Clinical reports also suggest that lung injuries induced by SFE are more indoluble in patients with pulmonary dysfunction or immature function, such as patients with ARDS or sepsis. In this study, because we used healthy guinea pigs, it is possible that we overlooked the potential of SFE to induce pulmonary injury in vulnerable lungs. Accordingly, without further study, we cannot conclude that the present findings are evidence of the overall safety of bolus-injected SFE.

In this study, we did not observe acute pulmonary dysfunction as a result of "bolus injection" of SFE. The low tox-
icity of SFE depends on the emulsification and low toxicity of soybean oil compared with OA. In addition, the potent pulmonary toxicity of OA may depend not only on pulmonary vascular embolization but also on pharmacological and/or inflammation-inducing properties. Our results suggest that the chemical form, for example, emulsification and the degree of saturability of the carbon chain, influence the pulmonary toxicities of lipids and fatty acids.

REFERENCES