Influences of Soybean Oil Emulsion on Stress Response and Cell-Mediated Immune Function in Moderately or Severely Stressed Patients

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OBJECTIVES: We previously reported that \(\omega-6\) fat emulsion increases cytokine production in burned rats. Effects of soybean oil emulsion on surgical stress responses and lymphocyte function according to the surgical severity have not been studied in detail. We investigated the effects of soybean oil emulsion, which contains 50% \(\omega-6\) fatty acid, on postoperative stress responses and cell-mediated immune function according to the severity of surgical stress.

METHODS: Eight patients who underwent gastric or colorectal surgery and nine who underwent esophagectomy were fed fat-free total parenteral nutrition. Ten patients who underwent gastric or colorectal surgery and seven who underwent esophagectomy were fed total parenteral nutrition with soybean oil emulsion. Total parenteral nutrition provided 1.5 g of protein and 40 kcal per kilogram every day from 7 d before surgery to postoperative day 14. Soybean oil emulsion (Intralipid) accounted for 20% of the total calories. Serum interleukin-6, C-reactive protein, glucagon, and concanavalin A– or phytohemagglutinin-stimulated lymphocyte proliferation were determined.

RESULTS: In the group of moderately stressed patients, soybean oil emulsion did not amplify the measured levels. In the group of severely stressed patients, soybean oil emulsion amplified the level of serum interleukin-6 and decreased concanavalin A– or phytohemagglutinin-stimulated lymphocyte proliferation.

CONCLUSIONS: Soybean oil emulsion amplifies the stress responses and possibly suppresses cell-mediated immune function induced by surgical stress in severely stressed patients, but not in moderately stressed patients.


KEY WORDS: soybean oil emulsion, interleukin-6, stress response, cell-mediated immune function, esophagectomy

INTRODUCTION

The incorporation of lipid into the nutritional regimen seems to optimize the provision of energy requirements in stressed patients. Lipids are inexpensive, provide essential fatty acids, and reduce the glucose load. However, commercially available lipid emulsion is limited to soybean oil emulsion, which contains more than 50% linoleic acid. Linoleic acid, one of the \(\omega-6\) polyunsaturated fatty acids (PUFAs), is the precursor of arachidonic acid, which in turn gives rise to dienoic prostaglandins and leukotrienes.\(^1\)\(^\text{-}\)\(^6\) Prostaglandin E\(_2\), which is derived from arachidonic acid, might be increased in a stressed state and suppress immune function. Therefore, \(\omega-6\) PUFA may be associated with adverse effects on inflammatory and immunologic responses. We previously reported that \(\omega-6\) PUFA–enriched fat emulsion amplifies stress responses and stress-induced immunosuppression in burned rats.\(^7\) We also demonstrated the effects of severity of surgical stress on whole-body protein kinetics and nitrogen balance.\(^8\)\(^,\)\(^9\) In those studies, we found unchanged or slightly decreased rates of whole-body protein synthesis with slightly increased breakdown in the group of patients who underwent gastric or colorectal surgery, whereas synthesis increased significantly with a greater increase of breakdown in patients who underwent severely surgical procedures such as esophagectomy for esophageal cancer.\(^8\)\(^,\)\(^9\) Those results suggested that the degree of stress responses and immune function depend on the extent of surgical stress. We measured interleukin-6 (IL-6), C-reactive protein (CRP), glucagon, concanavalin A (Con A)– or phytohemagglutinin (PHA)–stimulated lymphocyte proliferation and rapid turnover proteins after surgical operation. IL-6, CRP, and glucagon can be used to gauge the intensity of injury stress responses. Con A- or PHA-stimulated lymphocyte proliferation provides an index of cell-mediated immunity. Rapid turnover proteins such as transferrin, retinol-binding protein, and prealbumin are indices of the final sum of protein breakdown and protein neosynthesis. We investigated whether immune function is suppressed according to the extent of surgical stress and whether soybean oil emulsion amplifies stress responses and stress-induced immunosuppression regardless of the extent of surgical stress.
**PATIENTS AND METHODS**

**Patients and Clinical Protocol**

Thirty-four patients who underwent surgeries at the First Department of Surgery of Chiba University School of Medicine were prospectively enrolled into the study. Of these patients, 18 who underwent gastric or colorectal surgery were assigned to the group of moderately stressed patients. According to our previous study, they were fed exclusively by total parenteral nutrition (TPN), which provided 1.5 g of protein and 40 kcal per kilogram per day, from 7 d before surgery to postoperative day (POD) 14. They then were assigned to one of two TPN groups. One was fed fat-free TPN (group A-1, n = 8), and the other was fed TPN with soybean oil emulsion (Intralipid), which accounted for 20% of total calories (group A-2, n = 10).

The group of severely stressed patients consisted of 16 patients who had undergone esophagectomy with thoracotomy and three-field lymph node dissection. This was followed by reconstructive surgery using a gastric tube or colon replacement through the retrosternal route. They were fed exclusively with TPN providing the same energy and protein levels as TPN for the moderately stressed patients. They were assigned to one of two groups. One was fed fat-free TPN (group B-1, n = 9), and the other was fed TPN with soybean oil emulsion, which accounted for 20% of total calories (group B-2, n = 7). The soybean oil emulsion contained 51.6% linoleic acid and 7.7% linolenic acid. This prospective, randomized protocol was approved by the Ethics Committee of the Chiba University School of Medicine. Randomization was done by a resident opening a sealed envelope containing a card labeled fat or control. The cards were produced by a computer-generated randomization program in blocks of six to avoid the same assignment more than three times in a row. Informed written consent was obtained from all patients. All patients had normal hepatic and renal functions and were not diabetic. They did not receive preoperative chemoradiation therapy. They did not use drugs that influence immune function such as corticosteroids.

Serum IL-6 was measured before surgery; 1 and 2 h after surgery; and 1, 3, and 10 d after surgery. CRP, glucagon, and rapid turnover proteins were measured before surgery and on POD 1, 3, and 10. Con A- or PHA-stimulated lymphocyte proliferation was determined before surgery and on POD 7.
FIG. 3. Serum glucagon concentration of postoperative patients in different groups. Data are expressed as mean ± standard of the mean. Solid circles, group A-1; solid squares, group A-2; open circles, group B-1; open squares, group B-2. ‡P < 0.01 versus group A-2; †P < 0.05 versus group A-2; ‡P < 0.05 versus group A-1. POD, postoperative days; pre, preoperation.

Laboratory Analyses

Levels of serum IL-6 were measured with a commercial human cytokine enzyme-linked immunosorbent assay kit (Amersham, Buckinghamshire, UK). The absorbance of the sample was determined with 450 nm as the primary wavelength. Cytokines in unknown samples were quantitated by comparison with standard curves of recombinant cytokine.

Fasting glucagon was measured in plasma from venous blood that was mixed with Trasyrol immediately after collection in tubes containing ethylene-diaminetetraacetic acid and centrifuged at 4°C. The concentrations of glucagon were determined by using a double-antibody 125I radioimmunoassay kit (Glucagon kit, Daich RI, Tokyo, Japan).

Mononuclear cells were separated from venous blood by density gradient centrifugation on a Conray/Ficoll. Mononuclear cells removed from the interface were washed two times in phosphate-buffered saline. Then 1 × 10^5 cells in a final volume of 200 μL of RPMI 1640 with 10% fetal calf serum was plated on microtiter wells. The cells were incubated with Con A or PHA in final concentrations of 10 μg/mL. Assays were performed in duplicate, and unstimulated background control cultures were incubated with every assay. Cells were incubated at 37°C for 64 h with an 8-h pulse labeling with [3H]thymidine, 0.25 μCi/well. Cells were harvested onto glass-fiber filters and [3H]thymidine content and, hence, proliferation were determined by liquid scintillation counting. Stimulation indices were calculated by dividing the counts per minute of [3H]thymidine in mitogen-stimulated cells by the counts per minute in cells cultured without mitogens.

CRP and rapid turnover proteins such as transferrin, retinol-binding protein, and prealbumin were determined by radial immunodiffusion assay. This technique allows quantitative determination of human plasma proteins after 24 h of diffusion. Serum containing the protein to be tested was placed in a well on the test plate. The area of the resulting antibody–antigen precipitin zone was directly related to the concentration of the substance placed in the plate well.11

Urinary nitrogen were measured by a chemiluminescence method. Nitrogen in biologic samples is oxidized at 1100°C, yielding nitric oxide. On contact with ozone, a metastable nitrogen dioxide is generated, emitting photons on decay. The intensity of the emitted light is proportional to the nitrogen content of the sample.12

Statistical Analyses

All values are expressed as mean ± standard error of the mean. Statistical analyses were performed using Fisher’s protected least significant difference when the overall analysis of variance was significant. P < 0.05 was considered significant.

RESULTS

The clinical details of all patients are presented in Table I. There were no significant differences across groups in age, baseline nutrition data, preoperative cell-mediated immunity, and blood loss, although differences in sex distribution and surgical duration were significant (P < 0.01). The volume of blood transfusion in group B was more than that in group A. Although these factors might have affected the results, we believed that the influences of surgical stress and soybean oil emulsion were greater.

The profiles of IL-6 production are shown in Figure 1. In groups A-1 and A-2, serum IL-6 levels peaked 1 h after surgery (group A-1: 212 ± 37 pg/mL; group A-2: 153 ± 25 pg/mL) and returned to preoperative levels on POD 10 (group A-1: 7 ± 3 pg/mL; group A-2: 13 ± 4 pg/mL). There were no significant differences between groups A-1 and A-2. In groups B-1 and B-2,

### TABLE II.

<table>
<thead>
<tr>
<th>Group</th>
<th>Con A (SI) Before surgery</th>
<th>Con A (SI) POD 7</th>
<th>PHA (SI) Before surgery</th>
<th>PHA (SI) POD 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>105 ± 27</td>
<td>92 ± 25</td>
<td>136 ± 27</td>
<td>160 ± 53</td>
</tr>
<tr>
<td>A-2</td>
<td>145 ± 22</td>
<td>94 ± 23</td>
<td>143 ± 27</td>
<td>143 ± 35</td>
</tr>
<tr>
<td>B-1</td>
<td>142 ± 24</td>
<td>45 ± 7‡</td>
<td>175 ± 37</td>
<td>73 ± 12‡</td>
</tr>
<tr>
<td>B-2</td>
<td>130 ± 43</td>
<td>17 ± 5†‡</td>
<td>149 ± 44</td>
<td>25 ± 9†‡</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± standard error of the mean of stimulation indexes.
† P < 0.01 versus before surgery.
‡ P < 0.05 versus group B-1.
Con A, concanavalin A; PHA, phytohemagglutinin; POD, postoperative days.
SI, stimulation index.
the levels of serum IL-6 were two to three times as high as those of groups A-1 and A-2 and peaked 2 h after surgery (group B-1: 321 ± 21 pg/mL; group B-2: 569 ± 111 pg/mL). Serum IL-6 in group B-2 was significantly higher than that in group B-1 2 h and 1 d after surgery (P < 0.05). Normalization of serum IL-6 in group B was more prolonged than in group A.

The profiles of CRP are shown in Figure 2. CRP levels peaked on POD 3 in all groups. There were no significant differences between groups A-1 and A-2 and between groups B-1 and B-2. CRP levels were significantly higher in group B-2 than in group A-2 on POD 3 and 10 (P < 0.05). CRP levels in group B-1 were significantly higher than those in group A-1 on POD 3 (P < 0.05).

The profiles of glucagon are shown in Figure 3. Glucagon levels peaked on POD 1 in all groups. There were no significant differences between groups A-1 and A-2 and between groups B-1 and B-2. On POD 1, glucagon levels in group B-2 (298 ± 56 pg/mL) were higher than those in group B-1 (224 ± 22 pg/mL), but the difference did not attain statistical significance. On POD 3, glucagon levels in group B-2 (202 ± 21 pg/mL) were significantly higher than those in group A-2 (137 ± 10 pg/mL; P < 0.05), and those in group B-1 (187 ± 36 pg/mL) were significantly higher than those in group A-1 (120 ± 26 pg/mL; P < 0.05). On POD 10, glucagon levels in group B-2 (168 ± 18 pg/mL) were significantly higher than those in group A-2 (116 ± 12 pg/mL; P < 0.05).

Con A- or PHA-stimulated lymphocyte proliferation (stimulation index) decreased significantly on POD 7 compared with preoperative values in groups B-1 and B-2 (P < 0.05); such changes were not observed in groups A-1 and A-2. Con
A-stimulated lymphocyte proliferation in group B-2 was significantly lower than that in group B-1 (Table II). The profiles of the rapid turnover proteins transferrin, retinol-binding protein, and prealbumin are shown in Figure 4. There were no significant differences across groups.

The profiles of cumulative nitrogen balance are shown in Figure 5. In groups B-1 and B-2, cumulative nitrogen balance was significantly negative compared with that in groups A-1 and A-2. There were no significant differences between groups A-1 and A-2 and between groups B-1 and B-2.

DISCUSSION

We investigated the effects of soybean oil emulsion on postoperative stress responses and cell-mediated immunity according to the severity of surgical stress. Despite the degree of surgical stress, all groups received the same dose levels of protein and energy. We previously reported that the levels of protein and energy intake in the presented study are appropriate for severely stressed patients.\(^\text{10}\)

The cytokines IL-1\(\alpha\), IL-1\(\beta\), tumor necrosis factor-\(\alpha\), and IL-6 are often designated as proinflammatory cytokines. These cytokines mediate the responses of hosts to inflammatory stimuli.\(^\text{13,14}\)

In this clinical study, the levels of serum IL-6 in patients who underwent esophagectomy were several times as high as those in patients who underwent gastric or colorectal surgery. Serum levels of IL-1\(\beta\) and tumor necrosis factor-\(\alpha\) were below the minimal standards for detection (data not shown). These results suggest that the level of serum IL-6 reflects surgical stress responses very well.

Lipid emulsion that can be used clinically is limited to soybean oil emulsion. Linoleic acid, an \(\omega-6\) PUFA, is the main fatty acid in this lipid emulsion. Overactivation of the arachidonic acid pathway through the provision of an excessive amount of the precursor linoleic acid by soybean oil emulsion might have deleterious effects in critically ill patients. We previously showed that safflower oil emulsion rich in \(\omega-6\) PUFA increases cytokine production such as IL-6, IL-8, and IL-10 in burned rats.\(^\text{7}\) Mochizuki et al. reported that the administration of safflower oil emulsion, which accounted for 30% to 50% of non-protein calories, decreases muscle weight, serum protein, and nitrogen balance in burned guinea pigs.\(^\text{15}\) Yaqoob et al. found that safflower oil decreases IL-2 production by Con A-stimulated murine lymphocytes.\(^\text{16}\) However, the effects of soybean oil emulsion on the production of proinflammatory cytokines in surgically stressed patients have not been studied in detail. According to our clinical study, soybean oil emulsion increased the level of serum IL-6 in patients who received esophagectomy with thoracotomy, although that was not shown in the patients who received gastric or colorectal surgery. These results suggest that soybean oil emulsion stimulates IL-6 production and amplifies stress responses for only severely stressed patients. Although production of proinflammatory cytokines is believed to benefit the host defense system, overproduction of proinflammatory cytokines has been correlated with poor outcome in severe disease states such as septic shock\(^\text{7,18}\) and burn trauma.\(^\text{19}\) Hack et al. reported that serum IL-6 levels in patients with sepsis are markedly increased, particularly in patients who develop a fatal septic shock.\(^\text{20}\) In the present and previous studies,\(^\text{21}\) the level of serum IL-6 in patients who underwent esophagectomy with thoracotomy was much higher than in those who underwent gastric or colorectal surgery. These findings indicate that IL-6 production was easily overstimulated in the patients who underwent esophagectomy and were fed excessive soybean oil emulsion.

The concept that \(\omega-6\) PUFA regulates the immune system has been established.\(^\text{22}\) Levels of prostaglandin E\(_2\), derived from arachidonic acid, increase in a stressed state and suppress immune function. Several studies have reported that the administration of \(\omega-6\) PUFA increases the duration of transplant survival.\(^\text{23-27}\)

Alexander et al. found that enteral administration of safflower oil increases the release of prostaglandin E\(_2\) from splenic macrophages and decreases delayed-type hypersensitivity in burned guinea pigs.\(^\text{28}\) We also found that the administration of safflower oil emulsion suppresses delayed-type hypersensitivity in burned rats.\(^\text{7}\) In the present study, supplementation of soybean oil emulsion suppressed cell-mediated immune function in patients who received esophagectomy with thoracotomy. However, this immunosuppression was not observed in patients who received gastric or colorectal surgery. These results suggested that soybean oil emulsion suppresses cell-mediated immune function according to the extent of surgical stress.

\(\omega-6\) PUFA is essential for cell membrane phospholipids and critical to maintain cellular function. In this study, we demonstrated that excessive administration of soybean oil emulsion amplifies the stress responses and increases stress-induced immunosuppression especially in patients who had received major surgery.

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(For an additional perspective, see Editorial Opinions.)