Anaesthetics modulate tumour necrosis factor $\alpha$: effects of L-carnitine supplementation in surgical patients. Preliminary results.

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Introduction

Patients undergoing surgery may subsequently have life-threatening multiple organ failure (MOF). 1 The dysregulated release of cytokines by macrophages is likely to play a major role in the pathogenesis of this disorder. 2 Among macrophage derived cytokines, TNF$\alpha$ is pivotal since it is directly involved in mediating the organism adaptative response to injury, including surgery. 3, 4

Several anaesthetic agents are able to affect the pattern of cytokine production by monocytes/macrophages in vitro, including TNF$\alpha$, and several reports have confirmed that the confirmed administration of anaesthetics, profoundly derange the normal functions of the immune system in vivo. 5-9

Here, the results of experiments are reported, which indicate that anaesthetic drugs, most notably Pentothal, could strongly enhance under in vitro conditions the TNF$\alpha$ production by peripheral blood mononuclear cells (PBMCs) from healthy individuals as compared to both lipopolysaccharide (LPS) treated and control cells. In addition, in in vivo studies the authors found that serum levels of TNF$\alpha$ are significantly increased following surgery. The administration of L-carnitine resulted in the reduction of circulating TNF$\alpha$.

Materials and Methods

In vitro experiments:

PBMCs. PBMCs were obtained from healthy volunteers by standard methods, as described previously. 10

Cell cultures. Briefly, PBMCs were resuspended at the concentration of $2 \times 10^6$ cells/ml in RPMI 1640 (Gibco Bio Cult., Paisley, UK) containing 10% heat-inactivated foetal calf serum, 1% L-glutamine, and penicillin/streptomycin. PBMCs were cultured for 72 h in a 5% CO$_2$ atmosphere at 37$^\circ$C in the presence of either standard LPS concentration, as described previously 10 or anaesthetic drugs (Leptofen, Pavulon, Pentothal) at the optimal concentration of 10 $\mu$g/ml. At the end of the culture period, the supernatants were harvested and TNF$\alpha$ was measured.

Measurement of TNF$\alpha$. TNF$\alpha$ was quantified by a sandwich enzyme immunoassay (Biokine, T Cell Sciences Inc., MA, USA), according to standard methods. 11 The lower limit of TNF$\alpha$ detection in this immunoassay was 10 pg/ml. Serum TNF$\alpha$ measured in healthy volunteers was below this limit.

In vivo studies:

Patients. Twenty patients were admitted for surgery and randomly assigned to receive either...
anaesthetic technique. Premedication was performed with atropine sulphate (0.5 mg i.m.) and diazepam (10 mg i.m.) administered 40–50 min before the scheduled time of surgery. Then, anaesthesia was induced with droperidol (2.5 mg), fentanyl (0.1 mg) and thiopentone (3.5 mg/kg). Tracheal intubation was performed after administering pancuronium (0.08 mg/kg). Anaesthesia was maintained with 60% nitrous oxide in oxygen. Supplementary doses of fentanyl, droperidol, and pancuronium were given as needed. Mean doses were fentanyl, 0.056 ± 0.02 mg; pancuronium, 8.3 ± 0.6 mg; droperidol, 3.8 ± 0.5 mg. At the end of surgery, residual neuromuscular blockade was antagonized with a mixture of atropine and neostigmine. All anaesthetic drugs were administered by the same anaesthetist.

Post-surgery care. All patients in both groups were given buprenorphine 0.3 mg for analgesia on the day of surgery only.

l-carnitine treatment. In Group 2 patients, l-carnitine was administered i.v. at the dosage of 8 g at the end of surgery and 24 h afterwards.

Blood sampling. Samples of peripheral blood were obtained for TNFα measurement on the day before the admission to the study. Surgery was performed after administering pancuronium (0.08 mg/kg). Anaesthesia was maintained with 60% nitrous oxide in oxygen. Supplementary doses of fentanyl, droperidol, and pancuronium were given as needed. Mean doses were fentanyl, 0.056 ± 0.02 mg; pancuronium, 8.3 ± 0.6 mg; droperidol, 3.8 ± 0.5 mg. At the end of surgery, residual neuromuscular blockade was antagonized with a mixture of atropine and neostigmine. All anaesthetic drugs were administered by the same anaesthetist.

Statistical analysis: Results are expressed as the mean ± standard deviation. The differences between groups for paired and unpaired data were statistically significant for p less than 0.01.

Results

In vitro experiments: The LPS stimulation of PBMCs proved to strongly increase the TNFα release in culture media as compared to unstimulated PBMCs (p < 0.05), as expected (Fig. 1). However, Pentothal was significantly more effective with respect to LPS in inducing TNFα synthesis and release by PBMCs (p < 0.001) (Fig. 1). The stimulation of PBMCs with other anaesthetic drugs, such as Pavulon and Leptofen, did not result in increased TNFα production. In supernatants from Leptofen driven PBMC cultures TNFα levels were strongly reduced compared to both LPS driven and unstimulated cultures (p < 0.05), whereas in supernatants from Pavulon driven PBMC cultures TNFα was similar to that in unstimulated cultures (Fig. 1).

In preliminary experiments, the addition of l-carnitine at various doses to anaesthetic driven PBMC cultures was followed by conflicting effects on the pattern of TNFα production (data not shown). Further studies to establish both the optimal dosage and kinetics of adding l-carnitine to PBMC cultures in order to obtain a clear-cut modulation of anaesthetic driven TNFα production are therefore needed.

In vivo studies: Patients in Groups 1 and 2 were well matched for age, weight, height, types and/or
Anaesthetics, L-carnitine and tumour necrosis factor α in surgical patients

Duration of surgery. During surgery, blood loss ranged from 600 to 1 200 ml. Nevertheless, patients did not require blood transfusion and received conventional crystalloid or colloid (Dextran 70) fluids. Both Group 1 and Group 2 patients did not exhibit any complication throughout their course and the body temperature following surgery did not exceed 38.2°C. The pattern was similar in both groups. Finally, any treatment related adverse effect was recorded in patients receiving L-carnitine.

The effects of surgical/anaesthetic trauma on TNFα serum levels are shown in Table 1. At t0, all patients had circulating TNFα within the normal range. In Group 1, a significant increase of plasma levels of TNFα was found throughout t1 (40 ± 2 pg/ml; \( p < 0.01 \)) to t3 (62 ± 7 pg/ml; \( p < 0.001 \)), peaking at t2 (68 ± 8 pg/ml; \( p < 0.001 \)), with respect to serum levels at t0 (5 ± 2 pg/ml).

In Group 2 patients strongly increased (\( p < 0.01 \)) serum levels of TNFα were found only at t1 (30.3 ± 5 pg/ml) compared to t0 (6 ± 2 pg/ml); at t2 (15 ± 4 pg/ml) and t3 (22 ± 9 pg/ml) TNFα was not significantly different with respect to t0. Notably, serum levels of TNFα were significantly different between Group 1 and Group 2 patients only at t2 (\( p < 0.001 \)) and t3 (\( p < 0.01 \)).

Total, free, and short-chain carnitine levels were comparable in the two groups and within the normal range at t0 (Table 2). In Group 1 patients, no significant change of total, free, and short-chain carnitine was found at t2 and t3 (Table 2). However, in Group 2 patients serum carnitine levels were increased significantly at t2 and t3 with respect to baseline values, as expected (Table 2).

Discussion

The results indicate that anaesthetic drugs can affect TNFα production and release by monocytes/macrophages, under in vitro conditions. However, strong differences exist among anaesthetic drugs in their ability to modulate TNFα production, as suggested by the finding that Pentothal strongly enhanced the cytokine production compared to LPS, whereas both Pavulon and Leptofen did not. The demonstration that Pentothal is effective in inducing TNFα is consistent with the hypothesis that surgical patients treated with Pentothal could be at risk of MOF, since TNFα is

**Table 1. Serum levels of TNFα (pg/ml) following surgical/anaesthetic trauma in patients receiving placebo (Group 1) and L-carnitine (Group 2)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>t0</td>
<td>5 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>t1</td>
<td>40 ± 2*</td>
<td>30 ± 5*</td>
</tr>
<tr>
<td>t2</td>
<td>68 ± 8**</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>t3</td>
<td>62 ± 7***</td>
<td>22 ± 9</td>
</tr>
</tbody>
</table>

* \( p < 0.01 \) with respect to t0; ** \( p < 0.001 \) with respect to t0; *** \( p < 0.001 \) with respect to t0; \( p < 0.001 \) with respect to t0; \( p < 0.01 \) with respect to t0. For t0, t1, t2, and t3 see Materials and Methods.

**Table 2. Total, free, and short-chain carnitine (nmol/ml) in serum from patients receiving placebo (Group 1) and L-carnitine (Group 2)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Patients</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>Total carnitine</td>
<td></td>
</tr>
<tr>
<td>t0</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>t2</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>t3</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>Free carnitine</td>
<td></td>
</tr>
<tr>
<td>t0</td>
<td>34 ± 7</td>
</tr>
<tr>
<td>t2</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>t3</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>Short-chain carnitine</td>
<td></td>
</tr>
<tr>
<td>t0</td>
<td>6.5 ± 3.5</td>
</tr>
<tr>
<td>t2</td>
<td>5.1 ± 1.9</td>
</tr>
<tr>
<td>t3</td>
<td>4.5 ± 2.7</td>
</tr>
</tbody>
</table>

* \( p < 0.001 \) with respect to both t0 and Group 1. For t0, t2, and t3. See Materials and Methods.
directly involved in the pathogenesis of the disorder. However, anaesthetic drugs per se could also down-modulate the production of TNFα, as shown by Leptofen, or not exhibit any effect at all, as is the case of Pavulon. Therefore, the pathophysiological pathways leading to the increased serum levels of TNFα following surgery, which were demonstrated in the in vivo study, may not be strictly dependent on the immunomodulating properties of the anaesthetic drugs per se. Further studies are needed to fully understand the complex network of immune and endocrine events which account for the increased TNFα production following surgical injury. In fact, the recently reported ability of benzodiazepines to modulate IL-1, IL-6, and TNFα synthesis by human monocytes/macrophages further emphasizes the complexity of the pathophysiological phenomena occurring throughout anaesthesia and surgery.

In the patients enrolled in this trial, serum carnitine levels did not substantially change following surgery, whereas increased levels were found in patients receiving L-carnitine, as expected. In these latter patients, serum TNFα was strongly reduced at t2 and t3 compared to placebo treated subjects. These preliminary data clearly suggest that the treatment with L-carnitine could protect surgical patients against the dysregulated production of monocyte/macrophage derived cytokines, most notably TNFα, which can lead to MOF and death.

References