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Effect of low-dose methyl prednisolone on serum cytokine levels following extracorporeal circulation

Mert Yilmaz, **Serdar Ener** Department of Cardiovascular Surgery, **Halis Akalin** Department of Immunology, **Kadir Sagdic** Department of Cardiovascular Surgery, **O Akin Serdar** Department of Cardiology and **Mete Cengiz** Department of Cardiovascular Surgery, Uludag University Medical Faculty, Bursa

The systemic inflammatory response to cardiopulmonary bypass (CPB) is associated with increased production of cytokines.This systemic inflammatory response characterized by the activation of interleukin-6 (IL-6) and interleukin-8 (IL-8) during and after CPB is well documented. A prospective, randomized, double-blind study was performed so as to understand the effects of low-dose methyl prednisolone sodium succinate (MPSS) on the circulating levels of serum cytokines and clinical outcome.

Twenty patients were randomly divided into two groups on the basis of the administration of low-dose (1 mg/kg) MPSS ($n = 10$) and placebo ($n = 10$) into the pump prime solution. All patients were scheduled to undergo a primary elective coronary artery bypass grafting operation. Patients receiving concurrent corticosteroids, salicylates, dipyridamol or anticoagulants were excluded from the study. Other exclusion criteria were concurrent chronic obstructive pulmonary disease, chronic renal failure, insulin-dependent diabetes, congestive cardiac failure, peptic ulcer history, prior cardiac operations, recent (in a one-month period) myocardial infarction and steroid dependency. Mild systemic hypothermia (30–32°C, rectal) was assured during the CPB. Four blood samples were drawn from the radial artery catheter immediately before starting CPB (T1), following protamine administration (T2) and at 24 (T3) and 48 h (T4) after completion of CPB. In each sample, creatine kinase-myocardial band (CK-MB), white blood cell (WBC), IL-6 and IL-8 levels were measured. IL-6 and IL-8 concentrations were measured by enzyme immunoassay and enzyme-linked immunoabsorbant assay methods.

Address for correspondence: M Yilmaz, Kadir Has University, Florence Nightingale Hospital, Sisli, Istanbul, Turkey. E-mail: mertyilmaz@superonline.com

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Serum IL-6 T2 and serum IL-6 T3 levels were significantly higher than IL-6 T1 levels in both groups ($p < 0.001$) and ($p < 0.01$), and there was no significant elevation in serum IL-8 levels in either group. Serum IL-6 levels were significantly higher in the placebo group than in the MPSS group at T3 ($p < 0.009$). There was no significant difference in CK-MB T1 levels between the groups. Although there was no significant difference between CK-MB T1 and T2 levels in the MPSS group, the CK-MB T2 and CK-MB T3 levels were significantly higher than T1 levels in the placebo group ($p < 0.001$) and ($p < 0.05$).

There was significant elevation of WBC levels at T2 and T3 in both groups without notable difference between the groups ($p < 0.05$).

This study has shown that low-dose MPSS suppresses CPB-induced inflammatory response. Further clinical studies (on larger and higher risk groups) may reveal more information on relations between morbidity and cytokine levels which may have some predictive value on clinical outcome following CPB.

Introduction

After exposure of blood to artificial surfaces during cardiopulmonary bypass (CPB), an acute systemic inflammatory response is induced that is known as the pump or perfusion syndrome in cardiac surgical practice.[1–3] This systemic inflammatory response characterized by the secretion of cytokines (interleukin-6 (IL-6) and interleukin-8 (IL-8)) during and after CPB is well documented. These cytokines might be responsible for many adverse sequelae associated with CPB. For this reason, many investigators suggested using heparin-bonded circuits or ultrafiltration to reduce the generation of cytokines or to eliminate circulating cytokines during CPB. Engelman *et al*. have shown dramatic reductions of complement and cytokine levels with high-dose steroid treatment.[1] A prospective, randomized, double-blind study was performed so as to understand the effects of single low-dose methyl prednisolone sodium succinate (MPSS) on the circulating levels of serum cytokines and the clinical outcome.

Material and methods

Patients

After approval by the university ethics committee (12 December 1994), 25 patients were prospectively included in this study. No study patient had any preoperative sign of infection. All patients had isolated coronary artery disease and each was scheduled to undergo a primary elective coronary artery bypass grafting (CABG) operation. Patients receiving concurrent corticosteroids, salicylates, dipyridamol or anticoagulants were excluded from the study. Other exclusion criteria were concurrent chronic obstructive pulmonary disease, chronic renal failure, insulin-dependent diabetes, congestive cardiac failure, peptic ulcer history, prior cardiac operations, recent (30 days) myocardial infarction and steroid dependency. Of the 25 enrolled patients, 20 patients completed the study. Three patients who required blood transfusion during or after surgery and two patients who had impaired glucose tolerance were excluded from further analyses. Twenty patients were randomly divided into two groups. Details of the patients and operations are summarized in Table 1.

Operative technique

The patients were premedicated with diazepam (0.15 mg/kg i.m.). General anaesthesia was induced with fentanyl (2 μg/kg i.v.) and muscle relaxation was achieved using vecuronium (0.1 mg/kg i.v.). Ceftriaxone (1 g i.v.) was used for antimicrobial prophylaxis. Mechanical ventilation was controlled to maintain $PaCO_2$ at approximately 40 mmHg and anaesthesia was supplemented by inhalation of 1% isofluorane.

The extracorporeal circuit consisted of a centrifugal pump and cardiotomy reservoir, and a membrane oxygenator (Biomedicus Centrifugal Blood Pump, Eden-Praire, USA; Maxima Plus PRH, Medtronic Minneapolis, MN, USA). The

Table 1 Characteristics of the patients and results are expressed as mean ± SD

Groups	Placebo	MPSS
Male/female	8/2	8/2
Age	55.1 ± 6.06	49.6 ± 8.89
Weight (kg)	74.3 ± 8.88	76.4 ± 9.37
Body surface area (m^2)	1.81 ± 0.15	1.84 ± 0.14
Distal graft number	2.80 ± 1.22	2.70 ± 1.15
CPB time (min)	113 ± 52.2	109.7± 26.75
Crossclamp time (min)	62.1 ± 32.95	59.9 ± 13.38
Preoperative ejection fraction (%)	55.8 ± 12.13	54.2 ± 9.0
Angina classification (Canada)	3.1 ± 1.1	3.20 ± 1.13

circuit was primed with 1800 ml of Ringer's lactate solution containing 8.4% $NaHCO_3$ 8 mmol/l, 20% mannitol 2.5 ml/kg and heparin 5000 IU. The patients were divided into two groups on the basis of the administration of low-dose (1 mg/kg) MPSS ($n = 10$) and placebo ($n = 10$) into the pump prime solution. After a baseline activated clotting time (ACT) was obtained, anticoagulation was achieved using intravenous heparin (300 IU/kg body weight). Before bypass, supplemental heparin was administered to prolong the ACT above 480 s.

CPB was instituted with the use of a single two-stage venous cannula placed through the right atrial appendage and an arterial cannula placed in the ascending aorta. Transatrial retroplegia cannulae were placed in all cases. Nonpulsatile extacorporeal circulation was initiated at a flow of 2.4 l/kg/min. Mild systemic hypothermia (30–32°C, rectal) was assured during CPB. Ultrafiltration and cell saver were never used during the study. Haematocrit levels were maintained over 20% throughout CPB.

Myocardial protection was obtained using intermittent antegrade and retrograde cold-blood potassium cardioplegia and terminal warm blood cardioplegia. Heparin was neutralized with protamine sulphate, at a ratio of 1 : 1, within 5 min after the end of CPB.

Blood sampling

Four blood samples were drawn from the radial artery catheter immediately before starting CPB (T1), following protamine administration (T2) and at 24 (T3) and 48 h (T4) after completion of CPB. The samples were immediately cooled at 4°C, centrifuged (1500*g*, 20 min, 4°C) and serum stored at –80°C until blind bench analysis.

Assay technique

In each sample, creatine kinase-myocardial band (CK-MB), IL-6 and IL-8 levels were measured. IL-6 and IL-8 concentrations were measured by EIA (enzyme immunoassay (Kit IL-6, Immunotech International, France)) and ELISA (enzyme-linked immunoabsorbant assay (Immunotech International)) according to the manufacturer's procedure. The detector limits were 3 pg/ml for IL-6, 10 pg/ml for IL-8. Results were not corrected for haemodilution because of the postoperative homogene variation of the haematocrit levels.

Follow-up

Operative and postoperative variables (cross-clamp time, CPB time, cardiac index, postoperative inotropic requirement, body temperature, mechanical ventilation time, intensive care unit and postoperative hospital stay and other morbidity) were recorded. Postoperative transthoracic echocardiographic evaluation of left ventricular global and segmental function was performed before discharge (5–7th postoperative day).

Statistics

Statistical analysis was performed using GraphPad InStat for MS-DOS mode (GraphPad software V2.02 Dr Granger, LSU Medical Center). The Mann–Whitney *U*-test was used for comparison of values between the groups. Due to the statistically significant difference obtained from Freidman nonparametric repeated measures ANOVA test, Dunn's multiple comparisons tests were also performed for intragroup comparisons. A p-value less than 0.05 was considered significant. Correlations between peak cytokine values and different parameters were assessed by Spearman's rank correlations. All data are expressed as mean ± SD.

Results

Table 2 shows the clinical characteristics and operative data for MPSS ($n = 10$) and placebo ($n = 10$) groups undergoing CPB and there was no statistical difference (Mann–Whitney U, $p > 0.05$) observed between the groups in sex, age, weight, body surface area, number of distal grafts, CPB time, crossclamp time, preoperative ejection fraction (EF1), angina status (Canadian classification), mechanical ventilation time, intensive care unit (ICU) time, postoperative ejection fraction (EF2) and postoperative hospital stay.

IL-6 and IL-8 levels were detectable in four patients in the MPSS group and in five patients in the placebo group at T1. Serum IL-6 T2 and serum IL-6 T3 levels were significantly higher ($p < 0.001$ and $p < 0.01$) than IL-6 T1 levels in both groups and also serum IL-6 levels were significantly ($p < 0.009$) higher in the placebo group than the MPSS group at T3 (Figure 1). There was no significant elevation in serum IL-8 levels in both groups and also no statistically significant difference between the groups (Table 3).

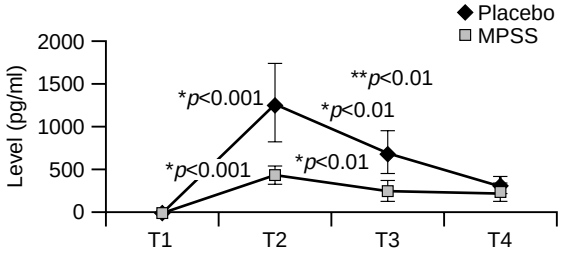

Figure 1 Serum IL-6 T2 and serum IL-6 T3 levels were significantly higher (*Friedman ANOVA test, $p < 0.001$ and $p < 0.01$) than IL-6 T1 levels in both groups and also serum IL-6 levels were significantly (**Mann–Whitney U-test, $p < 0.01$) higher in the placebo group than the MPSS group at T3. (Graph: mean ± SD.)

Table 2 Postoperative data are expressed as mean ± SD

Groups	Placebo	MPSS
Ventilation time (h)	11.7 ± 3.56	13.4 ± 11.05
ICU time (h)	34.2 ± 7.8	33.7 ± 17.3
Postoperative ejection fraction (%)	54.1 ± 6.8	50.0 ± 9.5
Postperative hospital stay (days)	9.2 ± 8.7	6.6 ± 0.9

Table 3 There was no significant elevation in serum IL-8 levels in both groups (Friedman ANOVA test) and also no statistically significant difference between the groups (Mann–Whitney U-test). Values (pg/ml) are expressed as mean ± (SD)

Groups	Placebo	MPSS
IL-8 T1	57.4 ± 10	67.8 ± 15.6
IL-8 T2	2366 ± 1546.8	121 ± 27.4
IL-8 T3	45 ± 6.9	92.6 ± 21.6
IL-8 T4	384 ± 320.6	150.6 ± 82.3

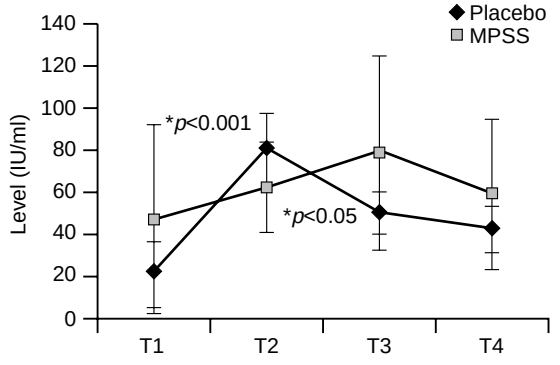

Figure 2 There was no significant difference in CK-MB T1 levels between the groups. Although, there was no statistically significant elevations between CK-MB levels in the MPSS group, the CK-MB T2 and CK-MB T3 levels were significantly (*Friedman ANOVA test, $p < 0.001$ and $p < 0.05$) higher than T1 levels in the placebo group. (Graph: mean ± SD.)

There was no significant difference in CK-MB T1 levels between the groups. Although there were no statistically significant elevations between CK-MB levels in the MPSS group, the CK-MB T2 and CK-MB T3 levels were significantly ($p < 0.001$ and $p < 0.05$) higher than T1 levels in the placebo group (Figure 2).

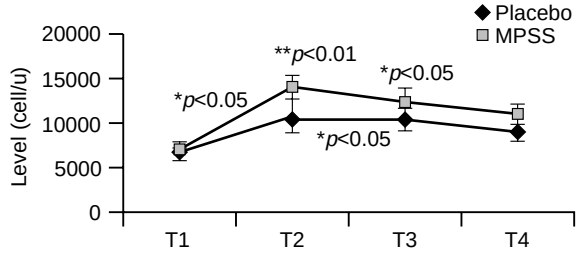

Figure 3 There was significant elevation of WBC levels at T2 and T3 in both groups. In the steroid group the p-value was less than 0.01 (**Friedman ANOVA test, $p < 0.01$) and in the placebo group the p-value was 0.05 at T2 (*Friedman ANOVA test, $p < 0.05$). (Graph: mean ± SEM.)

There was significant elevation of white blood cell (WBC) levels at T2 and T3 in both groups. In the steroid group the *p*-value was less than 0.01 and in the placebo group the *p*-value was 0.05 at T2 (Figure 3).

There was no correlation between peak levels of IL-6 or IL-8 and CPB or aortic crossclamping durations. The preoperative and postoperative (5–7th day) echocardiographic investigations showed no statistically significant difference in EF1 and EF2 between the groups.

One patient in the MPSS group and two patients in the placebo group required inotropic support in the early postoperative period. There was no significant difference in temperature follow-up between the groups, but two patients in the placebo group who had dramatically high IL-6 and IL-8 levels at T2 also had high fever (38.5°C, rectal); one of them was diagnosed as mediastinitis on the fifth postoperative day. Blood and pus culture revealed methicillin-resistant *Staphylococcus aureus*. This patient was treated with vancomycin and early surgical debridement and discharged with complete healing. The other patient required inotropic support postoperatively. No side-effects of MPSS were observed in the patients receiving this drug.

Discussion

The systemic inflammatory response to CPB is associated with increased production of cytokines. IL-6 and IL-8 levels have both been clearly demonstrated to be elevated during and after CPB.[1–5] Steinberg *et al*. had reported that IL-6 levels increased after protamine administration and reached a maximum at 3 h after bypass.[2] At 24 h after bypass, IL-6 levels remained above the levels measured at induction.[2] Our results have shown that the IL-6 levels were above the T1 at the T2 and T3 in both groups. We have also observed no change in IL-8 levels at any sampling point in either group. Finn *et al*.[3] reported that IL-8 release was correlated significantly with the length of CPB, although Frering *et al*.[6] reported that there was no correlation between peak levels of IL-6 or IL-8 and CPB or crossclamp times as we observed in our small number of patients.

The systemic inflammatory response following CPB may have a role in postoperative myocardial dysfunction. Hennein *et al*.[7] demonstrated a correlation between postoperative serum levels of cytokines and left ventricular wall motion abnormalities and myocardial ischaemia, suggesting that IL-6 and IL-8 might be responsible for myocardial depression.[7] Therefore, there is a strong possibility that myocardial stunning might be relieved if cytokine generation or neutrophil activation were inhibited.

As expected, the WBC counts at T2 and T3 were higher than the count at T1 in both groups. We cannot conclude from the higher significance of the WBC count difference in the steroid group compared to the placebo group that MPSS administration can reduce leukocyte sequestration. Our results should be supported by further investigation. There was no notable difference in preoperative and postoperative EF levels between the groups. CK-MB levels at T2 and T3 were significantly higher than T1 levels in the placebo group, but no level difference was observed in the MPSS administered group. CK-MB is not a very specific parameter for showing myocardial injury but in light of previous investigations[7] these data suggest that there may be a relationship between the administration of low-dose MPSS and reduced myocardial injury with lower cytokine levels. Troponin T assay should be included in a further study as a more specific marker of myocardial injury in this regard.

Inaba *et al*. reported that high-dose (30 mg/kg) MPSS significantly suppressed a CPB-induced increase in IL-6 levels.[8] Jorens *et al*. administered the same dose of MPSS to patients undergoing CPB in an effort to reduce CPB leukocyte sequestration and found significantly lower plasma IL-8 concentrations in patients treated with MPSS at the end of CPB compared to those without treatment.[5] Engelman *et al*. have also shown that high-dose steroid use significantly reduced IL-1 and IL-8 levels postoperatively.[1] In our study, IL-6 levels were significantly higher in the placebo group than in the MPSS group at the postoperative 24th hour. Markewitz *et al*. administered indomethasine and tymopentin to patients undergoing CPB in an effort to suppress CPB-induced increase in IL-6.[9]

Steinberg *et al*. showed that the use of heparin-bonded bypass circuits decreased the circulating levels of serum IL-6 and IL-8.[10] There was no possibility of comparing the groups regarding clinical outcome because of the small number of patients. However, two patients in the placebo group who

had significantly high IL-6 and IL-8 levels at T2 also had high fever and one of these patients was diagnosed as mediastinitis.

Recent studies have shown that platelet concentrates contain significant concentrations of IL-1, IL-6 and tumour necrosis factor (TNF).[11,12] The most notable difference of this study from the previous similar research projects was the exclusion of the patients requiring blood transfusion from this study.

In conclusion, this is the first study suggesting that 'low-dose MPSS' may help suppress CPB-induced inflammatory response. Further clinical studies are needed to reveal more information on relations between morbidity and cytokine levels which may have some value on clinical outcome following CPB.

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