Suppression by methylprednisolone of augmented plasma endotoxin-like activity and interleukin-6 during cardiopulmonary bypass

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SUMMARY

It has been reported that plasma endotoxin, measured by Limulus amoebocyte lysate assays, markedly increased during extracorporeal circulation (ECC). Therefore, this study was undertaken to see if pretreatment with methylprednisolone 30 mg kg⁻¹ modifies the endotoxaemia and associated increases in plasma cytokines in 17 patients undergoing cardiac surgery requiring ECC. We found that methylprednisolone suppressed significantly the ECC-induced increases in plasma endotoxin, measured by a conventional Limulus amoebocyte lysate assay (Toxicolor), and interleukin-6. Plasma concentrations of endotoxin, measured by a highly specific chromogenic Limulus test (Endospecy test), tumour necrosis factor-α and interleukin-1β did not increase significantly in either the control or methylprednisolone groups. (Br. J. Anaesth. 1994; 72: 348-350)

KEY WORDS

It is well known that cardiac surgery with extracorporeal circulation (ECC) may be associated with coagulopathy and dysfunction of vital organs [1]. The pathophysiological mechanisms of this “post-perfusion syndrome” remain to be clarified. Recently, several investigators have demonstrated that extracorporeal circulation is associated with increased plasma concentrations of endotoxin, as measured by Limulus amoebocytes lysate (LAL) tests [2, 3]. This finding may be important pathophysiologically, as endotoxin activates macrophages to produce tumour necrosis factor (TNF), interleukin-1β (IL-1β) and interleukin-6 (IL-6), which may be involved in the development of organ failure. However, the concentrations of endotoxin reported previously varied widely, probably because of differences in analytical methods. The diagnosis of endotoxaemia is difficult, as the conventional LAL tests are not specific for endotoxin [4].

Corticosteroid treatment has been recommended to minimize the risk of postperfusion syndrome [5] and pretreatment with corticosteroids suppresses the overall immune reactions to endotoxin or lipopolysaccharide. Therefore, the present study was conducted to see if pretreatment with methylprednisolone may modify endotoxaemia and circulating concentrations of TNF-α, IL-1β and IL-6.

METHODS AND RESULTS

The study was approved by our Institutional Committee. We studied 17 adult patients undergoing open-heart surgery for valvular replacement or coronary artery bypass grafting. We studied eight patients for changes in plasma concentrations of endotoxin, measured by a conventional LAL assay, and another eight for changes in plasma endotoxin, measured using specific antibodies. In each of these two groups, four patients were treated with methylprednisolone. In the remaining one patient who received methylprednisolone, plasma endotoxin concentration was determined by both conventional and specific LAL assays, and plasma concentrations of TNF-α, IL-1β and IL-6 were also measured. All patients in the two control groups underwent coronary artery bypass surgery. However, two patients in the methylprednisolone-treated groups underwent aortic, or mitral valve replacement, or both. The distributions of gender and age were similar in the two treatment groups. All patients received a laxative after dinner and an enema was given 2 h before admission to the operating room.

The patients were premedicated with atropine 0.5 mg i.m. and diazepam 0.15-0.2 mg kg⁻¹ i.m. General anaesthesia was induced with an infusion of fentanyl 10-20 µg kg⁻¹ i.v. for 5-10 min, slow administration of midazolam 10 mg i.v. and pancuronium 0.15-0.2 mg kg⁻¹ i.v. Anaesthesia was maintained with continuous infusions of fentanyl 500 µg h⁻¹, midazolam 1-2 µg h⁻¹ and pancuronium 1 mg h⁻¹. In those patients undergoing coronary artery bypass surgery, nitroglycerine and diltiazem were administered continuously at a rate of 0.3-0.5 µg kg⁻¹ min⁻¹.

A roller pump (Type MHS, Senko, Tokyo, Japan) and membrane oxygenator (Maxima 1380, Medronic, U.S.A. or Univox, Baxter, CA, U.S.A.) were used in the extracorporeal circuit. All tubing used in the circuit was made from polyvinylchloride. The priming solution comprised lactated Ringer solution, THAM, mannitol, aprotinin, antibiotics and stored...
autologous blood. Immediately before ECC, heparin 3 mg kg⁻¹ i.v. was administered. In the methylprednisolone-treated groups, methylprednisolone 30 mg kg⁻¹ was also administered. The initial pump flow was 80 ml kg⁻¹ min⁻¹. Body temperature was decreased to 26–28 °C. Myocardial protection was produced with cold Young’s solution and external cooling with sterile ice. After cardiac surgery was completed, body temperature was allowed to recover and the aortic cross-clamp was removed. Cardiopulmonary bypass was discontinued when the haemodynamic state was stable. Aortic cross-clamp time ranged from 74 to 248 min in the control groups and from 72 to 206 min in the methylprednisolone groups. Cardiopulmonary bypass time ranged from 105 to 374 min in the control groups and from 95 to 321 min in the methylprednisolone groups. In two control patients and two methylprednisolone patients, bypass time exceeded 240 min because of surgical difficulties.

Blood samples were obtained from a radial artery cannula immediately before induction of anaesthesia, immediately before initiation of ECC, 1 h after initiation of ECC, immediately after release of the aortic clamp, immediately after termination of ECC, 1 h after the end of ECC and 1 h after the end of operation. Arterial blood was also obtained 24 h after the end of operation. Blood samples were collected into sterile, heparin-coated, endotoxin- and β-glucan-free tubes, and centrifuged (150 g) for 10 min at 4 °C to obtain platelet-rich plasma.

Plasma endotoxin activity was measured by a conventional LAL assay in the first group and by a specific LAL assay in the second group. The conventional assay was performed using a commercially available chromogenic test kit (Toxicolor; Seikagaku Kogyo, Tokyo, Japan), and the specific LAL assay was undertaken using a kit (Endospecy; Seikagaku Kogyo, Tokyo, Japan) as described previously [5]. In the second group, plasma concentrations of TNF-α were measured by an enzyme-linked immunosorbent assay (ELISA) (Fuji Rebio, Tokyo, Japan) using anti-human TNF-α monoclonal antibody. Plasma concentrations of IL-1β were measured by a two-step sandwich method using two types of monoclonal antibody against human IL-6 (Fuji Rebio, Tokyo, Japan).

Differences between the two groups were analysed at each sampling point by the Mann–Whitney test. Changes in variables in each group were analysed by two-way analysis of variance (ANOVA). Differences were considered significant when probability (P) values were less than or equal to 0.05. All values in the figure and text are expressed as mean (SEM).

As shown in figure 1A, plasma endotoxin concentration, measured by the Toxicolor test, was increased significantly in the control groups to a very high concentration after the release of the aortic clamp. In the methylprednisolone groups, plasma endotoxin activity was not increased significantly by ECC (P = 0.063, two-way ANOVA). There were significant differences in plasma endotoxin concentrations between the two groups immediately after release of the aortic clamp (P = 0.0275), and immediately (P = 0.0275) and 1 h (P = 0.05, Mann–Whitney test) after the end of ECC.

Plasma endotoxin concentrations, measured by a specific LAL test (fig. 1B), and plasma concentrations of TNF or IL-1β (data not shown) did not alter significantly with ECC in either the control or methylprednisolone groups. Plasma concentrations of IL-6 increased significantly after release of the aortic clamp in the control groups. In the methylprednisolone groups, plasma IL-6 concentration was increased significantly after the end of ECC. There were significant differences in plasma IL-6 concentration between the two groups after release of the aortic clamp (P = 0.0275) and after the end of ECC (P = 0.05, Mann–Whitney test).

**Fig. 1.** Effect of methylprednisolone on ECC-induced increase in plasma activity of endotoxin, as measured by the Toxicolor or the Endospecy test, and interleukin-6 (IL-6) concentration. A: In the first group of patients, plasma endotoxin concentration was measured by the Toxicolor test, in the control (○) and methylprednisolone (●) groups (1) before induction of anaesthesia; (2) before initiation of ECC; (3) 1 h after initiation of ECC; (4) immediately after release of the aortic clamp; (5) immediately after the end of ECC; (6) 1 h after the end of ECC; (7) 1 h after the end of operation. B and C: In the second group of patients, plasma endotoxin and IL-6 concentrations were determined by the Endospecy test and a two-step sandwich method, respectively. Blood was sampled at points (1)–(7) as above (A) and at (8) (24 h after the end of operation). *P < 0.05 compared with the control group (Mann–Whitney test). Closed symbols represent significant changes from sampling point (2) in each group (P < 0.05, two-way ANOVA, followed by Dunnet’s test). Values are mean and SEM.
There were no significant differences in white blood cell and platelet counts 4 h after operation between the control and methylprednisolone groups. None of the patients, either in the control or methylprednisolone groups, had any postoperative complications or organ failure.

COMMENT
In this study, there are wide differences between plasma endotoxin concentrations measured by the conventional LAL (Toxicolor) test compared with the specific LAL (Endospecy) test. As described previously [2], ECC increased plasma concentrations of endotoxin (Toxicolor test) after release of the aortic clamp. However, ECC did not increase plasma endotoxin concentrations measured by the Endospecy test. A similar observation has been reported in patients undergoing dialysis [4]. Conventional LAL contains a (1-3)-β-D-glucan-sensitive factor G, and therefore the conventional LAL tests are not specific for endotoxin. The Endospecy test uses factor G-free LAL and chromogenic substrate to exclude the effects of LAL-reactive substances [4]. Thus it is suggested that ECC increases circulating LAL-reactive or endotoxin-like substances, but not endotoxin. The diagnosis of endotoxaemia is influenced not only by the specificity of the analytical method but also the presence of inhibitory factors in plasma and binding of endotoxin to plasma proteins. Careful consideration should be given to the diagnosis of endotoxaemia until a standardized analytical method is established. It should be noted that the patients in our study received both laxatives and an enema which lavaged the gut and may have minimized the risk of translocation of bacteria and endotoxin.

Pretreatment with high-dose methylprednisolone prevented the ECC-induced increases in LAL-reactive materials or endotoxin-like activity and plasma IL-6 concentration. It is debatable if the suppression of IL-6 by methylprednisolone was caused by inhibition of ECC-induced augmentation of endotoxin-like activity which may stimulate IL-6 production. Further studies are required to clarify the origin and pathophysiological nature of LAL-reactive substances.

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REFERENCES