EFFECTS OF CORONARY BYPASS SURGERY UNDER HIGH-DOSE FENTANYL ANAESTHESIA ON GRANULOCYTE CHEMILUMINESCENCE

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Many host defence mechanisms are affected by open-heart surgery. For example, cell-mediated (Roth et al., 1981) and humoral immunity (Eskola et al., 1984) and natural killer cell activity (Ryhanen, Huttunen and Ilonen, 1984) are depressed. In addition, complement activation has been documented (Haslam, Townsend and Branthwaite, 1980). However, the most important single defence mechanism against invading bacterial infections is phagocytosis with intracellular killing.

Measurement of chemiluminescence is a new, rapid method for the evaluation of bactericidal related oxidative mechanisms of phagocytically activated granulocytes (Horan, English and McPherson, 1982). The present study evaluated the changes in granulocyte chemiluminescence activity against zymosan, S. aureus, E. coli and N-formylmethionyl-leucylphenylalanine (FMLP) in patients after coronary artery bypass surgery. FMLP is a synthetic tripeptide, an analogue of bacterial chemotactic factors (Snyderman and Goetzl, 1981). Zymosan is a suspension of yeast particles. S. aureus and E. coli were chosen because they are responsible for the majority of the postoperative infections in our hospital.

PATIENTS AND METHODS

The study was undertaken in 12 patients admitted for elective coronary bypass surgery involving extracorporeal circulation (mean ± SD age 51 ± 6 yr, weight 77 ± 9 kg and height 170 ± 8 cm). No patient had a history of malignancy, and none was receiving immunosuppressive drugs. The operation was performed under high-dose fentanyl anaesthesia (induction dose of fentanyl $75 \mu g \text{kg}^{-1}$, total dose during operation $108 \pm 6 \mu g \text{kg}^{-1}$) with 40 % oxygen in air. Surgery was performed under moderate (30 °C) generalized hypothermia. The heart–lung machine with a bubble oxygenator was primed with 1500 ml of crystalloid solution, 1000 ml of gelatin solution and 3–4 units of packed red blood cells. The flow rate was 2.4 litre min$^{-1}$/m$^2$ body surface area. Patients received cefuroxime 1.5 g i.v. during the surgical procedure, and 750 mg three times daily for the 3 days following operation. As antithrombotic drugs, acetylsalicylic acid 250 mg once daily and dipyridamole 75 mg three times daily were given before and after the operation. The mean duration of surgery was 328 ± 59 min, and of extracorporeal circulation was 116 ± 26 min. After operation the lungs of all patients were ventilated artificially until the following morning.

One patient with an urinary catheter had an urinary tract infection (S. aureus), which was
recognized 5 days after operation. No other complications were observed.

**Blood samples**

Samples of heparinized peripheral blood were taken between 7.30 and 8.00 a.m. on the day before and on the day of operation, and on days 1, 3 or 4 and 6 or 7 after operation. The samples were processed and measured immediately. At least 200 cells were counted for leucocyte and differential counts, using May–Grünwald–Giemsa-stained smears.

**Isolation of granulocytes**

Granulocytes were isolated by a modification of the one-step. Percoll density gradient centrifugation method (Hjorth, Jonsson and Vretblad, 1981). An isotonic Percoll stock solution was made by diluting 9 vol of Percoll (Pharmacia, Fine Chemicals, Uppsala, Sweden) with 1 vol of NaCl 1.5 mol litre⁻¹. The 74% and 55% (v/v) Percoll solutions were made by diluting the isotonic stock solution appropriately with NaCl 0.15 mol litre⁻¹.

Heparinized blood 4 ml was carefully layered on top of the discontinuous Percoll gradient prepared with 55% isotonic Percoll 4 ml and, under it, 74% isotonic Percoll 4 ml. The gradients were centrifuged at 350 × g for 20 min at 4 °C. A clear band of granulocytes formed in the lower part of the gradient just above the band of erythrocytes. The cells were removed carefully with a Pasteur pipette, washed twice with Hanks’ balanced salt solution without phenol red (HBSS, Flow laboratories Ltd, Irvine, Scotland) with opsonizing plasma added. Finally, the granulocytes were resuspended to the final concentration of 2 × 10⁹ cells ml⁻¹. The purity of the granulocyte pool was evaluated on spread smears stained with May–Grünswald–Giemsa stain. The viability of the cells (over 95%) was confirmed with the trypan blue exclusion method.

**Opsonizing plasma**

Plasma from a blood group O Rh-negative healthy person without any medication was collected to opsonize zymosan and the microorganisms. The plasma was stored at −70 °C in 2 ml volumes until required.

**Preparation of opsonized zymosan**

Zymosan (Sigma Chemical Co., St Louis, Missouri, U.S.A.) suspended in phosphate buffered saline (PBS) to a concentration of 20 mg ml⁻¹ was boiled for 30 min. It was then opsonized by incubating it with opsonizing plasma at 37 °C for 30 min. After washing, zymosan was centrifuged and resuspended in PBS to a concentration of 20 mg ml⁻¹.

**Preparation of FMLP**

Chemotactic peptide, N-formylmethionyl-leucylphenylalanine (FMLP, Sigma Chemical Co.) was dissolved in dimethylsulphoxide (Sigma Chemical Co.) and diluted with HBSS to 7 × 10⁻³ mol litre⁻¹.

**Preparation of bacteria**

*Staphylococcus aureus* (strain 25923, American Type Culture Collection (ATCC), Rockville, Maryland, U.S.A.) and *Escherichia coli* (strain 25922, ATCC) bacteria were grown for 6 h in brain–heart infusion broth (Difco, Detroit, U.S.A.). The bacteria were then harvested in tryptone soya broth (Difco) containing 5% dimethylsulphoxide and stored in small aliquots at −70 °C. After thawing, the amounts of viable bacteria were measured by the limiting dilution method described by Koch (1981). The suspension was washed three times with HBSS and its concentration adjusted to 1 × 10⁸ bacteria ml⁻¹ with HBSS.

**Chemiluminescence assay**

To each test vial, 0.5 ml of granulocyte suspension (1 × 10⁸ cells) in 1.4% opsonizing plasma was added and the mixture was incubated for 30 min at 37 °C. After incubation, 100 μl of luminol 0.001 mol litre⁻¹ (Sigma Chemical Co.) was pipetted and the solution was incubated for 30 min at 37 °C. Immediately before measurement, 100 μl of the microorganisms (10⁷ bacteria), zymosan, FMLP or HBSS as blank solution was added to the appropriate vials. The vials were placed immediately in the light-proof chamber of Luminometer 1251 (LKB Wallac, Finland) and measurements started. Chemiluminescence in FMLP vials was measured at 10-s intervals and in other vials at 5-min intervals for 70 min. The resulting light output was measured in millivolts (mV). The results were read at peak activity, which was found to be comparable to the area under the chemiluminescence curve.

**Statistical analysis**

Changes in leucocyte and granulocyte counts, and in chemiluminescence values, were studied by
the analysis of variance for repeated measurements and by Dunnett's test. Logarithmic transformation was used for normalization of the distribution of chemiluminescence values.

RESULTS

Leucocyte and granulocyte counts were increased on the first day after operation \((P < 0.05)\) and on days 6–7 \((P < 0.01)\), but not on days 3–4 (table I).

The chemiluminescence responses of the granulocytes to zymosan were decreased on day 1 \((P < 0.01)\) and on days 3–4 \((P < 0.01)\); and those to \(S. aureus\) \((P < 0.01)\) and \(E. coli\) \((P < 0.05)\) were decreased on days 3–4 (table II). The values had returned to preoperative values by days 6–7 in the postoperative period. No changes were seen in chemiluminescence values for responses to FMLP or in unstimulated controls, nor were any differences observed between the two preoperative values in any of the responses.

DISCUSSION

A coronary bypass operation involving extracorporeal circulation is a procedure characterized by extensive surgical trauma, mechanical manipulation of blood, deep anaesthesia, hypothermia and blood transfusion; most of these can affect the function of granulocytes. Indeed the granulocyte chemiluminescence responses to zymosan, \(S. aureus\) and \(E. coli\) were depressed in this study following surgery. Depression of the metabolic activity of granulocytes has also been shown by the nitroblue tetrazolium dye test during and after anaesthesia and surgery (Bardosi and Tekeres, 1985), and depressed chemiluminescence responses have also been observed after general anaesthesia (Lippa et al., 1983). However, since the most marked depression of chemiluminescence in this study occurred on days 3 and 4 of the postoperative period, anaesthesia could not have been the only cause.

Of the drugs received by the patients, anti-inflammatory agents are able to inhibit granulocyte chemiluminescence (Van Dyke et al., 1979). However, the dose of acetylsalicylic acid was low and medication continued throughout the period of observation. Although the patients received prophylactic cefuroxime, cephalotin has been the only cephalosporin shown to inhibit chemiluminescence activity at high plasma concentrations (Welch, Davis and Thrupp, 1981; Milatovic, 1983). Any effect of serum suppressive factors such as those found in septic patients (Lanser et al., 1985) is also unlikely because, in our study, the granulocytes were washed several times during harvesting and we always used the same opsonizing plasma. One explanation might be a lack of some opsonic receptors on the surfaces of granulocytes, since different opsonic receptors (Fc and C3b receptors) are needed for phagocytosis of zymosan, \(S. aureus\) and \(E. coli\) (Hed and Stendahl, 1982; Horwitz, 1982). Another explanation may be a discharge of myeloperoxidase-containing granules and exhaustion of the granulocytes, because phagocytosis-induced and luminol-enhanced chemiluminescence depends on a myeloperoxidase-mediated reaction of the granulocytes (De Chatelet et al., 1982). Indeed, such granulocyte degranulation has been found after surgery.
especially in critically ill patients. Such an granulocyte microbicidal functions. This depression activity that occurred in this study after molecules (Snyderman and Goetzl, 1981). Since have specific high-affinity receptors for FMLP-like molecules, granulocytes unaffected in the present study. Granulocytes the chemiluminescence-producing chemoattractant response is mediated by proteins on the surface of the granulocyte cell (Allfred and Hill, 1978), their activity seems to remain unaffected following surgery. However, there are some reports of depressed chemotaxis after surgery and anaesthesia (Stanley et al., 1976; Wandall, 1982).

The depression of granulocyte chemiluminescence activity that occurred in this study after coronary bypass surgery reflects a disturbance in granulocyte microbicidal functions. This depression might contribute to postoperative infection, especially in critically ill patients. Such an infection was not, however, observed in any of the patients studied. The postoperative granulocytosis possibly compensated for the disturbance in granulocyte function.

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REFERENCES
