IMPAIRED B LYMPHOCYTE FUNCTION DURING OPEN-HEART SURGERY

Effects of Anaesthesia and Surgery

J. ESKOLA, M. SALO, M. K. VILJANEN and O. RUUSKANEN

B lymphocyte function in vitro was measured in patients undergoing open-heart surgery. Conventional balanced anaesthesia, or high-dose fentanyl anaesthesia was used. Pokeweed mitogen (PWM) induced lymphocyte transformation was depressed at the end of the operation, but the response to formalinized Staphylococcus aureus Cowan I (StaCw) was not. The numbers of immunoglobulin producing and secreting cells measured by an indirect protein A plaque-forming cell assay decreased after PWM-stimulation, but remained unchanged after StaCw stimulation at the end of the operation. IgG, IgM and IgA secretion by PWM- and StaCw-stimulated lymphocytes, into the culture medium, was depressed in the period after operation. Depressed immune functions occurred after open-heart surgery, but not in association with anaesthesia alone before surgery. The decreases were not mediated by hydrocortisone-sensitive suppressor cells. Minor differences between the two anaesthetic techniques were found in lymphocyte proliferative responses.

Anaesthesia and surgery affect the immune defence mechanisms, but it has been shown that anaesthesia per se has either none, or only minor immunological effects. In contrast, surgical trauma can cause immunosuppression of clinical importance (Salo, 1982a). However, the clinical effects of anaesthesia and surgery on cell-mediated immunity have been documented more extensively than those on humoral immunity primarily because of a lack of proper techniques for assessing human humoral immune functions. Recent developments in immunological techniques have made it possible to study B lymphocyte function in vitro. This study has investigated the effects of anaesthesia and open-heart surgery on B cell functions and lymphocyte responses to mitogens in vitro.

PATIENTS AND METHODS

Twenty male patients admitted for open-heart surgery were allocated to two groups (table I). Ten patients in group A underwent operation under conventional balanced anaesthesia using thiopen-tone, neuroleptic drugs, fentanyl (mean dose 17 ± 3 (SD) µg kg⁻¹) and nitrous oxide in oxygen. The patients in group B were anaesthetized using high-dose fentanyl (mean dose 72 ± 22 µg kg⁻¹). Six of the patients in group A underwent coronary bypass surgery, three underwent aortic and one, mitral valve, replacement. All the patients in group B underwent coronary artery bypass operations. The operations were performed under moderate (31°C) generalized hypothermia. The Rygg–Kyvsgaard heart–lung machine with a roller pump and disposable bubble oxygenator was primed for extracorporeal circulation with Haemaccel 1000 ml, Ringer’s lactate solution 1500 ml and four units of

---

**TABLE I. Patient data (means ± SD)**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>51.20 ± 8.9</td>
<td>47.50 ± 4.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.70 ± 9.9</td>
<td>75.20 ± 7.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 ± 0.08</td>
<td>1.73 ± 0.05</td>
</tr>
<tr>
<td>Duration of anaesthesia before surgery (min)</td>
<td>85 ± 15</td>
<td>75 ± 16</td>
</tr>
<tr>
<td>Duration of operation (min)</td>
<td>375 ± 56</td>
<td>371 ± 45</td>
</tr>
<tr>
<td>Duration of extracorp. circ. (min)</td>
<td>127 ± 45</td>
<td>108 ± 23</td>
</tr>
</tbody>
</table>
packed red blood cells, to which were added 50% glucose solution 20 ml and NaHCO₃ 88 mmol. The flow rates were calculated at 2.4 litre/m² body surface area. The durations of anaesthesia, surgery and extracorporeal circulation are given in table I and did not differ between the groups. Following operation, all patients were ventilated artificially overnight.

No anaesthetic or operative complications occurred. One patient in group B required surgery for an acute perforated appendicitis on the 7th day after operation and two patients suffered minor wound infections.

Blood samples were taken, before the induction of anaesthesia; during anaesthesia immediately before surgery; at the end or, for technical reasons, near the end of the operation, and on the 6–7th days after operation. The mean duration of anaesthesia before surgery for patients of group A was 85 ± 15 min and those of group B 75 ± 16 min.

The tests were made with separated lymphocytes obtained by gradient centrifugation of heparinized blood. In the lymphocyte transformation test, separated lymphocytes in RPMI1640 solution (Grand Island Biological Co., Grand Island, N.Y., U.S.A.) with human AB serum and gentamicin 50 μg ml⁻¹ were cultured in triplicate in humidified air and 5% carbon dioxide at 37°C, with mitogens added to the selected wells of flat-bottomed microtitre plates (Salo, 1982b). The mitogens were phytohaemagglutinin M (PHA, Difco Laboratories, Detroit, Mich., U.S.A.) 100 μg ml⁻¹, concanavalin A (Con A, Pharmacia Fine Chemicals, Uppsala, Sweden) 10 μg ml⁻¹, pokeweed mitogen (PWM, Grand Island Biological Co.) 2.5 μg ml⁻¹ and formalinized Staphylococcus aureus strain Cowan I bacteria (StaCw, prepared by us) 0.02% v/v. Eighteen hours before harvesting ¹²⁵I-labelled 5-iodo-2'-deoxyuridine 0.25 μCi (¹²⁵IUdR, specific activity 90–110 mCi mg⁻¹, The Radiochemical Centre, Amersham, United Kingdom) in 20 μl was added to each microculture together with 5-fluoro-2-deoxyuridine (FUrD, Fluka, Buchs, Switzerland) to a final concentration of 10⁻⁶ mol litre⁻¹. After a total incubation time of 90 h, the cultures were harvested and the radioactivities measured with a gamma-counter.

Polyclonal immunoglobulin synthesis was measured by enumerating the plaque forming cells (PFC, Ig-producing and secreting cells) by the protein A PFC assay method described by Fauci, Whalen and Burch (1980) with slight modifications (Ruuskanen et al., 1980) and by measuring the IgG, IgM and IgA concentrations secreted into the culture medium by the enzyme-linked immunoassay method (Wasserman et al., 1979; Viljanen, Eskola and Ruuskanen, in preparation). Separated lymphocytes in these tests were activated in cultures for 7 days by PWM 2.5 μl ml⁻¹ or StaCw 0.002% v/v. To inhibit hydrocortisone-sensitive suppressor cells, hydrocortisone was added at a concentration of 10⁻⁵ mol litre⁻¹ into PWM-activated culture replicates. After the activation period, the immunoglobulin producing and secreting cells were detected by incubating the activated lymphocytes, first, with Protein A-coated sheep erythrocytes in agarose for 2 h, then with the IgG fraction of rabbit anti-human polyvalent Ig for 16 h and, third, with guineapig complement for 1.5 h, after which the plaque forming cells were counted. The IgG, IgM and IgA concentrations were measured in the culture supernatants after the lymphocyte activation period.

The plasma cortisol concentrations were measured in the patients of group B by a modified

<table>
<thead>
<tr>
<th>Table II. Pokeweed mitogen- and Staphylococcus aureus-induced mitogenic responses of separated lymphocytes from patients operated on by open-heart surgery under conventional balanced (A) and high-dose fentanyl (B) anaesthesia. Means of ¹²⁵IUdR uptake (cpm) ± SEM. *P &lt; 0.05; **P &lt; 0.01, compared with values before operation (Student's t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
radioimmunoassay using 8-anilo-1-naphthalene sulphonate to inhibit the binding of cortisol to cortisol-binding globulins (Foster and Dunn, 1974).

Student's t test for paired means was used to compare lymphocyte transformation values and the Mann–Whitney U test for comparison of the numbers of PFC and immunoglobulin concentrations.

RESULTS

PWM-induced lymphocyte transformation did not decrease during the period of anaesthesia before surgery, but was diminished in both groups at the end of the operation (P<0.05). The values returned to those recorded before anaesthesia by the 6–7th days after operation. However, there was no decrease in lymphocytic responses to StaCw during anaesthesia or surgery (table II). Lymphocytic responses to PHA decreased during surgery, but the decrease in response to Con A was not statistically significant.

The numbers of PWM- and StaCw-stimulated plaque forming cells (PFC) did not decrease in either group during anaesthesia alone. In contrast, the numbers of PWM-stimulated PFC were significantly decreased in the patients of group B at the end of the operation (fig. 1). Although there was a decrease in PWM-stimulated PFC numbers in every patient of group A and in all except one in the presence of hydrocortisone, the changes were not statistically significant (Mann–Whitney U test). The decrease in the number of PWM-stimulated PFC was similar in the presence or absence of hydrocortisone. The numbers of StaCw-stimulated PFC were only decreased on the 6–7th days after operation.

No changes occurred in immunoglobulin concentrations secreted into the culture by PWM- and StaCw-stimulated mononuclear cells during anaesthesia alone in either group. As with most of the other variables, a decrease in IgG, IgM and IgA concentrations occurred at the end of the operation in both groups. This is shown in figure 2 as a decrease of IgG concentrations in patients of group A. The addition of hydrocortisone into the PWM-stimulated lymphocyte cultures did not restore immunoglobulin production to its value before operation.

No differences occurred between the three patients of group B with infective complications and the other patients in B-cell proliferative responses, in the number of PFC or in immunoglobulin synthesis.

The plasma cortisol concentrations in patients of group B were 428 ± 101 (SD) nmol litre⁻¹ before anaesthesia, 246 ± 131 nmol litre⁻¹ (P<0.01) during anaesthesia before surgery, 371 ± 101 nmol litre⁻¹ at the end of the operation and 635 ± 180 nmol litre⁻¹ (P<0.01) on the 6–7th days after operation.
Two types of anaesthetic technique were examined: conventional balanced anaesthesia, which induces a marked cortisol response (Salo et al., 1981) and high-dose fentanyl anaesthesia, which is not associated with any cortisol response. However, the proliferative responses of T lymphocytes, the numbers of antibody-producing and -secreting cells and immunoglobulin production in vitro were similar in patients receiving these two different anaesthetic techniques in association with open-heart surgery. The differences found in PWM- and StaCw-induced lymphocyte proliferative responses between the groups were probably not caused by anaesthetic technique since the observed increases in lymphocyte proliferative responses were irregular and no differences occurred in other immunological values. Anaesthesia itself did not cause any statistically significant decreases in the immunological variables during the 75–85 min period before the start of surgery. In contrast, PWM-induced lymphocyte transformation, the number of antibody-producing cells and immunoglobulin production were decreased at the end of the operation. Thus, these decreases probably resulted from the operation, including the surgical procedure and the extracorporeal circulation, blood transfusion and drugs, in a manner similar to the decreases induced in cell-mediated immunity (Salo, 1978).

The PHA- and ConA-induced lymphocytic responses as a measure of T-cell function decreased in both groups in this study but to a lesser extent than in our earlier study (Salo et al., 1981) or other studies (Ryhänen, Herva et al., 1979; Roth et al., 1980; Goodwin et al., 1981). This may be partly a result of the fact that the preoperative values were determined from a preinduction blood sample, possibly already affected by the psychological stress of surgery. Although the number of mononuclear cells is always constant in cultures of separated lymphocytes, the T/B cell ratio in postoperative blood samples is decreased (Ryhänen, Herva et al., 1979). However, if the T-cell numbers in cultures are adjusted to a constant value, postoperative T-cell mitogenic responses are still depressed as a sign of functional impairment of T-cells (Roth et al., 1980).

No mitogen-induced B lymphocyte proliferative responses were depressed during the operation. The PWM responses of lymphocytes were depressed at the end of the operation but these reflect both T- and B-cell function. In contrast, the responses to StaCw did not decrease during the operation, although a decrease was observed on the 6–7th days after operation. StaCw is principally a B-lymphocyte mitogen (Sirianni et al., 1981), but may also activate T-cells (Schuurman, Gelfand and Dosch, 1980).

The impairment of humoral immunity was demonstrated by decreased numbers of PWM-
stimulated plaque forming cells and by decreased immunoglobulin production into the culture media by PWM- and StaCw-stimulated lymphocytes. This was not caused by the hydrocortisone-sensitive suppressor cells. Whether there was a defect in B cells, in other T-cell regulatory mechanisms, or in both, remains unclear. Serum immunoglobulin concentrations decrease after open-heart surgery (Ryhänen, Leinonen et al., 1979), but this is caused primarily by sequestration of proteins, protein denaturation, aggregate formation and haemodilution, not by a defect in immunoglobulin synthesis. Therefore, although the methods used in this study measure activation of circulating lymphocytes by artificial stimulants, they can show a failure in immunoglobulin synthesis better than measurement of serum immunoglobulin concentrations.

In practice, these findings signify a decreased capacity of cell-mediated and humoral immunity after open-heart surgery. The immunological variables of the three patients with infective complications did not differ from those of the other patients, and no severe infections typical of immunosuppression were observed. Thus, the impairment of immune functions does not necessarily have clinical consequences.

ACKNOWLEDGEMENTS

We thank H.Doc. B. Laurent, M.D., Department of Clinical Chemistry, University Central Hospital of Turku, for the determination of the plasma cortisol concentrations.

This study was supported financially by the Finnish Academy of Sciences and a grant from the University of Turku.

REFERENCES


B LYMPHOCYTE FUNCTION


ALTERATION DE LA FONCTION DES LYMPHOCYTES B AU COURS DE LA CHIRURGIE A COEUR OUVERT

La fonction in vitro des lymphocytes B a été mesurée chez des patients subissant des actes de chirurgie à cœur ouvert, sous anesthésie balancée conventionnelle ou sous anesthésie au fentanyl à haute dose. La transformation lymphocytaire induite par le mitogène Pokeweed (PWM) était déprimée à la fin de l'intervention, mais pas la réponse au staphylocoque doré formé Cowan I (StaCw). Le nombre de cellules sécrétant et produisant des immunoglobulines, mesuré grâce à un dosage indirect de croissance cellulaire sur plaque protéique A, a diminué après stimulation par le PWM mais pas après stimulation par le StaCw dans les prélèvements de fin d'intervention. La sécrétion d'IgG, d'IgM et d'IgA par les lymphocytes stimulés par le PWM et le StaCw dans le milieu de culture était déprimée dans la période postopératoire. Les dépressions immunitaires qui surviennent après chirurgie à cœur ouvert ne sont pas induites par l'anesthésie seule avant tout acte chirurgical. Les cellules suppressives hydrocortisone sensibles n'étaient pas les agents de transmission de ces baisses. Nous avons retrouvé des différences mineures entre les deux techniques d'anesthésie pour ce qui est des réponses de prolifération des lymphocytes.
ZUSAMMENFASSUNG


SUMARIO

Se midió in vitro la función de los linfocitos B en pacientes sometidos a cirugía a corazón abierto. Se usó la anestesia equilibrada tradicional o la anestesia con altas dosis de fentanilo. La transformación de los linfocitos inducida por el mitógeno de la hierba carmin (PWM) se deprimió al final de la operación, pero no así la respuesta al Cowan I Staphylococcus aureus (StaCw) formalinizado. El número de las células que secretan y producen la inmunoglobulina, medido mediante un ensayo indirecto de célula formadora de plaquetas de proteína A, bajó después de estimulación por PWM, pero permaneció sin cambio después de estimulación por StaCw al final de la operación. La secreción de IgG, IgM e IgA por linfocitos estimulados por PWM y StaCw adentro del medio de cultivo fue deprimida durante el periodo siguiente la operación. Ocurren funciones de inmunidad deprimidas después de la cirugía a corazón abierto, pero no es en asociación con la anestesia sola administrada antes de la cirugía. Los descensos no fueron afectados por las células supresoras de sensibilidad a la hidrocortisona. Se observaron diferencias menores entre las dos técnicas anestésicas con respecto a las respuestas proliferativas de los linfocitos.