PLASMA CORTISOL, CORTICOSTERONE AND NON-PROTEIN-BOUND CORTISOL IN EXTRACORPOREAL CIRCULATION

By
T. Uozumi, H. Manabe, Y. Kawashima, Y. Hamanaka, Y. Monden and K. Matsumoto

ABSTRACT

The response of plasma cortisol, corticosterone and non-protein-bound cortisol in the extracorporeal circulation was investigated in 14 patients. The pre-perfusion levels of plasma cortisol, corticosterone and non-protein-bound cortisol were significantly elevated. During and immediately after perfusion, the levels of cortisol and corticosterone were found to decrease significantly from the pre-perfusion levels, while the percentage of non-protein-bound cortisol was shown to increase significantly. This indicates a marked decrease in cortisol binding capacity of plasma during extracorporeal circulation. Moreover in 200 plasma samples, it was demonstrated that the cortisol level increased markedly and the cortisol binding capacity decreased slightly during and shortly after major surgery without perfusion. It is concluded that stressful situations in major surgery with or without perfusion are associated with markedly increased levels of biologically active non-protein-bound cortisol. The elevated level of non-protein-bound cortisol in surgery seems to be dependent on the increase in the level of plasma cortisol as well as on the decrease in the cortisol binding capacity of plasma. Although the increased plasma cortisol plays the most important role in surgery with no perfusion, the decreased cortisol binding capacity may be the more effective factor involved during perfusion.

It has been clearly shown that the peripheral plasma levels of cortisol, corticosterone and non-protein-bound cortisol are elevated following anaesthesia as well as during and after surgery with no perfusion (Franksson & Gemzell).
However, the response of plasma cortisol, corticosterone and non-protein-bound cortisol under extracorporeal circulation has not been clarified. Following surgery with perfusion, the levels of plasma cortisol were found to rise in the same quantitative and qualitative manner as those following other operations (Britt et al. 1961; Hasner et al. 1961; Carveth et al. 1965). With regard to the response of plasma cortisol during perfusion, extracorporeal circulation has been reported to cause a decrease (Mittelman et al. 1959; Carveth et al. 1965) and no change (Siska et al. 1964) in peripheral cortisol levels. In dogs, no significant change in the peripheral cortisol level occurred during perfusion (Tala et al. 1967; Alexander et al. 1969). Concerning the change in plasma corticosterone and non-protein-bound cortisol during perfusion, no observations have so far been made.

In the present study, the response of plasma cortisol, corticosterone and non-protein-bound cortisol in extracorporeal circulation is described. Furthermore, a decreased cortisol binding capacity of plasma under surgery both with and without perfusion is demonstrated.

MATERIALS AND METHODS

Plasma samples

The investigations on extracorporeal circulation were carried out on fourteen patients, aged 15 to 58 years. Seven patients with atrial septal defect or ventricular septal defect were perfused with the bubble oxygenator and seven patients with mitral stenosis and insufficiency were perfused with the disc oxygenator. One hour before the operation which started at approximately 8 a.m., the patients were injected intramuscularly with 50 mg of operidine. The duration of surgery from incision to closure ranged from 5 to 9 hours. The perfusion was performed for 30 to 160 minutes. Anaesthesia was maintained with nitrous oxide, oxygen and penthrane following induction with thiopental and succinylcholine. The bubble oxygenator was primed with 1000 ml of low molecular dextran and 1000 ml of electrolyte solution, while the disc oxygenator was primed with 1000 ml of dextran, 1000 ml of electrolyte solution and 3000 ml of heparinized blood. The dilution ratio was maintained at 20 to 35 per cent during the perfusion. All the patients were perfused at rates from 2000 to 2400 ml/min/m². The temperature of the perfused blood was maintained at 35 to 37°C. Since the blood losses during the operations ranged from 1000 to 3000 ml, each of the patients received an infusion of 1200 to 3800 ml of blood during the operation. One thousand to 3000 ml of electrolyte solution was also infused. Preoperative samples of peripheral blood were taken on the day before the operation at 8 a.m. Subsequent samples of venous blood from the vena cava superior and vena cava inferior were obtained immediately before perfusion, after 10 minutes of perfusion, 10 minutes before the end of the perfusion and immediately after the end of the perfusion. Samples of blood were also drawn from the machine during extracorporeal circulation. Postoperative samples of peripheral blood were taken at the end of the operation.
The effects of major surgical operation without perfusion on the cortisol binding capacity in plasma were examined in 20 chronically ill patients aged from 17 to 62 years; 6 who were suffering from mitral stenosis underwent closed commissurotomy, 9 with carcinoma of the lung underwent lobectomy and 5 with carcinoma of the stomach underwent gastrectomy. All the operations, starting at 8 a.m., were carried out at room temperature and the duration of the operations ranged from 3 to 6 hours. Anaesthesia was maintained with nitrous oxide, oxygen and fluothane. From 150 to 1000 ml blood and 1000 to 3000 ml of electrolyte solution were infused into each of the patients during and shortly following the operation. Preoperative (control) plasma samples were taken on the day before the operation at 8 a.m. Subsequent samples were obtained from the patients immediately, 2, 4, 6, 8, 12, 18 and 24 hours following closure.

None of the patients described above had any clinical evidences of endocrine, liver or kidney disorders nor had they received ACTH or glucocorticoids. The blood was collected into heparinized containers. The plasma was separated immediately by centrifugation, stored at -20°C until required and used within 3 weeks. No significant changes were found in the cortisol-binding to plasma frozen for periods of up to 4 weeks.

In addition, the cortisol binding capacity in the plasma following ACTH administration and cortisol infusion was estimated. Nine preoperative patients were injected with 20 IU of ACTH on 2 consecutive days under non-stress conditions and plasma samples were taken for 3 days following the first injection. Six preoperative patients were injected intravenously with 50 mg of cortisol and plasma samples were taken immediately and 2, 4 and 6 hours after the end of infusion.

**Estimation of plasma cortisol and corticosterone**

The method for the quantitative determination of plasma cortisol and corticosterone has been described in a previous report (Hamanaka et al. 1970). The method is based on the fluorimetric determination of cortisol and corticosterone following purification and separation by elution chromatography on Amberlite IRC-50.

**Estimation of non-protein-bound cortisol**

In order to determine the percentage of non-protein-bound cortisol in plasma, equilibrium dialysis was performed according to the method of Slaunwhite & Sandberg (1959) with a slight modification as previously described (Hamanaka et al. 1970).

**Estimation of plasma protein**

Plasma total protein was measured using the biuret reagent. Protein patterns were examined with electrophoretic analysis using a cellulose acetate membrane.

**Results**

**Response of plasma cortisol, corticosterone and non-protein-bound cortisol in extracorporeal circulation**

Table 1 shows the pre-surgical, pre-perfusion, during-perfusion, post-perfusion and post-surgical levels of plasma cortisol, corticosterone and non-
**Table 1.**
Response of plasma cortisol, corticosterone and non-protein-bound cortisol in extracorporeal circulation.

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>Cortisol Mean ± SE (µg/100 ml)</th>
<th>Corticosterone Mean ± SE (µg/100 ml)</th>
<th>Non-protein-bound cortisol Mean ± SE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vena cava superior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before operation</td>
<td>14</td>
<td>8.7 ± 0.75**</td>
<td>0.3 ± 0.08**</td>
<td>2.7 ± 0.26**</td>
</tr>
<tr>
<td>Before perfusion</td>
<td>14</td>
<td>21.0 ± 1.22</td>
<td>3.1 ± 0.35</td>
<td>11.9 ± 1.19</td>
</tr>
<tr>
<td>During perfusion (1)</td>
<td>14</td>
<td>13.1 ± 1.14**</td>
<td>1.8 ± 0.23**</td>
<td>16.0 ± 1.56**</td>
</tr>
<tr>
<td>During perfusion (2)</td>
<td>14</td>
<td>12.9 ± 1.14**</td>
<td>1.8 ± 0.21**</td>
<td>18.4 ± 1.71**</td>
</tr>
<tr>
<td>End of perfusion</td>
<td>14</td>
<td>16.5 ± 1.38**</td>
<td>2.1 ± 0.26**</td>
<td>17.0 ± 1.36**</td>
</tr>
<tr>
<td>End of operation</td>
<td>14</td>
<td>22.2 ± 2.12</td>
<td>2.9 ± 0.36</td>
<td>19.9 ± 1.71**</td>
</tr>
<tr>
<td><strong>Vena cava inferior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before perfusion</td>
<td>10</td>
<td>23.3 ± 1.64</td>
<td>4.7 ± 0.432</td>
<td>16.2 ± 2.02</td>
</tr>
<tr>
<td>During perfusion (1)</td>
<td>10</td>
<td>17.2 ± 1.25**</td>
<td>2.5 ± 0.32**</td>
<td>19.4 ± 2.16</td>
</tr>
<tr>
<td>During perfusion (2)</td>
<td>10</td>
<td>16.2 ± 1.50**</td>
<td>2.4 ± 0.26**</td>
<td>22.1 ± 2.50**</td>
</tr>
<tr>
<td>End of perfusion</td>
<td>10</td>
<td>20.8 ± 2.04**</td>
<td>3.9 ± 0.77</td>
<td>23.0 ± 2.75**</td>
</tr>
<tr>
<td><strong>Machine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During perfusion (1)</td>
<td>14</td>
<td>13.3 ± 1.09</td>
<td>1.8 ± 0.28</td>
<td>17.3 ± 1.70</td>
</tr>
<tr>
<td>During perfusion (2)</td>
<td>14</td>
<td>13.4 ± 1.39</td>
<td>1.9 ± 0.33</td>
<td>19.8 ± 1.63</td>
</tr>
</tbody>
</table>

Differences from «Before perfusion» (P): * < 0.05, ** < 0.01.
Differences from «Vena cava superior» (P): 1 < 0.05, 2 < 0.01.
(A t-test for paired observations was used).
a: Plasma was obtained from peripheral vein blood.

protein-bound cortisol in 14 cases of extracorporeal circulation. The pre-perfusion levels of plasma cortisol were significantly elevated. This reflects an adrenal response to anaesthesia and surgical procedures. Ten minutes after the beginning of perfusion, the patients exhibited a significant fall in cortisol levels. This might have been the result of mixing various volumes of perfusate with the blood of the patients. The cortisol concentrations, 10 minutes before the end of perfusion did not differ from those obtained 10 minutes after the beginning of the perfusion. Immediately after the termination of the perfusion, the cortisol levels of most of the patients were still below the pre-perfusion determinations. The cortisol level returned to the pre-perfusion level at the end of the operation. The concentrations of cortisol in the plasma from the vena cava inferior were significantly higher than those from the vena cava superior and that in the machine. The response of the plasma levels of corticosterone in extracorporeal circulation was found to parallel the change in the levels of cortisol.
The percentage of non-protein-bound cortisol in the pre-perfusion plasma increased significantly from that in the pre-operation plasma concomitant with the rise in plasma cortisol. A further significant increase in the percentage of non-protein-bound cortisol was demonstrated during and immediately after the perfusion, though the levels of plasma cortisol decreased significantly. This indicates that the cortisol binding capacity of the plasma decreases during and shortly after extracorporeal circulation. The increased percentage of non-protein-bound cortisol remained almost unchanged at the end of the operation.

**Cortisol binding capacity of plasma during surgery**

The cortisol binding capacity of plasma in preoperative control patients, preoperative patients injected with ACTH or cortisol, and patients during surgery with or without perfusion is shown in Figs. 1 and 2. Since the per-

![Graph]

**Fig. 1.**

Cortisol binding capacity of plasma in extracorporeal circulation. Individual points relating proportion of non-protein-bound cortisol to plasma cortisol levels are plotted for the preoperative control patients and in patients with extracorporeal circulation. The points for the preoperative control patients were obtained by *in vitro* addition of 0, 0.1, 0.2 and 0.4 µg cortisol to 1 ml of control plasma. They are regarded as the normal range for cortisol binding capacity. Note that the points for specimens with decreased protein binding capacity of cortisol (patients in extracorporeal circulation) fall above the normal range.

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Cortisol binding capacity of plasma in major surgery without perfusion. Individual points relating proportion of non-protein-bound cortisol to plasma cortisol levels are plotted for patients in major surgery without perfusion and also for preoperative control patients injected with ACTH or cortisol. Each of the two pairs of lines indicate the limits of cortisol binding capacity of the normal plasma and the plasma under extracorporeal circulation, respectively. Most points for the preoperative control patients injected with ACTH or cortisol fall within the normal range. Most points for the patients in major surgery without perfusion fall above the normal range but fall below the range for extracorporeal circulation.

Percentage of non-protein-bound cortisol varied with the level of plasma cortisol, the effect of increasing the cortisol concentration by in vitro addition of cortisol to the preoperative control plasma on the percentage of non-protein-bound cortisol was examined. As shown in Fig. 1, points indicating the proportion of non-protein-bound cortisol to plasma cortisol concentrations for preoperative control plasma thus obtained are regarded as the normal range for cortisol binding capacity in the present study. Thus, cortisol binding capacity is represented by the percentage of non-protein-bound cortisol in relation to the plasma cortisol concentration. It was clearly demonstrated that the cortisol binding capacity of plasma in extracorporeal circulation decreased
Table 2.
Plasma protein in surgery.

<table>
<thead>
<tr>
<th>Protein content M ± SE (g/100 ml)</th>
<th>Albumin</th>
<th>(a_1)-Globulin</th>
<th>(a_2)-Globulin</th>
<th>(\beta)-Globulin</th>
<th>(\gamma)-Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surgery with perfusion (13 patients)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before operation</td>
<td>7.1</td>
<td>64.6</td>
<td>2.4</td>
<td>8.1</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>±0.11</td>
<td>±1.17</td>
<td>±0.17</td>
<td>±0.27</td>
<td>±0.23</td>
</tr>
<tr>
<td>During perfusion</td>
<td>4.2**</td>
<td>66.9**</td>
<td>2.0*</td>
<td>7.2*</td>
<td>10.9**</td>
</tr>
<tr>
<td></td>
<td>±0.11</td>
<td>±1.29</td>
<td>±0.23</td>
<td>±0.23</td>
<td>±0.59</td>
</tr>
<tr>
<td><strong>Surgery without perfusion (15 patients)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before operation</td>
<td>7.0</td>
<td>62.9</td>
<td>2.9</td>
<td>9.9</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>±0.11</td>
<td>±0.92</td>
<td>±0.24</td>
<td>±0.56</td>
<td>±0.46</td>
</tr>
<tr>
<td>After operation (2 h)</td>
<td>6.2**</td>
<td>63.2</td>
<td>3.0</td>
<td>8.8</td>
<td>11.0*</td>
</tr>
<tr>
<td></td>
<td>±0.16</td>
<td>±1.41</td>
<td>±0.32</td>
<td>±0.72</td>
<td>±0.73</td>
</tr>
</tbody>
</table>

Differences from «Before operation» (P): * < 0.05, ** < 0.01.
(A t-test for paired observations was used).

markedly and that during surgery without perfusion this decreased slightly. The cortisol binding capacity of plasma remained within the normal range following ACTH injection and cortisol infusion.

Changes in plasma protein during surgery are shown in Table 2. The protein content of plasma decreased markedly during and shortly after perfusion. A slight but significant decrease in protein content was also seen during surgery without perfusion. The percentage of the \(\alpha\)-globulin fraction was found to decrease slightly in surgical operation.

**DISCUSSION**

It was reported that the plasma levels of cortisol, corticosterone and non-protein-bound cortisol were found to be elevated during and after surgery without perfusion (Franksson & Gemzell 1953; Sandberg et al. 1954, 1960; Helmreich et al. 1957; Thomasson 1959; DeMoor et al. 1962; Murray 1967; Wendt 1967; Hamanaka et al. 1970). Although the changes in plasma cortisol in extracorporeal circulation have been reported by some investigators (Mittelmann et al. 1959; Brit et al. 1961; Hasner et al. 1961; Carveth et al. 1965; Siska et al. 1964), the response of plasma cortisol, corticosterone and non-
protein-bound cortisol during extracorporeal circulation seems to have been demonstrated in detail for the first time in the present study. The onset of extracorporeal perfusion was associated with a definite fall in plasma levels of cortisol and corticosterone (Table 1). Dilution with reservoir blood, plasma substitutes and electrolyte solution during perfusion is probably an important factor, although changes in the vascular bed of the adrenal gland, anterior pituitary depression or accelerated destruction of ACTH during perfusion have to be considered. Major surgical procedures are known to cause maximum stimulation of the pituitary-adrenocortical axis without any further increase in cortisol secretion after exogenous ACTH (Thomasson 1959; Hume et al. 1962). Hence it would appear that the adrenal cortex is unable to secrete a sufficient amount of cortisol and corticosterone to eliminate the dilution factor by blood and electrolyte solution from the machine during perfusion. However, the level of non-protein-bound cortisol seems to be maintained at a high level during perfusion, because the cortisol binding capacity in plasma is markedly decreased during perfusion, as shown in Table 1 and Fig. 1. It is believed that the decreased cortisol binding capacity can be caused by a marked decrease in the concentrations of plasma protein, especially a-globulin, during perfusion (Table 2). Only slight denaturation of plasma protein was found during prolonged extracorporeal circulation (Wright et al. 1962; Kekki et al. 1968). It is considered that dilution of the blood with perfusate seems to be a suitable means for maintaining elevated levels of biologically active non-protein-bound cortisol during perfusion.

In our previous report (Hamanaka et al. 1970), the response of plasma cortisol, corticosterone and non-protein-bound cortisol during surgery without perfusion was demonstrated in detail. Furthermore, there was some evidence which suggested a decreased cortisol binding capacity in major surgery. In the present study, a decreased cortisol binding capacity is clearly demonstrated during and shortly after major surgery without perfusion, as shown in Fig. 2. A slight but significant decrease in the concentrations of plasma protein (Table 2) is probably an important factor. However, this can not be the only reason for the decrease in the binding capacity of cortisol. It is considered that the cortisol binding activity by plasma may be influenced by some medications and transfusions during and after the operation. Partial saturation of cortisol binding protein with increased steroids other than cortisol may play some role. DeMoor et al. (1962) found normal levels of cortisol binding capacity in the plasma of patients after extensive abdominal surgery. On the other hand, Murray (1967) showed some evidence of a decrease in transcortin binding sites during shock following major stress.

It is concluded that stressful situations in major surgery with and without perfusion are associated with markedly increased levels of biologically active non-protein-bound cortisol. The elevated level of non-protein-bound cortisol
seems to be established by the increase in the level of plasma cortisol as well as by the decrease in the cortisol binding capacity of plasma in surgery. Although the increased plasma cortisol plays the most important role in surgery without perfusion, the decreased cortisol binding capacity may be the more effective factor during perfusion.

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