Perioperative plasma endothelin-1 concentrations and vasoconstriction during prolonged plastic surgical procedures

H. P. Tuominen, N. E. Svartling, I. T. Tikkanen, O. Saijonmaa and S. Asko-Seljavaara

Summary
The role of endothelin-1, a potent vasoconstrictor released by vascular endothelium, in the vasoconstriction that develops after prolonged operations is not clear. This study was performed in order to determine if there was any relationship between endothelin-1 and the degree of vasoconstriction during prolonged plastic surgery. Plasma concentrations of endothelin-1, skin-forearm temperature gradient ($T_{\text{grad}}$), rectal temperature, mean arterial pressure (MAP) and heart rate (HR) were measured at nine predetermined times before, during and after operation in nine women undergoing breast reconstruction with a pedicled transverse rectus abdominis musculocutaneous flap. Development of cutaneous or fat necrosis of the flap was assessed clinically and with ultrasound. Concentrations of endothelin-1 before induction were increased (median 8.9 (25-75% quartiles 5.5-12.5) pg ml$^{-1}$). During operation they were approximately 3 pg ml$^{-1}$ and after operation approximately 5 pg ml$^{-1}$. $T_{\text{grad}}$ was approximately 4 °C before induction and after operation, indicating marked vasoconstriction; during operation it was about zero, indicating vasodilatation. There was a statistically significant correlation between endothelin-1 concentrations and $T_{\text{grad}}$ (Spearman non-linear correlation) ($r = 0.32$, $P = 0.004$) and between endothelin-1 and MAP ($r = 0.25$, $P = 0.02$), but not between endothelin-1 and HR or development of minor cutaneous or fat necrosis of the flap (five patients). We conclude that increased plasma concentration of endothelin-1 is associated with the extent of peripheral vasoconstriction. (Br. J. Anaesth. 1995; 74: 661–666)

Key words

Peripheral vasoconstriction frequently develops during prolonged surgery, and it may last for several hours after operation. It is a common problem in reconstructive plastic surgery, where operations generally last from 4 to 12 h.

Endothelin-1 (ET-1), which was first described in 1988, is a 21-amino acid polypeptide, synthesized and secreted mainly by vascular endothelial cells [1, 2]. Its binding to smooth muscle cells causes intense, prolonged vasoconstriction [2]. ET-1 acts as a local hormone and is involved in the control of cardiovascular function by maintenance of vascular tone [2, 3]. The role of endothelin in intra- and postoperative vasoconstriction is not known.

We performed this study to see if any changes occur in plasma concentrations of ET-1 during and after prolonged plastic surgery and if the plasma concentrations of ET-1 correlate with peripheral vasoconstriction, changes in arterial pressure and heart rate and development of cutaneous or fat necrosis of musculocutaneous flaps.

Patients and methods
We studied 10 consecutive women undergoing breast reconstruction because of mammary ablation performed for breast cancer 2.5–11 yr earlier. Breast reconstruction was performed with a pedicled transverse rectus abdominis musculocutaneous (TRAM) flap. The study was approved by the Töölo Hospital Ethics Committee and the patients gave informed consent. Two of the patients smoked. Eight patients had received radiotherapy because of axillary node metastases. Two patients had received chemotherapy after the ablation. None took tamoxifen or any other medication at the time of the study. The patients were otherwise healthy.

The patients were premedicated with diazepam 0.15–0.21 mg kg$^{-1}$ approximately 75 min before induction of anaesthesia, which took place between 08:00 and 09:00. After i.v. boluses of fentanyl 0.1 mg, glycopyrronium 0.2 mg and precurarization with pancuronium 1 mg, anaesthesia was induced with thiopentone 5 mg kg$^{-1}$. Suxamethonium 1–1.5 mg kg$^{-1}$ was administered to facilitate tracheal intubation. Anaesthesia was maintained with 65% nitrous oxide and 0.2–1% isoflurane in oxygen. We aimed to maintain mean arterial pressure at 60–70 mm Hg during dissection of the flap and at 90–100 mm Hg after the flap had been moved to the bed.
mastectomy wound. The lungs were ventilated mechanically to normocapnia with a Servo 900 ventilator (Siemens-Elema, Sweden). Neuroumuscular block was produced with pancuronium 1–2 mg as needed after a bolus of 0.08 mg kg⁻¹. Fentanyl was given in doses of 0.05–0.1 mg. At the end operation, neuroumuscular block was antagonized with neostigmine 2.0 mg and glycopyrronium 0.4 mg. Isoflurane and nitrous oxide inhalation were discontinued after the wounds had been bandaged. The trachea was extubated when spontaneous ventilation was adequate.

In the recovery room, the patients breathed 35% oxygen throughout the study (3 h). Oxycodone in doses of 0.05 mg kg⁻¹ was given i.v. for pain relief. The need for pain medication was assessed by experienced anaesthesia nurses. No other medication was given in the recovery room.

Hydroxyethylstarch 120 6% (Plasmafusin, Leiras-Kabi Infusion Oy, Vantaa, Finland) (500 ml) was given after induction, and Ringer’s acetate was infused in order to maintain a stable haemodynamic state and mild hypervolaemic haemodilution, as recommended for microvascular surgery [4]. Packed cell volume (PCV) was kept at 0.30–0.35 and whole blood was transfused as needed. Dextran 40 (100 ml) (Rheomacrodex, Kabi Infusion A/S, Norway) was infused after operation until the next morning. All infusions were given via a venous cannula in the upper extremity on the side opposite the operative side.

The infusion bags were warmed in water at 37 °C before the infusion started. Ambient temperature was maintained at approximately 22 °C during operation and in the recovery room. Skin surface warming devices were not used during operation except for a water-filled warming mattress during operation.

Electrocardiogram, heart rate, intra-arterial pressure, pulse oximetry, urinary output, end-tidal carbon dioxide and inspiratory oxygen concentration were monitored continuously throughout anaesthesia. Neuroumuscular block was monitored with a nerve stimulator. A cannula was inserted in the radial artery of the arm with the infusion cannula.

The new breast was reconstructed with a pedicled TRAM flap, which is a typical musculocutaneous flap. The surgical technique was standard and is described in detail elsewhere [5]. All operations were performed by the same surgeon. In brief, the flap was formed of a symmetrical area of skin and subcutaneous tissue on both sides of the lower abdomen and one of the rectus abdominis muscles. The pedicled TRAM flap was based on the superior epigastric artery and vein running inside the rectus abdominis muscle. The flap was elevated from the abdomen, and the rectus muscle was cut at its caudal end. The flap was then rotated cranially 180° and tunnelled under the upper abdominal skin to the mastectomy wound.

MEASUREMENTS

Before induction of anaesthesia, a 16-gauge venous cannula was inserted without local anaesthesia into an antecubital vein of the arm on the side of the removed breast to obtain blood for measurement of plasma ET-1 concentrations. The cannula was closed with an obturator when not in use. Probes for peripheral temperature measurements (Exaco MC 8700 thermocouple probes, Exaco, Copenhagen, Denmark) were attached to the same limb, one at the tip of the index finger (T_i) and one on the radial side of the middle third of the antecubital area (T_c). I. v. fluids were not infused in this arm during the study. A rectal temperature (T RECT) probe was inserted and taped in place. The patients had received laxatives and liquid food on the day before operation in order to empty the rectum.

The arm on the mastectomy side was covered during operation with one cotton sheet. The rest of the patient was covered with double-thickness cotton drapes. In the recovery room, the arm with the measurement probes was exposed; otherwise the patient was covered with a hospital blanket and sheet.

Before induction, 1 and 3 h after induction of anaesthesia, at the end of operation, and 10 min, 30 min, 1, 2 and 3 h after arrival in the recovery room, mean arterial pressure (MAP), heart rate (HR), T_i, T_c and T RECT (first measurement immediately after induction) were measured and blood was sampled from the antecubital vein for measurement of plasma ET-1 concentrations. Arterial blood samples were obtained for measurement of oxygen tension and PCV 1 and 3 h after induction of anaesthesia and 30 min after arrival in the recovery room. The patients were monitored for shivering which was graded as none, minimal fasciculations, visible tremor or visible and generalized tremor.

TRAM flap survival was assessed clinically (colour and capillary refill of the flap skin) in the recovery room and for the next 6–7 days in the ward. A dark or blue zone on the TRAM flap margin was regarded as cutaneous necrosis. To check for the presence of fat necrosis areas, all flaps were examined with ultrasound before patients were discharged from hospital.

PLASMA ET-1 MEASUREMENTS

Venous blood (10 ml) was obtained in ice-chilled tubes containing Na₂ EDTA 15 mmol litre⁻¹ (final concentration). Plasma was separated by centrifugation and stored at −70 °C until assay for ET-1. Radioimmunoassay of ET-1 was performed as described previously [6] using synthetic ET-1 and ET-1 antiserum generated in rabbits. The antiserum showed 100% cross-reaction with ET-2 and ET-3 and < 0.1% cross-reaction with the 20–50, 74–91 and 171–201 sequences of preproendothelin and with big ET-1, sequences 1–38 and 33–38.

Before ET-1 radioimmunoassay, plasma samples were purified using Bondelut C18-OH analytical columns. Plasma (1 ml) was acidified with 4% acetic acid and injected onto a column. After the samples had been washed with distilled water, the absorbed peptide was eluted with 40% ethanol and 4% acetic acid. The eluted fraction was lyophilized and dissolved in assay buffer, 50 mmol litre⁻¹ of buffer...
pH 7.4, containing Na₂EDTA 1 mmol litre⁻¹, cystine 0.2 nmol litre⁻¹, 0.01 % mercaptoethanol, 0.1 % bovine serum albumin and 0.1 % triton x-100. The radioimmunoassay was performed using sequential incubation by adding ¹²⁵I-labelled ET-1 on the third day. Bound ligands were separated on the fourth day using the second antibody technique. The sensitivity of the assay was 0.8 pg/tube, and recovery of synthetic ET-1 added to plasma was 80 %. For external control, each ET-1 radioimmunoassay we measured three samples of pooled normal human plasma containing 0, 20 or 50 pg of synthetic human ET-1.

**STATISTICAL ANALYSIS**

Parametric data are given as mean (SD) and non-parametric data as median (25-75 % quartiles). Thermoregulatory vasoconstriction was evaluated by skin temperature gradient (T_grad). T_grad was determined according to Stoen and Sessler as the difference between T_ref and T_ad [7]. Statistical analysis for differences between the measuring times was performed with the Wilcoxon–Pratt test. The initial preoperative values were considered as the baseline values for comparison of the changes in ET-1 values, HR, MAP and temperature changes. We also compared postoperative values with values measured at the end of operation. Non-linear correlation between measured variables was tested with the Spearman rank test. P values less than 0.05 were considered significant.

**Results**

Data on nine patients were included in the final evaluation. Three of the plasma ET-1 determinations of one patient yielded exceptionally high results (highest 51 pg ml⁻¹, 7-22 SD above the mean of the plasma ET-1 concentrations of the other patients). All data on this patient were excluded from the study. This patient was a non-smoker who had a mastectomy performed 3 yr earlier and had received postoperative radiotherapy. Her flap healed without necrosis.

The characteristics of the nine patients and operations are presented in table 1. In five patients, duration of operation was prolonged by a separate procedure performed on the other breast.

The changes in T_ref and T_ad are shown in table 2. T_grad decreased during the course of the long operations. In the recovery room, it initially decreased further, but 30 min after the patients arrived in the recovery room it started to increase. The patients became slightly hyperthermic during the first 3 h after operation.

**Table 1** Characteristics of patients and operations (mean (sd) [range])

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>47 (9) [31-60]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>163 (7) [147-171]</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65 (10) [47-74]</td>
</tr>
<tr>
<td>Duration of operation (min)</td>
<td>282 (46) [230-360]</td>
</tr>
<tr>
<td>Ringer's acetate infused (litre)</td>
<td>2.8 (0.7) [2.0-4.5]</td>
</tr>
<tr>
<td>Blood transfused (u.)</td>
<td>2.3 (0.9) [1-4]</td>
</tr>
<tr>
<td>Preoperative PCV</td>
<td>0.39 (0.02) [0.36-0.42]</td>
</tr>
<tr>
<td>Postoperative PCV</td>
<td>0.30 (0.02) [0.27-0.33]</td>
</tr>
<tr>
<td>PO₂, 1 h before induction (kPa)</td>
<td>24.2 (3.2) [19.1-30.5]</td>
</tr>
<tr>
<td>PO₂, 3 h before induction (kPa)</td>
<td>24.6 (4.3) [15.2-29.9]</td>
</tr>
<tr>
<td>PO₂, 30 min after recovery (kPa)</td>
<td>16.8 (3.7) [8.6-23.7]</td>
</tr>
</tbody>
</table>

**Table 2** Mean (sd) [range] a changes in T_ref and T_ad during and after free TRAM flap breast reconstruction. RR = Recovery room.

<table>
<thead>
<tr>
<th></th>
<th>Before induction</th>
<th>1 h of anaesthesia</th>
<th>3 h of anaesthesia</th>
<th>End of operation</th>
<th>10 min in RR</th>
<th>30 min in RR</th>
<th>1 h in RR</th>
<th>2 h in RR</th>
<th>3 h in RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_grad (°C)</td>
<td>36.4 (0.5)</td>
<td>36.1 (0.4)</td>
<td>35.7 (0.6)*</td>
<td>35.7 (0.6)*</td>
<td>35.9 (0.7)†</td>
<td>36.2 (0.8)†</td>
<td>36.8 (0.9)†</td>
<td>37.4 (0.8)†</td>
<td>37.6 (0.7)†</td>
</tr>
<tr>
<td>[35.7-37.2]</td>
<td>[35.4-36.7]</td>
<td>[34.9-36.4]</td>
<td>[34.9-36.5]</td>
<td>[35-37]</td>
<td>[35.1-37.5]</td>
<td>[35.3-38]</td>
<td>[35.7-38.5]</td>
<td>[36.2-38.8]</td>
<td></td>
</tr>
<tr>
<td>T_ad (°C)</td>
<td>27.3 (1.5)</td>
<td>35.1 (0.7)*</td>
<td>34.6 (0.9)*</td>
<td>31.9 (2.5)*</td>
<td>28.3 (0.9)†</td>
<td>27.3 (1.2)†</td>
<td>26.8 (1.6)†</td>
<td>27.7 (3.4)†</td>
<td>28.6 (4)</td>
</tr>
<tr>
<td>[24.4-28.9]</td>
<td>[33.9-35.9]</td>
<td>[33.3-36]</td>
<td>[26.9-34.2]</td>
<td>[27.2-29.2]</td>
<td>[25.7-29.6]</td>
<td>[25-29]</td>
<td>[24.1-31.1]</td>
<td>[23.4-33.8]</td>
<td></td>
</tr>
</tbody>
</table>
Peripheral cutaneous vasoconstriction diminished, but some vasoconstriction still remained at the end of the study (fig. 2).

During anaesthesia, MAP was significantly lower than before induction, indicating the level of induced mild hypotension. After operation it differed from the preinduction level only at 2 h after arrival in the recovery room. All MAP values measured in the recovery room were significantly higher than those measured at the end of operation. HR remained at the preinduction level during operation. At all measurement times in the recovery room, it was significantly higher than the level before induction and also the level at the end of operation (fig. 3).

Shivering was noticed in three patients: minimal fasciculations in two and generalized and visible tremor in one. None of the patients suffered nausea or vomiting that required antiemetic medication after operation.

Four of the nine TRAM flaps healed uneventfully. There was minor skin necrosis in three and fat necrosis in two flaps. The three flaps with skin necrosis needed surgical revision. The final result was satisfactory in all flaps.

In the non-parametric Spearman rank correlation test, there was a statistically significant non-linear correlation between ET-1 and $T_{\text{grad}}$ ($r = 0.32, P = 0.004$), between ET-1 and MAP ($r = 0.25, P = 0.02$) and between ET-1 and $T_{\text{end}}$ ($r = -0.34, P = 0.003$) but not between ET-1 and HR. There was a statistically significant non-linear correlation between preoperative $T_{\text{grad}}$ and development of necrosis ($r = 0.81, P = 0.008$). There was no statistically significant correlation between development of necrosis and intra- and postoperative $T_{\text{end}}$ values, ET-1 concentrations or the characteristics of the patients and the operations shown in table 1, or between ET-1 concentrations and the occurrence of postoperative shivering.

**Discussion**

Recent work has shown that ET-1 has a physiological role in the regulation of peripheral vascular tone in humans [3]. The contribution of endogenous ET-1 secretion in specific clinical circumstances is still unclear. Our study has shown that increased plasma concentrations of ET-1 coincided with a marked degree of vasoconstriction before and after prolonged plastic surgery.

Plasma ET-1 concentrations of 0.5–5 pg ml$^{-1}$ have been measured in healthy humans [8]. In our study, slightly increased plasma concentrations of ET-1 were measured before and after operation. The highest values were noted before induction, when a significant correlation was found with $T_{\text{grad}}$. The cause of the increased value before operation is unclear. We did not use local anaesthesia before venous cannulation and it is not known if pain causes changes in ET-1 concentrations. However, it is known that ET-1 is released as a result of hypoxia, stretch, increased intramural pressure and cold [9]. In this study, intra- and postoperative systemic oxygenation was adequate, as assessed by arterial oxygen tensions. Before induction, the patients' peripheral oxygen saturations were normal (95–98%). The patients' central temperatures were normal before induction but peripheral temperatures were low compared with more proximal areas. It is possible that in our patients adrenergic stimulation produced by anxiety caused peripheral vasoconstriction, and that in turn this induced a relative peripheral hypoxia and hypothermia causing release of ET-1.

There is evidence that ET-1 is released locally. Plasma ET-1 concentration is thought to increase only when high amounts of ET-1 are released from the tissues [2, 9]. We obtained blood samples for measurement of plasma ET-1 from the limb where the $T_{\text{grad}}$ and $T_{\text{end}}$ probes were positioned. We assume that the increased concentrations of ET-1 observed before induction probably reflected considerable local release of ET-1.

We do not know if the increased initial ET-1 concentrations had any effects. Very small i.v. doses of synthetic ET-1 cause an increase in forearm blood flow in humans. As the dose of ET-1 increases, blood flow decreases as a result of intense vasoconstriction of the small arteries and arterioles, an effect that lasts...
The increased ET-1 values before and after operation [18-20]. Operation was caused at least partly by peripheral hypothermia. We assume that the patients' rectal temperature was very low and exceeded 4°C at 30 min and 1 h after arrival in the recovery room. In our study, peripheral vasoconstriction was not observed 1 h after induction, as judged by Tgrad. On the contrary, Tgrad was reduced, indicating vasodilatation, which lasted throughout surgery. It is possible that plasma ET-1 concentrations were high only transiently before induction of anaesthesia. Fast pulmonary clearance of ET-1 has been observed in some animal models, but not in humans [9]. The vasoconstrictive effect of the high preinduction levels of plasma ET-1 may have been prevented by the use of isoflurane during anaesthesia. The vasodilatatator effect of isoflurane is mediated via adrenergic receptors of vascular smooth muscle [11] but probably not via nitric oxide [12, 13]. We postulate that isoflurane-induced vasodilatation may have overcome the vasoconstrictor effect of ET-1 during anaesthesia. On the other hand, if the initial ET-1 release was transient, it could have caused vasodilatation by stimulating release of nitric oxide or prostacyclin [13].

The cause of the high ET-1 concentrations in the patient who was excluded remains obscure. Falsely high ET-1 values occur after incomplete lyophilization, which is unlikely, as tens of samples are handled simultaneously in our radioimmunoassay technique. The haemoglobin remaining in the sample may also lead to high ET-1 values, but our method of extraction removes all haemoglobin. The rectum was chosen as the measurement site for core temperature, because a rectal probe is tolerated better in the recovery room than nasopharyngeal or oesophageal probes. The rectum is a suitable site for core temperature measurement during long operations [14]. Trec decreased during anaesthesia, but not to very low levels. Considerably larger decreases in central temperature have been observed during isoflurane-nitrous oxide anaesthesia of shorter duration [15-17] and therefore it would appear that our techniques for conserving heat loss were satisfactory. After an initial vasodilatation, which typically occurs during isoflurane anaesthesia [11], Tin decreased slowly, and was 31.9 (2.5)°C at the end of operation. However, significant vasoconstriction defined by Stoen and Sessler as a Tgrad value between the lower arm and fingertip of 4°C or more [7] was not observed during anaesthesia. The most likely reason is that Trec did not decrease enough in our patients to reach the thermoregulatory threshold for isoflurane-nitrous oxide anaesthesia [7]. In the recovery room, the patients' Tgrad were very low and Tgrad exceeded 4°C at 30 min and 1 h after arrival in the recovery room. At the same time, plasma ET-1 concentrations were higher than during surgery. We assume that the postoperative increase in plasma ET-1 concentrations was caused at least partly by peripheral hypothermia. Surgical stress and manipulation of the tissues are also thought to cause increased ET-1 release after operation [18-20]. In our patients, there was a significant correlation between plasma ET-1 concentrations and MAP. The increased ET-1 values before and after operation were accompanied by high MAP levels. ET-1-induced vasoconstriction results in an increase in arterial pressure but does not affect HR [2, 9]. Increased ET-1 concentrations occur in several disease states with disturbed vascular control, for example Raynaud's phenomenon, pulmonary hypertension and subarachnoid haemorrhage [2, 9, 21]. There may be several mechanisms in the development of high arterial pressure before and after operation, and endothelin may be one.

We have not found any association between increased ET-1 concentrations and flap necrosis. Further studies are needed to define the role of ET-1 in circulatory derangement and necrosis in plastic surgical flaps.

We conclude that increased plasma concentrations of ET-1 observed before induction and after operation after prolonged plastic surgery are associated with peripheral vasoconstriction.

Acknowledgements

We thank the Einar and Karin Stroem Foundation (Helsinki), the Sigrid Juselius Foundation (Helsinki) and the Nordisk Forsknings Komite (Copenhagen) for financial support.

References

7. Stoen R, Sessler DI. The thermoregulatory threshold is inversely proportional to isoflurane concentration. Anesthesiology 1990; 70: 822-827.


