Infusion of hypertonic saline before elective hysterectomy: effects on cytokines and stress hormones

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Background. Infusion of hypertonic saline provides early haemodynamic benefits and may affect the immune system. It is unknown if infusion of hypertonic saline affects plasma cytokines and stress hormones after surgery.

Methods. Sixty-two women undergoing abdominal hysterectomy were randomized in a double-blind study to infusion of NaCl 7.5% (HS), NaCl 0.9% (NS4), both 4 ml kg \(^{-1}\), or NaCl 0.9% 32 ml kg \(^{-1}\) (NS32) over 20 min. Blood was collected at baseline, 1, 4, and 24 h after surgery (\(n=34\)) for the determination of interleukin (IL)-1\(\beta\), IL-6, IL-8, IL-10, IL-12, IL-1ra, and tumour necrosis factor-\(\alpha\). Serum cortisol and vasopressin were measured at these time points and 48 h after operation. Epinephrine and norepinephrine (\(n=26\)) were quantified at baseline, after infusion, 25 min after incision, 1, and 4 h after surgery. Finally, C-reactive protein was measured at baseline, 24, and 48 h after surgery.

Results. Surgery and anaesthesia induced well-reported changes in the concentrations of cytokines and hormones. The concentration of norepinephrine briefly increased after infusion of HS and NS32 but not NS4 (\(P<0.05\)). Epinephrine was increased 25 min after incision in Group NS32 compared with the other groups (\(P<0.05\)). No other differences were found between the groups.

Conclusions. Infusion of a clinically relevant dose of hypertonic saline before hysterectomy appears to have limited effect on the postoperative concentration of selected plasma cytokines and the hormonal stress-response.

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Like trauma, surgery activates the neuroendocrine stress response.\textsuperscript{1} Thus, we sought to establish whether infusion of hypertonic saline 7.5\%, 4 ml kg\textsuperscript{-1}, a clinically relevant dose and the dose used in most trauma trials,\textsuperscript{3} would affect the plasma concentration of common stress hormones and cytokines. We hypothesized that the infusion of hypertonic saline before abdominal hysterectomy would ameliorate the postoperative stress response and change the cytokine profile in an anti-inflammatory direction.

**Methods**

The data described here were collected during a study on the effect of hypertonic saline infusion on the cellular immune response after surgery.\textsuperscript{17} The study protocol has been described previously.\textsuperscript{17} The Regional Ethical Review Committee (VN 2000/145) and The Danish Medicines Agency (2612-1491) approved the study.

**Eligibility**

Patients, undergoing elective open abdominal hysterectomy, were invited to take part in the study. The criteria for inclusion were an age between 18 and 65 yr, non-malignant disease, and American Society of Anesthesiologists (ASA) Physical Status Scale class I–II. The exclusion criteria were: BMI above 35 kg m\textsuperscript{-2}, heart failure [New York Heart Association (NYHA) Group III–IV], renal failure (P-creatinine >120 \(\mu\text{mol litre}\textsuperscript{-1}\)), anaemia (B-haemoglobin <11.6 g dl\textsuperscript{-1}), diabetes mellitus, and use of medications known to affect immune function within 48 h of participation in the study. These included glucocorticoids, non-steroidal anti-inflammatory drugs, and histamine antagonists. Informed consent was obtained from all patients.

**Procedure**

Sixty-two women were included in the study. Subjects fasted overnight and received acetaminophen 1 g orally in addition to their daily medication. Patients aged 40 yr of age and older (\(n=19\)) received dalteparin 2500 IU s.c. for thromboprophylaxis. Oral diazepam 5 mg was allowed at the discretion of the attending nurses and was administered to six, four, and four patients in Groups HS, NS4, and NS32, respectively. Patients were studied in the operating theatre on the morning of surgery. A cubital vein of each arm was cannulated for blood sampling and fluid infusion. Study group allocations were placed in sealed, opaque, randomly assorted envelopes, which were opened by a hospital staff member who was not one of the study investigators. Patients were randomly assigned to receive either NaCl 7.5\%, 4 ml kg\textsuperscript{-1} (The Central Pharmacy, Odense University Hospital, Odense, Denmark, Group HS, \(n=21\)), NaCl 0.9\%, 4 ml kg\textsuperscript{-1} (Group NS4, \(n=21\)), or NaCl 0.9\%, 32 ml kg\textsuperscript{-1} (Group NS32, \(n=20\)) (The Hospital Pharmacies in Denmark, Copenhagen, Denmark) infused by pump at a constant rate over 20 min (IVAC 591, IVAC, CA, USA). Patients, surgeons, anaesthetists, nurses, and laboratory technicians were blinded to the type of the fluid given to each patient. Owing to risk of potential fluid overload, the maximal infused volume did not exceed a volume corresponding to 75 kg, which was the case in five, seven, and five patients in Groups HS, NS4, and NS32, respectively. Between termination of the test fluid infusion and extubation, we infused NaCl 0.9\%, 5 ml kg\textsuperscript{-1} h\textsuperscript{-1} which was reduced to 1.5 ml kg\textsuperscript{-1} h\textsuperscript{-1} during the stay in the recovery room.

We recorded heart rate, arterial pressure, and peripheral oxygen saturation every 2 min during the infusion and every 5 min during anaesthesia and surgery.

Patients were anaesthetized with fentanyl 3 \(\mu\text{g kg}\textsuperscript{-1}\) and thiopental 3–5 mg kg\textsuperscript{-1}. Intubation was facilitated by rocuronium 0.6 mg kg\textsuperscript{-1}. Anaesthesia was maintained with sevoflurane 1–3\% in oxygen and air and supplementary fentanyl 100 \(\mu\text{g}\) was given when judged necessary by the anaesthetist. Cefuroxime 1.5 g was administered i.v. as antibiotic prophylaxis to all patients.

Postoperative pain was treated with oral acetaminophen 1 g, four times daily, tramadol 50 mg, three times daily, and i.v. morphine, or ketobemidon. We treated nausea with i.v. ondansetron 1 mg and droperidol 0.5 mg.

**Blood chemistry**

Venous blood was collected at baseline (T0), after the saline infusion (T-inf), 25 min after incision (T-surg), and 1 (T1), 11 (T11), 24 (T24), and 48 h (T48) after skin closure for the determination of haemoglobin, plasma sodium, chloride, and potassium concentrations which were measured according to laboratory routines in our hospital. Plasma osmolality was measured at T0, T-inf, T1, T24, and T48 by freezing point depression (The Advanced Osmometer Model 3900, Advanced Instruments, MA, USA).

**Serum cortisol**

Cortisol was measured in serum from venous blood at T0, T-inf, T1, T4, T24, and T48 by a solid-phase, time-resolved fluoroimmunoassay (AutoDELFIA® Cortisol Kit, PerkinElmer Life and Analytical Sciences, Turku, Finland). The analytical sensitivity of this kit was 15 nmol litre\textsuperscript{-1}.

**Plasma C-reactive protein**

C-reactive protein (CRP) was determined by a DadeBehring BNII nephelometer (Marburg, Germany).
Serum vasopressin

We measured vasopressin in serum from venous blood at T0, T1, T4, T24, and T48 by radioimmunoassay after Sep Pak extraction (Waters Corporation, MA, USA) as described elsewhere.18 The antibody was kindly donated by Dr Peter Bie, Odense, Denmark. The detection limit was 0.26 pg ml⁻¹.

Plasma epinephrine and norepinephrine

Plasma epinephrine and norepinephrine were measured by a radioenzymatic assay described in detail elsewhere.19 The sensitivity of the assay was 0.3 and 0.5 pg ml⁻¹ for epinephrine and norepinephrine, respectively, calculated as three times the standard deviation of the analytical blank.

Plasma cytokines

The plasma concentrations of IL-1β, IL-6, IL-8, IL-10, IL-12, and TNFα were quantified by means of a cytometric bead array assay (Human Inflammation CBA, BD Biosciences Pharmingen, Copenhagen, Denmark) using a FACSCalibur flow cytometer (BD Biosciences, NJ, USA). The detection limits for these cytokines ranged from 1.1 to 4.1 pg ml⁻¹. The concentration of IL-1 receptor antagonist (IL-1ra) was measured by a previously described solid-phase sandwich ELISA using monospecific polyclonal rabbit antibodies to purified recombinant cytokines.20 The sensitivity limit of the ELISA was 60 pg ml⁻¹.

Statistical analysis

If a substance was non-detectable, the measurement was assigned a value corresponding to the detection limit of the test. This was the case for measurements of CRP, epinephrine, cytokines, and vasopressin. The frequency of non-detectable measurements in each group was compared by the χ² test at each time point. Friedman’s test for multiple related non-parametric data was used on each group to test for changes over time (SPSS 10.05, Chicago, IL, USA). Baseline characteristics, the median values at each time point, and the median changes between each of two consecutive time points were compared by the Kruskal–Wallis test. The hypothesis was that the preoperatively infused fluid affected the measured variables. Data are presented as median (inter-quartile range). P<0.05 was considered significant.

Results

Sixty-two patients were included and randomized. Four patients were excluded from the final analysis. One patient did not wish to finish the study (Group HS), one patient (Group HS) experienced an anaphylactoid reaction towards fentanyl or rocuronium (the patient was tested later and found to be allergic to these drugs), one patient was transferred to another hospital before finishing follow-up (Group NS4), and one patient underwent re-operation after 24 h due to postoperative bleeding (Group NS32). The study population baseline characteristics of the remaining 58 patients are shown in Table 1. None of the patients received any blood products during the study.

Plasma electrolytes, osmolality, and blood haemoglobin

The changes in plasma sodium, chloride, potassium, osmolality, and blood haemoglobin have previously been published.17 Briefly, median (IQR) plasma sodium concentration increased by 9 (8–11) mmol litre⁻¹ to a maximum of 151 (149–152) mmol litre⁻¹ after infusion of hypertonic saline corresponding to an increase in plasma osmolality of 18 (14–20) mmol kg⁻¹ to a maximum of 302 (299–306) mmol kg⁻¹. Both variables returned to normal after 24 h. Potassium concentration decreased 0.3 (0.2–0.5) mmol litre⁻¹ in all groups between 24 and 48 h after surgery. Haemoglobin concentration decreased a total of 1.9 g dl⁻¹ (1.3–2.3) in all groups within the first 24 h with a temporary increase 1 h after surgery and stabilization between 24 and 48 h in all groups.

Serum cortisol

The cortisol concentration (Fig. 1A) changed over time in a similar fashion in all groups (P<0.001 for the effect of time in all groups). It decreased after test fluid infusion, peaked 1 h after surgery, and returned towards baseline.

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Table 1 Baseline characteristics. Data are median (25–75th percentile). BMI, body mass index. *Between termination of test fluid infusion and extubation. †P<0.05, ††P<0.01, Kruskal–Wallis test

<table>
<thead>
<tr>
<th></th>
<th>NaCl 7.5%, 4 ml kg⁻¹</th>
<th>NaCl 0.9%, 4 ml kg⁻¹</th>
<th>NaCl 0.9%, 32 ml kg⁻¹</th>
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<tr>
<td>n</td>
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<td>Age (yr)</td>
<td>46 (43–51)</td>
<td>45 (39–48)</td>
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<td>Weight (kg)</td>
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<td>BMI (kg m⁻²)</td>
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<td>Volume of test fluid (ml)</td>
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<td>282 (261–300)</td>
<td>1920 (1790–2570)</td>
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<td>administration (number of patients)</td>
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<td>11 (9–13)</td>
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<td>NaCl 0.9% infused*</td>
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<td>Surgery</td>
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<td>400 (250–500)</td>
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<td>Blood loss*† (ml)</td>
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<td>5 (2–6)</td>
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<tr>
<td>Blood loss†† (ml kg⁻¹)</td>
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<tr>
<td>Duration (min)</td>
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<td>65 (60–71)</td>
<td>77 (55–98)</td>
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</table>
after 24 h. The decrease after test fluid infusion was greater in Group NS32 than in the other groups ($P<0.01$).

**Plasma CRP**

CRP (Fig. 1B) changed over time in all groups ($P<0.001$, for the effect of time in all groups) without significant differences between the groups at any time. The concentration increased from 4 (4–4) mg litre$^{-1}$ in all groups to 57 (42–73) mg litre$^{-1}$, 86 (45–107) mg litre$^{-1}$, and 61 (31–91) mg litre$^{-1}$ after 48 h in Groups HS, NS4, and NS32, respectively.

**Plasma vasopressin**

Vasopressin (Fig. 1C) changed over time in all groups ($P<0.001$ for the effect of time in all groups) without significant differences between the groups at any time. The concentration rose from 0.76 (0.42–1.43) pg ml$^{-1}$ at baseline to 5.3 (3.0–10.6) pg ml$^{-1}$ 1 h after surgery and was still more than double baseline after 48 h.

**Plasma epinephrine and norepinephrine**

The concentrations of epinephrine and norepinephrine changed significantly over time in all groups (Fig. 2). The concentration of both hormones peaked 1 h after surgery. In patients who received normal saline 32 ml kg$^{-1}$, the concentration of epinephrine increased significantly during surgery when compared with the other groups ($P<0.05$). The concentration of norepinephrine increased significantly after infusion in patients who received hypertonic saline or normal saline 32 ml kg$^{-1}$ whereas it was unchanged after infusion of normal saline 4 ml kg$^{-1}$. There were no other differences between the groups at any time point. The concentrations of both hormones were measured at 24 and 48 h in six women, two in each group, in addition to the time points shown in Figure 2. Plasma norepinephrine concentrations were decreased at 24 h and returned to baseline at 48 h, whereas epinephrine concentrations had returned to baseline at 24 and 48 h with no obvious difference between the groups (data not shown).

**Cytokines**

Changes in cytokine concentrations are shown in Figure 3. The concentrations of IL-6, IL-8, and IL-1ra increased significantly in all groups after surgery. IL-10 concentrations only increased significantly after surgery in Group NS4. The concentration of IL-8 was higher at baseline and 1 h after surgery in Group HS compared with the other groups ($P<0.01$). No other differences between the groups were seen for any of the cytokines. TNF$\alpha$, IL-1$\beta$, and IL-12 were undetectable in 30–80% of the measurements at each time point with no significant difference in the
frequency of this between the groups (P>0.05 for all, $\chi^2$ test). Six pilot samples, two in each group showed that there was little difference in the measured cytokines between 24 and 48 h. Consequently, we did not measure cytokines at 48 h.

**Discussion**

To our knowledge, this is the first study to investigate the effects of hypertonic saline infusion in a clinical relevant dose on circulating cytokines and stress hormones after surgery. Abdominal hysterectomy under general anaesthesia elicited a balanced and self-limiting pro- and anti-inflammatory cytokine response. CRP increased and a classic hormonal stress response was observed with temporary increases in plasma cortisol, vasopressin, epinephrine, and norepinephrine. The response seemed largely unaffected by the volume and tonicity of the preoperatively infused fluids.

As in the present study, previous studies found no significant effect of hypertonic saline infusion on pro- or anti-inflammatory cytokine concentrations in plasma from healthy women\(^{16}\) or in the supernatant above monocytes from hypotensive trauma patients.\(^{15}\) In contrast, Rizoli and colleagues\(^ {14}\) found that a single dose of 250 ml of NaCl 7.5%, Dextran-70 6%, added to the standard resuscitation regimen of patients sustaining traumatic haemorrhagic shock, resulted in a reduced intracellular production of the pro-inflammatory cytokine, TNF$\alpha$, and increased synthesis of the anti-inflammatory cytokines, IL-10 and IL-1ra, in peripheral blood monocytes. Methodological differences may help to explain these different findings, that is, plasma and the supernatant do not necessarily reflect changes in intracellular cytokines.\(^ {21}\) However, it may also be, as suggested by Rizoli, that the anti-inflammatory properties of hypertonic saline are only evident in the presence of an intense inflammatory stimulus. This notion is corroborated by recent studies in severely haemorrhaged rats\(^ {22}\) and isolated human immune cells.\(^ {10}\) The latter study suggests that hypertonicity impedes cytokine synthesis in LPS-stimulated cells but not in quiescent cells.\(^ {10}\) It is worth noting that the concentration of IL-6 in trauma patients, which correlates with injury severity, exceeds the maximum concentration measured in this study many times.\(^ {23}\) Thus, the inflammatory stimulus elicited by abdominal hysterectomy during general anaesthesia may be too small to show an anti-inflammatory effect of hypertonic saline.

The primary sources of cytokines include the different leucocyte subtypes.\(^ {24}\) The circulating number of these cells may therefore affect the concentration of the measured cytokines. This is unlikely to be the case in the present study. We have reported elsewhere that only small differences were found between the groups in the number of circulating neutrophils, monocytes, natural killer cells, and lymphocyte subtypes.\(^ {17}\) It is notable, though the groups differed slightly at baseline, that the increase in IL-8 and the concentration 1 h after surgery seemed higher
in the HS group than in the other groups. This is consistent with studies on isolated human peripheral blood mononuclear cells, which showed that hypertonicity dose-dependently increases IL-8 gene expression and IL-8 protein synthesis. IL-8 is a chemokine that attracts neutrophils. There was, however, no effect on the number of neutrophils in the blood.

We observed brief peaks in the concentration of circulating norepinephrine after infusion of hypertonic saline and normal saline 32 ml kg⁻¹. It has recently been shown that infusion of hypertonic saline 3%, 9 ml kg⁻¹ over 60 min in healthy volunteers, raising plasma osmolality to levels comparable with those seen in our study, increased the concentration of plasma norepinephrine. Our data show that this increase may be a result of increased intravascular volume rather than an effect of the induced hypertonicity alone since the plasma norepinephrine increased in both the HS and the NS32 groups (Fig. 2). It is conceivable that the increased intravascular volume facilitates microperfusion and thereby a washout of norepinephrine from neuroeffector junctions. In contrast, Boldt and colleagues found that doubling the wedge pressure by the infusion of hydroxyethyl starch 6% in hypertonic saline 7.2% in cardiac patients attenuated the increase in plasma norepinephrine during cardiopulmonary bypass. Furthermore, Rizoli and colleagues found that infusion of NaCl 7.5%, Dextran-70 6% prevented the rise in circulating norepinephrine elicited by haemorrhage and resuscitation. The available data suggest that infusion of hypertonic saline in relatively unstressed individuals may increase plasma norepinephrine, whereas the increase, caused by major stress such as cardiopulmonary bypass and haemorrhagic shock, is blunted.

Blood loss was greater and the duration of surgery was longer in the NS32 group compared with the other groups (Table 1). We cannot exclude the possibility that this may have affected our result and it may explain the observed increase in epinephrine during surgery in Group NS32. Previous studies suggest that the infusion of large amounts of isotonic saline may increase bleeding and use of blood products during surgery.

We observed a seven-fold increase in vasopressin to a maximum of 5.3 (3.0–10.6) pg ml⁻¹ 1 h after surgery. The increase was unrelated to the tonicity of the infused fluid. We have previously measured circulating vasopressin after infusion of hypertonic saline. Twenty healthy women were randomized to infusion over 10 min of NaCl 7.5% or normal saline, both 4 ml kg⁻¹ were randomized to infusion over 10 min of NaCl 7.5% or after infusion of hypertonic saline. Twenty healthy women underwent a standardized surgical procedure under general anaesthesia. Abdominal hysterectomy induces well-described changes in the inflammatory cytokines and stress hormones similar to those seen after trauma and other kinds of major surgery. It is generally accepted that the anaesthesia may influence the stress response, but that the extent of the surgical trauma is more important. In order to minimize bias from these factors, the surgery and anaesthesia were standardized and performed by persons blinded to the treatment allocation. Our study confirms that abdominal hysterectomy offers a valid and reproducible means of studying the stress response. The results of this study, however, may not be extrapolated to situations with a more extensive inflammatory stimulus such as major trauma, ischaemia/reperfusion, or massive bleeding.

In conclusion, this study shows that infusion of a clinically relevant dose of hypertonic saline before abdominal hysterectomy appears to have limited effect on the post-operative concentration of selected plasma cytokines and the hormonal stress-response.

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