Differential modulation of interleukin-6 and interleukin-10 by diclofenac in patients undergoing major surgery

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Background. Prostaglandins modulate cytokine release through increases in cAMP, regulating interleukin (IL) 6 and IL-10. Diclofenac inhibits cyclo-oxygenase activity and hence prostaglandin production. We hypothesized that diclofenac would affect release of IL-6 and IL-10 and modulate the immune response.

Methods. In a randomized, double-blind, placebo-controlled study, we investigated the effect of diclofenac in patients undergoing major urological surgery. Patients were randomized to receive either diclofenac (50 mg orally every 8 h the day before surgery and 75 mg i.m. every 12 h on the day of surgery, n=23) or placebo (n=23). Standardized combined general anaesthesia and epidural analgesia was administered. Serum IL-6, IL-10 and cortisol were measured before surgery and 30 min and 2, 6, 12 and 24 h after skin incision. Temperature, leucocyte count and C-reactive protein concentration were measured before surgery and after 24 h.

Results. IL-6 and IL-10 concentrations increased, reaching peak levels at 12 and 6 h respectively in both groups. At 12 h, the IL-6 concentration was significantly lower in patients receiving diclofenac than in those receiving placebo (P=0.003). In contrast, IL-10 concentration at 6 h was higher in diclofenac-treated patients (P=0.008), and this was associated with less pyrexia (P=0.03), a lower leucocyte count (P=0.0002) and a lower C-reactive protein concentration (P=0.0039). Serum cortisol concentration was similar in the two groups of patients until 24 h, when the concentration was lower in patients who received diclofenac (P=0.002). Cortisol concentration correlated with IL-6 concentration at 24 h.

Conclusions. Administration of diclofenac was associated with lower IL-6 and higher IL-10 concentrations, and lower leucocyte count, C-reactive protein concentration and temperature. Diclofenac may have an anti-inflammatory role in major surgery.

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Cytokines are a heterogeneous group of proteins which orchestrate the inflammatory response after surgery. Circulating T helper (Th) lymphocytes are capable of wide-ranging cytokine expression and are prompted into a more restricted pattern of cytokine production depending on initial signals. Th1 cells secrete cytokines associated with cellular immunity, including interferon γ (INF-γ) and interleukin (IL) 2, whereas Th2 cells secrete IL-4, IL-5 and IL-10 and are associated with immunosuppressive responses.1 IL-6 is also able to promote Th2 phenotypic responses and its actions can be classified as both pro- and anti-inflammatory. The local balance of IL-6 and IL-10 is an important determinant of subsequent immune responses. Th2 responses predominate in critically ill patients and after surgery.2,3

Prostaglandins increase intracellular cAMP, which regulates IL-6 and IL-10 release and promotes Th2-type responses.4 IL-6 is a multifunctional protein released early during surgery which mediates the release of acute-phase proteins. IL-10 is an acute inflammatory mediator which
down-regulates IL-6 and other proinflammatory cytokines through the transcription factor nuclear factor kappa B (NFκB) [5]. Persistently elevated IL-10 release leads to postoperative monocyte dysfunction, termed immune paralysis, which may predispose to subsequent sepsis. It is thought that Th1- rather than Th2-type responses may promote recovery, and indeed therapeutic strategies involving administration of the Th1 cytokine interferon γ (IFN-γ) are beneficial. Diclofenac is a cyclo-oxygenase inhibitor, which decreases prostaglandin release and hence the cAMP concentration, but the effects of diclofenac on IL-6 and IL-10 are not known. We hypothesized that diclofenac treatment during major surgery would alter IL-6 and IL-10 release and beneficially modulate the inflammatory response. We therefore undertook a randomized controlled trial of perioperative diclofenac administration during major urological surgery.

Patients and methods

After ethical committee approval and written informed consent, patients scheduled for major urological surgery at the Alexandria Main University Hospital, Egypt, were included in a prospective randomized, double-blind, placebo-controlled trial. Patients with known sensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) and those with a history of peptic ulceration or renal, cardiac, endocrine and/or haematological abnormalities were excluded. Patients who had received NSAIDs within 14 days before enrolment were also excluded. Consecutive patients scheduled for surgery within a predefined period of 1 yr were screened for suitability for enrolment. Randomization was performed using computer-generated random numbers. In the study group, 24 patients were randomized to receive perioperative diclofenac as 50 mg tablets every 8 h the day before surgery and 75 mg i.m. every 12 h on the day of surgery. Twenty-four patients were randomized to receive identical placebo tablets and i.m. saline at the same dosing intervals. All patients received oral ranitidine 300 mg on the day before surgery and 50 mg i.m. 8-hourly on the day of surgery. All staff were unaware of which patients received diclofenac or placebo.

All patients received a standardized anaesthetic technique comprising premedication with 10–20 mg oral diazepam 2 h before surgery and a combined general/epidural technique. Patients were fluid-loaded with hydroxyethyl starch 12–14 ml kg⁻¹ and an epidural catheter was sited at L2–3. A test dose of 0.5% bupivacaine 3 ml alone was then given, followed by 4 ml increments of 0.25% bupivacaine with fentanyl 5 μg ml⁻¹ to produce bilateral loss of sensation to pinprick and cold from S5 to T4. The block was maintained with 0.25% bupivacaine and fentanyl 5 mg ml⁻¹ at 5–10 ml h⁻¹ throughout the surgical procedure. General anaesthesia was induced with thiopental 4–7 mg kg⁻¹ and fentanyl 2 μg kg⁻¹ and patients also received pancuronium 0.08–0.1 mg kg⁻¹. Patients were ventilated to maintain normocapnia, and anaesthesia was maintained with 0.5–1% isoflurane and 60% nitrous oxide in oxygen. Central venous catheters were inserted routinely before surgery.

At the end of surgery, neuromuscular blockade was reversed with neostigmine 40 μg kg⁻¹ and glycopyrrrolate 10 μg kg⁻¹. Central venous pressure was maintained at 8–12 cm H₂O and blood transfusion was only given if blood loss exceeded 20% of the empirically estimated blood volume (75 ml kg⁻¹ body weight). In the recovery area, the level of sensory block was reassessed and increments of 0.125% bupivacaine mixed with fentanyl 2 μg ml⁻¹ were given if required, to achieve optimal sensory blockade and control of pain.

After fulfilling the usual criteria for discharge, patients were transferred to a postanaesthetic care unit, where pain was controlled with epidural bupivacaine/fentanyl. The total volume used, the level of sensory block, and the degree of motor power were assessed hourly. Respiratory rate, tympanic temperature, mean arterial pressure and heart rate were recorded and pain intensity was assessed using a visual analogue scale 1 h after discharge, every 2 h for 4 h and every 4 h thereafter. The level of sedation was also assessed using a four-point scale on which 1 indicated awake, 2 easily arousable, 3 difficult to arouse and 4 unarousable. The anaesthetist in charge was called immediately if the sedation score was 3 or 4, if the respiratory rate was less than 10 b.p.m. or if there was any degree of motor loss in the lower extremities. Failure to institute and/or maintain adequate epidural analgesia constituted an exclusion criterion. Urine output was measured every 2 h and if oliguria of <0.5 ml kg⁻¹ h⁻¹ developed, furosemide 20 mg was given i.v. If oliguria persisted in spite of adequate central venous pressure, diclofenac/placebo was stopped and the patient was excluded from the study. The decision for this latter exclusion is based on the ethical consideration of not exposing these patients to further renal insult.

Blood samples were collected at 08.00 h on the day of surgery, and 30 min and 2, 6, 12, and 24 h after skin incision for IL-6 and IL-10 and cortisol measurement. Blood was centrifuged and serum stored at −20°C until assay. Cytokines were measured using an in-house enzyme-linked immunosorbent assay using optimized reagents and antibody pairs and human recombinant IL-6/IL-10 as calibration standards (OptEIA; PharMingen, San Diego, CA, USA). The detection limits for IL-6 and IL-10 were 4.7 and 7.8 pg ml⁻¹ respectively, and between-assay precision was consistently below 10% coefficient of variation. Cortisol was measured using an enzyme immunoassay (Diagnostic Systems Laboratories, Webster, Texas, USA). The detection limit was 0.1 μg dl⁻¹ and precision was a coefficient of variation <12%.

Blood samples were also collected at 08.00 h on the day of surgery and 24 h after skin incision for total leucocyte count and C-reactive protein determination. The C-reactive protein concentration was measured with the latex agglu-
Statistical analysis

A power calculation was performed on the basis of an expected difference of 20% in IL-6 values between patient groups, at a power of 80%, \( P < 0.05 \) (standard deviation 220 pg ml\(^{-1}\), based on previous studies). This indicated that 24 patients per group would be required. Normally distributed data are presented as mean (SD) and were analysed using Student’s \( t \)-test and two-way analysis of variance as appropriate. Non-parametric data are presented as median (range) and were analysed with the Mann–Whitney \( U \)-test, Friedman analysis of variance and the Wilcoxon signed ranks test as appropriate. Correlations were assessed using the Spearman rank test and \( P < 0.05 \) was taken as statistically significant. Correction for multiple comparisons was undertaken whenever appropriate using the Bonferroni method.

Results

Patient groups were similar with respect to age, sex, blood loss, transfusion requirements, fentanyl consumption and type and duration of surgery (Table 1). All but three patients underwent surgery for malignancy. One patient was withdrawn from the control group because of surgical bleeding that necessitated reoperation, and one patient was withdrawn from the diclofenac group because of oliguria unresponsive to fluid maximization and furosemide. In the latter case, adequate urine output was re-established within 48 h of exclusion from the study and did not require any further renal support interventions.

Circulating IL-6 concentrations increased during surgery and peaked at 12 h in both groups of patients (\( P < 0.0001 \); Fig. 1). Post hoc testing showed that concentrations 2, 6, 12 and 24 h after skin incision were significantly higher than baseline values in both diclofenac- and placebo-treated patients (\( P < 0.0001 \); Fig. 1). At 12 h, IL-6 concentrations were significantly lower in the diclofenac group \([385 (67–1291) \text{ pg ml}^{-1}] \) compared with the placebo group \([681 (201–1606) \text{ pg ml}^{-1}; P = 0.003; \text{Fig. 1}] \). Peak IL-6 concentrations correlated with the duration of surgery in both groups \( (r_S = 0.76; P < 0.0001 \text{ in the diclofenac group}; r_S = 0.68, P = 0.001 \text{ in the placebo group}) \).

Circulating IL-10 concentrations also increased during surgery and peaked slightly earlier than IL-10, at 6 h, in both groups of patients \( (P < 0.0001; \text{Fig. 2}) \). Post hoc testing showed that concentrations 2, 6, 12 and 24 h after skin incision were significantly higher than baseline values in both diclofenac- and placebo-treated patients \( (P < 0.0001; \text{Fig. 2}) \). At 6 h, IL-10 concentrations were significantly higher in the diclofenac-treated patients \([136 (44–442) \text{ pg ml}^{-1}] \) compared with the placebo group \([84 (4–218) \text{ pg ml}^{-1}] \).
Discussion

We have shown increases in circulating IL-6 and IL-10 concentrations during and after major surgery, which were significantly altered by diclofenac treatment. Peak concentrations of IL-6 were significantly attenuated by diclofenac, whilst peak IL-10 concentrations were found to be higher. Other measures of the inflammatory response (cortisol, leucocyte count, C-reactive protein and temperature) were also lower in patients treated with diclofenac.

IL-6 concentrations increased in all patients during major surgery and correlated with the duration of the surgical procedure, as reported previously [8]. Cruickshank and colleagues [9] reported a similar correlation, although the extent of tissue trauma rather than the duration of surgery was thought to be the primary determinant of the IL-6 response. All but three of the patients in our study underwent surgery for malignancy. Although increased concentrations of both IL-6 and IL-10 have been reported to correlate with cancer staging [10, 11] we found no evidence of a pre-existing IL-10-mediated inflammatory response, as baseline concentrations of both cytokines were within ranges reported for healthy subjects [10, 11]. Others have reported that IL-6 concentrations increase during surgery independently of the presence of cancer and again suggest that the extent of trauma and the duration of surgery are more important [12].

Although specific anaesthetic agents may affect cytokine release, there is little evidence for clinically relevant modulation of the inflammatory response by anaesthesia. Cytokine release during surgery is also related to the magnitude of the neuroendocrine stress response. Smaller increases in cortisol in conjunction with abrogated IL-6 release have been reported in patients undergoing general anaesthesia with large doses of opioids [13], and other immune functions are also affected. However, combined epidural/general anaesthesia, as used in the present study, did not result in reduced IL-6 concentrations compared with general anaesthesia alone [14]. In our study, IL-6 concentrations increased in all patients during surgery, but peak concentrations were significantly lower in patients who received diclofenac treatment and were associated with decreased cortisol concentrations 12 h later. Similar reductions in IL-6 concentrations were found in patients undergoing cholecystectomy who were treated with ibuprofen [15]. IL-6 is known to stimulate ACTH and cortisol release [16], and glucocorticoids inhibit IL-6 release [17]. However, cortisol concentrations increase earlier than circulating concentrations of IL-6; this increase is blocked during regional anaesthesia without affecting IL-6 [14], suggesting that IL-6 does not initiate the cortisol response to surgery. In our study, decreased IL-6 in diclofenac-treated patients preceded changes in cortisol in this group, suggesting that diclofen-
nac-mediated changes in IL-6 occurred independently of cortisol.

In vitro studies using fibroblasts have shown that release of IL-6 is preceded by increases in cAMP. Prostaglandins are known to increase intracellular concentrations of cAMP, and NSAIDs such as diclofenac are potent inhibitors of IL-6 expression in several human cell lines. Decreased IL-6 release in the present study may therefore have been mediated by reduced prostaglandin production and decreases in cAMP. However, other mechanisms should also be considered. The transcription factor NFκB exists in cytoplasm in an inhibited state by virtue of the formation of a complex with the inhibitory subunit, IκB. The NSAID tepoxalin has been shown to inhibit IL-6 release from astrocytes through decreased NFκB activation as a result of stabilising the IκB, and diclofenac may therefore inhibit the release of IL-6 at the transcriptional level.

Interleukin-10 concentrations increased in this study during major surgery, as described previously, and peaked slightly earlier than IL-6, at 6 rather than 12 h. This earlier peak is perhaps surprising as it might be expected that IL-6 release would precede that of IL-10. Early IL-10 release has been described in patients undergoing other major surgery, such as coronary artery bypass grafting. Our study certainly suggests that IL-10 release occurs before IL-6 and we also showed that diclofenac treatment increased IL-10 release. This is unexpected, as in vitro prostaglandins prime T cells for production of IL-10, and the regulation of cytokine balance in lymphocytes and macrophages depends on cyclo-oxygenase-2 activity for the up-regulation of IL-10 and down-regulation of IL-12 production. However, studies using murine peritoneal macrophages showed that several NSAIDs increased IL-10 production, and indomethacin administration before experimental sepsis in rats was also associated with increased IL-10 production. In addition, another in vitro study showed that prostaglandin E1 suppressed IL-10 production by lipopolysaccharide-stimulated mononuclear cells, and certainly, because IL-10 is an anti-inflammatory agent, it might be expected that prostaglandins would decrease its release and the inhibition of prostaglandins would be associated with increased IL-10.

As peak concentrations of IL-6 occurred later than those of IL-10, it is possible that diclofenac treatment increased IL-10 production, which in turn down-regulated IL-6, in addition to any direct effects of cyclo-oxygenase inhibition on IL-6 via either cAMP or NFκB. IL-10 has consistently been shown to regulate proinflammatory cytokines, including IL-6, at the transcriptional level. The mechanism for the effect can be attributed either to IL-10-mediated decreased prostaglandin E2 formation or to IL-10-mediated stabilization of IκB. NFκB is one of several transcription factors involved in the regulation of IL-6, but the effects of IL-10 and diclofenac on other transcription factors, such as nuclear factor-IL-6, are not known. The differential effect of diclofenac on IL-6 and IL-10 during major surgery may be mediated through a combination of several mechanisms.

The attenuated rise in body temperature observed in the diclofenac group can be attributed simply to the antipyretic effect of the non-steroidal anti-inflammatory agent, as reported previously. This effect is thought to be mediated through inhibition of prostaglandins and IL-6, both of which are endogenous pyrogens. Other anti-inflammatory effects of diclofenac were also observed in the present study, namely decreased leucocytosis and a smaller increase in C-reactive protein. IL-6 up-regulates the release of acute phase proteins, including C-reactive protein, and the effect of diclofenac may be mediated by IL-6. The low pain scores and the similarity of scores in the two groups of patients probably reflect the fact that the epidural was highly effective, as we intended, leaving little scope for the analgesic action of diclofenac. This indicates that our findings are unrelated to differences in pain-induced stress responses between the groups, and it is clearly crucial.

In summary, we have shown that diclofenac administration during major surgery has differential effects on IL-6 and IL-10 release which may affect the inflammatory response. The concentration ratio of circulating IL-6 to IL-10 and the IL-10 concentration alone have been shown to correlate with injury severity score in patients after trauma.

### Table 3

<table>
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<th></th>
<th>Before surgery</th>
<th>Time after discharge from recovery area (h)</th>
<th>P*</th>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td>3</td>
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<tr>
<td>MAP (mm Hg; mean (SD))</td>
<td></td>
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<tr>
<td>Diclofenac</td>
<td>93 (9)</td>
<td>85 (9)</td>
<td>85 (10.5)</td>
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<tr>
<td>Placebo</td>
<td>95 (7)</td>
<td>86 (1)</td>
<td>87 (9.0)</td>
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<tr>
<td>Heart rate (beats min⁻¹; mean (SD))</td>
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<tr>
<td>Diclofenac</td>
<td>87 (8)</td>
<td>86 (8)</td>
<td>87 (8.2)</td>
</tr>
<tr>
<td>Placebo</td>
<td>89 (9)</td>
<td>85 (9)</td>
<td>86 (9.3)</td>
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<tr>
<td>Pain score (median range)</td>
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<tr>
<td>Diclofenac</td>
<td>–</td>
<td>1 (1–3)</td>
<td>2 (0–3)</td>
</tr>
<tr>
<td>Placebo</td>
<td>–</td>
<td>1 (0–3)</td>
<td>2 (0–3)</td>
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</table>
Higher IL-10 concentrations are also associated with subsequent development of sepsis, possibly because of the host’s diminished antimicrobial defences. However, our findings support the concept that altering the balance between IL-6 and IL-10 by treatment with diclofenac may be beneficial, and this is suggested by the observed effects on leucocyte count, pyrexia and the acute phase response. Clearly, further large trials are required to determine the effects of this simple therapeutic strategy on morbidity and mortality.

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