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Effects of Preemptive Analgesia on Pain and Cytokine Production in the Postoperative Period

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Background: **The postoperative period is associated with increased production of proinflammatory cytokines, which are known to augment pain sensitivity, among other effects. In a previous study, the authors found that patients treated with patient-controlled epidural analgesia (PCEA) exhibited attenuated proinflammatory cytokine response in the postoperative period. In the present study, the authors examined whether preemptive analgesia continued with PCEA may further attenuate the proinflammatory cytokine response and reduce pain sensitivity in the postoperative period. They compared cytokine production in two groups of patients, one receiving PCEA, the other receiving preemptive epidural analgesia continued by PCEA.**

Methods: **Female patients hospitalized for transabdominal hysterectomy were randomly assigned to one of two pain management techniques: PCEA or preemptive epidural analgesia followed by PCEA (PA + PCEA). Postoperative pain was assessed using the visual analog scale. Blood samples were collected before, 24, 48, and 72 h following surgery. Production of the following cytokines was assessed *ex vivo* in stimulated peripheral blood mononuclear cells: interleukin (IL)-1β, tumor necrosis factor α, IL-6, IL-1ra, IL-10, and IL-2.**

Results: **Patients of the PA + PCEA group exhibited lower pain scores throughout the 72 h postoperatively, compared with patients of the PCEA group. In patients of the PA + PCEA group in the postoperative period, production of IL-1β, IL-6, IL-1ra, and IL-10 was significantly less elevated, while IL-2 production was significantly less suppressed.**

Conclusions: **Proinflammatory cytokines are key mediators of illness symptoms, including hyperalgesia. The present results suggest that preemptive epidural analgesia is associated with reduced postoperative pain and attenuated production of proinflammatory cytokines.**

PAIN management remains a major concern in the postoperative period. Pain affects various systems, including cardiac, pulmonary, and metabolic, and thus, it may affect surgical outcome.[1] Although it has been shown in animal studies that pain can contribute to postoperative immune suppression,[2] little clinical evidence is available showing the effects of postoperative pain management on the immune response.

We have recently examined the effects of three postoperative pain management techniques on the immune response in patients undergoing abdominal surgery (submitted for publication). Patients were randomly assigned to one of three postoperative pain management techniques: systemic administration of opiates on intermittent regimen, patient-controlled analgesia, and patient-controlled epidural analgesia (PCEA). In the immediate postoperative period, patients of the PCEA group exhibited reduced postoperative pain, reduced suppression of lymphocyte mitogenic response, and attenuated proinflammatory cytokine response.

Preemptive analgesia is a commonly used pain management technique in which analgesic treatment begins prior to the surgical incision. Preemptive analgesia, continued into the postoperative period, can reduce both the incisional and inflammatory pain and in this way can reduce peripheral and central sensitization.[3] Although the beneficial effects of preemptive analgesia have been demonstrated in animal studies, the clinical value of this technique remains indecisive because of methodological problems, flaws in the design of clinical studies, and lack of objective measures.[4]

Extending our previous findings, the present study sought to examine whether preemptive analgesia continued with PCEA may attenuate proinflammatory cytokine production in the postoperative period. We also examined the effects of preemptive analgesia on the production of antiinflammatory cytokines and on interleukin (IL)-2. The present study compared *ex vivo* cytokine production in two groups of patients, one receiving PCEA, the other receiving preemptive epidural analgesia continued in the postoperative period with PCEA.

Materials and Methods

Patients

Healthy female patients (American Society of Anesthesiologists physical status I or II) aged 40–70 yr old were included in this study after obtaining approval from the Hospital Human Studies Committee and informed consent from the patients. Patients were hospitalized to undergo transabdominal hysterectomy (for details, see table 1). On the preoperative visit by the anesthesiologist, patients were randomly assigned to one of two perioperative pain management techniques (treatment

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Table 1. Characteristics of Patients According to Postoperative Pain Management Group

Variable	PCEA	PA + PCEA
Patients (N)	20	21
Body weight (kg)	70 ± 2.45	67 ± 2.54
Age (yr)	53 ± 1.78	54 ± 2.05
Surgery duration (min)	116 ± 8.83	103 ± 6.57
Total fentanyl (mg/48 h)	1.38 ± 0.04	1.36 ± 0.02

Values are mean ± SEM. There were no significant differences between groups with respect to body weight, age, surgery duration, and total fentanyl consumption.

PA = preemptive analgesia; PCEA = patient-controlled epidural analgesia.

lasted until 48 h postoperatively): One group (N = 20) received PCEA in the postoperative period; the other (N = 21) received preemptive epidural followed by PCEA (PA + PCEA). On the same visit, patients were familiarized with the visual analog pain scale (VAS) and were instructed on the use of the PCEA pump.

Anesthesia

Patients were premedicated with lorazepam (1–2 mg orally) 90 min before induction of anesthesia, followed by 2–3 mg intravenous midazolam on arrival in the operating theater. An epidural catheter was placed in all patients *via* the L2–L4 interspaces and was advanced 3 to 4 cm cephalad. The position of the epidural catheter was tested with 3 ml lidocaine, 2%. Patients of the PA + PCEA group received an epidural mixture of 12 ml bupivacaine (0.5%) plus fentanyl (50–100 μg) 20–25 min before incision.

General anesthesia was induced using 2–3 μg/kg fentanyl, 4–6 mg/kg thiopental, and 0.1 mg/kg vecuronium, intravenously. Anesthesia was maintained with nitrous oxide, isoflurane, and additional fentanyl. Mean arterial blood pressure was maintained within 20% of baseline values with isoflurane and fentanyl. Patients received upper body forced-air warming, and intravenous fluids were warmed to 37°C. Patients requiring blood transfusion during the perioperative period were not included in this study.

Postoperative Pain Management

On arrival to the postanesthesia care unit, patients of both groups were connected to the PCEA pump (Pain Management Provider; Abbott, Chicago, IL) and received 3 ml bupivacaine (0.1%) plus 2 μg/ml fentanyl per demand (lockout time, 10 min), with continuous background infusion of 6 ml/h.

A 10-cm VAS (with end points labeled "no pain" and "worst possible pain") was used to assess pain intensity in rest and after coughing at 4, 8, 12, 24, 48, and 72 h after completion of surgery.

Immunologic Assays

Venous blood samples (15 ml) were collected on the morning of the surgery and at 24, 48, and 72 h following surgery. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood using a histopaque (Sigma-Aldrich, Rehovot, Israel) gradient centrifugation. PBMCs were washed twice in RPMI-1640 medium containing 1% penicillin, streptomycin, and nystatin and supplemented with 10% fetal calf serum (designated complete medium). Cells were suspended in fetal calf serum containing 10% dimethyl sulfoxide (Sigma) and were frozen at −70°C until used. On the day of assay, cells were thawed quickly and washed three times in complete medium, and their viability was tested by trypan blue dye exclusion. The viability was over 95%.

Cytokine (IL-1β, IL-2, IL-6, IL-10, IL-1ra, and TNF-α) Production

Peripheral blood mononuclear cells (2×10^6) suspended in 1 ml RPMI-1640 supplemented with 5% fetal calf serum were incubated for 24 h in the presence of 10 ng/ml lipopolysaccharide (*E. coli*; Sigma-Aldrich) for IL-1β, IL-1ra, IL-6, IL-10, and tumor necrosis factor (TNF)-α. For IL-2 production, 2×10^6 PBMCs were suspended in 1 ml complete medium and were incubated for 48 h with 1% phytohemagglutinin (PHA-M; Difco Laboratories, Detroit, MI). Following the incubation period, culture media were collected, cells were removed by centrifugation, and the supernatants were kept at −70°C until assayed for cytokine content.

Cytokine concentration in the supernatant was assessed using ELISA kits specific for human IL-1β, IL-1ra (Biosource International, Camarillo, CA), IL-6, (Pharmingen, San Diego, CA), IL-2 (R&D Systems, Minneapolis, MN), and IL-10 (Genzyme Corporation, Cambridge, MA), as detailed in the guidelines provided by the manufacturers. The detection levels of the cytokines in the assays were 30 pg/ml for IL-1β and IL-2, 15 pg/ml for IL-6, and 60 pg/ml for IL-1ra and TNF-α.

Statistical Analysis

The number of observations varies slightly among assays as a result of an occasional missing sample or an occasional problem in a particular assay. Data were analyzed for each measure, using analysis of variance with repeated measures (time periods before and after surgery).[5] A *post hoc* Bonferroni procedure was conducted as appropriate, correcting for multiple comparisons.[5] Probability values of $P < 0.05$ were considered significant. The results are expressed as mean ± SEM.

Results

The groups were similar in body weight, age, duration of surgery, and total fentanyl consumption in 48 h postoperatively (table 1).

Postoperative Pain

Pain scores reveal similar trends between the measures taken at rest or during coughing. There were significant

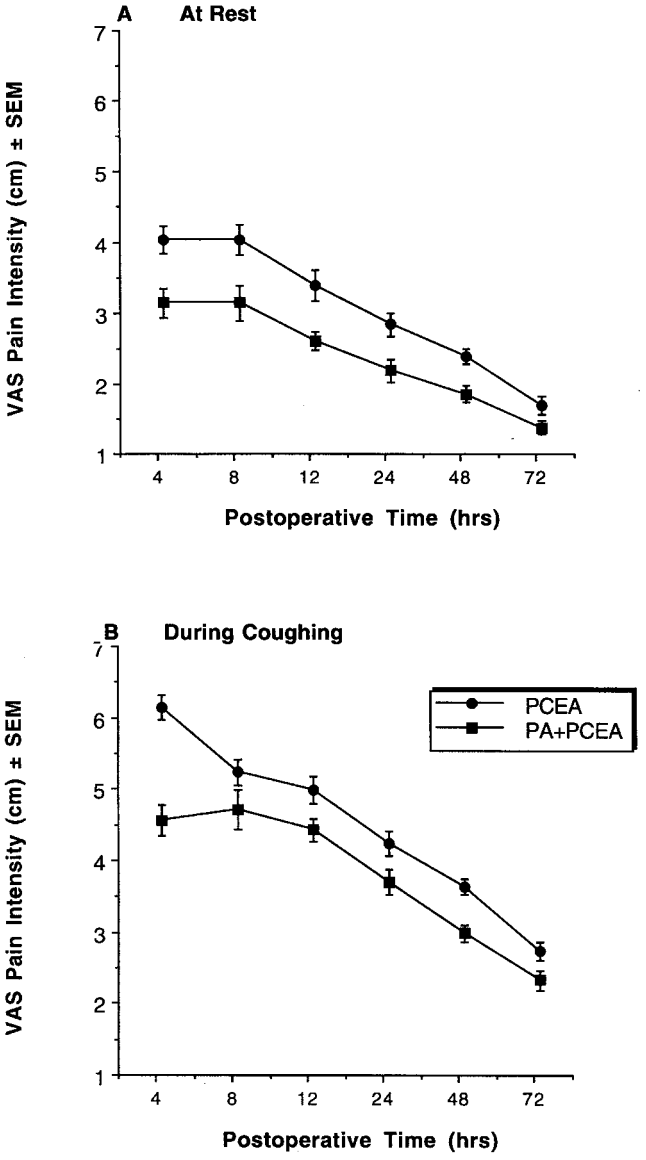

Fig. 1. Visual analog scale (VAS) pain scores at rest (*A*) and during coughing (*B*) beginning 4 h and up to 72 h postoperatively, for patients of the two groups: patient-controlled epidural analgesia (PCEA) or preemptive analgesia (PA) plus PCEA. Pain intensity was significantly greater in patients of the PCEA group throughout the observation period, both at rest and during coughing. Values are mean (±SEM).

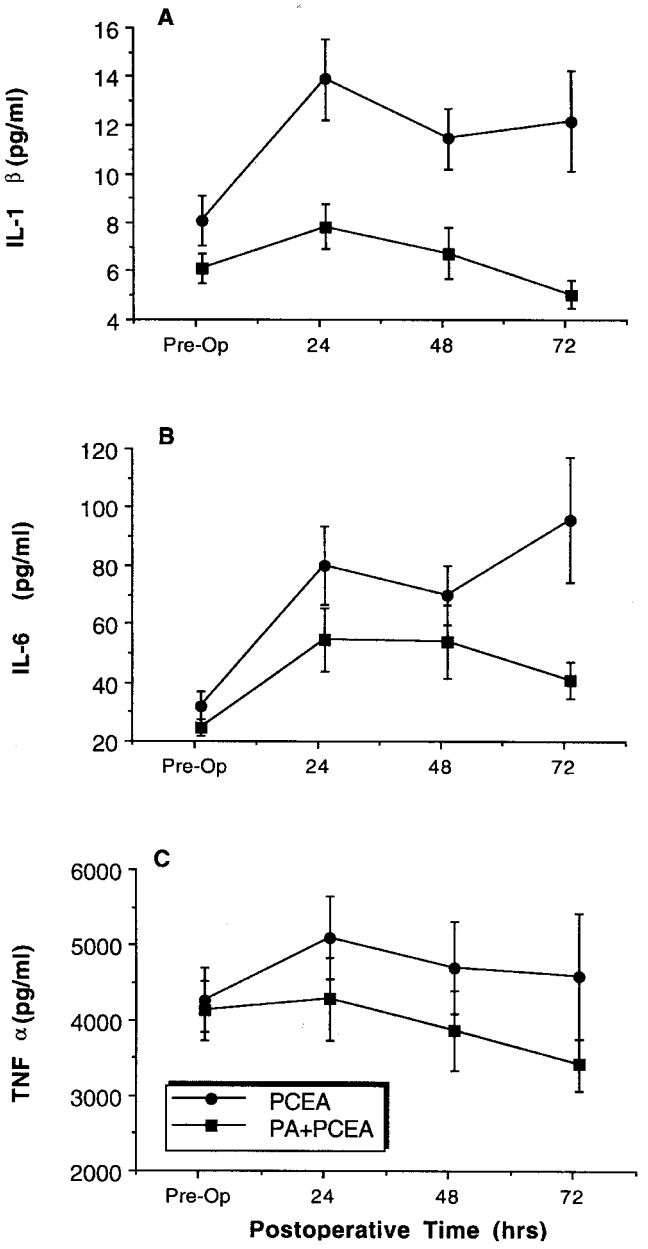

Fig. 2. Production of interleukin (IL)-1β (*A*), IL-6 (*B*), and tumor necrosis factor (TNF) α (*C*) by peripheral blood mononuclear cells in the two study groups. Levels of the proinflammatory cytokines were more elevated in the patient-controlled epidural analgesia (PCEA) group. Values are mean (±SEM). PA = preemptive analgesia.

differences among the study groups ($F_{1,39}$ = 20.71 or 23.67; $P < 0.0001$ for VAS at rest or VAS during coughing, respectively; figs. 1A and B). Patients of the PA + PCEA group experienced less severe postoperative pain throughout the postoperative observation period.

Ex Vivo *Cytokine Production*

Analysis of variance with repeated measures for IL-1β levels revealed significant main effects of groups (PCEA *vs.* PA + PCEA; $F_{1,39}$ = 13.63, $P < 0.001$) and trials (preoperative, 24, 48, and 72 h postoperative; $F_{3,117}$ = 6.08, $P < 0.001$). There was also a significant interaction (group × trial; $F_{3,117}$ = 3.11, $P < 0.03$). The analysis of variance test for IL-6 revealed significant main effects of groups ($F_{1,39}$ = 4.25, $P < 0.05$) and trials ($F_{3,117}$ = 9.72, $P < 0.001$), and significant interaction (group × trial; $F_{3,117}$ = 2.89, $P < 0.04$). These results indicate that proinflammatory cytokine production by stimulated PBMCs was elevated in the postoperative period and that such elevation was significantly less pronounced in patients of the PA + PCEA group compared with patients of the PCEA group for both IL-1β and IL-6 (figs. 2A and B, respectively). Levels of TNF-α exhibited a similar

trend, which was not statistically significant (fig. 2C). It is known that the maximal release of TNF-α by lipopolysaccharide-stimulated PBMCs occurs earlier than 24 h. This may account for the lack of significant group differences at the times observed in the present study.

Ex vivo production of antiinflammatory cytokines was also assessed by stimulated PBMCs. IL-10 and IL-1ra levels were elevated in the postoperative period (significant trial effects in both groups), but this elevation was significantly less pronounced in patients of the PA + PCEA group (group effects: $F_{1,38} = 6.16$, $P < 0.02$ and $F_{1,36} = 5.22$, $P < 0.03$, respectively; figs. 3A and B). The IL-1ra/IL-1β ratio exhibited a slight, but not significant, linear increase from the preoperative to the 72 h postoperative measures (0.74–1.23). There were no significant main effects or a significant interaction. That is, the two groups did not differ in the IL-1ra/IL-1β ratio at any time point (data not shown).

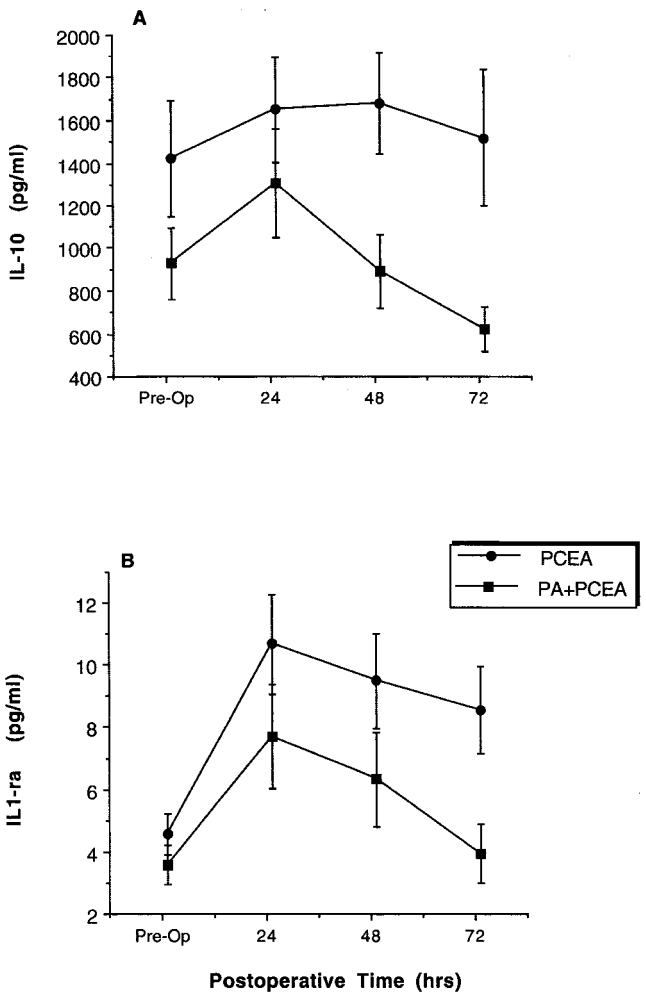

Fig. 3. Production of interleukin (IL)-10 (*A*) and IL-1ra (*B*) by peripheral blood mononuclear cells in the two study groups. Levels of the antiinflammatory cytokines were more elevated in the patient-controlled epidural analgesia (PCEA) group. Values are mean (±SEM). PA = preemptive analgesia.

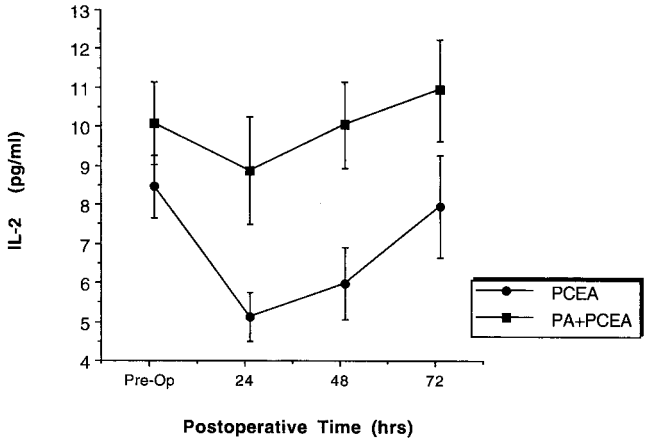

Fig. 4. Production of interleukin (IL)-2 by peripheral blood mononuclear cells in the two study groups. Levels of IL-2 were more suppressed in the patient-controlled epidural analgesia (PCEA) group. Values are mean (±SEM). PA = preemptive analgesia.

Ex vivo IL-2 production was assessed in PHA-stimulated PBMCs. There was a significant trial effect ($F_{3,117} = 6.18$, $P < 0.001$), indicating a significant suppression of IL-2 levels in 24 and 48 h postoperatively, examined over the two study groups. There was a significant difference between the study groups in IL-2 levels ($F_{1,39} = 5.41$, $P < 0.025$; fig. 4). IL-2 levels remained almost unchanged in the PA + PCEA group, while they were significantly suppressed in the PCEA group in the first 48 h postoperatively, gradually recovering by 72 h.

Discussion

Patients of the PA + PCEA group exhibited less severe postoperative pain, compared to the PCEA group. *Ex vivo* stimulated PBMCs of these patients produced less elevated levels of both proinflammatory and antiinflammatory cytokines in the postoperative period. In addition, production of IL-2 by stimulated PBMCs was significantly less suppressed in the PA + PCEA group compared to the PCEA group. The findings of increased production of proinflammatory cytokines and suppressed production of IL-2 observed in the postoperative period in our present and previous studies[6] are in agreement with reports of alterations in cytokine serum levels following surgery (*e.g.*, Baxevanis *et al.*[7]).

Surgery-associated tissue injury sets off a cascade of related events, including nociception and inflammatory reaction. Tissue and peripheral nerve injury leads to local inflammatory reaction, accompanied by elevated levels of proinflammatory cytokines, including IL-1β and IL-6.[8] These cytokines can induce peripheral and central nerve system sensitization, leading to pain augmentation (hyperalgesia).[8] Peripherally, IL-1β induces long-lasting synthesis and release of substance P from peripheral

nerve terminals of primary afferent neurons, which may contribute to neurogenic inflammation.[9,10]

Within minutes of injury, glial cells in the central nervous system respond with increased production of immune factors, including proinflammatory cytokines.[11] IL-1β can induce central sensitization *via* IL-1 receptors on neurons[12] or *via* activated glia cells, which produce pain mediators, including substance P, glutamate, and nitric oxide synthase,[13] all of which can alter pain processing within the central nervous system. Elevated IL-1β in the central nervous system also leads to the production of COX2 by neurons in the brain and spinal cord and further synthesis of PGE2, which is known to increase pain sensitivity.[14] Similarly, IL-6 levels are also elevated following nerve injury, both peripherally and centrally, contributing to hyperalgesia by direct spinal nociceptive mechanisms or by glial activation.[15,16] IL-1β and IL-6 are involved in the mechanisms of allodynia and possibly in the development of postoperative neuropathic and chronic pain.[11,17]

It has been argued that preemptive analgesia, commencing before surgery and continuing in the postoperative period, prevents the establishment of peripheral and central sensitization.[3] In the present study, patients receiving preemptive analgesia exhibited less severe pain and attenuation of proinflammatory cytokine production throughout the postoperative period. It is difficult to determine whether blocking the pain contributes to the reduced production of proinflammatory cytokines or, *vice versa*, reduced production of proinflammatory cytokines results in less severe pain experience.

It is noteworthy that patients receiving preemptive analgesia also exhibited less suppression of IL-2 production in the postoperative period. Since IL-2 is secreted by the TH1 subset of lymphocytes, the present finding may suggest attenuated suppression of the TH1 phenotypic response in the PA + PCEA pain management group. It has been shown that IL-2 structurally resembles opiate peptides and has analgesic effects in both the peripheral and central nervous system.[18] Thus, it is possible that less suppressed IL-2 levels seen in the PA + PCEA group contributes to the less severe pain reported by these patients.

In conclusion, preemptive analgesia results in reduced pain intensity and attenuated production of proinflammatory cytokines by stimulated PBMCs in the postoperative period. These findings are interrelated, demonstrating correlation between subjective measure of pain and objective measures of immune reactivity. Proinflammatory cytokines are key mediators of illness symptoms, including fever, reduction in synthesis and release of acute phase proteins, deceased food and water intake, and hyperalgesia. This study suggests that reduced secretion of proinflammatory cytokines following preemptive analgesia may contribute to reduced postoperative pain and possibly to attenuated illness response.[19]

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