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# Cytokine and Hormonal Changes After Cholecystectomy

## Effect of Ibuprofen Pretreatment

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### Objective
Surgical stress induces hormonal and cytokine responses proportional to the extent of the injury. Therefore, the authors assessed the effect of ibuprofen pretreatment on metabolic and hormonal changes after surgery.

### Summary Background Data
Postoperative administration of cyclo-oxygenase inhibitor reduces cytokine production and nitrogen losses.

### Methods
The authors studied the plasma hormones and metabolic and cytokines changes after perioperative ibuprofen administration in 22 patients undergoing cholecystectomy under inhalational anesthesia. Suppositories containing ibuprofen (500 mg) or placebo were administered 12 and 2 hours before surgery, and every 8 hours until the third postoperative day. Blood samples were collected 24 and 2 hours before surgery and 2, 4, 6, 24, 48, and 72 hours after surgery for glucose, C-reactive protein, leukocytes, adrenocorticotropic hormone (ACTH), cortisol, tumor necrosis factor, and interleukin-1 and interleukin-6 determinations.

### Results
In both groups, plasma cortisol levels remained elevated for 3 days, whereas plasma ACTH levels returned to the basal level at day 1. The ACTH ($p < 0.01$), cortisol ($p < 0.01$), and glucose changes ($p < 0.001$) were smaller in the ibuprofen group and their duration was shorter. The interleukin-6 levels increased gradually after skin incision until the sixth hour and were significantly lower ($p < 0.05$) in the ibuprofen group.

### Conclusion
Ibuprofen pretreatment in perioperative course is able to reduce the endocrine response and cytokine release. Therefore, ibuprofen may be useful in decreasing the stress response in severely surgical patients.

In response to surgical stress, plasma concentrations of interleukins increase.[1,2] Associated with hormonal changes, this stress response can go beyond its protective objectives and become harmful to the body, resulting in glucose intolerance, negative nitrogen balance, and immunologic impairment, which increase postoperative morbidity.

Regional anesthesia[3] and high doses of opioids[4] can suppress or attenuate the stress response to lower abdominal surgery, whereas the response to upper abdominal surgery is only slightly or not at all modulated by the anesthetic technique.[5,6]

Previous studies have shown that pretreatment with cyclo-oxygenase inhibitors attenuates the toxic effects of cytokines or endotoxin administration or both.[7-9] After upper abdominal surgery, one study showed that postoperative administration of cyclo-oxygenase inhibitor reduce cytokine production and nitrogen losses, but so far no study examined the effect of pretreatment with cyclo-oxygenase inhibitors on the stress response to surgery.[10]

To investigate the effects of cyclo-oxygenase inhibitor pretreatment on the response to surgical stress in humans, we studied the changes in plasma hormones, cytokines, and metabolism after ibuprofen administration in patients undergoing cholecystectomy.

## PATIENTS AND METHODS

In a prospective randomized, double-blind protocol approved by the Ethics Committee of Claude Bernard University, 22 patients who gave written informed consent and were scheduled for a routine cholecystectomy were enrolled in the study. All were American Society of Anesthesiology (ASA) physical status I and no patients were receiving nonsteroidal anti-inflammatory drug (NSAID).

Patients with a history of peptic ulcer, gastrointestinal bleeding, or allergy were excluded. Patients were allocated randomly to receive suppositories containing 500 mg of ibuprofen (n = 11) or placebo (n = 11). The suppositories were administered 12 hours and 2 hours before surgery, and every 8 hours until the third postoperative day.

The same anesthetic technique was used in all patients. After an overnight fast, patients were premedicated with oral hydroxyzine (100 mg; UCB Pharma, Nanterre, France) and alprazolam (1 mg; Upjohn, Paris, France) 2 hours before surgery. Anesthesia was induced with propofol (2 mg/kg; Zeneca, Cergy, France), fentanyl (3–5 μg/kg; Janssen, Boulogne Billancourt, France) intravenously and maintained using end-tidal isoflurane 0.7% with 50% nitrous oxide in oxygen and fentanyl (0.5 or 1 μg/kg) when necessary. Tracheal intubation was facilitated with atracurium (0.6 mg/kg). Neuromuscular block was produced with continuous infusion of atracurium (0.5 mg/kg/hour).

Ringer's lactate solution was infused at a constant rate of 10 mL/kg/hour during surgery through a peripheral intravenous cannula. After surgery, nutritional support consisted of dextrose 5% (40 mL/kg) for 3 days. Only 0.3 mg subcutaneous injection of buprenorphine was allowed for analgesia when necessary.

Blood samples were collected from an antecubital vein as follows: at 9 o'clock in the morning the day before surgery except for cytokines, at premedication, 1 hour after skin incision, 2 hours after skin incision, 4 hours after skin incision, 6 hours after skin incision, 24 hours after skin incision except for tumor necrosis factor (TNF-α) and interleukin-1 (IL-1), 48 hours after skin incision except for cytokines, and 72 hours after skin incision except for cytokines.

Plasma concentrations of glucose, C-reactive protein (CRP) leukocytes, adrenocorticotropic hormone (ACTH), cortisol, TNF-α, IL-1, and interleukin-6 (IL-6) were measured. Plasma glucose levels were determined by a glucose oxydase method. Serum concentration of CRP (normal range < 8 mg/L) was measured by an immunoprecipitation method (Array Protein System, Beckman, Brea, CA). The plasma concentrations of ACTH were determined by immunoradiometry (Biomérieux, Lyon, France; normal range < 35 ng/mL) and cortisol by radioimmunoassay (normal range = 340–640 nmol/L at 8 o'clock).

The plasma TNF-α (normal range = 6.3 ± 4.2 pg/mL) and IL-1 levels (normal range = 12 ± 7.1 pg/mL) were determined by an immunoradiometric assay (Eria-Medgenix Diagnostics, Fleurus, Belgium) and IL-6 concentration (normal range ≤ 20 pg/mL) by the enzyme-linked immunosorbent assay method (Easia, Medgenix Diagnostics, Fleurus, Belgium). Rectal temperature was recorded at 8 o'clock, 4 o'clock, and midnight in each patient.

Data are presented as mean ± standard error of the mean. Repeated-measures analysis of variance and paired t test were used to evaluate changes within each treatment. Data were analyzed by a Mann–Whitney test between ibuprofen and control groups. A p value < 0.05 was considered statistically significant.

## RESULTS

Table 1 summarizes the demographic data and drug doses. Both groups were comparable. Before surgery, endocrine and metabolic variables were similar in both

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**Table 1. DEMOGRAPHIC DATA AND ANESTHESTIC REQUIREMENTS**

| | Placebo (n = 11) | Ibuprofen (n = 11) |
|---|---|---|
| Sex ratio (M/F) | 2/9 | 5/6 |
| Age (yr) | 56.6 ± 5.8 | 43.1 ± 3.9 |
| Weight (kg) | 59.5 ± 3.7 | 68.4 ± 3.7 |
| Height (cm) | 160 ± 1.8 | 167 ± 2.8 |
| Body mass index | 23.1 ± 1.31 | 24.3 ± 1.2 |
| Surgical procedure (min) | 95 ± 10.6 | 89.5 ± 12.1 |
| Propofol induction (mg/kg) | 1.92 ± 0.14 | 1.73 ± 0.08 |
| Total fentanyl (μg/kg) | 6.13 ± 0.33 | 5.9 ± 0.52 |
| Total atracrium (mg/kg) | 0.886 ± 0.073 | 0.807 ± 0.061 |

Data are expressed as mean ± standard error of the mean. There is no significant difference between the two groups in any parameter.

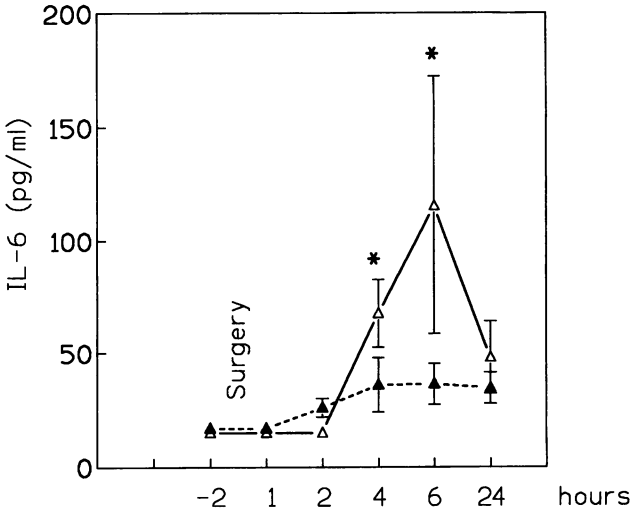


**Figure 1.** Changes in interleukin levels during and after cholecystectomy in patients with (black triangle) or without (white triangle) preoperative treatment with ibuprofen (± standard error of the mean); *p < 0.05.

groups. When compared with preoperative values, surgery induced significant changes for all endocrine and metabolic parameters in both groups. After skin incision, plasma ACTH and cortisol levels increased rapidly after the first hour of surgery. Whereas the plasma ACTH level decreased after the first hour, the plasma cortisol level remained elevated for 72 hours.

In the ibuprofen group, the responses for ACTH ($p < 0.01$) and cortisol ($p < 0.01$) were smaller (Table 2). The results of plasma TNF-α and IL-1 levels were too scattered, and no significant changes were observed in this study. Conversely, the IL-6 levels increased gradually after skin incision until the sixth hour and were significantly lower ($p < 0.05$; Fig. 1) in the ibuprofen group.

Glycemia and leukocytes count increased from the first hour until the third day. In the ibuprofen group, the glycemia ($p < 0.001$) and leukocytes count ($p < 0.001$) rose slightly and remained close to the normal values.

**Table 2. METABOLIC AND HORMONAL CHANGES FOLLOWING CHOLECYSTECTOMY**

| | | Surgery↓ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | −24 hr | −2 hr | 1 hr | 2 hr | 4 hr | 6 hr | 24 hr | 48 hr | 72 hr |
| Glucose (mmol/L) | P | 5.4 (0.4) | 5.2 (0.3) | 7.4 (0.5) | 7.7 (0.5) | 8.8 (0.5) | 8.3 (0.6) | 7.6 (0.3) | 7.4 (0.3) | 6.2 (0.3) |
| | I | 5.3 (0.1) | 4.8 (0.2) | 6.1 (0.3)* | 6.8 (0.4) | 6.9 (0.4)† | 6.2 (0.4)† | 6.7 (0.4)* | 5.2 (0.4)‡ | 5.7 (0.3) |
| Leukocytes (g/L) | P | 4.2 (0.5) | 3.7 (0.5) | 4.4 (0.4) | 7.3 (0.8) | 15.1 (1.1) | 14.9 (1.1) | 10.9 (0.9) | 9.4 (0.9) | 6.7 (0.5) |
| | I | 3.7 (0.3) | 3.4 (0.4) | 3.3 (0.4) | 5.1 (0.6) | 10.5 (1)† | 10 (0.8)‡ | 7.9 (0.6)† | 6.1 (0.6)† | 5.1 (0.6)* |
| Temperature (C) | P | 36.9 (−1) | 36.6 (0.1) | | | | 37.3 (0.2) | 37.8 (0.1) | 37.7 (0.2) | 37.3 (0.1) |
| | I | 37.1 (0.1) | 36.7 (0.1) | | | | 37.1 (0.2) | 37.2 (0.1)‡ | 37.1 (0.1)† | 36.9 (0.2) |
| TNF (pg/mL) | P | | 43.1 (33) | 54.3 (37.4) | 15.6 (6.4) | 13.1 (5.1) | 10.2 (1.9) | | | |
| | I | | 8.6 (1) | 22.2 (13.5) | 43.7 (34.4) | 82.9 (74.8) | 34.2 (26.3) | | | |
| IL-1 (pg/mL) | P | | 4.3 (0.5) | 4.7 (0.7) | 9.4 (2) | 8.5 (2) | 6.8 (1.3) | | | |
| | I | | 5.5 (2.4) | 5.5 (1.9) | 5.2 (1) | 7 (1.8) | 6.8 (1.8) | | | |
| ACTH (ng/mL) | P | 28.8 (7.7) | 30.8 (6.4) | 151.6 (34.5) | 143.1 (29.2) | 103.9 (28.8) | 57.9 (17.9) | 15.8 (2.1) | 20 (3.5) | 21 (4.1) |
| | I | 29.3 (8.7) | 32 (7.9) | 66.6 (20.1)* | 76 (16.8) | 79.3 (18.8) | 37.7 (7.8) | 36.3 (10) | 24.3 (7.6) | 25.3 (7.7) |
| CRP (mg/L) | P | 0 | 0 | 0 | 0 | 0 | 0 | 49.7 (10.1) | 109 (20) | 06.4 (18.4) |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 44.8 (9.3) | 100 (19.7) | 01.6 (20.4) |
| Cortisol (nmol/L) | P | 642 (100) | 679 (81) | 1357 (64) | 1417 (113) | 1763 (152) | 1788 (166) | 918 (123) | 882 (122) | 748 (68) |
| | I | 637 (66) | 781 (80) | 977 (138)* | 1407 (84) | 1506 (132) | 1345 (179) | 634 (141) | 468 (66)† | 622 (65) |

P = placebo; I = ibuprofen.
Values are mean standard error of the mean.
* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$, placebo *vs.* ibuprofen treatment.

Serum CRP levels increased only from the first day to the third day but without differences in both groups. Temperature increased in both groups after surgery, but the increase was significantly lower and its duration was shorter in the ibuprofen group ($p < 0.001$; Table 2). There were no side effects related to ibuprofen administration.

## DISCUSSION

We have found that metabolic, neuroendocrine responses and IL-6 synthesis are significantly diminished in the ibuprofen group. There were no differences in anesthetic requirements, and thus, these effects could be allocated to ibuprofen pretreatment. Cholecystectomy induces neuroendocrine and metabolic changes. As previously described, there is a neuroendocrine response in the first phase with an early rise of ACTH and cortisol from the first hour after skin incision,[11,12] then cytokines release with acute phase protein synthesis occurs in the second phase.[12-14] The plasma cortisol levels remained elevated for 3 days, whereas plasma ACTH levels returned to the basal level at day 1. Naito et al.[11,12] also have observed these biphasic changes. They showed that in the later phase, one of the mechanisms responsible for the hypercortisolemia is the increased responsiveness of the adrenal cortex to ACTH.[12] Nevertheless, cytokines could be responsible for this glucocorticoid synthesis, but there is no direct evidence of the action of cytokines on glucocortical cells. The site of these actions seems to be through a hypothalamus or a direct pituitary secretion.[15] Plasma IL-1 concentrations rise slightly from the first hour with a peak at 2 hours. Plasma IL-6 levels increase only from the fourth hour with a maximum at 6 hours. The CRP plasma level increases from the first day up to day 3. The CRP synthesis is induced mainly by IL-6 secretion 20 or 30 hours after hepatocytes stimulation.[16] This kinetics also is observed by other authors.[11,14] The IL-6 is the main cytokine released by mononuclear cells after elective surgery.[17] The IL-6 response is correlated with the importance of surgical process and duration of the surgery.[13] In the present study dealing with cholecystectomies, the secretion of cytokines remains low, as found also by Cruickshank et al.[13] Cholecystectomy induces a smaller response in IL-6 than vascular or colorectal surgery.[13] The IL-6 also has been reported as a marker of postoperative complications when its serum levels are more than 400 pg/mL.[18] In the ibuprofen group, the low plasma level of IL-6 is unable to affect significantly the CRP synthesis. Perhaps the IL-6 response remains too high to inhibit CRP synthesis. Joris et al.[14] have shown that cholecystectomy performed by laparoscopy decreases the acute phase response with an attenuation of plasma IL-6 levels and CRP synthesis, but this surgical procedure has no effect on the neuroendocrine changes. The hyperglycemic response is mediated initially by neuroendocrine stimulation.[1] In previous studies, indomethacin could not affect the hyperglycemic response, but these different results can be explained by several reasons: anesthesia was a regional anesthesia, the administration started 1 hour only before surgery or even after surgery, and the plasma concentrations of indomethacin were probably insufficient.[10,19,20] The elevation of temperature nearly is abolished in the ibuprofen group. This effect on fever is reported by other workers[10,19-21] who explain the improvement in nitrogen balance observed in their studies by this mechanism. Ibuprofen, like other NSAID, is an effective antipyretic. The mechanism by which NSAID blocks the fever is direct with an inhibition of prostaglandin release and indirect with a decrease in IL-1, TNF-$\alpha$, and IL-6 secretion (endogenous pyrogens).[22] After surgery, an increase of leukocytes occurs at the second hour and is in relation with the rise in corticosteroids, catecholamines, and cytokines plasma levels.[23] In agreement with previous studies,[10,19] this increase is attenuated as well as neuroendocrine and humoral responses in the ibuprofen group.

In summary, we observed two successive responses after a surgical stress: a neuroendocrine one in a first phase, then a cytokine release. Ibuprofen treatment in the perioperative course is able to attenuate but not suppress these two responses. Therefore, ibuprofen may be useful in decreasing this stress response in severely surgical patients.

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