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Effect of surgical stress on endogenous morphine and cytokine levels in the plasma after laparoscopoic or open cholecystectomy

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Abstract
Background: Endogenous morphine in the brain leads to various biological responses after surgery. The aim of this study was to determine whether morphine levels in the plasma would be enhanced by open laparotomy rather than by laparoscopic procedures.
Methods: We compared 19 patients who underwent laparoscopic cholecystectomy with five patients who underwent resection of the gallbladder by open laparotomy. Morphine levels in the plasma were measured by an electrochemical detection system.
Results: Postoperative endogenous morphine levels were higher with open laparotomy than with the laparoscopic technique (three h after surgery: open, 200 ± 52.6 fmol/ml vs laparoscopy, 17.6 ± 3.7, $p < 0.01$). This morphine elevation accounted for higher levels of cytokine, greater pain scores, and longer duration of fasting in open laparotomized patients than in laparoscopic cholecystectomy patients. Stress hormone levels in the plasma were also higher with open laparotomy than with laparoscopy.
Conclusion: Morphine synthesis was enhanced by open laparotomy, resulting in greater biological response postoperatively than that seen with laparoscopic cholecystectomy.

Key words: Cytokine — Surgical stress — Endogenous morphine — Laparoscopic cholecystectomy — Gallbladder

A significant advantage for laparoscopic cholecystectomy (LC) is a shorter hospital stay than that required after conventional open cholecystectomy [4]. The main reasons for the earlier recovery with LC are a less acute phase response and less pain than is experienced with open surgery. Indeed, postoperative interleukin-6 levels in the plasma and pain scores have been reported to be lower with LC than with open cholecystectomy [2, 5].

Sympathetic nerve stimulation by pain is a stress that creates acute phase responses after surgery, which may be harmful to surgical patients [14]. In fact, fentanyl analgesia can block elevation of stress hormone levels compared with pentobarbital anesthesia in traumatized rats [18] and with conventional anesthesia in patients with esophagectomy [19]. Furthermore, Anand et al. [1] reported that sufentanil analgesia reduced surgical complications after cardiac surgery in neonates. However, the mechanism by which pain initiates the acute phase response in the central nervous system is still unclear.

We have previously shown that intracerebroventricular (ICV) injection of TNF-α causes an increase in glucose production [15]. This altered glucose metabolism was attributed to an increase in endogenous morphine levels in the brain associated with an increase in ACTH, corticosterone, and catecholamine levels in the plasma [15]. Similar to the ICV injection of TNF-α, Molina et al. [10] reported that ICV injection of morphine caused an increase in the stress hormone levels in the plasma, resulting in an elevation of glucose production. Taken together, these studies suggest that morphine is involved in changes in the hormonal milieu initiated by cytokine as a second messenger. In addition, Molina et al. [11] reported that morphine levels in the brain but not in the plasma were increased in fasting rats. These results prompted us to determine whether endogenous morphine levels in the plasma after cholecystectomy were lower with laparoscopic procedures than with open laparotomy, because pain scores are higher and the fasting period is longer [4] with the open procedure than with LC.

Materials and methods

The subjects were 24 patients with cholecystolithiasis who were scheduled for routine laparoscopic cholesystectomy, because none of these patients preferred the open technique to LC. There was no evidence of cholecystitis based on leukocyte count and C-reactive protein levels in the plasma at their admission to our hospital (data not shown). The ethics committee of

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Table 1. Patient profiles and pain scores[a]

	Open laparotomy	Laparoscopy
n (male/female)	5 (3/2)	19 (10.9)
Body weight (kg)	60.0 ± 4.3	58.3 ± 10.5
Total protein levels (g/dl)	7.5 ± 0.1	7.4 ± 0.2
Albumin levels (g/dl)	3.7 ± 0.1	3.8 ± 0.2
Duration of operation (min)	126 ± 16	115 ± 27
Time to resume oral intake after surgery (h)	36.0 ± 9.6	15.4 ± 4.8[a]
Pain score (postoperative day 1)	3.8 ± 0.4	3.1 ± 0.2[a]
Pain score (postoperative day 2)	3.0 ± 0.2	1.8 ± 0.2[a]

Data expressed as mean ± SEM
[a] $p < 0.05$ vs the open laparotomy group

the Kurume University Hospital approved the experimental protocol to collect blood from the patients over sequential periods, and written informed consent was obtained from all the patients.

The laparoscopic approach with pneumoperitoneum was attempted, but five of 24 patients were converted to open laparotomy due to adhesive changes around the gallbladder caused by previous inflammation. In two patients, previous adhesions around the gallbladder and great omentum forced us to discontinue laparoscopic procedures. The three other patients had accidental bleeding during dissection of the adhesive tissues of the gallbladder. Thus, a total of 19 patients were enrolled in the laparoscopic group. The patient profiles on age, plasma total protein levels, plasma albumin levels, and body weight were identical for the two groups, and the duration of surgical procedures was equivalent for the two groups (Table 1).

The same anesthetic technique was used for all patients. After overnight fasting, the patients were premedicated with a 25-mg muscular injection of hydroxyzine hydrochloride (Pfizer Pharmaceutical Co., Fukuoka, Japan) and 0.5 mg of Atropine sulfate (Tanabe Pharmaceutical Co., Tokyo, Japan) 2 h before surgery. The epidural catheter was placed by tapping the thoracic vertebrae between 9 and 10, and 0.5% of marcain was injected (1 mg/kg; Fujisawa Pharmaceutical Co., Tokyo, Japan) when necessary. There were no differences between the two groups in the amount of bupivacaine used during the surgical procedures (open, 0.26 ± 0.12 mg vs LC; 0.22 ± 0.06, n.s.). Anesthesia was induced by intravenous injection of isozol (Yoshitomi Pharmaceutical Co., Osaka, Japan) and maintained using end-tidal isoflurane (Dainippon Pharmaceutical Co., Tokyo, Japan) and 50% nitrous oxide in oxygen. No differences were found between the two groups in the amount of gas anesthesia inhaled (nitrous oxide: open, 468 ± 196 ml vs LC; 370 ± 122, n.s.; isoflurane: open, 76 ± 36 ml vs LC; 68 ± 24 ml, n.s.). Tracheal intubation was done by an i.v. injection of vecronium bromide (0.8 mg/kg), and neuromuscular block was maintained with additional injections of vecronium bromide (0.2 mg/kg; Sankyo Pharmaceutical Co., Tokyo, Japan) when necessary. During the surgical procedures, Ringer's lactate solution was infused through peripheral venous catheters at a constant rate of 10 ml/kg/h.

After the surgery, fluid infusion consisted of Ringer's lactate (20 ml/kg/day) and maintenance fluid plus 10% glucose (Physiosol-3; Yoshitomi Pharmaceutical Corp., Osaka, Japan; 20 ml/kg/day). Pain management after surgery was done by a continuous injection of 0.5% Marcain (0.08 mg/h) and a muscular injection of pentazocin (25 mg, Sankyo Pharmaceutical Co.) when necessary. More frequent injections of pentazocin were requested by the open laparotomy group than by the LC group during the first 24 h after the surgery (open; 45.0 ± 14.5 mg vs LC; 4.5 ± 3.0 mg, $p < 0.01$). Neither morphine nor a related opiate was administered for pain control.

The first postoperative meal was given after the bowel flatus, and the period of taking the first meal was recorded. Postoperative pain scores were evaluated through a questionnaire using a scale devised by Whaley and Wong [17] at 5 PM on postoperative days 1 and 2.

Blood samples were collected from an antecubital vein in intervals as follows: at 8:00 AM prior to surgery, immediately after the surgery, 3 h after the surgery, and 24 h after the surgery.

Plasma concentrations of C-reactive protein (CRP), ACTH, cortisol, morphine, IL-6, IL-1 receptor antagonist (IL-1ra), and soluble TNF receptor-I (sTNFr-I) were measured. CRP was measured by enzyme immunoassay (Special Reference Laboratories Inc., Tokyo, Japan). The plasma concentration of ACTH was determined by radioimmunoassay (Alegro ACTH kit; Nippon Medics Inc., Tokyo, Japan), and cortisol was measured by a gamma coat cortisol kit (Baxter Travenol, Inc., Tokyo, Japan), as described previously [20]. IL-6, IL-1ra, and sTNFr-I concentrations were measured by ELISA, using kits for the measurement of human IL-6, IL-1ra, and sTNFr-I, respectively (R & D Systems, Inc., Minneapolis, MN, USA).

Morphine levels in the plasma were measured according to the modification of the two previously published methods [8, 12]. Briefly, 20 μl of 1 N NaOH and 125 μl of 1 N $NaHCO_3$ were added to 1 ml of plasma. Then, 1-butanol and benzene (15:85) was added to the aliquot and vortexed for 1 min. The organic layer was collected, dried, and reconstituted with the mobile phase buffer (150 μl) and filtered (LCR13-LH; Millipore Japan Inc., Osaka, Japan). Eighty microliters of this aliquot was injected into a coulometric analytical system equipped with an L-7100 pump (Hitachi Inc., Tokyo, Japan), an MCM column (ODS 5 μm, 4.6 ϕx 150 mm; MC Medical Inc., Tokyo, Japan), a recorder (Chromelon Chromatography Data Systems; Gynkotek HPLC Inc., Munich, Germany), and an electrochemical detector (Coulochem II 5200 A; ESA Inc., Bedford, MA, USA) with a 5022 guard cell and a 5011 analytical cell (ESA Inc.).

Statistical analysis

Data are presented as mean ± SE. Statistical analysis of the data was done by the Student's *t*-test and the Wilcoxon test for parametric and nonparametric analysis, respectively, using Statview 412 applied by Abacus Concepts (Berkeley, CA, USA). Differences between means were considered to be significant at $p < 0.05$.

Results

Pain scores were significantly lower in LC patients than in open patients on postoperative days 1 and 2 (Table 1). Resumption of oral intake after the surgery was earlier with LC patients than with open laparotomy patients (open; 36.0 ± 9.6 h vs LC; 15.4 ± 4.8, $p < 0.05$) (Table 1).

Adrenocorticotropic hormone (ACTH) levels in the plasma were higher with open laparotomy patients than with LC patients both immediately after the cholecystectomy (open; 643.0 ± 241.0 pg/ml vs LC; 156.0 ± 32.5, $p < 0.05$) (Table 2) and 3 h after surgery (open; 214.0 ± 61.1 pg/ml vs 36.0 ± 17.5, $p < 0.05$). In association with ACTH, cortisol levels in the plasma were greater in open laparotomy than in LC patients at 3 h after surgery (open; 683.0 ± 381.2 μg/dl vs LC; 171.2 ± 42.5, $p < 0.05$) (Table 2). Both ACTH and cortisol returned to the normal range at 24 h after surgery in both groups.

Open laparotomy caused a greater increase in IL-6 levels in the plasma than LC at the end of surgery (open; 22.2 ± 3.6 pg/ml vs LC; 13.8 ± 1.7, $p < 0.05$) (Table 2) and 3 h after surgery (open; 79.9 ± 22.1 pg/ml vs LC; 26.2 ± 2.7, $p < 0.05$). Similarly, IL-1ra and sTNFr-I levels in the plasma were significantly higher for open laparotomy than for LC at the end of surgery, 3 h after surgery, and 24 h after surgery. In association with an increase in IL-6 levels, CRP levels in the plasma were significantly higher in the open group than the LC group at 24 h after surgery (open; 90.5 ± 21.1 μg/ml vs LC; 22.4 ± 16.4, $p < 0.05$) (Table 2). Furthermore, morphine levels in the plasma were higher in the open laparotomy patients than in the LC patients immediately after surgery (open; 96.3 ± 28.7 fmol/ml vs LC; 29.4 ± 7.0, $p < 0.05$) (Table 2), 3 h after surgery (open; 200.5 ± 52.6 fmol/ml vs LC, 17.6 ± 3.7, $p < 0.01$), and 24 h after surgery (open; 114.7 ± 26.0 fmol/ml vs LC; 22.7 ± 10.5, $p < 0.01$).

Table 2. The chronologic changes in plasma morphine, stress hormone, cytokine, and C-reactive protein levels in patients with open laparotomy or laparoscopic cholecystectomy

	Group	Preoperation	At end of operation	3 h after operation	24 h after operation
ACTH (pg/ml)	Open	6.7 ± 1.5	643.5 ± 241.0	214.0 ± 61.1	7.0 ± 1.2
	Laparoscopy	8.5 ± 1.2	156.0 ± 32.5[a]	36.0 ± 17.5[a]	8.2 ± 1.0
Cortisol (μg/dl)	Open	13.8 ± 1.8	26.2 ± 1.4	30.0 ± 2.4	21.7 ± 2.7
	Laparoscopy	12.3 ± 1.0	20.7 ± 3.2	21.3 ± 1.2[a]	16.7 ± 5.8
sTNFR-I (pg/ml)	Open	1,355.1 ± 75.7	2,516.6 ± 449.7	3,751.0 ± 343.0	2,878.4 ± 240.5
	Laparoscopy	1,249.0 ± 130.6	1,602.7 ± 174.1[a]	1,923.1 ± 196.1[a]	2,045.2 ± 232.3[a]
IL-1ra (pg/ml)	Open	318.4 ± 133.6	659.0 ± 340.0	2,381.2 ± 191.8	963.7 ± 367.1
	Laparoscopy	223.1 ± 21.4	296.7 ± 52.7[a]	487.6 ± 85.4[b]	255.1 ± 38.1[a]
IL-6 (pg/ml)	Open	9.6 ± 1.1	22.2 ± 3.6	79.9 ± 22.1	20.1 ± 1.9
	Lapaproscopy	8.0 ± 0.6	13.8 ± 1.7[a]	26.2 ± 2.7[a]	17.7 ± 3.5
CRP (μg/ml)	Open	3.0 ± 0.7	4.1 ± 2.4	11.4 ± 8.2	90.5 ± 21.1
	Lapaproscopy	3.1 ± 1.1	3.4 ± 0.9	9.8 ± 7.5	22.4 ± 16.4[a]
Morphine (fmol/ml)	Open	6.5 ± 1.7	96.3 ± 28.7	200.5 ± 52.6	114.7 ± 26.0
	Laparoscopy	8.2 ± 3.7	29.4 ± 7.0[a]	17.6 ± 3.7[b]	22.7 ± 10.5[b]

Data expressed as mean ± SEM
[a] $p < 0.05$ vs the open laparotomy group
[b] $p < 0.01$ vs the open laparotomy group

Discussion

In the present study, IL-6 levels were lower following LC than with open laparotomy. This finding was associated with lower levels of CRP in the LC group, suggesting that the conversion from LC to open and the open cholecystectomy enhanced the acute phase response rather than the LC procedure alone. Furthermore, IL-1ra concentrations increased following open cholecystectomy but not with the LC procedure. Similar to our data on IL-1ra, Pruitt et al. [13] reported that IL-1ra levels in plasma were greater in patients who underwent abdominal aneurysm repair and surgical resection for inflammatory bowel disease than in patients who had laparoscopic cholecystectomy. In addition to the increased levels of IL-1ra in the plasma, sTNFr-I was also higher with the open procedure than with the LC in the present study. We previously reported that transthoracoabdominal esophagectomy caused a greater increase in sTNFr-I levels in the plasma and that it was associated with much greater excretion of sTNFr-I in the urine than is seen following gastrectomy [19]. Taken together, these data suggested that IL-1ra and sTNFr-I levels in the plasma reflect the severity of surgical trauma, as does IL-6.

Karayiannakis et al. [5] reported that peak levels of ACTH and cortisol were equivalent for LC, and the open procedure. Contrary to their report, peaks of ACTH and cortisol levels in the present study were higher with the open than with the LC. This is probably because of the attempt at laparoscopic cholecystectomy that preceded open laparotomy in the present open group. Hence, the conversion procedure from LC to open laparotomy may enhance acute phase response more than open laparotomy alone.

The most important finding of this study was an elevation of morphine levels in the plasma in the open group but not in the LC group. Endogenous morphine is excreted in the discretion area of brain and pancreas [6], while glucuronidation from morphine to morphine-3-glucuronide and morphine-6-glucuronide occurs in the liver and kidney [9]. Morphine in the brain is probably produced by stimulation from cytokines in the brain, because cytokines are not only synthesized in the brain [7] but can also reach the brain through the blood/brain barrier [3]. Furthermore, TNF-α injection into the intracerebroventricular space causes a significant increase in morphine levels in the brain [15]. Molina et al. [11] demonstrated that fasting causes an increase in morphine levels in the brain, but they did not observe a significant increase in morphine levels in the plasma.

In the present study, however, morphine levels in the plasma were increased with the open procedure but not the LC. One reason for this increase in morphine in the plasma was probably a greater cytokine production in the brain and/or the entry of more cytokines into the brain from the systemic circulation [3]. Another reason is that higher pain scores were recorded for patients undergoing open rather than LC, even though the open group patients had more pentazocin than LC group. In addition, oral intake was begun within 24 h after LC, but the patients in the open group were still fasting on i.v. therapy 24 h after surgery. Thus, these three combined factors probably contributed to higher levels of morphine in the plasma for the patients in the open group at 24 h after the surgery.

Thorell et al. [16] have reported that insulin sensitivity is impaired by open surgery but not by LC. Morphine injection into the intracerebroventricular space enhanced the excretion of such anti-insulin hormones as epinephrine and cortisol, resulting in an increase in glucose production. Thus, an increase in morphine levels in the plasma with open cholecystectomy may explain how insulin resistance occurs with open surgery rather than LC.

In summary, morphine levels in the plasma were elevated by open laparotomy because of higher cytokine production, higher pain scores with open surgery than with LC, and fasting in the open group, resulting in higher ACTH and cortisol levels in the plasma of the open group.

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