ORIGINAL ARTICLE

Decreased Peritoneal Expression of Active Transforming Growth Factor β1 During Laparoscopic Cholecystectomy With Heated Carbon Dioxide

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Background: Laparoscopic surgery involves the establishment of a pneumoperitoneum, mostly using carbon dioxide. Cooling of the peritoneum, due to insufflation, may traumatize the peritoneum and disturb local biological processes. The current study was performed to assess the effect of the temperature of carbon dioxide on peritoneal transforming growth factor β1 (TGF-β1) expression.

Design: Patients were randomized into 2 groups. In one group, a pneumoperitoneum was created with carbon dioxide at room temperature; in the other, with carbon dioxide at body temperature. Peritoneal biopsy specimens were taken at the start and end of surgery.

Setting: Community hospital.

Patients: Thirty patients scheduled for laparoscopic cholecystectomy.

Main Outcome Measures: Tissue concentrations of total and active TGF-β1 were measured using enzyme-linked immunosorbent assays.

Results: At the start of surgery, there were no significant differences between groups in the total and active fractions of TGF-β1. At the end of the procedure, the peritoneal active TGF-β1 concentrations were significantly lower ($P = .03$) in patients receiving carbon dioxide at body temperature. In contrast, the concentrations of total TGF-β1 did not differ between groups. A slight, nonsignificant increase in total and active TGF-β1 levels was observed in patients receiving unheated carbon dioxide. The ratio of active to total TGF-β1 did not change during procedures, and there were no differences between groups.

Conclusions: Heating of carbon dioxide, used for insufflation, to body temperature decreases the expression of active TGF-β1 in the peritoneum. Considering the broad biological effects of TGF-β1, including the regulation of peritoneal healing and oncological processes, this observation might have clinical repercussions.

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LAPAROSCOPIC SURGERY INcludes the establishment of a pneumoperitoneum to create adequate space in the abdominal cavity. Carbon dioxide is the most commonly used gas for this purpose. The introduction of gases into the peritoneal cavity has been shown to diminish body and intraperitoneal temperatures, possibly increasing postoperative pain.[1,2] During laparoscopy, the intra-abdominal temperature has been shown to decrease to temperatures as low as 27.7°C.[3] On the tissue level, insufflation with carbon dioxide induces peritoneal acidosis and the release of various cytokines and causes morphological changes.[4,5] As with open surgery, retraction and bulging of mesothelial cells and exposure of the basal lamina were found during laparoscopic procedures.[6,7]

Gases used for creation of a pneumoperitoneum are typically inflated at room temperature. Heating of the insufflated carbon dioxide to body temperature has been introduced in an attempt to minimize the decrease in body temperature. Clinical studies, unfortunately, have failed to demonstrate an effect of heating on core temperature or postoperative pain sensation.[1,2] Few studies have focused on the effects of the temperature of insufflation gases on peritoneal biological and local wound-healing processes. Hypothermia might affect peritoneal macrophage functions; patients undergoing a laparoscopic procedure with pneumoperitoneum at room temperature were shown to have higher levels of tumor necrosis factor, interleukin 1β, and interleukin 6 in their peritoneal fluid compared with those who underwent the procedure with pneumo-

peritoneum at body temperature.[8] In a previous study,[9] we observed that heating of carbon dioxide decreases the expression of plasminogen activator inhibitor 1 (PAI-1) in peritoneal tissue. Plasminogen activator inhibitor 1 is an important protein in peritoneal repair processes. In another experimental model, we found that the choice of dissection device and the light intensity used in laparoscopic surgery may affect the peritoneal transforming growth factor β1 (TGF-β1) concentrations.[10]

Transforming growth factor β1 is a naturally occurring growth factor that is involved in numerous biological processes. It is released as an inactive peptide that can be activated in several ways, including proteolytic cleavage and pH and temperature changes.[11,12] Transforming growth factor β1 regulates chemotaxis, mitogenesis, and angiogenesis and thereby is involved in dissemination processes.[13] Secretion of TGF-β and activation of TGF-β signaling pathways have been associated with increased aggressiveness of several types of tumors, including those of the pancreas, colon, stomach, lung, endometrium, prostate, breast, brain, and bone.[14,15] Literature on the relation between laparoscopic surgery and peritoneal dissemination and port-site metastasis is still controversial. Besides its involvement in oncological processes, TGF-β1 appears to be a major regulator of peritoneal wound-healing processes and adhesion formation, mainly by increasing the peritoneal production of PAI-1, which is the main inhibitor of fibrinolysis and a key factor in adhesiogenesis.[16] Transforming growth factor β is a major stimulator of extracellular matrix deposition by inducing the production of collagen, fibronectin, and integrins.[17,18] Increased TGF-β concentrations have been observed in peritoneal fluid of patients with adhesions and in adhesion tissue itself.[19] Moreover, postoperative intraperitoneal administration of TGF-β increased adhesion formation in mice, whereas its inactivation reduced the occurrence of adhesions.[20]

The present study was conducted to evaluate the hypothesis that the temperature of carbon dioxide affects peritoneal TGF-β1 expression. Considering the involvement of TGF-β1 in oncological and peritoneal repair processes, this may have clinical consequences.

METHODS

Thirty consecutive patients scheduled for elective laparoscopic cholecystectomy for symptomatic gallbladder stone disease were randomized into 2 groups. In the first group (n=15), the pneumoperitoneum was created with carbon dioxide at room temperature. In the second group (n=15), the carbon dioxide was heated to a temperature of 37°C using an insufflator (Thermoflator; Karl Storz GmbH & Co, Tuttlingen, Germany). Institutional review board approval was obtained, and written informed consent was given before enrollment.

OPERATIVE PROCEDURE

In all patients, a uniform technique of videolaparoscopic cholecystectomy was applied, including the use of 4 trocar ports in the American technique and a 0° optic scope. The gallbladder hilum and the Calot triangle were dissected, and metal clips were used for the cystic duct and artery. Two biopsy samples of the parietal peritoneum were taken with forceps and scissors without electrocautery. The first sample was taken immediately after carbon dioxide insufflation and the second after 45 minutes of surgery. When the procedure was finished before 45 minutes had passed, the second biopsy sample was taken just before deflation.

TISSUE SAMPLING AND PROCESSING

The peritoneum was carefully dissected, with care taken not to include the underlying muscle. The tissue specimens were snap frozen in liquid nitrogen and stored at −70°C until further processing. Before homogenizing, a sample of thawing peritoneal tissue was cut off before being blotted and weighed. Each biopsy specimen was rinsed with phosphate-buffered saline with 0.5M sodium chloride (pH, 7.4), cut into small pieces, and placed into ice-cold homogenization buffer (phosphate-buffered saline with 0.01% Triton X-100 [Sigma-Aldrich Corp, St Louis, Missouri]) in a final concentration of 40 mg of tissue per milliliter of buffer. The tissue was homogenized for 60 seconds on ice using a homogenizer (Ultra Thurrax IKA T-25; Janke & Kunkel, Staufen, Germany) and centrifuged at 10 000*g* for 4 minutes at 4°C, and the supernatant was stored at −70°C until further analysis. Tissue processing and assays were performed in batches.

BIOCHEMICAL ASSAYS

Concentrations of active and total TGF-β1 were measured using commercially available enzyme-linked immunosorbent assays (R&D Systems, Abingdon, England). The active and total forms of TGF-β1 were measured because TGF-β is inactive when produced, and it has to be activated to become an active cytokine. The active and total levels of TGF-β1 were measured in separate steps. First, the active fraction of TGF-β1 was assayed directly in the enzyme-linked immunosorbent assay plate; second, the total amount of TGF-β1 was assayed by acidifying the samples with 1M hydrogen chloride to a pH of 3, followed by a 15-minute incubation at 22°C, resulting in an activation of TGF-β1. To neutralize samples, 1M sodium hydroxide was supplemented before addition to the assay plate, according to the instructions from the manufacturer. The lower detection limit for the TGF-β1 assay was 32 pg/mL. The intra-assay variation was 3.3% to 4.5%, and the interassay variation was 7.6% to 19.1%. In addition, results were normalized to total protein content using a commercial protein assay (Bio-Rad Laboratories, Hercules, California).

STATISTICAL ANALYSIS

Values are presented as mean (SD). Analysis of differences between groups was performed using the Friedman test and the Mann-Whitney test. All tests were 2-tailed. $P < .05$ was considered significant.

RESULTS

CLINICAL RESULTS

There were no differences in sex (4 [27%] male and 11 [73%] female patients in each group) or age (52.0 [15.7] years) between groups. The overall incidence of previous laparotomies was 23% (7 patients), with no difference between groups. Moreover, there was no difference in the occurrence of intraperitoneal adhesions

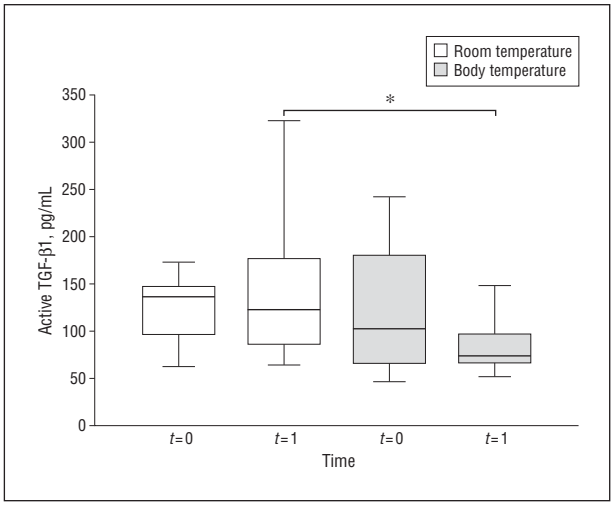

Figure 1. Active transforming growth factor β1 (TGF-β1) concentrations in peritoneal samples measured immediately after initiation of the procedure (t=0) and after 45 minutes (t=1) in patients undergoing pneumoperitoneum with carbon dioxide at room and body temperatures. Results are illustrated as median (horizontal lines), interquartile range (boxes), and 10th and 90th percentiles (error bars). *P=.03.

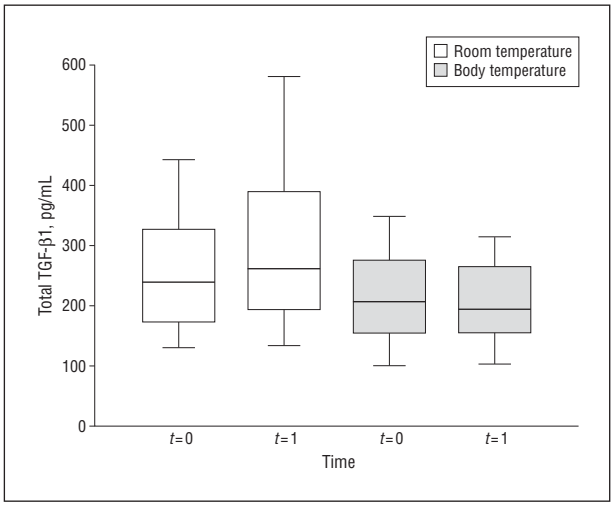

Figure 2. Total transforming growth factor β1 (TGF-β1) concentrations in peritoneal samples measured immediately after initiation of the procedure (t=0) and after 45 minutes (t=1) in patients undergoing pneumoperitoneum with carbon dioxide at room and body temperatures. Results are illustrated as median (horizontal lines), interquartile range (boxes), and 10th and 90th percentiles (error bars).

between groups. The timing of the second biopsy was equal in both groups (40.6 [9.7] minutes).

BIOCHEMICAL RESULTS

Active TGF-β1 Concentrations

Immediately after initiation of the procedure with carbon dioxide at room temperature, the peritoneal concentrations of active TGF-β1 in samples taken were 123.3 (41.2) pg/mL (**Figure 1**). During the procedure, there was a nonsignificant 20% increase to 147.2 (87.5) pg/mL. When heated carbon dioxide was used, the initial peritoneal concentration of active TGF-β1 was within the same range as that found in samples from patients undergoing pneumoperitoneum with unheated carbon dioxide. During the procedure, the levels of active TGF-β1 decreased to 85.4 (33.0) pg/mL, which is significantly lower compared with samples from patients undergoing pneumoperitoneum with carbon dioxide at room temperature (P=.03).

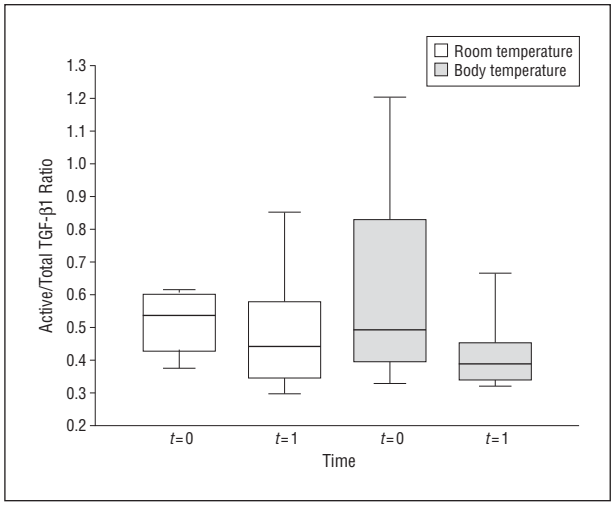

Figure 3. Ratio of active to total transforming growth factor β1 (TGF-β1) in peritoneal samples measured immediately after initiation of the procedure (t=0) and after 45 minutes (t=1) in patients undergoing pneumoperitoneum with carbon dioxide at room and body temperatures. Results are illustrated as median (horizontal lines), interquartile range (boxes), and 10th and 90th percentiles (error bars).

Total TGF-β1 Concentrations

When carbon dioxide at room temperature was used, the initial peritoneal concentrations of total TGF-β1 were 258.2 (126.4) pg/mL (**Figure 2**). During the procedure, a 20% increase was observed to 310.2 (178.9) pg/mL (P=ns). When using heated carbon dioxide, the initial levels of total TGF-β1 were similar to those found in samples from patients undergoing pneumoperitoneum with unheated carbon dioxide. During the laparoscopic cholecystectomy, the concentrations remained at the same level.

Active to Total TGF-β1 Ratio

In both surgical groups, there was no significant difference between the ratio of active to total TGF-β1 at the start of surgery compared with the ratio at the end of the procedure (**Figure 3**). There were also no differences in the ratios between different treatment groups at both times.

COMMENT

In the present study we have demonstrated that the temperature of the carbon dioxide used for insufflation of the peritoneal cavity may affect peritoneal biological processes by altering the peritoneal concentrations of active TGF-β1. Heating the carbon dioxide causes a lower active TGF-β1 expression compared with unheated carbon dioxide.

The observation that differences in the insufflated gas temperature may change local biological processes in the peritoneal organ has previously been published by our group.[9] We found that insufflation with carbon dioxide at room temperature caused significantly higher concentrations of PAI-1 in peritoneal specimens. Plasminogen activator inhibitor 1 is an antifibrinolytic protein involved in peritoneal wound healing and adhesion formation.[16] Various studies have shown that TGF-β1 is an important stimulator of PAI-1 production by the peritoneum. The observed higher active TGF-β1 concentrations in specimens from patients receiving carbon dioxide at room temperature may partly explain the higher PAI-1 levels caused by unheated carbon dioxide. The observations that peritoneal active TGF-β1 and PAI-1 levels are both lower in patients receiving heated carbon dioxide might indicate that cooling of the peritoneum traumatizes the peritoneal layer, leading to decreased fibrinolytic activity. Whether the subsequent reduced hypofibrinolysis in patients receiving heated carbon dioxide will also lead to a further reduction in the formation of postsurgical adhesions remains to be investigated. Although the technique of heating is easy to implement in daily surgical practice and investments are low, it seems to be premature to advocate the use of heated carbon dioxide during all laparoscopic procedures.

In contrast to the observation that heating of carbon dioxide caused significantly lower local active TGF-β1 levels in the peritoneum, we did not find any effect on the total concentrations of TGF-β1. The active fraction represents the equilibrium between the total concentrations of the cytokine and its inhibitors. In the active and total fractions of TGF-β1, a nonsignificant 20% increase was observed in the group receiving carbon dioxide at room temperature. The lack of significance may well be caused by the relatively small sample sizes. Nevertheless, these data suggest that increased production of TGF-β1 in the unheated group and an enhanced production of its inhibitors in the heated group may be responsible for the lower concentrations of active TGF-β1 in the latter group. On the other hand, the fraction of activated peptide level could have been activated owing to the changes in temperature because temperature has previously been shown to activate TGF-β1.[11,12] This might be an explanation for detecting changes in the active fraction but not the total amount of TGF-β1. This hypothesis may be supported by our observations on the ratio of active to total TGF-β1 that showed a 30%—but not statistically significant—decrease in the heated group only. Additional physiological experiments are indicated to elucidate the involved mechanisms. Moreover, experimental studies comparing the peritoneal response with laparoscopic and open surgical procedures may further elucidate the local peritoneal response to various aspects of laparoscopic surgery.

The minor increase in peritoneal TGF-β1 levels in patients receiving carbon dioxide at room temperature is in accord with our previous observation that active TGF-β1 levels significantly increase during a laparoscopic right hemicolectomy.[21] In that study, these levels were significantly increased already after 26 minutes of surgery. In contrast, during a laparoscopic gastric bypass, the TGF-β1 levels did not increase throughout a procedure lasting more than 2 hours. Surgical trauma to the peritoneal surface, rather than a prolonged pneumoperitoneum, seemed to affect the local concentrations of active TGF-β1 in that study. Nevertheless, in the present study we found that the group receiving heated carbon dioxide had a decrease in active TGF-β1 expression of 30%, which indicated that cooling may also be important. The effect of the intra-abdominal temperature was not a variable in the other studies mentioned and therefore remains to be further investigated.

The decreased TGF-β1 expression using heated carbon dioxide might have some important clinical consequences. Transforming growth factor β is involved in a range of biological processes, including chemotaxis, mitogenesis, and angiogenesis, all important in oncological processes.[22-24] An increasing percentage of laparoscopic procedures is performed for oncological and pathological examinations, including colonic resection, nephrectomy, and hysterectomy. Various experiments have shown that laparoscopy is correlated with decreased intraperitoneal tumor growth compared with open surgery, whereas insufflation of carbon dioxide may, in turn, promote peritoneal tumor growth compared with gasless laparoscopy.[25,26] However, Lécuru et al[27] did not find any deleterious effect of carbon dioxide insufflation on ovarian tumor growth when compared with gasless laparoscopy or midline laparotomy in a rat model. Only few clinical data exist to allow assessment about whether these experimental concerns may be translated into clinical problems. Velanovich[28] found no effect of laparoscopy on the occurrence of trocar-site disease or peritoneal disease progression of pancreatic cancer. The possible role of the temperature of insufflation gases on these oncological processes has, to our knowledge, never been studied. Our results warrant further studies focusing on the possible clinical repercussions of an altered active TGF-β1 concentration in the peritoneal tissue. Moreover, its relation to the quality of laparoscopic manipulation technique should be studied because that may be the most important clinical factor for oncological outcome in terms of port-site metastases and peritoneal tumor progression.

In conclusion, we have demonstrated that heating of carbon dioxide used for insufflation to body temperature decreases the expression of active TGF-β1 in the peritoneum. Considering the broad biological effects of TGF-β1, this observation might have clinical repercussions.

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