Journal of Surgical Research 125, 73–77 (2005)
doi:10.1016/j.jss.2004.11.029

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Electron-Microscopic Alterations of the Peritoneum after Both Cold and Heated Carbon Dioxide Pneumoperitoneum

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Submitted for publication August 17, 2004

Background. Carbon-dioxide (CO_2) is used universally as an insufflation agent to create a laparoscopic pneumoperitoneum. In this study, we aimed to examine the electron and light microscopic alterations of the peritoneum after both cold-dry and heated-humidified CO_2 pneumoperitoneum.

Materials and methods. Thirty male Sprague-Dawley rats were used in this study. The rats were separated into three groups each comprising 10 rats. Group-I: (Control group): Gas insufflation was not applied to these animals. Group-II: These animals received standard cold-dry (21°C, 2% relative humidity) CO_2. Group-III: These animals received heated-humidified (40°C, 98% relative humidity) CO_2. In groups II and III, peritoneal gas was emptied 2 h after pneumoperitoneum application. All rats were killed after 12 h. Peritoneal samples were examined both by scanning electron and light microscopy by two different pathologists who were not aware of the groups.

Results. According to light microscopic examination; in group II and III, cellular response (increased lymphocyte) was significantly higher than the control group ($P < 0.01$). Similarly, in group II cellular response was significantly higher than group III. ($P < 0.01$). There was no difference in increased capillarity among all groups. ($P > 0.05$). According to scanning electron microscopic examination, in group I, normal peritoneum was covered by a sheet of flat mesothelial cells densely covered with microvilli. No intercellulary clefts and no free basal lamina were detected. In group II, drastic alterations of the surface layer were seen. The mesothelial cells had extreme desquamation, and the basal membrane was clearly visible. In group III, the mesothelial cells had bulged up to the surface layer and retracted. Intercellulary clefts become visible, but the basal lamina was not seen.

Conclusions. Electron and light microscopic examination revealed that heated-humidified CO_2 results in less peritoneal alteration than cold-dry CO_2. Accordingly, we believe that heated-humidified CO_2 is more suitable for pneumoperitoneum application in laparoscopic surgery especially in selected cases. © 2005 Elsevier Inc. All rights reserved.

Key Words: laparoscopy; pneumoperitoneum; heated-humidified CO_2; electron-microscopy.

INTRODUCTION

Carbon-dioxide (CO_2) is used universally as an insufflation agent to create a laparoscopic pneumopertoneum [1]. Examinations of pneumoperitoneum pathophysiology have shown that the cold-dry CO_2 used during laparoscopic surgery may alter the peritoneal surface and also irritate the peritoneum [1–3]. There are few studies about the alterations of the peritoneum after standard cold-dry CO_2 pneumoperitoneum [1, 2], as far as we know, there are no studies about the effect of heated-humidified CO_2 pneumoperitoneum on the peritoneum dealing with light and electron microscopic examination.

The creation of a capnoperitoneum has been shown to be associated with structural changes in the peritoneal mesothelial surface layer that are visible with scanning electron microscopy [3, 4]. These alterations may reflect changes secondary to CO_2, or they may because of desiccation of the peritoneal surface by cold insufflation gas [1]. The aim of this study was to examine the characteristic alterations of the peritoneum after both cold-dry CO_2 and heated-humidified CO_2 pneumoperitoneum.

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TABLE 1

Lymphocyte and Capillarity Count of the Groups (mean and SD)

Parameters	Group I $n = 10$ mean ± SD	Group II $n = 10$ mean ± SD	Group III $n = 10$ mean ± SD	P value
Lymphocyte count	7.8 ± 1.2	20.7 ± 4.0[a]	15.1 ± 3.2[b]	$P < 0.05$
Capillary count	2.3 ± 0.8	2.3 ± 0.7	2.4 ± 0.9	$P > 0.05$

Note. No significant change was observed in capillarity in all groups $P > 0.05$ (Tukey HSD).
[a] $P < 0.05$ according to control group (Tukey HSD); [b] $P < 0.05$ according to group II. (Tukey HSD).

MATERIALS AND METHODS

This study was established at the Experimental Research Center of Selçuk University with the permission of the Ethical Committee. Light microscopic examination was done in the Department of Pathology of Selcuk University. Electron microscopic examination was done in the Department of Histology and Embryology of Ankara University.

Thirty Sprague-Dawley type rats weighing 250 to 290 g (range: 275 ± 17 g) were used in this study. The rats were separated into three groups each comprising 10 rats.

Group-I: Control group: Gas insufflation was not applied to these animals.

Group-II: Cold-dry CO_2 group: These animals received standard cold-dry CO_2 (21°C, 2% relative humidity) at 10 mmHg pressure for 2 h.

Group-III: Heated-humidified CO_2 group: These animals received heated-humidified CO_2 (40°C, 98% relative humidity) at 10 mmHg pressure for 2 h.

Insufflation Technique

Pneumoperitoneum consisted of the following procedure, as described previously [5]. After anesthesia with ketamin hydrochloride (60 mg/kg), the animals were restrained in a supine position and shaved under sterile conditions. A thin needle (gauge 22) was inserted into the right lower abdomen of all groups.

Gas insufflation was not applied to the control group. Standard cold-dry CO_2 (21°C, 2% relative humidity) was applied with an insufflator in group II. Every 15 min cold-dry gas was desufflated and reinsufflated. The abdominal pressure was maintained at 10 mmHg for 2 h. In group III, before entering the needle, gas flowed through a chamber containing a temperature-humidity probe connected to an indicator unit (Datoscope Gm BH, Passport XG, Bensheim ±1% relative humidity) that monitored the temperature and humidity of gas entering the animal throughout the experiment. Heated-humidified (40°C, 98% relative humidity) CO_2 was applied to group III. In this group, a heater-humidification chamber was placed in series upstream from the temperature-humidity probe. The consistency of the temperature and humidity output using this apparatus had previously been confirmed by Bessell *et al.* [5]. Every 15 min heated-humidified gas was desufflated and reinsufflated. The abdominal pressure was maintained at 10 mmHg for 2 h.

Normal saline solution 20 ml/kg body-weight was administered subcutaneously at this time to replace insensible losses. In group II and III, peritoneal gas was emptied 2 h after pneumoperitoneum application. Because peritoneal reaction peaked after 12 h in another previous study [1], all rats were killed after 12 h by an over dose of ketamin hydrochloride. Peritoneal samples were collected immediately and were examined both by electron and light microscopy. The injection site of the peritoneum was not included in the evaluation.

Light Microscopic Examination

Peritoneal materials of all rats were dissected immediately, and fixed in 10% of buffered paraformaldehyde. Tissue specimens were prepared in an autotechnicon, embedded in paraffin and sectioned with microtome. The sections (5 μm) were stained with Hematoxylen and Eosin. Stained specimens were investigated by a Nikon Eclipse E400 light microscope (Nikon, Melville, NY). For each specimen, the same area was photographed after staining by using a Nikon Coolpix 5000 photograph attachment. The photograph of Nikon micrometer microscope slide was also taken during the procedure. All photographs were then transferred into PC environment and analyzed by using Clemex Vision Lite 3.5 Image Analysis program (Clemex, Longueuil, CA). The length was calibrated by comparing the photograph of specimen with the photograph of Nikon micrometer microscope slide, which was taken under the same magnification. 0.1 square millimeter area was designated with using Clemex Vision Lite 3.5 Image Analysis program then, lymphocytes, and capillaries were marked with the same Image Analysis program in totally 0.1 square millimeter area (four different areas). Damaged cells were not evaluated. The marked cells and capillaries were counted automatically with the same Image Analysis program. The reader was masked to the origin of the specimen.

Preparation for Scanning Electron Microscope

The specimens were fixed in 2.5% gluteraldehyde in 0.1 M sodium phosphate buffer at pH = 7.3 for 6 h. After fixation the specimens

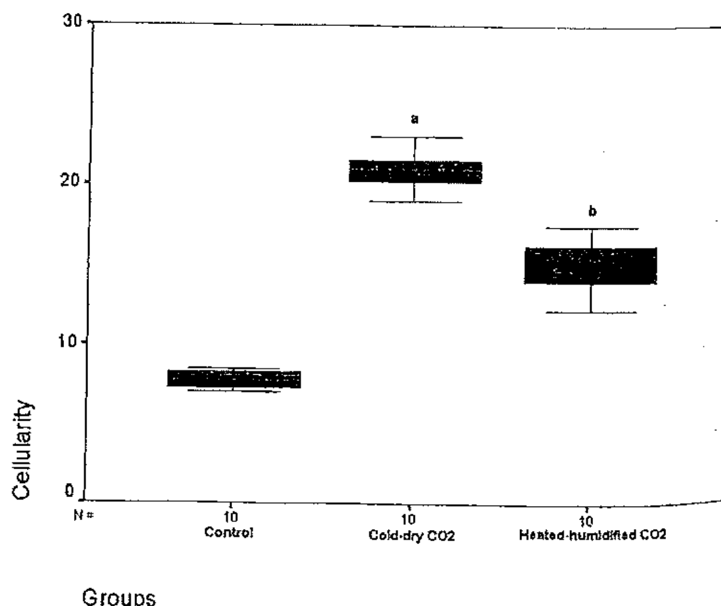


FIG. 1. Increased lymphocyte count significantly diminished by heated-humidified CO_2 application. (a) $P < 0.05$ according to control group, (b) $P < 0.05$ according to group cold-dry CO_2 group. (Color version of figure is available online.)

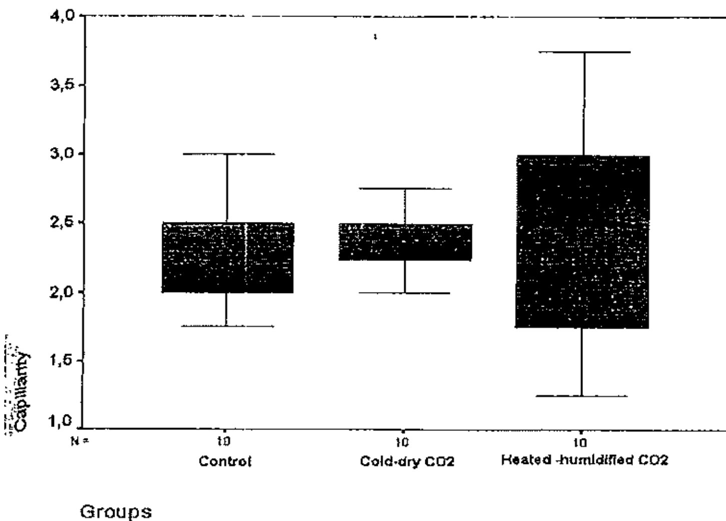


FIG. 2. No significant change was observed in capillarity in all groups ($P > 0.05$). (Color version of figure is available online.)

were rinsed with 0.1 M sodium phosphate buffer at pH = 7.3 for 30 min. Then they were post-fixed in 1% OsO4 in 0.1 M sodium phosphate buffer at pH = 7.3 for 2 h. They were then dehydrated in ascending grades of ethanol [%50 for 15 min, %75 for 15 min, %95 for 15 min, %100 for 15 min(×2)]. After the final ethanol step the specimens were dried in an Emitech K850 critical point drying apparatus. Then they were coated with gold-palladium in an Emitech K 550X sputter coater and examined in a Leo 438 VP scanning electron microscope.

The peritoneal surface was examined regarding two criteria.

1. Changes in mesothelial cells (destruction, bulging up, intercellular clefts, and changes of microvilli).
2. The visibility of the basal lamina.

Statistical Analysis

One-way ANOVA using Tukey's *post-hoc* test was used to determine the significance between groups. A P value <0.05 was regarded as significant.

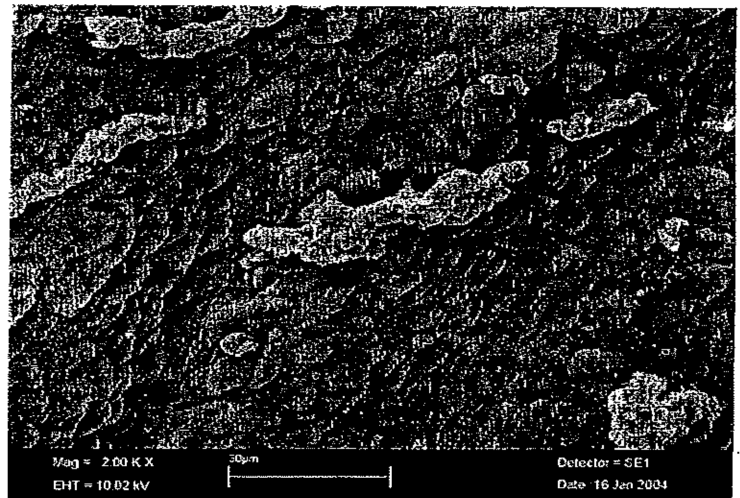
RESULTS

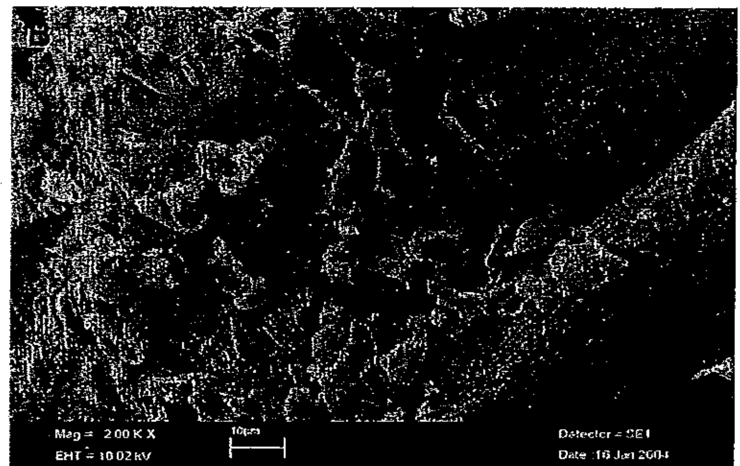
No rat died before completion of study. According to light microscopic examination; in groups II and III, cellular response (increased lymphocyte) was significantly higher than the control group ($P < 0.05$). Similarly, in group II, cellular response was significantly higher than group III. ($P < 0.05$). There was no difference in capillarity among all groups ($P > 0.05$) (Table 1, Figs. 1 and 2).

According to scanning electron microscopic examination; in the control group, normal morphology of the mesothelium was observed by flat mesothelial cells and intercellular clefts were seen slightly between the bulging-up cells. The basal lamina couldn't be seen. Microvilli were common (Fig. 3A).

After application of standard cold-dry CO_2, the mesothelial cells became strongly desquamate, and detachment of mesothelial cells became clearly visible, and intercellular clefts were not seen. The basal lamina was clearly seen because of massive desquamation. Desquamated mesothelial cells were gathered together with the macrophages and microvilli were not seen (Fig. 3B).

In the heated-humidified CO_2 applicated group, the





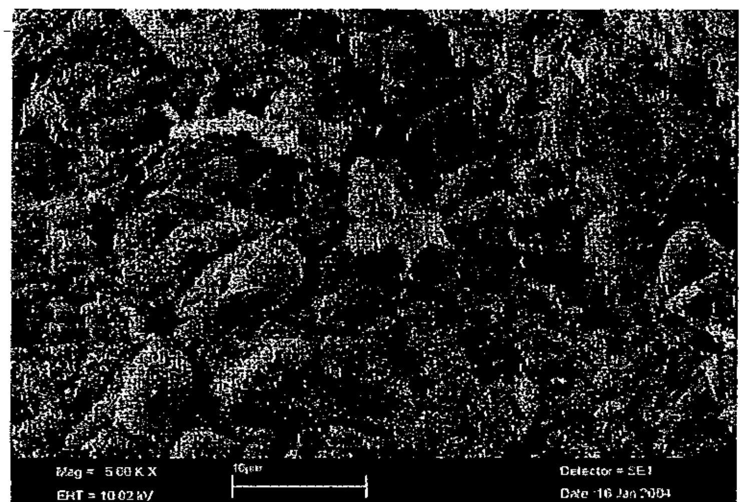


FIG. 3. (A) In control group: All cells are bulged up, basal lamina is not seen. (B) In group II: Basal lamina is clearly seen because of massive desquamation. Also desquamated mesothelial cells are seen. (C) In group III: Bulging cells are visible. Basal lamina is not seen because of the absence of desquamation.

TABLE 2

Scanning Electron Microscopic Findings

Groups	Detachment of mesothelial cells	Bulging-up	Intercellular cleft	Macrophage	Basal lamina	Microvili
Gr-I	---	++	+	---	---	++
Gr-II	++	---	---	+	++	---
Gr-III	---	+	++	---	---	+

---, non or slight; +, moderate; ++, marked.

mesothelial cells strongly retracted and bulged-up, so intercellular clefts became visible, but the basal lamina was not seen because of absence of desquamation. Depression of the microvilli was partial, detachment of mesothelial cells was slight (Fig. 3C). All these findings are given in Table 2.

In group II, detachment of the mesothelial cells was clearly seen, but there was no detachment in groups I and III. In group II, bulging-up cells and intercellular clefts were not seen because of massive desquamation of the mesothelial cells.

DISCUSSION

Standard cold-dry CO_2 is currently the insufflation gas of choice for laparoscopy. It fulfills most of the requirements for an ideal insufflation gas, being colorless, non-inflammable, and rapidly excreted from the circulation [1]. Volz *et al.* [2] were the first to describe characteristic alterations of the peritoneum after standard cold CO_2 pneumoperitoneum dealing with electron microscopic examination. They found that CO_2 pneumoperitoneum induced morphologically recognizable alterations in the superficial layer of the peritoneum. However, as far as we detected, there are no studies about the effects of heated-humidified CO_2 pneumoperitoneum on the peritoneum dealing with light and electron microscopic examination.

After 12 h of standard cold-dry CO_2 application, Volz *et al.* [2] found that uncovered basal lamina was seen nearly in the entire peritoneum. They detected the mesothelial cells had partially retracted and strongly bulged up, and intercellular clefts had become clearly visible and increased in size. However, in our study, after standard cold-dry CO_2 application, we detected the mesothelial cells had become strongly desquamate, and detachment of the mesothelial cells was visible, but intercellular clefts were not seen. The basal lamina was clearly seen because of massive desquamation. After heated-humidified CO_2 application normal peritoneal morphology was partially preserved. In this group, the mesothelial cells bulged-up, so intercellular clefts became visible, but the basal lamina was not seen because of the absence of desquamation. When compared to standard cold-dry CO_2 and heated-humidified CO_2 application, unfavorable findings were clearly seen in standard cold-dry application for the organism. At the same time, light microscopy showed that after standard CO_2 application cellular response was higher than heated-humidified CO_2 application.

It has been shown that patients who receive warm, humidified gas during laparoscopic procedures, have less postoperative pain than patients who received cold-dry gas [6], and the standard cold-dry CO_2 application increased risk of adhesion formation, hypothermia [5, 7]. We found that after standard CO_2 application cellular response and peritoneal damage in the peritoneum were increased. Thus, the question of whether standard cold-dry CO_2 application is of benefit to the patient is still an open.

Peritoneal macrophages play an integral role in the primary inflammatory response to infection within the abdominal cavity [1]. In our study, with standard cold dry CO_2 application, macrophages were gathered together with desquamated mesothelial cells.

The peritoneal changes after application of standard CO_2 were shown to be transient in the performed studies [2, 4]. However, laparoscopic intervention in patients with peritonitis may results in disruption of the integrity of the endothelial layer and removing the barrier function of the peritoneum. This condition will lead to extremely bad results in the organism with peritonitis. Bloechle *et al.* [8] revealed that in scanning electron microscopy analysis of the peritoneum, premature distortion, and disintegration of the mesothelial cell layer was observed in animals exposed to increased abdominal pressure in addition to gastric perforation induced peritonitis. We think that, laparoscopic intervention in patients with peritonitis, pneumoperitoneum formed by heated humidified CO_2 will be less harmful and more protective than cold CO_2.

In this study, we investigated the peritoneal alterations by heated humidified CO_2 throughout both by electron and light microscopy. After standard cold dry CO_2 application, in the light microscope; we observed increased cellulary response (increased lymphocyte). Reduction of this cellular response by heated humidified CO_2 application is a gain for the organism. Increased distance between the mesothelial cells, visible basal lamina, and destruction of mesothelial cells were

shown by electron microscopy. We think that these findings are sufficient for investigation of changes in the peritoneal barrier function. However, other parameters relating with peritoneal inflammatory changes and cytokine response in association with wound healing were not included in this study. We believe that these parameters could be investigated in other studies and that additional studies might lead to a better understanding of this subject.

At present, there are no complaints from surgeons about the application of cold CO_2. We do not advocate the application of heated humidified CO_2 in all laparoscopic interventions. In addition, a previous study revealed that the effects of CO_2 on the peritoneum were reversible [2]. However, we want to emphasize the benefit of heated humidified CO_2 in selected cases. Patients with peritonitis, high risk for the development of peritonitis, malignancy, and hypothermia in newborns and children may be considered as selected cases. Accordingly, we believe that the application of heated-humidified CO_2 might be rather useful in selected cases. After application of heated-humidified CO_2, we believe that less corruption of the peritoneal continuity may hinder entrance of microorganisms to the systemic circulation, and it may decrease the chance of tumor cells from entering the systemic circulation or adhering to the peritoneal surface.

In this study, we did not evaluate the injection site of the peritoneum. If we had investigated the probable changes at the site of injection we might have been able to explain port site metastasis in malignant cases. We believe that this subject should be evaluated in further studies.

In conclusion, application of heated humidified CO_2 leads to less cellular response than standard CO_2 application. Reduction of this cellular response by heated humidified CO_2 application is a gain for the organism. Peritoneal damage in pneumoperitoneum is less in heated humidified CO_2 than in standard cold dry CO_2 application. As a result, we believe that heated humidified CO_2 application is physiologically more suitable for the establishment of pneumoperitoneum in laparoscopic surgery, especially in selected cases.

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