

## Analysis of the bactericidal effect of ozone pneumoperitoneum<sup>1</sup>

### Análise do efeito bactericida do pneumoperitônio de ozônio

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#### ABSTRACT

**Purpose:** To assess the bactericidal action of ozone pneumoperitoneum, and to compare the results with CO<sub>2</sub>. **Methods:** It was used 36 Wistar rats. The animals, under anesthesia, were inoculated with 2ml of *E. coli* ATCC at a concentration of 10<sup>10</sup>UFC, and 1ml of BaSO<sub>4</sub>, into the peritoneal cavity. They were divided into three groups: Group 1, CO<sub>2</sub> pneumoperitoneum was performed for 15 minutes; Group 2, ozone pneumoperitoneum was performed for 5 minutes at a concentration of 42µg/ml, and Group 3, ozone pneumoperitoneum was performed for 5 minutes at a concentration of 62µg/ml. Six animals from each group were sacrificed after the experiment, and the remaining 6 observed for 24 hours. Material was collected from the cavity of all animals for microbiological study. **Results:** Ozone presented a greater bactericidal effect than CO<sub>2</sub> in those animals sacrificed immediately after pneumoperitoneum. In the animals studied 24 hours after pneumoperitoneum evidenced no difference in bactericidal effect between the two gases. Moreover, no difference in mortality was observed. **Conclusion:** Ozone has a more potent bactericidal effect than carbon dioxide gas, although this did not influence survival of the animals.

**Key words:** Peritonitis. Ozone. Laparoscopy. Peritoneum. Rats.

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#### RESUMO

**Objetivo:** Avaliar a ação bactericida do pneumoperitônio de ozônio comparando-o à ação do CO<sub>2</sub>. **Métodos:** Foram utilizados 36 ratos Wistar. Após anestesia e inoculação de 2ml de *E. coli* ATCC na concentração de 10<sup>10</sup> UFC e 1ml de BaSO<sub>4</sub> na cavidade peritoneal, os animais foram divididos em três grupos: Grupo 1, realização de pneumoperitônio de CO<sub>2</sub> por 15 minutos; Grupo 2, realização de pneumoperitônio de ozônio durante 5 minutos na concentração de 42µg/ml, e Grupo 3, realização de pneumoperitônio de ozônio durante 5 minutos na concentração de 62µg/ml. Seis animais de cada grupo foram sacrificados após experimento e os outros seis foram observados por 24 horas. Em todos os animais colheu-se material da cavidade para estudo microbiológico. **Resultados:** O ozônio teve maior efeito bactericida em comparação ao CO<sub>2</sub> nos animais sacrificados logo após pneumoperitônio. Nos animais estudados após 24 horas não houve diferença do efeito bactericida entre os gases. Também não se observou alteração da mortalidade. **Conclusão:** O ozônio tem efeito bactericida mais potente que o gás carbônico, embora não tenha influenciado a sobrevida dos animais.

**Descritores:** Peritonite. Ozônio. Laparoscopia. Peritônio. Ratos.

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#### Introduction

Ozone gas (O<sub>3</sub>) is a component found in the atmosphere produced by the action of ultra-violet rays from sunlight or can be created artificially using an ozone generator. The action of ultra-violet rays on air at the earth's surface promotes the formation of a protective ozone layer, which is fundamental to the survival of all life forms on Earth<sup>1</sup>.

Ozone is not only a potent oxidizing agent but is also considered an important disinfectant. Its potent bactericidal effect stems from direct attack of microorganisms with oxidation of biological material. The bactericidal power of the gas can reach 3,500 times the speed of chlorine<sup>1</sup>.

Peritonitis remains a constant challenge for surgeons. Commonly used therapeutic measures include crystalloid replacement, administering of broad spectrum antibiotics, rapid surgical control of the contamination source, and peritoneal cavity lavage with a variety of drugs and solutions<sup>2</sup>.

Recently, the use of diagnostic and therapeutic videolaparoscopy in peritoneal infections has shown to be a therapeutic alternative given its benefits<sup>3</sup>. Early diagnosis, less invasive intervention and safety application, even in more severe cases, has led to an increase in its indication. Investigations concerning the effect of gaseous distension of the peritoneal cavity in the presence of infection have become the focus of increased interest<sup>4-6</sup>.

Experimental studies have demonstrated the bactericidal effects of CO<sub>2</sub> and helium – the most frequently used gases in pneumoperitonium – when employed in peritoneal contamination models<sup>7-9</sup>. However, to date, no significant clinical benefits have been verified regarding the use of any particular gas.

Several studies have shown bacteria, spores and viruses to be sensitive to ozone, whereby only a few minutes of exposure is required to inactivate these agents. Therefore, it is believed that ozone has anti-septic powers chiefly due to its oxidizing properties<sup>1,2,3</sup>.

With a view to surgically treating patients with diffuse peritonitis using videolaparoscopy, it was decided to assess the bactericidal effect of ozone introduced into the peritoneal cavity and to compare results with carbon dioxide, envisaging future therapeutic applications.

The objective of this study was to assess the bactericidal action of ozone pneumoperitonium based on microbiological study and survival, following inoculation of a known quantity of *Escherichia coli* into the peritoneal cavity of rats. The influence of use of different ozone concentrations was also analyzed and compared with the effect of carbon dioxide gas.

## Methods

The study was carried out at the Surgical Technique and Experimental Surgery Unit (UTECE) of the Medical Sciences Faculty of Santa Casa Hospital, Sao Paulo and it was approved by the Experimental Ethical Committee.

A total of 36 Wistar rats were used, weighing between 200 and 500 grams and kept under standard conditions of conventional biotério, housed in cages containing 6 animals each and offered specific ration with free access to water.

All 36 animals underwent the following procedures:

1- Intra-muscular anesthesia performed to the right hind foot of the animal using Ketamine/Xylazine (25mg/kg and 10mg/kg, respectively);

2- Non-invasive monitoring of pulse oximetry, heart rate and temperature measured with a thermometer via the rectal route, during Ozone and CO<sub>2</sub> pneumoperitonium. Respiratory frequency was monitored by direct observation;

3- Tricotomy of the abdominal wall;

4- Anti-sepsis using polyvinylpyrrolidone iodine;

5- A small incision was made into the abdominal wall to introduce an 18 abocath-type catheter which was affixed using polyglycolic acid 3-0 thread above the umbilical scar;

6- Inoculation with 2.0ml of a solution containing *Escherichia coli* ATCC at a concentration of 10<sup>10</sup>UFC and 1ml of barium sulfate;

After this stage, animals were divided into 3 groups of 12 animals and underwent the following experiments:

- **Group 1:** CO<sub>2</sub> pneumoperitoneum for 5 minutes at a constant intra-abdominal pressure of 3mmHg;

- **Group 2:** Ozone pneumoperitoneum for 5 minutes at a concentration of 42µg/ml and constant intra-abdominal pressure of 3mmHg;

- **Group 3:** Ozone pneumoperitoneum for 5 minutes at a concentration of 62µg/ml and constant intra-abdominal pressure of 3mmHg;

The Ozone source employed was a Canadian Yanco Industries LTD model OL80F/DST ozone generator.

Upon completion of the respective insufflation periods, 6 animals were sacrificed from each group of 12 animals and submitted to microbiological study. Microbiological study was performed on the remaining 6 animals after 24-hour observation. This period also served to assess differences in animal survival.

For the purposes of microbiological study, median laparotomy was performed and intra-abdominal secretions collected using a sterile handle, for subsequent cultures. This material was cultured on a blood-agar plate. Colony counts were subsequently performed.

Statistical analysis of results was carried out using the Kruskal-Wallis non-parametric test.

## Results

Results depicted in Table 1 show the colony counts obtained in animals sacrificed immediately after the pneumoperitoneum procedure in groups 1, 2 and 3, respectively.

Bactericidal effect of ozone was greater than that of carbon dioxide (p<0.05). In addition, it was verified that the concentration of 62µg/ml yielded a greater bactericidal effect than 42 µg/ml, where this comparative analysis also attained statistical significance (p<0.05).

**TABLE 1** - Number of *E.coli* ATCC colonies in UFC/ml obtained after CO<sub>2</sub> and ozone pneumoperitoneum procedures

Animal	Colonies UFC/ml (CO <sub>2</sub> )	Colonies UFC/ml (O <sub>3</sub> , 42µg/ml)	Colonies UFC/ml (O <sub>3</sub> , 62µg/ml)
<b>R1</b>	100,000	1,000	1,000
<b>R2</b>	100,000	1,000	1,000
<b>R3</b>	50,000	1,000	1,000
<b>R4</b>	100,000	10,000	1,000
<b>R5</b>	30,000	1,000	100
<b>R6</b>	15,000	100,000	1,000
<b>Mean Colonies</b>	<b>65,833</b>	<b>19,000</b>	<b>850</b>
<b>Standard Deviation</b>	<b>39,040.6</b>	<b>39,844.7</b>	<b>367.4</b>

Results of microbiological study performed 24 hours after pneumoperitoneum are shown in Table 2. No differences between the bactericidal effects of ozone and carbon dioxides

gases were observed.

Also, no difference in animal survival was found amongst the three groups over the period.

TABLE 2 - Result of *E.coli* colony concentration in UFC/ml obtained after 24-hour observation

Animal	Colonies UFC/ml (CO <sub>2</sub> )	Colonies UFC/ml (O <sub>3</sub> , 42µg/ml)	Colonies UFC/ml (O <sub>3</sub> , 62µg/ml)
R1	1,000,000	1,000,000	1,000,000
R2	100,000	100,000	1,000,000
R3	1,000,000	1,000,000	1,000,000
R4	1,000,000	1,000,000	100,000
R5	100,000	1,000,000	1,000,000
R6	100,000	1,000,000	1,000,000
<b>Mean of colonies</b>	<b>550,000</b>	<b>850,000</b>	<b>850,000</b>
<b>Standard Deviation</b>	<b>492,950.00</b>	<b>367,423.5</b>	<b>367,423.5</b>

## Discussion

The treatment of patients with peritoneal infection remains a challenge. Its high frequency and rates of morbidity/mortality in routine clinical practice have driven the search for new therapeutic methods, drugs and surgical approaches in a bid to improve outcome. The use of coadjuvant topical substances in cavity washes such as antibiotics, antiseptics, immunostimulants, heparin and others remains controversial.

Video assisted surgery is also employed in the treatment of peritonitis. However, the use of pneumoperitoneum in infections remains a controversial issue. Sare *et al.*<sup>9</sup> and Ipek *et al.*<sup>10</sup> demonstrated in an experimental study that pneumoperitoneum induced greater bacteremia than the laparoscopic technique<sup>9,10</sup>.

In contrast, Benoit *et al.*<sup>2</sup> who conducted a prospective analysis of patients operated using the videolaparoscopic approach for acute appendicitis, with and without peritonitis, observed no positive blood cultures after performing the procedure, nor incidence of septic complications, and reported no increase in morbidity<sup>2</sup>. Similarly, Barbaros *et al.*<sup>11</sup> found no significant differences between CO<sub>2</sub> pneumoperitoneum and laparotomy in terms of clinical evolution of induced peritonitis in animals.

In view of the advantages of application of laparoscopic surgery in peritoneal infection patients, it was investigated the possible effects of ozone as an adjuvant substance. Ozone is a potent oxidizing agent characterized by the ability to destroy a large variety of microorganisms, although its therapeutic application remains controversial<sup>1,2</sup>.

In addition, some studies have shown toxic effects caused by excessive doses of ozone, particularly in connection with inhalation of the gas through the airways, as well as toxicity to the endocrine, reproductive and central nervous systems<sup>12</sup>.

Ozone has been administered using several routes: oral, nasal, auditory tube, vaginal, vesical, colorectal, cutaneous, intra-articular, intratissue, intramuscular and subcutaneous<sup>13</sup>. Nevertheless, little is known about the use of intra-peritoneal ozone or the repercussions on intra-abdominal organs and tissues.

Santa Casa de Sao Paulo Faculty Medical Sciences has been a pioneer in the study of intra-peritoneal administration of ozone, which experience at using in gaseous form showed to be a potent inhibitor of *in vitro* bacterial growth, as compared to carbon dioxide and helium. In this Institution was also established pneumoperitoneum model involving an oxygen-ozone mix using continuous flow in which no significant histopathological alterations were observed as a result of direct contact of the mixture in specimens of the liver, spleen, terminal ileum and fatty tissue enveloping the seminal vesicle and showed toxic effects of high ozone concentrations (120µg/ml) when infused for longer than ten-minute periods.

Schulz *et al.*<sup>14</sup> found promising results on proving the efficacy of repetitive pre-treatment using intra-abdominal ozonized oxygen in reducing mortality in animals submitted to lethal polymicrobial peritonitis.

The present study has shown that infusion of ozone concentrations of less than 65µg/ml for periods of up to 5 minutes causes no evident toxic effects and that the bactericidal effect of ozone exceeds that of carbon dioxide gas. Despite the increased

bactericidal effect observed, no impact on animal survival was observed since both those receiving ozone and carbon dioxide died within 24 hours of bacterial inoculation.

The death of these animals can be attributed to the peritonitis resulting from injection of an exorbitant number of bacteria, rather than to the toxic effect of the gas tested. This is evidenced by the fact that both groups of animals, CO<sub>2</sub> and ozone, did not survive the procedure 24 hours after the experiment.

Therefore, one can assume that the bactericidal effect of ozone is significant. However, further studies are warranted to establish the true adjuvant effect of ozone in managing peritonitis infection, as well as to investigate its mechanism of action, in order to enable its safe use in peritonitis patients.

The results of the present study allow to draw the following conclusions: ozone has a bactericidal effect, and this effect is relative to the concentration used and it is greater than the effect of carbon dioxide gas. This effect however, did not guarantee survival of the animals.

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