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# Therapeutic Effect of Ozone Gas on Bovine Mastitis

**Hyun-Joo Kwon, Jianzhu Liu, Sung-Nam Jo, Kun-Ho Song, Duck-Hwan Kim[1], Moo-Hyung Jun*, Sung-Whan Cho**, Myung-Cheol Kim*** and Hyo-In Yoon******

*Laboratory of Veterinary Internal Medicine, *Laboratory of Veterinary Microbiology*
***Laboratory of Veterinary Pathology, ***Laboratory of Veterinary Surgery, ****Laboratory of Veterinary Pharmacology,*
*College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea*

**Abstract:** The potential therapeutic effect of ozone gas on bovine mastitis was investigated. Eighteen quarters from 18 lactating cows with chronic mastitis were included. The 18 quarters were assigned to the control group (treatment with antibiotics for 3 days), experimental group I (0.1 ppm ozone treatment, for 7 days) and experimental group II (1 ppm ozone treatment, for 3 days). In experimental group I, milk somatic cell counts were lower on day 7 after ozone treatment, compared to the pretreated counts, but were higher than the control counts. In experimental group II, somatic cell counts were significantly decreased ($p<0.05$) on day 7 compared to the pretreatment counts, and they were lower than the control counts. There were no changes in leukocyte, neutrophil, or lymphocyte numbers, N/L ratios, or serum total protein in the control and experimental groups. We concluded that ozone gas treatment (1 ppm for 3 days) might be effective for treatment of bovine mastitis.

***Key words***: therapeutic effect, ozone gas, bovine, mastitis.

## Introduction

Bovine mastitis is an inflammation of the mammary gland caused by infection with microorganisms [3]. It is one of the common diseases of dairy cows and is difficult to control. Total economic losses to mastitis were estimated to be approximately $200 per cow per year in the United States[1,3]. The economic impact of bovine mastitis is also an issue in Korea[15,22].

Bovine mastitis can be classified into clinical and subclinical mastitis[3]. Asymptomatic subclinical mastitis is more important than clinical mastitis because of subclinical mastitis may be left untreated[13,17,18].

In addition, *Staphylococcus aureus* and *Streptococcus spp.* are considered to be major pathogens of contagious mastitis[8,9,16,26]. Infection with one organism may lead to clinical mastitis with another[9].

The intramammary administration of antibiotics is the most common method of treatment for bovine mastitis. Treatment with antibiotics during the lactation period has a low recovery rate because of the many kinds of resistant pathogens such as *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae*[5,19]. In addition, the presence of residual antibiotics in milk has become an issue in the dairy industry[14].

Ozone ($O_3$) is polymerized oxygen generated by the treatment of air or oxygen with high-energy electrodes using an ozone generator or by ultraviolet light. Ozone has a strong disinfecting effect, but this may have an inconsequential role in therapy because it can be immediately converted to oxygen[7,19]. Ozone is currently used for sterilization of foods, water and wastewater[19].

[1]Corresponding author.
E-mail : dhkim@cnu.ac.kr

In human medicine, Andredula *et al.*[2] reported that periganglionic injection of oxygen-ozone and periganglionic injection of steroids had an effect on lumbar disk herniation. In addition, Tafil-Klawe *et al.* [23] found that ozone therapy administered by intravenous infusion and aerosol oxygen-ozone baths of the lower extremities yield much better therapeutic results than classical balneology. Jordan *et al.* [12] evaluated the effectiveness of ozone treatment for skin radiotherapy. Tylicki *et al.* [24] reported the influence of ozonated autohemotherapy on oxidative stress in hemodialyzed patients with atherosclerotic ischemia of the limbs. Further Daulaeva and Baizakova[6] reported that ozone therapy applied to wounds of the face and neck increased the sensitivity of microorganisms to antibiotics.

In veterinary medicine, Grundner and Erler[10] reported that ozone damaged the metabolism and reproductive capacity of mouse Ehrlich ascites tumor cells. Ogata and Nagahata[19] applied intramammary infusion of ozone gas to acute clinical mastitis in dairy cows. Ducusin *et al.* [8] examined ozone therapy in vitro to explore a possible healing mechanism by ozone gas. However, prior to this study, the veterinary use of ozone therapy in Korea has not been reported.

To establish the therapeutic effect of ozone therapy on bovine mastitis, we investigated its influence on somatic cell counts in milk, blood cell counts and blood chemical values, at different concentrations and using different infusion protocols.

## Material and Methods

### Experimental animals

Eighteen quarters from 18 lactating cows with chronic mastitis were selected for study. They were raised on dairy farms in the Gongju, Boun and Paju areas in Korea, respec-

tively. The 18 quarters were assigned to a control group (5 quarters), experimental group I (5 quarters) and experimental group II (8 quarters).

**Treatments**

Control quarters were treated with norfloxacin ointment (Nopazin®, Daesung Microbiological Co., Korea), based on the result of sensitivity twice a day for 3 days.

Experimental group I was treated with ozone gas (0.1 ppm) (ozone generating equipment made by Myunghua(MH) Co., Korea). Ozone gas was infused into the inflamed quarter via the teat canal, twice a day for 7 days. Total infusion volume was 250 ml per quarter.

Experimental group II was treated with ozone gas (1 ppm). The infusion volume was 50ml per quarter for 3 days. Ozone gas was infused into the inflamed quarter via the teat canal, twice a day for 3 days.

**Analysis of milk**

About 10 ml of milk samples were collected from each inflamed quarter under aseptic conditions into sterilized conical tubes for somatic cell counts.

The determinations of somatic cell counts in milk were performed by an auto-counter for somatic cells in milk (Fossmatic-90, Foss Electric Co., Denmark).

**Analysis of blood samples**

Blood samples were taken from the mammary vein and caudal vein. A 5 ml sample of blood was withdrawn into a tube containing EDTA as an anticoagulant for hematological analysis, while another 5 ml sample was withdrawn into plain tubes with no anticoagulant to provide serum samples. The hematological analyses were performed shortly after sampling. The serum samples were stored frozen at -20°C until analyzed. Total white blood cells and differential counts were calculated using an automated blood cell counter (HEMA Vet, CDC Technologies Inc., USA). Serum total protein was analyzed by use of an automated serum chemistry analyzer (SM-4000 plus®, B.S. Biochemical systems, Italy).

**Statistical analysis**

Significant difference between control group and experimental group was analyzed using paired Student's t-test with a database (SPSS v. 12.0, K). The data were expressed as mean±S.D..

**Table 1.** The changes of milk somatic cell counts in control and experimental groups

| Groups | Day | |
|---|---|---|
| | 0 | 7 |
| Control | 4,065±2,827* | 2,493±1,884 |
| Exp.I | 4,508±2,827 | 4,041±1,755 |
| Exp.II | 3,898±5,149 | 673±475** |

*: $\times 10^3$/ml.
**: $p<0.05$

## Results

**Changes in somatic cell counts in milk**

The numbers of somatic cells in milk at 0 and 7 days after treatment with antibiotics were 4,065±2,827×$10^3$/ml and 2,493±1,884×$10^3$/ml, respectively, in the control group. This difference was not statistically significant.

The number of somatic cells in milk at 0 day was 4,508±2,827×$10^3$/ml in experimental group I and the value had decreased to 4,041±1,755×$10^3$/ml by 7 days after treatment with ozone gas (0.1 ppm, 250 ml). This value was higher than that of the control. The decrease in Group I milk somatic cell counts was not statistically significant (Table 1).

The number of somatic cells in milk at 0 and 7 days after treatment with ozone gas (1 ppm, 50 ml) were 3,898±5,149 ×$10^3$/ml and 673±475×$10^3$/ml, respectively in experimental group II. The somatic cell counts in milk were significantly ($p<0.05$) decreased by ozone gas, compared to that of pretreatment in experimental group II (Table 1).

**Hematology**

There were no significant changes in WBC counts, neutrophils, lymphocytes, or N/L ratios in control and experimental groups (Table 2).

**Changes in blood chemistry**

There were no significant changes in total protein in either the control or experimental groups (Table 2).

## Discussion

Bovine mastitis is a troublesome disease in the dairy industry, and the economic loss caused by bovine mastitis is large, due to the appearance of resistant pathogens and residual antibiotics in milk[3,19,20].

**Table 2.** The changes of WBC, neutrophils, lymphocytes, N/L ratio and total protein in control and experimental groups (D=day)

| Groups | WBC (×$10^3$/μl) | | Neutrophils (×$10^3$/μl) | | Lymphocytes (×$10^3$/μl) | | N/L ratio | | Total protein (g/dl) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 D | 7D | 0 D | 7D | 0 D | 7D | 0 D | 7D | 0 D | 7D |
| Control | 13.3±7.1 | 13.7±8.3 | 6.8±2.5 | 6.3±2.1 | 7.7±4.7 | 8.0±6.5 | 1.1±0.7 | 1.2±0.9 | 7.9±0.6 | 7.9±0.6 |
| Exp.I | 13.6±2.2 | 16.2±4.4 | 4.4±1.2 | 6.8±2.7 | 8.3±3.4 | 8.8±4.8 | 0.7±0.4 | 0.9±0.6 | 8.2±0.8 | 8.6±1.0 |
| Exp.II | 11.2±5.1 | 11.5±4.4 | 4.8±3.0 | 3.8±1.3 | 5.9±3.1 | 6.6±3.2 | 0.9±0.6 | 0.7±0.3 | 7.7±0.7 | 7.6±0.6 |

Ozone is a kind of active oxygen and is important in the oxidation of lipids, but is not a radical itself[19]. Ozone has been used in human medicine and many diseases have been safely treated with ozone[19].

Combined gas therapy with ozone gas and $O_2$ has been used for treatment of fistula, bedsores and ulcers of the leg, in addition to diseases of body cavities including fistulae, colitis, tumor, and gynecological and urinary diseases[11]. The intramuscular injection, subcutaneous injection, intravenous injection, intra-arterial injection, intra-articular injection, auto-hemotherapy, and rectal insufflation with ozone gas or ozonated water have been widely used for treatment of various human diseases[1].

The somatic cell count includes leukocytes and epithelial cells in milk, and is used to monitor the health of the bovine udder in bovine mastitis control programs[3,7]. In addition, de Haas *et al.*[7] reported that the somatic cell count always remained elevated after the occurrence of pathogen-specific clinical mastitis. In the present study, we investigated the therapeutic effect of ozone gas on bovine mastitis. Somatic cell counts in milk were decreased by infusion of ozone gas (1 ppm) after 7 days, similar to the counts of the control group. This was similar to results reported by Ducusin *et al.*[8] and Shiratori *et al* [21] in bovine mastitis. Ducusin *et al.*[8] found that polymorphonuclear leukocyte phagocytosis was increased after ozone gas administration in vitro in milk from cows with mastitis. It is well known that ozone has bactericidal, fungicidal and virucidal effects[7,19]. Decrease of somatic cell counts in milk was observed in the experimental group II treated with ozone gas (1 ppm). We consider this to be caused by the healing mechanism of ozone. Ozone gas destroys the capsid or exterior protein shell protecting nucleic acid via oxidative breakdown, so that the DNA or RNA structures of the microorganism are affected[11]. Ozone inactivates viruses through oxidation and inhibition of viral surface proteins, including cell receptors, and effects intracellular transduction of peroxides[11]. Alternatively, since ozone is immediately converted to oxygen, the polymorphonuclear leukocytes may have used the available oxygen for normal metabolic processes[21].

The effective dosage and concentration of ozone gas were investigated in this study. We found that 1 ppm of ozone gas, given in two infusions of 50 ml per day for 3 days, was effective for bovine mastitis. Shiratori *et al.*[21] reported that ozone has an inflammatory or anti-inflammatory action and can be used to modulate flogosis, depending on the concentration. High concentrations of ozone gas have been used for the treatment of various human diseases. Ozone gas (70-90 ppm) was used to treat fungal infection and ulcerative colitis. Ozone gas (40-70 ppm) was used to treat fistula, bedsores and ulcer of the leg. Ozone gas (0-40 ppm) has been used to treat cancer, allergy, rheumatic arthritis, viral infection and intestinal disease. The effects of high concentrations of ozone on bovine mastitis should be examined in the future.

Ozonated water and ozonated ointment have been used for the treatment of various bacterial, fungal and viral diseases in humans[4]. Ozonated water was widely used for disinfection and hemostatic of oral cavities[7]. In addition, ozonated ointment was used for various human dermatological and gynecological diseases. The therapeutic effects of ozonated water and ozonated ointment should be investigated in many kinds of bovine diseases, including bovine mastitis, in the future.

The toxicity of ozone gas has been documented[19,25,27]. High concentrations cause fatal pulmonary alveolar damage in humans[25]. The toxicity of ozone arises from its ability to accelerate the conversion of alcohols to aldehydes[27]. This toxicity was not investigated in this study, however, alveolar changes in the bovine mammary gland should be examined.

## Conclusion

We concluded that ozone gas treatment(1 ppm for 3 days) might be effective for treatment of bovine chronic mastitis.

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# 젖소 유방염에 대한 오존가스의 치료효과

**권현주 · 유건주 · 조성남 · 송근호 · 김덕환[1] · 전무형 · 조성환 · 김명철 · 윤효인**

충남대학교 수의과대학

**요 약**: 오존가스의 젖소 유방염에 대한 치료효과를 구명할 목적으로 본 실험을 수행하였다. 만성유방염에 이환된 비유우 18분방을 대상으로 하였다. 실험분방은 대조군(항생제 투여군: norfloxacin 10g/tube, 2회/일, 3일간: 5분방), 실험군 I (오존가스 0.1 ppm, 250 ml, 2회/일, 7일간: 5분방) 및 실험군 II(오존가스 1 ppm, 50 ml, 2회/일, 3일간: 8분방)로 각각 나누어 공시하였다. 우유 중 체세포수, 혈액충 백혈구수, 호중구수, 림프구수, 호중구/림프구(N/L)비 및 혈청 단백량의 변화를 처치 전 및 처치 후 7일에 각각 비교 검토하였다. 그 결과, 실험군 I에서는 오존가스 주입 후 7일에 주입 전에 비하여 우유 중 체세포수가 감소하였으나 대조군 보다 높은 수치를 나타내었다. 실험군 II에서는 오존가스 주입 후 7일에 주입 전에 비하여 현저한 감소소견을 나타내었으며($p<0.05$), 대조군 보다 낮은 수치를 나타내었다. 그러나 대조군 및 실험군 간의 유의성은 인정되지 않았다. 또한 말초혈액 백혈구수, 호중구수, 림프구수, N/L비 및 혈청 단백량의 변화에서는 대조군 및 실험군에 있어서 각각 유의한 변화를 나타내지 않았다. 이상의 결과를 종합해 볼 때, 오존가스는 젖소 유방염의 치료에 유효할 것으로 판단된다.

**주요어**: 치료효과, 오존가스, 소, 유방염