

## A Therapeutic Effect of Ozonated Oil on Bovine Mastitis

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**Abstract:** Forty-nine quarters from 24 lactating cows with chronic mastitis were selected. The cows were raised on dairy farms in Gongju, Jochiwon and Yeongi in Chungnam province, and Iksan in Jeonbuk province, Korea. The 49 quarters with bovine mastitis were divided into control (7 quarters) and experimental (42 quarters) groups. The experimental quarters were assigned to experimental group A (10 quarters, somatic cell count:  $50-100 \times 10^4/\text{ml}$ ), experimental group B (14 quarters, somatic cells count:  $100-300 \times 10^4/\text{ml}$ ), and experimental group C (18 quarters, somatic cells count:  $> 300 \times 10^4/\text{ml}$ ), according to the number of the somatic cells in their milk. The quarters of control group were treated with norfloxacin ointment (10 g/tube) based on the result of sensitivity, twice a day for 3 days. The quarters of experimental groups were infused 10 ml of ozonated oils twice a day for 3 days. After treatment, the milk of the control group contained non-significantly lower numbers of somatic cells and bacteria on day 7, compared with pretreatment levels. Experimental groups A, B and C had lower somatic and bacterial cells in their milk on day 7, compared with pretreatment levels. Experimental groups B and C had significantly lower numbers of somatic cells in their milk on day 7 than before treatment ( $p < 0.01$ ). However, no significant difference in somatic cell numbers was detected between the control and experimental groups. It was concluded that ozone therapy with ozonated oil applied on bovine mastitis might be effective.

**Key words:** therapeutic effect, ozonated oil, bovine, mastitis

### Introduction

Ozone was discovered by Christian Friedrich Schonbein, a German chemist. Since its bactericidal action was reported by Fox, ozone had been used for water purification<sup>12</sup>. Ozone therapy was developed mainly in Germany: there are reports that it is effective in treating various human diseases such as bacterial, viral and fungal diseases<sup>2,7,11</sup>.

In clinical practice, ozonated water is used for its bactericidal and anti-inflammatory effects in dental diseases. Ozone gas therapy combined with  $\text{O}_2$  cab is used in the treatment of the fistulas, bedsores and ulcers of the limbs. Intra-articular injection is used for treatment of rheumatoid arthritis. In addition, major autohemotherapy (autotransfusion with a large volume of blood treated with pure ozone gas) can be used for the treatment of herpes infections, hepatitis, blood circulation disorders, rheumatoid diseases and allergic diseases. Minor autohemotherapy (intramuscular injection of a small volume of blood treated with ozone gas) is also used in the treatment of inflammatory disease, rheumatoid diseases and mild arterial circulatory disturbances. Rectal insufflation with ozone gas and local application of ozonated oil can be used for the treatment of inflammatory diseases<sup>12</sup>.

Ogata and Nagahata<sup>11</sup> reported that infusion of ozone gas into the inflamed quarters had a therapeutic effect in bovine mastitis, but the therapeutic effect of ozonated oil on bovine

mastitis has not been investigated until now. We have monitored somatic cells and bacterial numbers in milk in order to determine if ozonated oil has a therapeutic effect on bovine mastitis.

### Material and methods

#### Experimental animals

Forty-nine quarters from 24 lactating cows with chronic mastitis which were not responsive to treatment over one month were selected for this study. The cows were raised on dairy farms in the Gongju (11 quarters from 10 heads), Jochiwon (7 quarters from 3 heads) and Yeongi (13 quarters from 6 heads) areas in Chungnam province, and in the Iksan (17 quarters from 5 heads) area in Jeonbuk province in Korea.

The 49 quarters were divided into control (7 quarters) and experimental groups (42 quarters) and the somatic cell and bacteria in the milk were counted. The experimental groups experimental group A (10 quarters, somatic cell count:  $50-100 \times 10^4/\text{ml}$ ), experimental group B (14 quarters, somatic cell counts:  $100-300 \times 10^4/\text{ml}$ ) and experimental group C (18 quarters, somatic cell count:  $> 300 \times 10^4/\text{ml}$ ).

#### Preparation of ozonated oil

Ozonated oil was prepared by bubbling ozone gas (200 ppm) into vegetable oil for 3 days, using a bubbling apparatus (ozone generating equipment, Myunghua(MH) Co., Korea). The ozonated oils were stored in syringes (10 ml each) and kept refrigerated at 4°C.

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### Treatment

The control group was treated with norfloxacin ointment (Nopazin<sup>®</sup>, Daesung Microbiological Co., Korea: 10 g/tube), based on the result of sensitivity, twice a day for 3 days. The ozonated oil was infused into the inflamed quarters twice a day for 3 days in the experimental groups.

### Determination of somatic cells and bacteria in milk

About 10 ml of milk samples were collected from each inflamed quarter under aseptic condition into sterilized conical tubes for somatic cell and bacteria counts. These were determined by use of an automatic cell counter (Fossmatic-90, Foss Electric Co., Denmark).

### 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 $\pm$ SD.

## Results

### The effect of ozonated oil on somatic cell numbers in milk

The changes in somatic cells in milk are presented in Fig 1. In the control group, cell numbers were low on the third day ( $1,685.9 \pm 1,079.8 \times 10^3/\text{ml}$ ) and then slightly high on day 7 ( $4,019.4 \pm 4,652.0 \times 10^3/\text{ml}$ ), compared with pretreatment level ( $4,358.0 \pm 2,753.9 \times 10^3/\text{ml}$ ). However, these differences were not significant.

Somatic cells in experimental group A showed a slight decrease on day 7 ( $595 \pm 541.9 \times 10^3/\text{ml}$ ) after treatment with ozonated oil compared with the pretreatment number ( $662 \pm 195.8 \times 10^3/\text{ml}$ ) without significance. Somatic cells in experimental group B were decreased significantly on day 7 ( $1,258.0 \pm 823.9 \times 10^3/\text{ml}$ ) after treatment with ozonated oil, compared with the pretreatment level ( $1,762.1 \pm 601.9 \times 10^3/\text{ml}$ ) ( $p < 0.01$ ). Somatic cell in experimental group C showed a marked significant decrease on the day 7 ( $1,276.1 \pm 973.5 \times 10^3/\text{ml}$ ) after treatment with ozonated oil, compared with the pretreatment counts ( $7,515.9 \pm 3,604.0 \times 10^3/\text{ml}$ ,  $P < 0.01$ ). However, significant difference in change of milk somatic cell was not detected between control (antibiotics) and experimental groups.

### Effect of ozonated oil on bacterial numbers in milk

The numbers of bacteria in milk are presented in Fig 2. The control group had slightly decreased numbers of bacteria on day 7 ( $32.8 \pm 46.3 \times 10^3/\text{ml}$ ) after treatment with antibiotics, compared with the pretreatment level ( $40.0 \pm 53.4 \times 10^3/\text{ml}$ ). This difference was not significant.

In experimental group A, bacterial numbers were slightly low on day 7 ( $25.7 \pm 38.2 \times 10^3/\text{ml}$ ) after treatment with ozonated oil, compared with the pretreatment numbers ( $28.6 \pm 26.1 \times 10^3/\text{ml}$ ) without significance. The bacterial numbers from

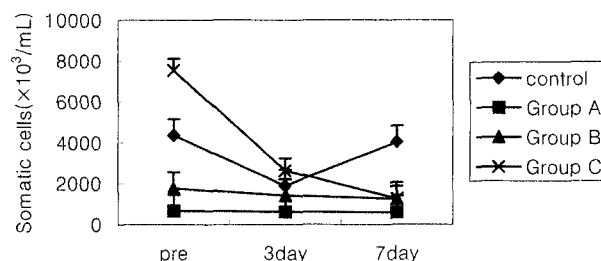


Fig 1. Changes in somatic cell numbers in milk after infusion with ozonated oil.

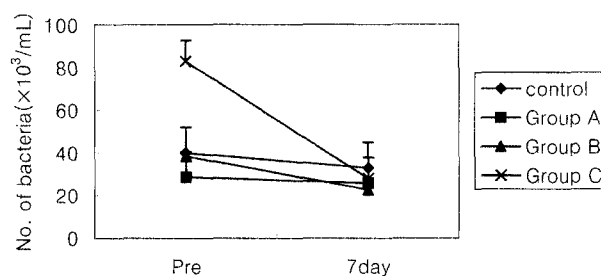


Fig 2. Changes in bacterial cell numbers in milk after infusion with ozonated oil.

experimental group B was low on day 7 ( $22.5 \pm 21.6 \times 10^3/\text{ml}$ ) after treatment with ozonated oil, compared with the pretreatment level ( $38.5 \pm 50.2 \times 10^3/\text{ml}$ ) without significance. The bacterial numbers in experimental group C showed a decrease on day 7 ( $27.8 \pm 32.5 \times 10^3/\text{ml}$ ) after treatment with ozonated oil, compared with the pretreatment count ( $83.0 \pm 101.6 \times 10^3/\text{ml}$ ) without significance.

## Discussion

Bovine mastitis is a major disease of dairy cows. The economical loss it causes is high. The disease has traditionally been treated with antibiotics. However, the appearance of resistant bacterial species and chronic mastitis make treatment difficult. Residual antibiotics in milk associated with long term use can cause serious public health problem. Accordingly, improved therapies for bovine mastitis are urgently needed<sup>6,11</sup>.

Ogata and Nagahata<sup>11</sup> reported that 9 out of 15 cows with acute clinical mastitis treated with ozone therapy did not require any antibiotics for recovery, based on the data of clinical symptoms, CMT score, and electronic conductivity of milk and somatic cell counts in milk. In addition, Kwon<sup>8</sup> pointed out that ozone gas therapy could be used as an alternative method for the treatment of chronic bovine mastitis based on the changes in the numbers of somatic cells and bacteria in milk.

Other researchers have described therapeutic effects of ozonated oils in human gynecological and skin diseases<sup>4,10</sup>. In the present study, ozonated oil was infused into the inflamed

quarters with bovine chronic mastitis. As a result, decreased somatic cell and bacterial numbers in milk were observed in the treatment groups. The somatic cells in group C especially showed a significant marked decrease, compared with the pretreatment value. We concluded that the decrease in numbers of milk somatic cells was caused by an anti-inflammatory action of the ozonated oil similar to that proven in the results of human diseases<sup>5</sup>.

It has been reported that Gram-negative bacteria including *E coli* are the most sensitive to the bactericidal action of ozone. Gram-positive bacteria such as *Staphylococcus aureus* are resistant to ozone. Long term treatment and a higher concentration of ozone are necessary for bactericidal action on Gram-positive bacteria<sup>19</sup>. Considering that elimination of bacteria was not completely accomplished in the present study and common causal agents of bovine mastitis are *E coli*, *Staphylococcus aureus*, *Streptococcus agalctiae* and *Streptococcus uberis* etc.<sup>2,11,12</sup>, it is evident that the applied concentrations and volumes of ozone are very important in clinical practice. An inhaled overdose of ozone can cause the alveolar damages in the lung<sup>12</sup>. However, medical ozone produced by using pure O<sub>2</sub> caused few side effects in human patients<sup>12</sup>. In the present study, 10 ml of ozonated oil were infused into the inflamed quarters. The most effective volumes of ozonated oil and treatment periods should be better defined by future investigation. The contained ozone concentration and stability in ozonated oil could not be determined here, more precise research about this should be performed. Further study on pathological changes by infusion with ozonated oil into the bovine mammary gland should be made in the near future.

### Conclusion

It was concluded that ozone therapy with ozonated oil might be effective for treatment of bovine chronic mastitis.

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## 젖소 유방염에 대한 Ozonated oil의 치료효과

조성남 · 유건주 · 이상은 · 홍민성 · 김덕환<sup>1</sup> · 김명철 · 조성환 · 전무형

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**요 약:** 충남 공주, 조치원, 연기 및 전북 익산 지역에서 사육 중인 만성유방염에 이환된 비유우 24두로부터 49분방을 선발하였다. 유방염에 이환된 49 분방은 대조군(항생제 투여군: 7분방) 및 실험군(ozonated oil 투여군: 42분방)으로 각각 구분하였다. 또한 실험군의 분방은 우유 중 체세포를 기준으로 실험군 A(체세포 수  $50-100 \times 10^4/\text{ml}$ : 10분방), 실험군 B(체세포 수  $100-300 \times 10^4/\text{ml}$ : 14분방) 및 실험군 C(체세포 수  $>300 \times 10^4/\text{ml}$ : 18분방)로 각각 구분하였다. 대조군의 분방은 항생제 감수성시험의 결과를 토대로 norfloxacin(10 g/tube)을 2회/1일, 3일간 유두내로 주입하였고, 또한 실험군의 분방에는 ozonated oil(200 ppm 농도, 3일간 폭기) 10 ml를 2회/1일, 3일간 유두내로 주입하였다. 대조군 및 실험군간 우유 중 체세포 수 및 세균수의 변화를 각각 비교 검토하였다. 그 결과 대조군의 분방은 처치 7일째 우유 중 체세포 수 및 세균수가 각각 감소 소견을 나타내었으나, 처치 전에 비하여 유의성이 인정되지 않았다. 한편 실험군의 분방은 처치 후 7일째 처치 전에 비하여 체세포 수 및 세균수의 감소소견을 각각 나타내었으며, 특히 실험군 B( $p < 0.01$ ) 및 실험군 C( $p < 0.01$ )에서 우유 중 체세포수는 처치 후 7일째에 처치 전에 비하여 각각 유의한 감소소견을 나타내었다. 그러나 대조군 및 실험군 양 군간에는 체세포 수 및 세균수의 변화에 있어 유의성이 인정되지 않았다. 이상의 결과를 종합해 볼 때, ozonated oil을 이용한 오존요법은 만성 유방염에 이환된 분방에 치료효과를 발휘할 수 있는 것으로 판단된다.

**주요어:** 치료효과, ozonated oil, 소, 유방염.