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 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), experimental group B (14 quarters), and experimental group C (18 quarters), according to the number of the somatic cells in their milk. The control group was treated with norfloxacin ointment based on the result of sensitivity, twice a day for 3 days. The experimental groups were received 10 mL of ozonated oils infused into the inflamed quarters 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 fewer 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 inflamed quarters afflicted with bovine mastitis might be effective. This therapy might also be used as an alternative to antibiotics for the treatment of bovine mastitis.

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. Ozone therapy was developed mainly in Germany: there are reports that it is effective in treating various human various diseases such as bacterial, viral and fungal diseases.

In clinical practice, ozonated water is used for its bactericidal and anti-inflammatory effects in dental diseases. Ozone gas therapy combined with O2 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.

Ogata and Nagahata 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 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 and bacterial cells in the milk were counted. The experimental groups experimental group A (10 quarters, somatic cell count: 50-100×10^4/ml), experimental group B (14 quarters, somatic cell counts: 100-300×10^4/ml) and experimental group C (18 quarters, somatic cell count: >300×10^4/ml).

**Preparation of ozonated Oil**

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

**Treatment**

The control group was treated with norfloxacin ointment (Nopazin, Daesung Microbiological Co., Korea), 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.

**Cell counts**

About 10 ml of milk samples were collected from each inflamed quarter under aseptic condition into sterilized conical tubes for somatic and bacterial cell counts. These were determined by use of an auto-counter (Fossmatic-90, 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±S.D..

**Results**

The effect of ozonated oil on somatic cell numbers in milk

The changes in somatic cell counts in milk are presented in Fig. 1. In the control group, cell numbers were lower on the third day (1,685.9±1,079.8×10^3/ml) and then slightly higher on day 7 (4,019.4±4,652.0×10^3/ml) compared with pretreatment level (4,358.0±2,753.9×10^3/ml). However, these differences were not significant.

Experimental group A showed a slight decrease on day 7 (595±541.9×10^3/ml) after treatment with ozonated oil, compared with the pretreatment number (662±195.8×10^3/ml), but this difference was not significant. Experimental group B’s somatic cells were decreased significantly on day 7 (1,258.0±823.9×10^3/ml) after treatment with ozonated oil, compared with the pretreatment level (1,762.1±601.9×10^3/ml) (p<0.01). Experimental group C showed a marked significant decrease on the day 7 (1,276.1±973.5×10^3/ml) after treatment with ozonated oil, compared with the pretreatment counts (7,515.9±3,604.0×10^3/ml, P<0.01). However, no significant differences in somatic cell numbers in milk between control and experimental groups were detected.

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

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

In experimental group A, bacterial counts were slightly lower on day 7 (25.7±38.2×10^3/ml) after treatment with ozonated oil, compared with the pretreatment counts (28.6±26.1×10^3/ml). This difference was not significant. The bacterial count from experimental group B was lower on day 7 (22.5±21.6×10^3/ml) after treatment with ozonated oil, compared with the pretreatment level (38.5±50.2×10^3/ml). This difference was not significant. Experimental group C showed a decrease on day 7 (27.8±32.5×10^3/ml) after treatment with ozonated oil, compared with the pretreatment count (83.0±101.6×10^3/ml),
though this difference was not significant. Significant differences in the change of milk bacterial numbers were not detected among groups.

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.

Ogata and Nagahata 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 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 and bacterial cells in milk, and on hematological findings.

Other researchers have described therapeutic effects of ozonated oils in human gynecological and skin diseases. However, no report on their use in veterinary clinical practice has been available until now. 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 experimental groups. Group C especially showed a significant marked decrease in the somatic cell count in milk compared with the pretreatment value for the group. We conclude that the decrease in numbers of somatic cells in the milk was caused by an anti-inflammatory action of the ozonated oil similar to that proven in the context of other human diseases. The therapeutic effect of ozonated oil on bovine mastitis has thus been clarified in veterinary clinical practice.

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 spore-forming *Staphylococcus aureus* are resistant to ozone. Long term treatment and a higher concentration of ozone are necessary for bactericidal action on spore-forming Gram-positive bacteria. 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 agalactiae* and *Streptococcus uberis*, etc., 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. However medical ozone produced by using pure O₂ caused few side effects in human patients. 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 oil's ozone concentration and stability of ozonated oil could not be determined here, but should be the subject of future work. Further study on pathological changes in the bovine mammary gland and the effects of infusion with ozonated oils should be made in dairy cows with bovine mastitis.

It was concluded that ozone therapy with ozonated oil applied inflamed quarters afflicted with bovine mastitis might be effective. This therapy might also be used as an alternative to antibiotics for the treatment of bovine mastitis.

Reference


Figure legends

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.