



RESEARCH ARTICLE

The effect of N-acetylcysteine on the treatment of clinical endometritis and pregnancy rate in dairy cows

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Özet

Tras B, Dinc DA, Uney K. Süt ineklerinde klinik endometritisin tedavisi ve gebelik oranı üzerine N-asetilsistein'in etkisi.

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Amaç: Bu çalışmada süt ineklerinde klinik endometritisin tedavisi ve gebelik oranı üzerine N-asetilsistein (NAS)'in etkisinin belirlenmesi amaçlandı.

Gereç ve Yöntem: Çalışma, ticari bir süt işletmesinde 50-60 günlük gönüllü bekleme süresinden sonra endometritisin klinik bulgularını gösteren 36 inek üzerinde gerçekleştirildi. Klinik endometritis, vajinada belirlenen uterus akıntısının değerlendirilmesiyle tanımlandı. İneklerden servikal svap örnekleri mikroorganizma ve tedavi için en etkili antibakteriyel ajanı belirlemek için toplandı. İnekler rastgele NAS tedavisi yapılan (NAS-T) ve yapılmayan (nNAS-T) olmak üzere iki gruba ayrıldı. NAS-T grubuna (n=18) %2'lik NAS uygulamasından 12 saat sonra amoksisilin trihidrat+potasyum klavulanat (3500 mg+875 mg) uygulaması yapıldı ve bu uygulama üç gün tekrar edildi. nNAS-T grubuna (n=18), NAS-T grubunda uygulanan tedavi protokolündeki NAS uygulaması yerine 100 mL serum fizyolojik uygulaması yapıldı. Tedavi oranı, tedaviyi takiben gerçekleşen ilk doğal östrusta klinik endometritisin bulgularını göstermeyen (vaginal muayenede şeffaf veya yarı şeffaf mukus) ineklerin oranı olarak tanımlandı. Hayvanlar tedaviden sonraki ilk doğal östrusta suni yolla tohumlandı.

Bulgular: NAS-T grubunda tedavi (%83.3) ve gebelik (%66.7) oranlarının nNAS-T grubuna (sırasıyla %55.5 ve %27.8) göre önemli derecede yüksek olduğu belirlendi (P<0.05).

Öneri: İneklerde klinik endometritisin tedavisinde NAS kullanımını, tedavi başarısını ve gebelik oranını artırabilir.

Anahtar kelimeler: İnek, klinik endometritis, N-asetilsistein, gebelik oranı

Abstract

Tras B, Dinc DA, Uney K. The effect of N-acetylcysteine on the treatment of clinical endometritis and pregnancy rate in dairy cows.

Aim: The objective of this study was to investigate the effect of N-acetylcysteine (NAC) on the treatment of the clinical endometritis and pregnancy rate in dairy cows.

Materials and Methods: This study was performed on 36 cows with clinical signs of endometritis after VWP (voluntary waiting period, 50-60 days) on a commercial dairy farm. The clinical endometritis was diagnosed by evaluation of uterine discharge detected in the vagina. Cervical swab samples were collected to determine microorganism and the most effective antibacterial agent for the treatment. Cows were randomly allocated to NAC treatment (NAC-T) and non-NAC treatment (nNAC-T) groups. The NAC-T group (n=18) received an intra-uterine infusion of amoxicillin trihydrate+potassium clavulanate (3500 mg+875 mg) in 100 mL dosages after 12 hours of an intrauterine infusion of 2% NAC during three days. Saline (total volume 100 mL) to the nNAC-T group (n=18) was given instead of NAC in the same protocol of the NAC-T group. The clinical cure rate was described as the percentage of cows with no signs of clinical endometritis (the clear or translucent mucus at vaginal examination), at the examination in first natural estrus following treatment. Animals were artificially inseminated following the first natural estrus after treatment.

Results: The clinical cure (83.3%) and pregnancy rates (66.7%) in the NAC-T group were significantly higher than those (55.5% and 27.8%, respectively) of nNAC-T group (P<0.05).

Conclusions: The usage of NAC for the treatment of the clinical endometritis in cows may increase the success of therapy and the improvement of pregnancy rate.

Keywords: Cow, clinical endometritis, N-Acetylcysteine, pregnancy rate





Introduction

The clinical endometritis of dairy cows influences the reproductive performance and causes economic losses (Leblanc et al 2006, Azawi 2008). Also, it causes the reduction of the body condition of dairy cows, which in turn suppresses fertility (Runciman et al 2008). It has been determined that it has the prevalence ranging from 5.0 to >30% affecting about 20.0% of lactating dairy cows (Leblanc et al 2002, McDougall et al 2007, Galvao et al 2009). In clinical endometritis, the purulent (>50% pus) or mucopurulent (50% pus, 50% mucus) uterine discharges is determined in the vagina after 21 days and more postpartum or after 26 days postpartum, respectively, and systemic signs are not observed (Sheldon et al 2006). It is usually diagnosed by evaluation of uterine discharge detected in the vagina (Leblanc et al 2002).

Abscess formation and other inflammation products throughout the infection are capable to alter pharmacokinetics and pharmacodynamics of antibacterial agents. Abscess leads up to an acidic and anaerobic environment, therefore it is usual to see dormant bacteria and enzymes such as beta-lactamase. The drainage of abscess plays a key role to enhance the drug efficiency due to the decreasing efficiency of alkaline antibacterial agents such as aminoglycosides (Wagner et al 2006). In uterine infections, a uterine discharge including abscess material and tissue fragments which may decrease the drug efficiency is commonly existent. Moreover, *Trueperella (Actinomyces) pyogenes*, a pyogenic bacterium, is isolated together with other pathogenic bacteria in all uterine infections (Azawi 2008). Pyogenic bacteria causing abscess formation, such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* play a role in uterine infections. Importantly, *Klebsiella* spp. and *S. aureus* induce focal micro-abscess formation in the tissue (Smith and Risco 2005, Le Blanc 2008, Tras and Uney 2011).

Staphylococcus epidermidis, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus* spp., *E. coli*, *Helicobacter pylori*, yeasts and fungi cause a biofilm formation which may impair the drug exposure, contribute to chronic infection and drug resistance. The biofilm is extracellular polymeric substances and generally composed of DNA, proteins, polysaccharides and lipids. Some antibacterial agents such as macrolides and clindamycin are not capable of treating the infection against the bacteria producing biofilm, unlike these agents have more efficiency than other bacterial agents (Le Blanc and Causey 2009, Tras and Uney 2011). It has been reported that the combination of antibiofilm and antibacterial agents may induce the drug synergism due to the increasing sensitivity of biofilm producing bacteria to antibacterial agent by deformation of biofilm (Cammarato et al 2012).

N-acetylcystein (NAC) is a derivative of L-cystein and the pre-

cursor of glutathione. NAC acting to reduce mucus viscosity by splitting disulfide bonds linking proteins present in the mucoproteins removes purulent or non-purulent secretions from the body. It is not ineffective on fibrin and living tissue and its effect does not decrease in the presence of DNA. NAC is used for mucolytic therapy and in the treatment of obstructive pulmonary diseases, tuberculosis and cystic fibrosis in human medicine. In addition to the mucolytic effect of NAC, it also has antioxidant (Boothe 2001, AHFS Drug Information 2010), cytoprotective and antiinflammatory effects (Kim et al 2000, Olivos-Garcia et al 2007, Tsai et al 2010, Melkus et al 2012). Moreover, it has been indicated that NAC has preventive and detractive effects on biofilm formation and bactericidal effects at the concentration of 80 mg/mL by deformation of uncolonized bacteria (Cammarato et al 2012). NAC promotes the entrance of antibacterial agents into the mucus and its mucolytic effect does not depend upon the route of administration (Boothe 2001). It has a good drug distribution potential all over the body including the uterus. Milk or meat withdrawal times of NAC are not required following its administration as its legal withdrawal time according to the data of EMEA is 0 days (CVMP 1996). Several authors stated that the intrauterine NAC administration in mares with endometritis may be beneficial in improving pregnancy and treatment rates and NAC has no adverse effects on the endometrial function (Gores-Lindholm et al 2009, Le Blanc 2010, Melkus et al 2012, Witte et al 2012).

In consideration of our knowledge about NAC and uterine infections, this study was carried out to develop the hypothesis that NAC might be useful to treat the animals with clinical endometritis and might improve the reproductive parameters such as pregnancy rate, calving to pregnancy interval and calving interval.

Materials and Methods

This study was carried out on cows with clinical signs of endometritis after VWP (voluntary waiting period/50-60 days) in a private commercial dairy farm (Tekirdag/Turkey). The clinical endometritis was diagnosed by evaluation of uterine discharge [purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) discharge] detected in the vagina, which is confirmed during cervical swab samples collected from each cow to determine microorganism and the most effective antibacterial agent for the treatment. Accordingly, a sterile cervical cotton swab was passed as far into the cervix as possible via a Polansky vaginal speculum. Cervical swab samples were transferred to the microbiology laboratory for isolation and identification of bacteria and then, antibiogram was performed for each sample. In the cervical swab samples of cows, *Corynebacterium* spp., *S. aureus*, *E. coli* and *Bacillus* spp. were isolated. The antibiogram profile of bacterial isolates showed that all bacterial isolates were sensitive to amoxicillin trihydrate + potassium clavulanate (Table 1).



Table 1. Antibiotic susceptibilities of bacteria species isolated from cervical swab specimens of dairy cows.

| Antibiotics | Isolated Bacteria | | | |
|--------------------------------|-----------------------------|------------------------------|-------------------------|----------------------|
| | <i>Corynebacterium</i> ssp. | <i>Staphylococcus aureus</i> | <i>Escherichia coli</i> | <i>Bacillus</i> ssp. |
| Amoxicillin + Clavulanic acid | +++ | +++ | ++ | +++ |
| Ampicillin | ++ | ++ | - | +++ |
| Cephalexin | + | + | + | ++ |
| Ceftriaxone | +++ | ++ | +++ | +++ |
| Enrofloxacin | +++ | ++ | ++ | +++ |
| Erythromycin | + | + | +++ | ++ |
| Gentamycin | - | - | ++ | + |
| Oxytetracycline | + | - | + | ++ |
| Penicillin G | ++ | +++ | - | +++ |
| Trimethoprim+Sulphamethoxazole | +++ | ++ | +++ | ++ |

Cows were randomly allocated to NAC treatment (NAC-T) and non-NAC treatment (nNAC-T) groups. Cows in the NAC-T group (n=18) received an intrauterine infusion of amoxicillin trihydrate+potassium clavulanate (3500 mg+875 mg) in 100 mL dosages after 12 hours of an intrauterine infusion of 2% NAC during three days. Saline (total volume 100 mL) to cows in the nNAC-T group (n=18) was given instead of NAC in the same protocol of the NAC-T group. The clinical cure rate was described as the percentage of cows with no signs of clinical endometritis (the clear or translucent mucus at vaginal examination) at the examination in first natural estrus following the treatment. These cows were artificially inseminated. The Ethics Committee of the Faculty of Veterinary Medicine (University of Selcuk, Konya, Turkey, report no: 2011/068) approved the study protocol. The clinical cure and pregnancy rates between groups were compared using the Two Proportions test.

Results

The proportion of cows with no signs of clinical endometritis (the clear or translucent mucus at vaginal examination) at the examination in first natural estrus following the treatment were found different between groups ($P<0.05$). The clinical cure rate in NAC-T group (83.3%) was significantly higher than nNAC-T group (55.5%). Pregnancy rates of the cows artificially inseminated following the first natural estrus was found significantly different between NAC-T (66.7%) and nNAC-T (27.8%) groups ($P<0.05$).

Discussion

In cows with clinical endometritis, the efficacy of NAC was firstly evaluated in this study. We showed that NAC administration is favorable effects on the treatment and the pregnancy rate in cows with clinical endometritis. Results of the clinical investigation involved an intra-uterine infusion of a 3.3% solution of NAC in repeat breeder mares have indicated

that pregnancy rates of NAC treated mares were 81 and 85% for different years (Le Blanc 2012). However, the pregnancy rate of NAC treated cows (66.7%) in this study was relatively lower than that determined in mares (Le Blanc 2012). It has been stated that a 0.3% solution of NAC had no influence on the oxidative burst of equine neutrophils but a 3% solution decreased (Le Blanc 2012). The difference in pregnancy rates between cows and mares treated with NAC can be attributed to differences in the doses of NAC used in treatment. It has been stated that the NAC use in the treatment of endometritis increases the treatment success and pregnancy rates with the removal of excessive mucus and biofilm, changing the viscosity of the mucus and improving sperm transport with excessively viscous mucous secretions (Le Blanc 2010, 2012). In addition, it is proposed that the preventive, anti-inflammatory and immunomodulatory potential of NAC on focal abscess formation and oxidative stress may be contributed to increased pregnancy rates. Moreover, it is indicated that the combination of NAC with antibacterial agents may increase the treatment success of the endometritis and conception rate when our results are compared to treatment results obtained on this farm and veterinary practice.

In a report from human medicine, it has been indicated that 24 patients suffering from upper respiratory tract infection caused by biofilm producing bacteria were treated with the combination of antibacterial agent and NAC and 87.5% of these patients were successfully treated (Macchi et al 2006). Moreover, in another study, it has been reported that 40 untreated patients following at least four therapies for *Helicobacter pylori* were divided into two groups to evaluate the efficiency of NAC. *Helicobacter pylori* in the first group treated with the combination of antibacterial agent and NAC were eradicated at the rate of 65%, whereas the eradication rate was 20% in the second group treated with only antibacterial agent. The authors have postulated that the high treatment rate in the first group might be caused due to the mucolytic effect of NAC. It has also been suggested that NAC might play





a key role in the prevention of biofilm formation, decreasing the oxidative stress and the modulation of immune response (Cammarota et al 2010).

Conclusions

It is suggested that NAC may be beneficial for treating genital tract infections presenting purulent discharge such as clinical endometritis due to the clinical features of NAC for cost saving, and has got no illegal residues in edible tissues of farm animals. Further studies included the large number of animals in different dosage regimens (parenteral route of administration) that are needed to clarify the efficiency of NAC for this indication.

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