Biodistribution of fluorescent nanoparticles in goat mammary gland - Gern J.C.[1]*, Guimarães A.S.[1], Andrade P.V.D.[1,2], Brandi R.R.[1,3], Silva S.R.[1,3], Lange C.C.[1], Brandão H.M.[1]*

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The treatment of mastitis in goats are not always successful and new therapies must be tested in order to better control this disease, which alters milk composition and reduces its quality. In this study we evaluated the glandular tissue of lactating goats in order to determine the biodistribution of fluorescent nanoparticles. Four lactating goats were slaughtered 48 hours after intramammary administration of a solution containing nanoparticles labeled with fluorescein. The mammary glands were removed and histologic sections of the glands were made for observation of fluorescence, which is indicative of the presence of the nanoparticles. The results showed a wide distribution of fluorescence in both normal tissues and those with infectious process, 48 hours after application, reaching more dorsal areas and the interior of the gland abscesses. This study showed that the nanoparticles have spread throughout the mammary tissue and can potentially be used in intramammary formulations for this species.

Key-words: goat mastitis, intramammary therapy, fluorescent nanoparticles

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Biodistribution of chitosan nanoparticles in goat mammary gland

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INTRODUCTION

The mastitis is one of the main factors affecting the composition of milk. Intramammary infections cause a decrease in milk production and promote large increases in the somatic cell count and the concentration of plasma components originating from the milk, beyond the reduction of products synthesized by the gland, mainly casein and lactose [1].In dairy goats, among the main agents in milk from animals with mammary infections are *Streptococcus agalactiae* and *Staphylococcus aureus* [2], but many other agents may also be involved, such as coagulase-negative staphylococci, mycoplasma and environmental agents such as coliform [3] or caused by *S. aureus, Streptococci*, enterobacteria and *Actinomyces pyogenes* [2]. New therapies have been developed to prevent and treat bovine mastitis and it is possible that these therapies may also be used in treatment and control of intra-mammary infections in goats. In this study, the objective was to evaluate, in mammary glands of lactating goats, the distribution of nanoparticles with potential use for a carrier of antibiotics in healthy tissue or natural infection.

MATERIALS AND METHODS

Chitosan conjugated to fluorescein- isothiocyanate (FITC) was obtained from 100 mg of FITC solubilized in 150 ml of dried methanol to which was added 100 mL of 1% chitosan in 0.1 M CH 3 COOH. After three hours of reaction in the dark environment, the chitosan was precipitated by raising the pH. For removal of free FITC, the precipitate was cycled repeated washing and centrifugation (40.000 G during 10 min) until the lack of fluorescence in supernatant (NanoDrop Spectrometer Thermo Scientific). After solubilization in 80 ml of 0.1M CH 3 COOH, chitosan-FITC was dialysed for three days in the dark environment, on 5L of distilled water, water was replaced daily [5].

Nanoparticles were prepared by ionotropic gelation. To this, it was followed by the ratio of 2 mL of a TPP-containing sodium chloride in 4 ml of a chitosan solution (acetic acid 1%) with magnetic stirring [6,7]. Then the nanoparticles were lyophilized and then characterized by particle size and zeta potential (Zetasizer NANO ZS,Malvern Instruments Limited).

Animal experimentation was conducted with four multiparous lactating goats. The health status of each mammary gland was determined by means of bacterial isolates in duplicate [8,9].

A healthy goat received no treatment (negative control), while the others received in the two glands, one injection intramammary 300 mg of nanoparticles chitosan-FITC.

The animals were slaughtered 48 hours after application of nanoparticles for removal of the mammary gland. Glandular tissue samples were collected 5, 10 and 15cm from the base of the teat for the preparation of histological sections on a freezing microtome (HYRAX-25, Carl Zeiss) and subsequent evaluation by fluorescence microscopy (Axioplan I Carl Zeiss).

RESULTS AND DISCUSSION

Chitosan nanoparticles were presented cationic, with zeta potential +23,5 mV, in pH 7,2. The average particle size was 220.2 nm. In infected mammary quarters were isolated *Actinomyces pyogenes*(01), *Staphylococcus* coagulase negativo (02) e *Staphylococcus aureus* (01). The other quarters were healthy.

Qualitatively, there was indication that cationic nanoparticles were distributed to the dorsal part of the mammary glands, both in healthy and infected glands, being evidenced by the increase in fluorescence, mainly in the glandular ducts.

The high fluorescence intensity in ducts justified by this structure to be connected to the gland cisterns, local deposition of nanoparticles. Abscesses difficult to increase the antibiotic concentration in this compartment to be coated and having rich tissue inflammatory infiltrate[10]. The high fluorescence in the region rich in inflammatory cells indicates the targeting of nanoparticles to the biological compartment, which can be explained by the cationic nature of the nanoparticle, which promotes phagocytosis [11].

CONCLUSION

Chitosan nanoparticles exhibit similar biodistribution, both in healthy glands and in glands with mastitis. In addition, it was identified direction of the nanoparticles to regions where conventional antibiotics typically have limited access, which may favor its use as a carrier for pharmaceuticals for the treatment of mastitis.

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