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Case Report

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Experimental treatment of recurrent otitis externa

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Abstract
The aim of this research is to determine the effect of the hybrid material based on polyvinyl alcohol and silver nanoparticles (PVA/AgNps) in the treatment of the otitis externa as an additional component in the commercial product "Betazon Trio". It was established that the experimental creamy formula with silver concentration 600 mg/L is suitable for recovery of the microbial homeostasis when it is administrated once daily in dose 1 ml over a period of 14 days.
Keywords: Atopic dermatitis, Food allergy, Otitis externa, Silver nanoparticles.

Introduction

Otitis externa, is an inflammation of the pinna and external ear canal. It may occur as a result of a predisposing factor and also accompanying a primary problem. Very often the organism forms antibodies from class IgE in a generalized response to a specific allergen and by this way the outer ear canal is involved as well. IgE antibodies coat mast cells (Olivry *et al*., 2010). Their role is to protect the organism by releasing chemicals (mast cells mediators) when a foreign protein enters the organism. In allergic animals this entire system is oversensitive and the release of mast cell mediators in the skin occurs inappropriately to apparently innocuous substances (atopic dermatitis, food hypersensitivity). Therefore, the skin barrier gets wrecked and becomes an easy target for bacterial multiplication. Unfortunately, nowadays this is one of the main and common problems in companion animals. There is an increased trend for reduced usage of antibiotics, veterinary pharmaceuticals, and hormones in animal production. Recently, a public consultation has been started regarding the request of the European Medicines Agency from the European Commission for scientific advice on the impact of public and animal health regarding the use of antibiotics for animals.

It was found that out of all metals with antimicrobial properties, silver had the most effective antibacterial action and lower toxicity to animal cells (Galdiero *et al*., 2011; Rai *et al*., 2014). Their simple synthesis and highly observed effective antibacterial activity make them a very attractive form of silver administration. Numerous studies on the properties and possibilities of the application (Pencheva *et al*., 2010, 2011, 2012) of the hybrid PVA/AgNps material, proved its advantages for a topical therapeutic use in the experimental creamy formula with silver, suitable for recovery of the microbial homeostasis in animals.

The aim of this research is to determine the effect of the hybrid PVA/AgNps material for the treatment of otitis externa as an additional component in the commercial product "Betazon Trio".

Case details

Subject of the experimental treatment is a ten years old Samoyed male dog named Ian. Frequent recurrent ear infection in the last few years leads to discomfort in the life of the dog. In the last instance the dog was affected by an outer ear infection due to allergen and it was submitted to an experimental treatment. The clinical signs were painful ears, unpleasant odor and itching. The examination established waxy yellowish discharge, filling the ear canal and running down the coat (Fig. 1). In the otoscope view the canal and the pinna were inflamed.

The treatment was performed using the following materials: silver nanoparticles stabilized in polyvinyl alcohol (PVA/AgNps) with a silver concentration of 2000 mg/L (ICP-OES). The preparation of PVA/AgNps is described earlier (Pencheva *et al*., 2012). Betazon Trio (Antibiotic, Razgrad, Bulgaria) (Betamethasone 0.500 mg/g plus Clotrimazole 10 mg/g plus Gentamicin 1 mg/g ointment); Epi-Otic Cleaner (Virbac); Depedin (Veyx-Pharma GmbH, Germany) (1 ml contains: 7.5 mg of prednisolone acetate, dexamethasone, 2.5 mg, 0.1 mg of ethyl 4-hydroxybenzoate, 0.1 mg of propyl 4-hydroxybenzoate), Eukanuba dermatosis.

The treatment of the dog was complex since it was necessary to overcome the bacterial infection and to reduce to a minimal re-exposure to the allergen. The destroyed skin barrier need to be reconstructed and the activity of the cells releasing mediators need

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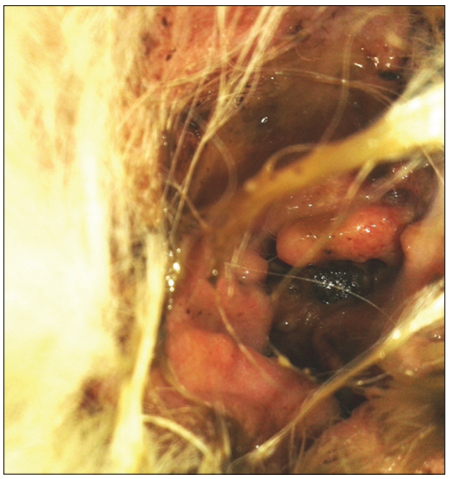

Fig. 1. Otitis externa of Ian, a 10 years old male Samoyed dog.

to be reduced. Therefore, the treatment of dog Ian was combined. The ears were treated 14 days with experimentally prepared material containing 5 ml of PVA/AgNps hybrid material (silver concentration 2000 mg/L) to 11.5 ml of the commercial product "Betazon Trio" - "Antibiotic-Razgrad".

The sample from the ear discharge was stained with Diff Quick and Gram stain and this revealed the presence of Gram-positive coccus-like bacteria. In the microbiological test of the ear wax swab, the presence of pathogenic microbes was not established. The predominant isolates were identified with conventional microbiological methods of identification and the software program "PIB" (Probabilistic Identification of Bacteria). *Staphylococcus capitis* and *Micrococcus luteus* were with a semi-quantitative evaluation considered as significant. Based on our results, we hypothesized a disturbed microbial homeostasis due to allergic irritation.

Estimation of cytotoxicity was essential before starting the application of one new substance in order to evaluate it as a potential anti-bacterial agent. To assess the cytotoxical *in vitro* effect of PVA/AgNps, Madin-Darby canine kidney cell line (MDCK) was used for testing. Cell viability was estimated by a modification of the MTT assay (Mosmann, 1983), which determines the metabolically active mitochondria of cells. Cells were seeded in 96-well microplates (Nunclon) at a concentration of 5×10^4 cells/well. Confluent monolayers were washed, covered with media containing the PVA/AgNps in concentrations from 70 mg/ml to 0.0005 mg/ml and cultured at 37°C for 24h and 48h. Cells grown in medium without the compound served as a control. After 48h incubation, the medium was replaced with MTT (Sigma-Aldrich) and dissolved at a final concentration of 5 mg/ml in serum-free medium, for further 4h incubation. Then, the MTT-formazan product was solubilised in ethanol: DMSO (1:1), and the optical density was measured at a test wave length of 540 nm maximal nontoxic concentration (MNC) was expressed as a % from the control cells taken as 100%. Using dose-response curves the concentration of tested compound able to reduce cell viability on the 24h and 48h with 50% (CD_{50}) was determined. Each experiment was performed in triplicate. The maximal concentration, which altered neither the morphology of monolayer nor the cell survival rate, was recognized as MNC. The CD_{50} of tested compound was calculated from the dose-response curves.

Both MNC and CD_{50} values were evaluated simultaneously by morphological and by cell survival criteria. According to both criteria silver nanoparticles in polyvinyl alcohol PVA/AgNps exhibited a low cytotoxicity in a concentration range from 0.0005µg/ml to10 µg/ml. This result was dose-dependent manner. The dose – response curves obtained after 24h and 48h culturing in modified media are presented in Fig. 2. Based on the data from cytotoxicity experiments we calculated the CD_{50} to MNC ratio – therapeutic efficacy or index (TE, TI). The ratio characterizes the tolerable concentration range in which the particular compound could be applied avoiding significant cell alterations. According to literature (Von Roemeling, 1991) any compound which is with TI greater than 3 is considered to be a promising chemotherapeutic agent. In our case the PVA/AgNps has a TI = 90×10^3. That puts it as a very promising agent with good tolerance and cell toxicity. These data gave us further reason to use the preparation for topical treatment for bacterial infections *in vivo* using a laboratory model dog.

Discussion

Otitis in dogs is a common problem and during the warmer months the frequency of ear infections increased. Reasons for development of this condition are varied and often predisposing factor lies at the root (dropping ears, often get water into, foreign bodies, parasites, tumor or allergen). All this leads to increased secretion of underlying auricular glands and subsequent modification of the normal microflora. Changed environment becomes conducive to the multiplication of various kinds of microorganisms and fungi (Harvey *et al*., 2001).

The product "Betazon trio" is a combination of a corticosteroid, antibiotic and antifungal elements. Betamezon corticosteroid helps to reduce swelling and soothe the mucous membrane in the presence of an allergen or after removal of the fallen foreign body. Gentamicin is an antibiotic with activity mainly against gram negative, and less against gram positive organisms. Included Clotrimazole has a well pronounced action against *Malassezia* spp. After the adding of the PVA/AgNps toward the experimental cream the received final concentration of gentamicin is even more insignificant

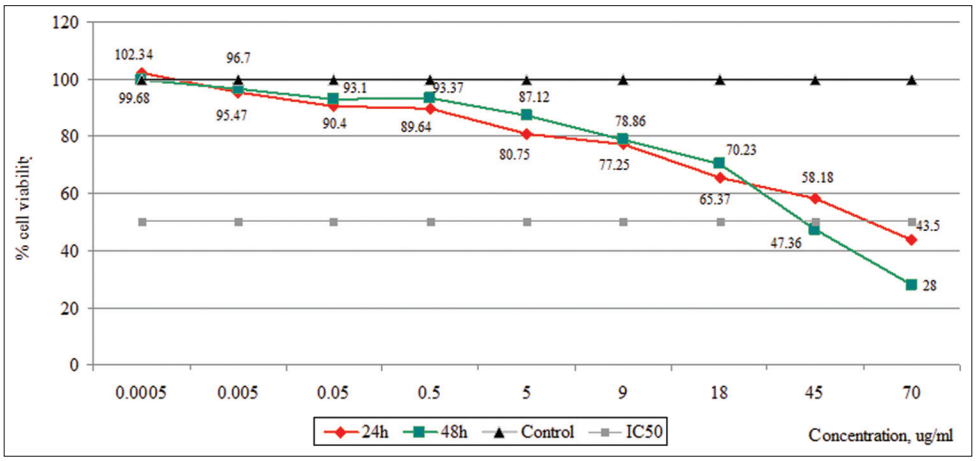

Fig. 2. Cytotoxic activities of PVA/Ag Nps on MDCK cell line at 24h and 48h.

and the antibacterial effect is result almost only on the bactericidal properties of the hybrid material. The aim was also to broaden the antifungal spectrum, include *Malassezia* spp. (Crespo *et al*., 2002). Combining the hybrid material with the commercial product is expected to increase the bactericide, fungicide and anti-inflammatory effects (Tallarida, 2001). The astringent action of the silver nanoparticles will assist in the mucus drying and will help for fast healing if ulcers are present during the ear infection (Rai *et al*., 2014). The main reason for the development of otitis media in our case was an allergen, which as a result of a breach of the skin barrier makes the environment in the ear canal suitable for infection. The combination of "Betazon Trio" with hybrid material had a positive effect on treatment. It reduced the reaction to the allergen and limit the amount of amplified micro-organisms through enhanced by silver nanoparticles effect of gentamicin. This confirms that the combination would be suitable also for other types of ear infections with bacterial, fungal or allergic nature. Synthesis and biological activity of hybrid materials are tested in numerous of *in vitro* and some *in vivo* studies (Pencheva *et al*., 2010, 2011, 2012). The determined value of the MBC for most of the tested in advance Gram-positive and Gram-negative bacteria and the value of MFC for some fungal strains (Pencheva *et al*., 2012) was under the value of established CD_{50} for MDCK cell line. The experimental ointment with creamy consistency is applied with a syringe in a volume 1 ml once a day in each ear canal after cleaning the ear with "Epi-Otic Cleaner". The preparation contains 0.2% salicylic acid which helps for the cleaning of the ear canal, removing crusts and wax. So after the cleaning, the ear canal got ready for the treatment with the experimental ointment. To reduce the activity of the cells releasing mediators was included also corticosteroids medicine "Depedin". It was administrated intramuscular in 48 hours with decline dosage calculated on the basis of the Prednisolone as follows – 1st day 0.5 mg/kg, 2nd day 0.3 mg/kg and 3rd day 0.2 mg/kg. Furthermore, it was established that the reason for the allergic state was faulty diet. The food was changed with hypoallergenic, we used the commercial food "Eukanuba Dermatosis" on the 5th day was established improvement. The discharges, the pain and the odor were significantly reduced. The duration of treatment course with experimentally added hybrid material was 14 days.

The treatment was successful. The experimental innovative creamy product with added PVA/AgNps in silver concentration of 600 mg/L into the commercial product "Betazon Trio" confirmed our expectation. The microbial homeostasis was repaired and it provided positive effect of the outer ear canal infection when the treatment was applied once daily over the period of 14 days.

Statement of novelty

For the first time, the well investigated hybrid material based on polyvinyl alcohol and silver nanoparticles (PVA/AgNps) was tested clinically on a mammal. The case is specific because recurrent otitis occurred as a result of allergic reactions and impaired microbial homeostasis.

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