e - ISSN - 2349 - 8005

INTERNATIONAL JOURNAL OF ADVANCES IN CASE REPORTS

Journal homepage: www.mcmed.us/journal/ijacr

ALTERATION OF THE BRONCHIOLI AND SMALL PULMONARY ARTERIES IN A PUPPY TREATED WITH SHAMPOO CONTAINING CHLORHEXIDINE - A CASE REPORT

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Article Info
Received 15/12/2014
Revised 27/12/2014
Accepted 2/01/2015

Key words:
Chlorhexidine - Dog - Lung – Alteration - Histopathology.

ABSTRACT
Four months old Yorkshire terrier suffered from *seborrhea oleosa,* and therefore was repeatedly, twice a week, treated with shampoo containing chlorhexidine diacetate. No adverse symptoms were noticed after first three baths. Immediately after the fourth bath the animal suddenly died. Necropsy disclosed only acute hyperaemia of the lung and mild dilatation of the both cardiac ventricles. Histological examination revealed hydropic degeneration and mild hyperplasia of the epithelial cells in the bronchioli. Plump to cuboidal endothelial cells, marked edema and hyperplasia of the subendothelial tissue, and hypertrophy and hyperplasia of the smooth muscle cells of the tunica media that caused stenosis or obliteration of lumen were present in the arteries of small diameter, situated in the neighborhood of the bronchi and bronchioli.

INTRODUCTION

Chlorhexidine is a disinfectant that is used in veterinary medicine for cleansing wounds, skin, instruments and equipment. In concentrations above 100µg/ml it is bactericidal, virucidal, and fungicidal, causing cell wall decomposition and leading to the loss of the cell's components [1]. Chlorhexidine binds strongly to skin and mucosa and therefore it is poorly absorbed after oral or topical application. In combined 6-month and 12-month safety studies, two dogs had to be euthanized due to bronchopneumonia. Irritation and redness of the conjunctiva, nausea and stomach pain after contact with the substance, irritation of the throat with feeling of tightness in the chest after inhalation were recorded in human beings [2]. According to California Department of Pesticide Regulation Report [3] the acute health risks from exposure to chlorhexidine gluconate are minimal due to its low mammalian toxicity. This report present a case of sudden death of a puppy that occurred shortly after repeated washing its hair with shampoo containing chlorhexidine diacetate.

MATERIALS AND METHODS

A female Yorkshire terriere, four months of age, about 2 kg of body weight, suffered from *seborrhea oleosa*. Veterinary physician recommended repeated bath in canine shampoo containing 5 mg/ml chlorhexidine diacetate. No other drug was used for therapy of the dog. The puppy was treated with the shampoo twice a week. During first three baths the owner did not noticed any adverse reactions. However, immediately after the fourth bath the animal suddenly died.

A complete necropsy was performed about four hours after the death and disclosed only acute hyperaemia of the lung and mild dilatation of the both cardiac ventricles. No developmental anomalies or macroscopically apparent pathological lesions in other organs and pleural and peritoneal cavities were observed. Samples of the cerebral hemisphere, brain stem, cerebellum, pons, heart, lung, liver and kidneys were collected for histopathological examination.

The samples were fixed in 10% neutral buffered formalin and processed by the common paraffin technique. Histological sections 5 µm thick, were stained with hematoxylin and eosin. Sections of the lung were in addition stained with PAS and with aldehydfuchsine-orcein-light green (AFOLG).

RESULTS

Histological examination revealed pathological changes only in the lung. They consisted in moderate, acute congestion in the capillaries and in the veins of small diameter. Hydropic degeneration and disruption of apical pole was diagnosed in epithelial cells of the bronchioli. Figure 1 In some of the bronchioli also mild hyperplasia of the epithelium was observed. PAS reaction did not prove mucin in vacuolated epithelial cells. Accumulation of alveolar macrophages with foamy cytoplasm was observed around some of the affected bronchioli. Epithelium in the bronchi was not damaged. Plump to cuboidal endothelial cells, marked edema and hyperplasia of the subendothelial tissue, hypertrophy and hyperplasia of the smooth muscle cells of the tunica media causing stenosis or obliteration of the lumen were observed in the arteries of small diameter situated in the neighborhood of the bronchi and bronchiole. Oedema of the adventitia, associated with lymphocytic and histiocytic infiltration were present in majority of the affected arteries. Figure 2, 3 Veins and venules were not damaged. Staining with AFOLG demonstrated intact both *lamina elastica interna* and *lamina elastica externa*. Figure 4.

Figure 1. Vacuolar degeneration and disruption of apical pole of epithelial cells in the bronchiole (arrow). Staining with HE

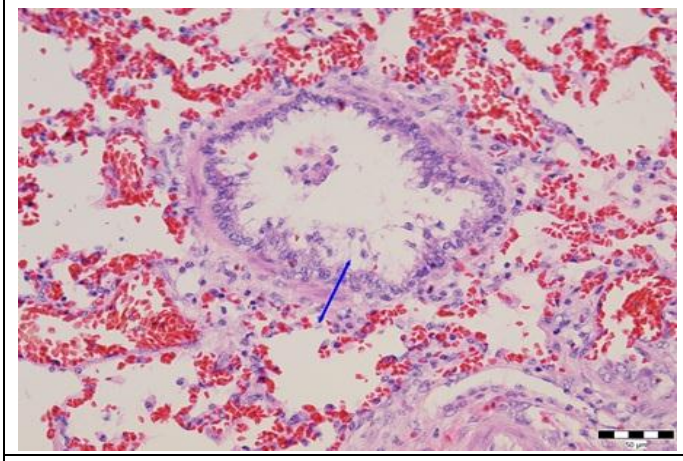


Figure 2. Acute venostatis, oedema of the subendothelial tissue, oedema, hyperplasia, and inflammatory cellulation in the adventitia of small arteries (arrows) situated in proximity of the bronchioli. Staining with HE

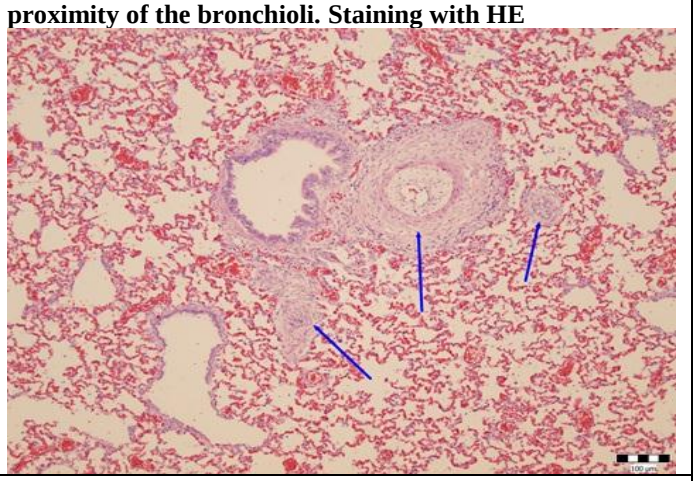


Figure 3. Edema of the subendothelial tissue (blue arrow), edema , hyperplasia and mild inflammatory cellulation of the adventitia. Obliteration of arterial lumen (red arrows) Staining with HE

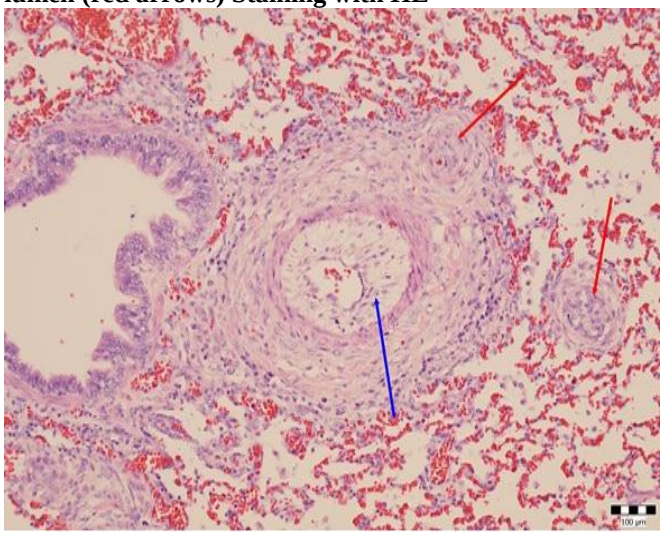
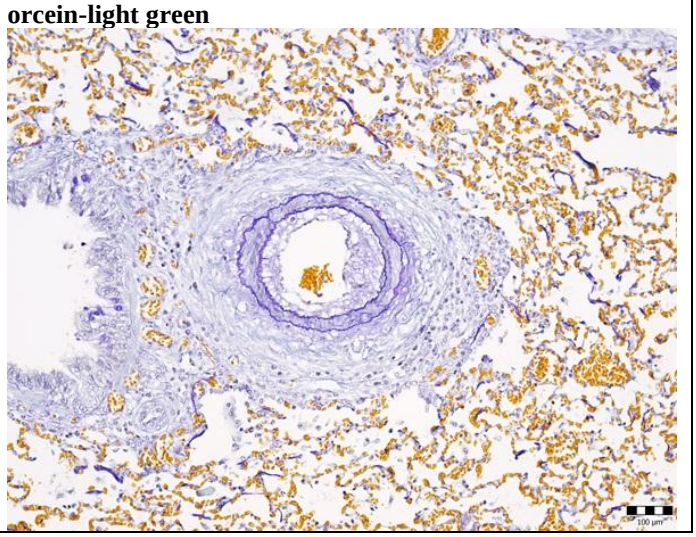


Figure 4. *Lamina elastica interna* and *lamina elastic externa* are unaffected. Hyperplasia of the subendothelial tissue and edema with inflammatory cellulation of the adventicia are present. Staining with aldehydefuchsine-orcein-light green



DISCUSSION

Accidental intra-airway exposure of dogs to pure oleic acid causing *brochiolitis obliterans* and bronchopneumonia was described by Li et al [4]. In the present case the lesions were more subtle, restricted to epithelium of the bronchioli and to small diameter arteries in their proximity. Cankaya et al [5] proved that repeated topical application of chlorhexidine to the rabbit nasal mucosa induced neutrophil infiltration, ciliary loss and metaplasia of epithelium. Yuca et al [6] noted hyperplasia, fibrosis, low-grade dysplasia, congestion or edema of the oral mucosa in mice after repeated topical application of three antibacterial products, including chlorhexidine.

The epithelial lining of the bronchiolar region is exquisitely susceptible to injury by infection or noninfectious agents, including toxic substances. Presumably this is due to high vulnerability of the bronchiolar epithelium to oxidants and free radicals, owing to presence of Clara cells that are rich in mixed-function oxidases, able to locally generate toxic metabolites, and also due to tendency of pulmonary alveolar macrophages and leukocytes to accumulate in this region [7]. The endothelial cells represent a macromolecular barrier and are important in maintaining vessel integrity. Endothelial cells are primary targets for the actions of pro-inflammatory cytokines such as tumor necrosis factor (TNF). TNF normally acts as an activator of endothelial cells but may change from an activator to a killer when it is combined with agents that release ceramide, such as UV irradiation or cytotoxic drugs [8]. In rats treated with the plant toxin monocrotaline, the endothelial cells were an important target [9]. The smooth muscle cells of the media are another target of chemically induced vascular injury [10]. In rats treated with allylamine a smooth muscle hypertrophy and proliferation of the subendothelial connective tissue were recorded in the medium-sized arteries. Acute endothelial injury and fibrocellular intimal obliteration of arteries, veins, and lymphatics were documented in intermediate and late-stage of adult patients suffering from respiratory distress syndrome [11]. In the presented case the lesions in arteries were similar to ones in human respiratory distress syndrome, however they were restricted only to small arteries. Administration of allylamine to dogs caused progressive edema, necrosis, medial hypertrophy, and subintimal proliferation in small and medium muscular arteries in the lung [12]. With exception of necrosis, the vascular lesions in our puppy were almost identical and vessels of the same type were affected.

CONCLUSION

The treatment of the dog by shampoo containing chlorhexidine diacetate was undoubtedly the reason of alteration of the lung and the sudden death immediately after the fourth bath. Repeated inhalation of small quantities of shampoo foam was very probably instrumental in pathogenesis of the pathological process. There was no evidence of accidental aspiration of the liquid during the last bath and development of histologically diagnosed pathological changes required several days.

ACKNOWLEDGEMENT

Author would like to thank L. Panek, DVM, Veterinary Clinic, Kolin (CZ) for providing clinical data concerning of the case.

Author‘s declaration

The author state that the manuscript, or part of it, have not been and will not be submitted elsewhere for publication. The author declares that he has no conflict of interest with respect to this authorship or publication of this article. This study was not supported by any grant or other funding.

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