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LACTOFERRIN AND CHLORHEXIDINE: A POTENTIAL APPLICATION OF THE SYNERGISTIC EFFECT IN A SUPPORTIVE THERAPY OF PERIODONTAL DISEASES IN DOGS AND CATS

Katarzyna Jodkowska, DVM
Dentistry Section of PSLWMZ
Private veterinary practice

Periodontitis and gingivitis affect more than 77% of dogs over 2 years of age under veterinary care. In cats, these illnesses occur earlier in life and are reported in over 80% of cats in Poland (1). It may be thus tempted to say that these diseases are one of the commonest health problems in small companion animals.

The bacterial plaque and specifically the pathogenic bacteria it contains are the causative factor in periodontopathy, namely periodontal diseases. The dental plaque’s microbes trigger inflammation and damage to the soft tissues via their toxins. Long-lasting inflammatory conditions produce damage to the connective tissue and bones. As a result, the attachment apparatus of teeth become traumatised, with a consequent teeth loss. In the light of the studies completed in the last several decades, a significant role in the pathogenesis of periodontal diseases is assigned to genetic predispositions as well as some external factors, for example, stress. Periodontal diseases also impact systemic illnesses, such as diabetes, renal insufficiency, cardiac diseases, or immune-impaired conditions. Therefore, a comprehensive therapeutic approach to periodontal diseases has to include the elimination of bacterial plaque (active and passive hygiene) and a reduction of the inflammatory response in the oral cavity.

On the market, there are products with lactoferrin and chlorhexidine for adjunctive therapy of the inflammatory condition in the oral cavity. The potential for the combined effect of both substances in one product seems very attractive. This combination is potentially very effective since chlorhexidine is an antiseptic compound with a broad spectrum of action and is commonly used in dentistry. It has a strong effect on Gram-positive bacteria, with a slightly lesser action on Gram-negative bacteria and on some viruses, fungi (yeasts) and parasites (protozoans). Bactericidal action of chlorhexidine involves damaging the cell membrane of the bacterium, which results in its death. The effects of lactoferrin, which also destabilizes the cell membranes of microbes, can be complemented in some ways. A potential of a negatively charged molecule of chlorhexidine to attach to the surface of oral mucosa and bacterial tooth plaque, from where it is subsequently released, plays a vital role. It extends its action to approximately 8 hours during the days and as many as 12 hours at night if the product is administered before sleep. It thus prevents bacterial colonisation and growth on the enamel surface, inhibits deposition of the dental plaque (by 50-60%) and has an anti-inflammatory effect (reducing the severity of gingivitis by 30-45%), and in saliva, it lowers the number of bacteria by as much as 95% (2). The advantage of chlorhexidine consists also in its very low harmfulness; apart fr4om some exceptional cases of individual hypersensitivity, a toxic effect on the epithelium and the other tissues in the oral cavity have not been found (3).

In dentistry, chlorhexidine is used to treat gingivitis and periodontitis, applied in endodontic treatments as a rinsing medium (especially in root canal treatments), as a disinfectant during some dental procedures (scaling, teeth extractions) and to maintain good oral hygiene following surgical procedures. The application of chlorhexidine products such as solutions or gels accelerates wound healing and reduces the inflammatory condition of the oral tissues (4).

Lactoferrin (LF) is a multifunctional glycoprotein from the transferrin family, namely the proteins capable of transporting iron and other metals, yet with a lower affinity (5). It is synthesized by some exocrine glands and neutrophilic granulocytes affected by infection or inflammation (6). It is found in many internal organs, mucosae, and body fluids (7).

It has been shown that lactoferrin impedes iron processing by e.g., bacterial cells. Because of this, it demonstrates a bacteriostatic effect and even a bactericidal action, under some conditions, on Gram-positive and Gram-negative bacteria, aerobic and anaerobic, and yeasts (8). Lactoferrin is also capable of binding to specific receptors in the tissues and on the surface of many micro-organisms. Its positively charged

Lactoferrin is an immunomodulator, and thanks to its specific characteristics, it can be called “a super cytokine” or “an intelligent cytokine” (10). This substance - via granulocytes, NK (natural killer) cytotoxic cells and LAK (Lymphokine-Activated Killer cells), complement system, dendritic cells, B and T cells, and macrophages - enhances a poor immune response and reduces an exaggerated immune reaction by “detecting” the actual activity of the immune system (10). Lactoferrin is also involved in iron transport and storage since it impacts reduction and oxidation reactions, and therefore, it has an indirect effect on the reduction of oxidative stress, preventing thereby cell damage (11).

All these mechanisms, of which some are still poorly elucidated, summarise into unspecific antibacterial, antiviral, anti-fungal, anti-parasitic, anti-inflammatory, and anti-neoplastic effects of lactoferrin. These effects of lactoferrin are included in medicinal and nutritional products used as an aid in the treatment and prophylaxis of many medical conditions. These characteristics allow administering lactoferrin with antimicrobial medications, for instance with antibiotics or chlorhexidine in a combined treatment. For antibiotics, it allows us to reduce their dosage whereas, for chlorhexidine, it enhances its potency and efficacy. Lactoferrin forms a protein-enzymatic complex with lysozyme and peroxidase that, apart from its non-specific

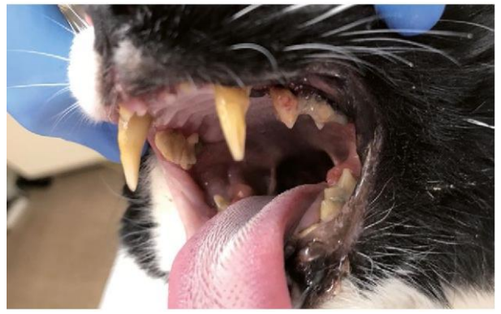

Fig. 1. Symptoms of stomatitis-gingivitis syndrome in an 8-year-old cat. Stomaferin Ultra had been administered to alleviate inflammation before full-mouth extraction.

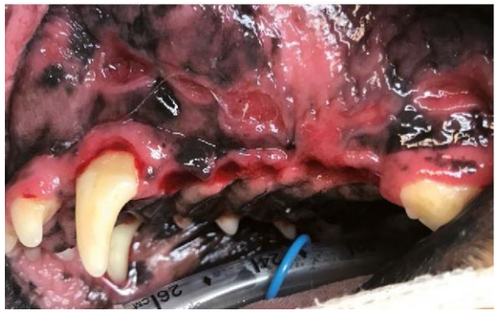

Fig. 2. A 6-year-old dog during a dentistry procedure, with the so-called “kissing syndrome” symptoms: reactions on the oral mucosa caused by contact with the bacterial plaque on teeth. Local administration of lactoferrin and chlorhexidine after the procedure together with antibiotic treatment and anti-inflammatory therapy improves the patient’s well-being and accelerates healing.

fragment interacts electrostatically with negatively charged surface glycosaminoglycan structures on the bacterial cell membranes. Binding with them, it protects the body against infections in a dual way: on one side, it attaches to the cellular receptors and abates a chance for a bacterial invasion into the cell, and on the other side, it blocks the pathogen's receptors and impedes its attachment to the host cells (9). Furthermore, it influences the carbohydrate metabolism in bacteria and can destabilise their cellular membranes.

immunological effect, plays a vital role in inhibiting multiplication and adhesion of bacterial cells to hydroxyapatite, which is the main mineral component of the enamel. With its insufficient activity in the saliva, bacteria attach to the enamel surface more easily and build there a biofilm that is subsequently colonized by further layers of bacteria, including the pathogenic ones, such as *Streptococcus mutans* and other species responsible for medical conditions in the periodontium. Importantly,

bacteria as plankton in the oral cavity are many times less resistant to antibiotics than those that grow as a biofilm (13). With time, such biofilm builds a bacterial plaque on the enamel, and the plaque may be secondarily saturated with calcium and magnesium salts and develop into tartar (14). Lactoferrin in the saliva coats the oral tissues, and by this, it impedes this process which is a crucial phase in bacterial growth and survival in the plaque and tartar. Many Gram-positive and Gram-negative bacteria are equipped with specific adhesins that allow them to attach to the epithelial cells in the host. Lactoferrin prevents this phenomenon since, by itself, it binds at these locations with the host’s cells. Because of this, it protects against gum and periodontal diseases and reduces biofilm built-up (15, 16).

In terms of oral cavity health, a very interesting clinical trial on a local application of lactoferrin and piroxicam in stomatitis-gingivitis syndrome in cats has been conducted. An improvement of the clinical condition and lessening of the clinical symptoms in 77% of the investigated animals: a reduction in the severity of lesions in the oral cavity, improvement in the quality of life, and an increase in body weight. It has been concluded that lactoferrin combined with piroxicam was effective in the treatment of stomatitis-gingivitis syndrome. Any

significant treatment-associated adverse reactions, including the impact of renal and liver functions, have not been reported. The reduced severity of the clinical symptoms was associated with a decrease in the number of macrophages in the oral mucosa, which resulted in decreased expression of pro-inflammatory cytokines and consequently brought about a suppression of the inflammatory response. Finally, it ended up in a significant improvement of the clinical condition of the investigated animals (17).

SUMMARY

The products dedicated to animals that contain lactoferrin (with a complex effect on the non-specific immune response and a direct effect on the cell membranes of microbes) and chlorhexidine (a potent antiseptic with a broad spectrum of action) can be a very useful tool in treating the inflammatory condition of the oral cavity. These synergistic and complementary effects of both substances allow for adding them as adjunctive therapy in treatments of the oral diseases, in which a significant role is attributed to an exaggerated immune response, such as chronic gingivitis, periodontopathies, canine ulcerative contact stomatitis, plaque-associated stomatitis, stomatitis-gingivitis syndrome in cats, and feline juvenile stomatitis.

Fig. 3. A 9-month-old cat with severe juvenile periodontitis. Before the dental procedure, lactoferrin and chlorhexidine were applied topically. A reduction of unpleasant smell in the oral cavity and increased appetite were reported.

Fig. 4. One of the indications for a local application of the combination of lactoferrin and chlorhexidine includes aggressive chronic periodontitis (a 2-year-old miniature schnauzer before teeth extraction procedure).

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