Antipruritic application of ovocystatin in atopic dermatitis in dogs - preliminary study

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Abstract

The study was an attempt to determine the possibilities of using ovocystatin, a component of a new generation product of natural origin, in local therapy of atopic dermatitis in dogs by suppressing pruritus during illness. Chicken egg cystatin was used locally in the interdigital spaces of forelimbs of dogs used in the experiment. The degree of pruritus and clinical changes in the animals were defined using CADESI-03 scale before and after the beginning of the experiment. The results obtained proved that ovocystatin may be used as a substance suppressing pruritus in atopic dermatitis.

Keywords: dogs, ovocystatin, pruritus, atopic dermatitis.

Introduction

Pruritus is defined as an autonomous feeling, independent of pain, which occurrence is related to the activity of specific mediators, spinal neurons, and cerebral cortex areas. The occurrence of pruritus in atopic dermatitis involves the activity C fibers, opioids, and psychogenic factors. Traditional methods of treatment include application of glicocorticosteroids. However, new therapeutic solutions are sought. Not only efficient medications are searched for but also the ones of the lowest toxicity and the weakest adverse reaction. Thus, chicken egg cystatin has been chosen as one of the substances which can be an alternative to traditional methods of treatment. Because of its natural occurrence, it is widely available and the methods of its efficient obtaining have been discussed in details (1, 8, 23).

Cystatin is a natural substance, which is found, for example, in chicken egg white, being a humoral factor of non-specific defence. It is an inhibitor of cysteine proteinases, such as papain and ficin. Chicken egg white cystatin belongs to the type 2 of cystatin family together with human cystatin C and D (24). It is a protein composed of 116 amino acids, which has no carbohydrate component and has two disulfide bonds (7). This is a protein of 12.7 kDa molecular mass and its inhibitory properties of cystein proteinase activity result from the presence of three conservative domains (13). The first domain contains a conservative amino acid residue Gly 9 and the remaining ones create two loops of “hair pin” type. The loops influence the inhibitor functionality, stabilise it and bond the structure consisting of two α-helixes and five β-coils (25). The significance of three conservative domains in inhibiting bacterial cysteine proteinases activity was shown in experimental tests (24). It is a highly stable protein (no loss of activity after 30 min heating at 100°C, nor change of pH) (22). Freezing and thawing can cause a decrease in cystatin activity therefore it is stabilised in the glycerol solution of pH 7.5 (22, 8). It is a barrier for bacterial and viral cysteine peptidase (7, 25). Intercellular and extracellular control of protein decomposition is a very important function of cystatin (2).

The objective of the study was to determine the effectiveness of ovocystatin use in local therapy of atopic dermatitis (AD) in dogs to suppress pruritus during illness.

Material and Methods

Cystatin C monomer, in the concentration of 50 µg/mL in PBS with 10% glycerol, was obtained
from the Chair and Department of Pharmaceutical Biochemistry of the Wroclaw Medical University. Ovocystatin was obtained using affinity chromatography method with immobilised carbomethylolopapain on Sepaharose 4b basis from egg white from green-legged partridge (8).

Twenty dogs (10 females and 10 males) of different sex, 1 - 6 years of age, and of the following breeds: Labradors (six), boxers (two), beagles (three), and one of each briard, bulldog, fox terrier, jack russell terrier, Belgian shepherd, poodle, Scottish terrier, west highland white terrier, and German pointer were used in the experiment. The dogs were qualified based on the following criteria: dogs with clinical symptoms of atopic dermatitis without suppressive complications, and dogs showing reactions to the tested allergens in skin tests (Agroskin RTU 20 set North/Central Europe) and IgE screening test performed using FceRIα receptor method (Allercept Test, IDEXX Laboratories).

Cystatin was used at a concentration of 50 mcg/mL in10% glycerol solution with PBS once a day for two weeks in interdigital spaces of the right forelimb at an amount of 1 mL. Blind probe was run in the interdigital spaces of the left forelimb using 10% glycerol solution with PBS at an amount of 1 mL.

Full haematological (flow cytometry, IDEXX LaserCyte) and biochemical (Konelab PRIME 30i) tests were performed in all dogs twice – before and after ovocystatin application. The following parameters were measured in haematological tests: number of red blood cells (RBC), haematocrit number (Hct, Ht), haemoglobin level (HGB, Hb), percentage of reticulocytes (%RETIC) and their absolute value (RETIC), number of platelets (PLT), absolute values and percentage of white blood cells (WBC, %WBC), neutrophils (NEU, %NEU), lymphocytes (LYM, %LYM), monocytes (MONO, %MONO), eosinophils (EOS, %EOS), and basophils (BASO, %BASO). Additionally, MCV, MCH, and MCHC were calculated. The following biochemical parameters were determined: activities of alanine transaminase (ALP, GPT), aspartate transaminase (AST, GOT), alkaline phosphatase (ALP, AP, FA), amyylase, and lipase, and contents of total protein, albumin, urea, creatinine, cholesterol, and triglycerides. Diagnostic assessment using the criteria of recognising atopic dermatitis according to Favrot et al. (6) and Olivry (19) was applied in all the patients qualified for the experiment. Dogs used in the experiment needed to fulfil at least three criteria essential for the study: onset of signs under three years of age, glicocorticoïd-responsive pruritus, and pruritus without lesions at onset. Most of the dogs met five or more Favrot’s criteria. After the assessment, the dogs received points related to the positive answers to the medical interview questions, which were used for statistical analysis. At the same time, the dogs that were qualified for the experiment underwent intradermal allergy tests using Agroskin RTU 20. Additionally, vein blood was taken in order to obtain serum used in the IgE screening test performed using FceRIα receptor method. A number of Favrot’s criteria with both positive allergy tests were essential for dogs classification and qualification for the experiment. Additionally, all tested animals were on elimination diet (at least 6 weeks of diet) to eliminate animals with coexisting food allergy/intolerance. During the experiment, no drugs were used (systemic and non-systemic medications) and no immunotherapy was applied.

Assessment of pruritus intensity, based on the changes of animal behaviour (10, 21) before ovocystatin application and two weeks after its application immediately after completing the experiment, was performed for each dog. The following symptoms of pruritus were considered: scratching, licking, biting, and rubbing.

The intensity of pruritus was additionally marked with numbers in order to facilitate the statistical analysis.

Clinical assessment of skin according to CADESI-03 (Canine Atopic Dermatitis Extent and Severity Index) was performed for all dogs qualified for the experiment (17,18). Sixty-two areas of body were assessed. The assessment was conducted before ovocystatin application and two weeks after completing the experiment. Apart from the assessment of the whole animal body, we concentrated on the points of the dorsal and palm surface of the left and right forelimb fingers to present possible differences between the places of ovocystatin application and the places of blind probe. Presented results refer to the dorsal ans palm surface of the forelimb fingers.

The statistical analysis of all results was made using Statistica 9.1 by StatSoft.

There was a consent of the 2nd Local Ethical Commission for Experiments on Animals to conduct the tests.

**Results**

Changes of morphological and biochemical parameters within the group before and after the therapy were analysed. Lack of statistically significant differences between all tested parameters before and after the therapy were found. The tests aimed at excluding coexisting diseases (it would eliminate the animal from the experiment) and showing/excluding possible influence of ovocystatin on circulatory blood parameters. In all the qualified dogs, there were no changes in blood chemistry and biochemistry parameters both before and after ovocystatin application, so the results are not published in the study but are available from the authors.

All dogs qualified for the experiment obtained at least two out of eight points possible to gain from positive answers to the questions. According to Favrot et al. (6), obtaining five positive answers allows to
prove atopic dermatitis in an animal with 80%-85% sensitivity and with 79%-85% specificity. In the present experiment, however, the dogs were not assessed independently, but in relation to the data obtained from the interview, history of sickness, clinical symptoms, results of allergic skin tests, and the IgE screening test performed using FcεRIα receptor method. In both kinds of tests, only whole year allergens were taken into consideration. Marking the reaction to selected all year allergens, but not confirming if the dog is atopic or not, was the aim of the present study. The data did not undergo a statistical analysis as it was not the aim of this experiment.

Analysis of pruritus intensity according to behavioural assessment described by Hill was made, therefore, it was impossible to use the parametric test (data in the ordinal scale) to compare the intensity of symptoms before and after the therapy. Rank Wilcoxon test was used for the matched pair at the 5% significance level. Statistically significant decrease in itching was observed in the dogs before and after the therapy (P = 0.005).

The results concerning the efficiency of the therapy (CADESI-03) for interdigital spaces of the right forelimb (ovocystatin applied) within the group were analysed. The Student t-test was used for independent tests on the 5% significance level. The following results were obtained: erythema – statistically significant difference was shown between the averages (improvement) in the group before and after the therapy (P = 0.012) (Fig. 1); lichenification (P = 0.771), excoration (P = 0.350027), self-induced alopecia (P = 0.337) - there were no statistically significant differences between the averages in the group before and after the therapy.

Blind probe conducted in the interdigital spaces of the left limb showed no statistically significant differences (P = 0.430) between the averages in the group before and after the therapy.

Additionally Fig. 2 shows the percentage of dogs showing selected cutaneous conditions in the interdigital of the right forelimb (CADESI-03). The degree of erythema decrease in the interdigital spaces of right forelimb in dogs before and after cystatin administration were compared. The following division into the classes was adopted:

- 0.0-1.999 class 1 of the reaction intensity
- 2.0-3.999 class 2 of the reaction intensity
- 4.0-7.999 class 3 of the reaction intensity
- 8.0-10.0 class 4 of the reaction intensity

Because of nonparametricity of the data, U Mann-Whitney test at a 5% level of significance was used and the result proving statistically significant difference between the class where the reactions were found before and after the therapy was obtained (P = 0.001).

Fig. 1. Erythema – interdigital space of the right forelimb before and after ovocystatin application
Discussion

Atopic dermatitis, one of the most often diagnosed pruritic skin diseases, causes the necessity of finding new therapeutic solutions. It is caused by adverse drug reactions to administered medicines and lack of their efficiency, as well as by constant search for the “golden mean” which would encompass efficiency and safety of its use. Innovative initial tests on antipruritic application of ovocystatin clearly have shown the efficiency of the tested substance. Ovocystatin applied at 50 µg/mL concentration once a day for two weeks efficiently decreased pruritus intensity in dogs. At the same time, the blind probe showed the lack of antipruritic action of the substance carrier (10% glycerol with PBS), which excludes the possibility of the results being distorted by the ovocystatin carrier. Additionally, it was presented that egg white cystatin diminished erythema in the places of its application, which may be connected with the pruritus symptoms (11). However, the influence on diminishing the intensity of lichenification, self-induced alopecia, and excoriation has not been observed. It may result from the fact that these clinical changes were not diagnosed as often as erythema in dogs qualified for the experiment (Fig. 2). This explains the choice of the individuals, who do not have advanced dermatological conditions resulting from complications of e.g. bacterial infections. Qualification of the animals aimed at choosing the dogs with pruritus without supportive complications or very advanced dermatological changes, which would make the assessment of pruritus itself impossible. The test also showed the lack of side effects of ovocystatin application. There was no clinical evidence of cutaneous atrophy or secondary infection. It is especially significant in the case of working out new medicines for animals suffering from atopy in which, because of adverse/allergic reactions, many of the preparations cannot be administered. In some cases of atopic dermatitis in dogs, localised medications can be administered, e.g. hydrocortisone aceponate, but their application is limited to the cases of localised pruritus of possibly hairless areas. Despite high level of safety of the medicament in case of its long term use it may lead to skin thinning (4, 9, 16). In the conducted studies, hydrocortisone aceponate was used for 70 d. Positive and rapid therapeutic effect was described as partial clinical improvement by 14 d with further improvement on day 28. Some owners also found spray difficult to apply (13). In contrast, ovocystatin was used only for 14 d in liquid formulation, what made it easy to apply. It is worth noting that glicocorticosteroids are contraindicated in dogs with diagnosed deep pyoderma. Topical glicocorticosteroids also have disadvantages. They are absorbed systemically, resulting in local and systemic adverse reactions especially after long-term application. These medications should not be used for a long-term treatment, as well as extensive application. There is no such contraindication in the case of ovocystatin. It has been proven that such proteases as chymase, tryptase and carboxypeptidase may affect PAR-2 receptor (proteinase - activated receptor 2) discovered in nerve fibres. It is activated by tryptase and other proteases, among others by chymotrypsin coming from mast cells, which leads to itching (12). The studies on this process are still conducted. In relation to its properties, ovocystatin evokes a great interest and studies on its application as a medicament are in progress. Attempts of local applications of active substances such as tacrolimus (calcineurin inhibitor) are described in literature. However, despite the promising
effects, these preparations often cause skin irritation and long waiting time for the effects, therefore, they are not recommended in acute states. Therapeutic effects also appear after longer time of its application; therefore, tacrolimus is not recommended in acute atopic dermatitis (3, 14, 20). Tacrolimus is recommended in a long-term therapy of chronic atopic dermatitis, especially in dogs with localised AD, but its application can be followed by signs suggesting irritation. Probably it is more beneficial in elimination or reduction of skin lesions than in pruritus decreasing (14). Otherwise, cystatin proved to be more effective in pruritus decreasing. In human medicine, attempts of cyclosporin A application in localised skin treatment have been undertaken, but they have not been so successful (5, 15). The conducted experiments showed the effectiveness of ovocystatin in decreasing the intensity of pruritus in the animals qualified for the test. At this stage of tests, its effectiveness was not compared to the steroid drugs applied locally or to tacrolimus. Safety of use, as no adverse reactions were observed during its application, is an advantage of the chicken egg cystatin. Ovocystatin, as a substance for localised application, may be used in case of small pruritus, which is a frequent situation in dogs with atopic dermatitis. In such cases, it would probably limit the application of systemic drugs, e.g. glicocorticosteroids, or would allow lowering their dose or frequency of application. Because of high costs of ovocystatin resulting from a laboratory method of its obtaining, the authors had no possibility to apply a higher concentration or higher frequency of its application (e.g. 2-3 times a day). Such promising results of chicken egg white cystatin application suggest that number of dogs in the test group should be increased as well as the concentration and/or frequency of the substance application.

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References