EVALUATION OF THE IMMUNOMODULATORY EFFECT OF GENERAL TREATMENT WITH SUB-ANTIMICROBIAL DOSES OF DOXYCYCLINE IN CHRONIC EXPERIMENTAL-INDUCED PERIODONTITIS

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Summary
In the present study, we intend to utilize an experimental model in order to evaluate the effect of general treatment with doxycycline in chronic periodontitis.

The study involved 22 mature female rats, to which the periodontal pathology was induced by summing up daily repeated acute gum lesions, for 16 days. On D 16, serum and saliva samples were harvested, as well as gingival specimens from two animals, sacrificed for this particular purpose. Serum and salivary levels of MMP-8 were measured using the ELISA technique and the histopathological aspect of the gum was evaluated on Masson trichrom, modified by Goldner stain technique. Eventually, the rats were randomly divided in two equal groups and had two different treatments: group M (n=10) benefited of hygienisation treatment, while group D (n=10) benefited of hygienisation treatment associated with doxycycline per os, in sub-antimicrobial doses: 1.2 mg/kg body/day, for 12 days (D 17-28). After the treatment, the immunological and histopathological examinations were resumed.

The obtained results showed a decrease of seric and salivary MMP-8 levels after the treatment and healing of the pathological process in both groups, but there were differences depending on the applied treatment. In group D there were significantly reduced values of seric MMP-8 levels in comparison to group M, and the resolution of the inflammation was also accentuated in the doxycycline treated group.

General administration of doxycycline in sub-antimicrobial doses has a immunomodulatory effect by reducing the seric levels of MMP-8 and by limiting the collateral tissue lesions.

Key words: doxycycline, periodontitis, immunomodulatory, MMP-8.

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Introduction
In periodontology, animal experimental models are necessary for testing some potential innovative therapies and establishing the therapeutic effect of a certain product (Oz and Puleo, 2011).

Among the factors involved in the periodontopathic pathogenesis is the matrix metalloproteinase-8 (MMP-8), secreted by PMN, which is responsible for producing collateral tissue lesions: damage of the extracellular matrix of the gum chorion (Gursoy et al., 2010; Sorsa et al., 2011). Therefore, the utilisation of some immunomodulatory therapeutic means, with an inhibitor effect on this enzyme would be salutary (Sorsa et al., 2011; Griffin et al., 2011).

Multiple clinical studies have shown a decrease in the seric and salivary levels of
MMP-8 in human patients and a deduction of clinic periodontal index after the administration of doxycycline in sub-antimicrobial doses (Marcaccini et al., 2009; Gorska et al., 2006).

Although valuable, the data obtained by immunological investigations and clinic examinations, do not provide complete information regarding the effect of this therapy. For a better understanding of the mechanism of action of doxycycline as a collagenase inhibitor, the supplementation of the present knowledge with histopathological analysis, that can appreciate objectively the changes taking place in the evolution of the inflammatory periodontal process, after this medication, is necessary.

Material and methods

The experimental study was a 28 days long pilot study. The animals used were 22 mature female rats, to which the chronic periodontitis was induced in 16 days (D 1-16). The induction of periodontal pathology consisted in daily provoked acute lesions, in gums, in the inferior incisive area. On D 16, serum and saliva samples were harvested from all animals in order to determine MMP-8; for the measurement of the enzymatic levels the ELISA technique was used. Two animals were sacrificed for harvesting gingival specimens for histopathological examination; the Masson tricrom, modified by Goldner stain technique was used.

After this period, the rats were randomly divided in two equal groups and had two different, 12 days long (D 17-28), treatments depending on the group.

Animals from group M, control (n=10) benefited of hygienisation treatment. The hygienisation consisted of removal of the alimentary detritus and soft deposits on the surface of the teeth and gum with a haemostat and cotton buds soaked in hydrogen peroxide, in order to favour healing.

Animals from group D, (n=10) benefited of hygienisation treatment associated with doxycycline. The general treatment with sub-antimicrobial doses consisted of oral administration of 1.2 mg/kg body/day, meaning that each rat was given 0.2 mg/day, unique dose, through a gastric probe.

Treatments were applied for 12 days (D 17-28). At the end of this period (D 28), the same immunological and histopathological examinations were made to evaluate the effect of the applied treatment.

The treatment response was traced daily and the following clinical parameters were registered: the degree of inflammation of the gum and the presence of bleeding at the sulcus probing.

In order to appreciate the treatment efficiency, certain clinical and histopathological aspects were monitored. The aspects considered to be clinically significant were: reduction of erythema and gingival oedema, organisation of the fibrino-leukocytic deposit on the epithelium surface and the reattachment of gum to the teeth surface. The aspects considered as significant histopathologically are: oedema and inflammatory infiltrate reduction, reorganisation of the connective tissue and reepithelisation of the superficial lesions.

The utilized methods for the statistical analysis were: Student’s t-Test for paired samples and ANOVA analysis completed with Post Hoc test and Bonferroni correction.

Results

Clinical and histopathological results:

Before the treatment (D 16), the severe gingivitis stage was relieved clinically (Fig. 1), and the advanced lesion stage, histopathologically (Fig. 2).

After the treatment (D 28), in control group M, the gum appeared to have a larger volume clinically, the gingival margin was thickened, the gingival furrow broad, an increase in the crevicular fluid and the presence of a purulent exudate were observed. A migration of the gum towards
the coronal region and an attempt to reattach on the tooth surface were remarked. The erythema was discrete and on the gum surface, the ulceration evolved towards healing, being covered by an adherent stable deposit (Fig. 3). The periodontal inflammation degree: discrete signs of inflammation. The presence of bleeding at the sulcus probing: presence of bleeding.

Histopatologically, the repariring processes were present, but not in a very advanced stage (Fig. 4). The inflammatory infiltrate was significantly more reduced than before treatment and present only in the superficial area. More profoundly, there were fibroblasts and very fine collagen fibers present, which showed some gradual connective consolidation processes in progress (from inside to the superficial area). The oedema was more reduced, the fundamental substance restructured and the blood vessels were dilated in the profound chorion. The hemorrhagic and necrosis area were very limited. At the periphery of the lesion, the epithelial proliferation was observed, as an attempt of covering the lesion.

Group D treated with doxycycline:
Clinically, the gum had a more discrete erythema than the control group, the gingival furrow was broad, with the presence of a purulent exudate in the profound area. The free gingival margin was thickened, covered by slough. Unlike the control group, in some areas there was a reattachment of the gum on the tooth surface (Fig. 5). The periodontal inflammation degree: discrete signs of inflammation. The presence of bleeding at the sulcus probing: presence of bleeding.

Histopathologically, the regeneration of the tissue in the intervening area was in a more advanced stage than the control group, aspect suggested by the presence of collagen fibers in the profound chorion, in the repairing processes progression area. The fibers appeared to be slightly thicker that those in the control group. All the other aspects were comparable with the control group (Fig. 6).

Immunological results:
Both groups presented a statistically significant decrease in seric and salivary values of MMP-8 after the treatment (p<0.001: Student t-Test for pair samples). The results are shown in graphs 1 and 2.

There was no significant statistical difference concerning the seric and salivary values of MMP-8 between groups before the treatment (p>0.05 – Post Hoc Test with Bonferroni correction). There was a significant statistical difference between groups after the treatment (ANOVA Test).

After the treatment, the seric values of MMP-8 had a highly significant statistical decrease in group D in comparison to group M (p<0.001 – Post Hoc Test with Bonferroni correction).

Instead, the salivary values of MMP-8 did not present a significant statistical difference in group D in comparison to group M (p>0.05 – Post Hoc Test with Bonferroni correction).

The results are presented in graph 3.

Discussion
Our study’s results have shown the efficiency of applied periodontal treatments, materialized through an adequate clinical and histopathological evolution, with healing of the periodontal lesions.

A significat decrease of the seric and salivary MMP-8 values was also highlighted in both groups after the treatment (p<0.001 – Student t-Test for pair samples). Our results are comparable, in many respects, with the ones reported by other researchers, after conducting studies on experiential animals and human patients (Sors et al., 2011; Marcaccini et al., 2009; Gorska et al., 2006).

For an extrapolation of this results to human, we intended to apply to the animals, similar therapeutic means to the ones administered to patients: the hygienisation treatment we used corresponds to the conventional periodontal
treatment and the doxycycline doses were chosen according to the speciality literature in order to exert an immunomodulatory effect (Griffin et al., 2011).

The length of the treatment was established depending on the clinical observation obtained after some preliminary researches, establishing as a standard element the period of spontaneous healing of periodontal lesions. By doing so, we tried to eliminate the possible sources of error, taking into account the reactivity particularities of the species used in the experiment, because of its well-known resistance towards the periodontal diseases (Graves et al., 2008).

After the removal of the causing factor through hygienisation, healing conditions were created in both groups, but the evolution was more favourable in group D, which benefited of doxycycline treatment.

The organisation of a fibrino-leukocytic deposit on the surface of the lesion was a barrier in the path of microorganisms and other particles. This assured the premises for the healing processes in the chorion and the optim condition for reepithelisation.

The resolution of the pathologic process consisted in the reduction of the inflammatory infiltrate and oedema, which corresponded with the clinical aspect: volume reduction of the gum and the tendency to return to its initial conformation. The high consistency and the resistance of the gum were due to the appearance of the collagen fibers in the inflammatory focus.

This connective reorganisation of the chorion was an attempt to limit the inflammatory process and was due to the decrease of PMN in the chorion and of the quantity of proteolytic enzymes discharged in the periodontal tissues. Consequently, the inflammation resolution led to the reduction of salivary and seric levels of MMP-8.

Clinically and histopahtologically, a more advanced stage of gum lesion healing and a more efficient limitation of the inflammatory process was noticed in the doxycycline treated group, in comparison to the control group. The thickness and organisation of the collagen fibers in the gingival chorion determine a reattachment of the gum to the tooth surface in some areas, an aspect not found in the control group. Recovery of the collagen fibers was the proof of a more efficient reduction of MMP-8 levels in the profound chorion in group D, in comparison to the control group.

Immunologically, in the doxycycline treated group, the seric enzymatic levels are significantly lower in comparison to the control group (p<0,001 – Post Hoc Test with Bonferroni correction). These observations are similar to other data presented in the speciality literature.

However, the salivary levels do not display a significant statistical decrease in group D, in comparison to the control group. The explanation is given by the persistency of the inflammatory infiltrate in the superficial chorion and dischargement of MMP-8 in periodontal tissues, followed by the enzyme diffusion in the saliva. A comparison of this results to othe studies was not possible because we did not find any similar researches in the speciality literature. Consequently, we interpreted the results based on the data obtained in the present study.

Our study’s results indicate that doxycycline administered generally, determines the sistemic inhibition of MMP-8 activation and, by default, the accentuated decrease of seric levels of the enzyme. The local effect, on periodontal tissues, is more reduced, the reason why the differences of the salivary values of the enzyme in comparison to the control group are insignificant.

The fluctuations of seric and salivary values of MMP-8 after the treatment and the differences observed based on the applied terapeutical means, picture the role of the enzyme in evaluating the efficiency of the therapy.
Conclusions
The parodontal treatment determines a healing of the gingival lesions, clinically and histopathologically proven and a decrease of seric and salivary values of MMP-8.

The seric and salivary fluctuations are in conformity with the clinical and histopathological aspects.

The clinical, histopathological and immunological evolution differs based on the treatment used.

The seric and salivary levels of MMP-8 are a display of the of the treatment efficiency.

The general treatment with sub-antimicrobial doses of doxycycline associated with hygienisation treatment, determine a significant decrease in the seric levels of MMP-8.

The general treatment with sub-antimicrobial doses of doxycycline associated with hygienisation treatment, does not determine a significant decrease in the salivary levels of MMP-8.

The general treatment with sub-antimicrobial doses of doxycycline, exerts an immunomodulatory effect by reducing the seric levels of MMP-8 and by limiting the collateral tissue lesions.

References
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Fig.1. Clinical aspect of the gum before the treatment D 16.

Fig.2. Histopathological modifications D 16. (Tricrom Goldner, ob. 10X)

Fig.3. Control group M at the end of the treatment (D 28). Clinical aspect of the gum.

Fig.4. Control group M after the treatment (D 28). Histopathological modifications (Tricrom Goldner, ob. 10X)

Fig.5. Group D – doxycycline – D 28. Clinical aspect of the gum.

Fig.6. Doxycycline treated group – D 28. (Tricrom Goldner, ob. 10X)
Graph 1. Evolution of the seric values of MMP-8 expressed in ng/ml in both groups after the treatment.

Graph 2. Evolution of the salivary values of MMP-8 expressed in ng/ml in both groups after the treatment.

Graph 3. Comparison between seric and salivary values of MMP-8 expressed in ng/ml in both groups before and after the treatment.