Experimental morphological study of reparative processes in oral mucosa erosive lesions

Eksperymentalna praca badawcza z zakresu morfologii procesów naprawczych zmian erozyjnych błony śluzowej jamy ustnej

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Abstract
The urgency of the problem under consideration is due to a relatively high prevalence of erosive lesions in the oral mucosa characterized by prolonged disease progression. Given a considerable arsenal of various medications and their combinations, a medical specialist today finds him/herself in an ever-difficult situation of having to make the right choice of medicine in order to quickly and efficiently stop an oral lesion, speed up the healing process and facilitate recovery of the reparative function in the affected tissue. The goal of the study is to substantiate the effectiveness of using platelet auto plasma to heal erosive lesions in the oral cavity. The leading method of this study is morphological analysis of qualitative and semi-quantitative parameters, allowing us to reveal and specify the state of oral mucosa. The study results demonstrated that, in morphological terms, the best aspect of disease was observed in the group of patients receiving autohaemotherapy, where no traces of lesion symptoms were found. In the reference group, during this observation period, there were signs of non-specific non-chronic inflammation in certain zones.

Keywords: experimental study, erosion, regeneration, inflammation

Streszczenie
Aktualność omawianego tu zagadnienia jest spowodowana stosunkowo wysoką frekwencją zmian erozyjnych w obrębie błony śluzowej jamy ustnej z przedłużonym procesem chorobowym. Dysponując całym szeregiem leków i ich możliwych połączeń lekarz musi dokonać właściwego doboru leku, aby szybko i skutecznie powstrzymać rozwój zmiany, przyspieszyć proces gojenia i ułatwić odtworzenie funkcji naprawczych zmienionych tkanek. Celem badania jest dowiedzenie skuteczności zastosowania osocza bogatopłytkowego pobranego od pacjenta w gojeniu zmian erozyjnych. Wiodącą metodą w tym badaniu jest analiza morfologiczna jakościowych i semiilościowych parametrów umożliwiająca kwalifikację stanu błony śluzowej.

W wyniku badań pokazały się, że z morfologicznego punktu widzenia najlepiej wypadły pacjenci otrzymujący autohemoterapię, u których nie stwierdzono śladów zmian. W grupie referencyjnej, w okresie obserwacji, ujawniono oznaki niespecyficznych, nieprzewlekłych stanów zapalnych w niektórych obszarach błony śluzowej.

Hasła indeksowe: badanie eksperymentalne, erozja, regeneracja, zapalenie
Introduction

Today, prevalence of gerontologic conditions is rather high and shows no signs of a downward trend. The high rate of oral cavity manifestations force patients to seek dental assistance with a range of problems: from banal traumas to systemic pathologies with oral complications. One of the most frequent oral mucosa conditions (OMC) are erosive-ulcerous processes that are characterized by a recurrent, persistent course of disease having lots of various manifestations and forms. The relatively high prevalence of erosive-ulcerous conditions is related to the fact that the oral mucosa has limited morphology, therefore it responds uniformly – with lesions and ulcers – to various impacts of different nature. When a dentist is confronted with erosive-ulcerous lesions of different localization/intensity in the oral mucosa, aggravated by bacterial impact, fast spread of the pathologic process to underlying tissues, and a possibility of malignization and deterioration of the general state of the patient – all this makes it a must to rely on the state-of-the-art diagnostics followed by a plausible and efficient therapy.

Alas, modern therapeutic measures cannot always ensure satisfactory outcomes; treatment usually drags on for long periods. Due to ever more widespread allergic and toxic-allergic syndromes accompanying administration of many medications at a time, the improvement of drug-free therapies for erosive lesions in the oral cavity seems particularly urgent, aiming at correcting the key pathogenetic components in order to speed up healing and facilitate recovery of the reparative function in the oral mucosa.

The physician’s arsenal at hand for curing erosive lesions in the oral cavity is relatively large; yet complications caused by this disease urge us to constantly seek new treatment protocols and sequences facilitating initiation and acceleration of recovery and regeneration of oral mucosa tissue. In this regard, the high prevalence of erosive-ulcerous conditions in the oral cavity is one of the high priority problems in modern day dentistry, along with the quest for new methods, means and their combinations enhancing the efficiency of therapeutic effect upon pathologic inflammations in the affected tissue; these methods and measures are to combine in themselves top level safety for patients, high bioactivity upon body tissue and should be based on home-grown technology.

As a procedure of choice, injections of platelet auto plasma may be used to enhance phagocytic and germicidal capabilities of immune cells and to support collagenation and synthesis of other proteins. However, insufficient knowledge of this procedure’s effectiveness when compared with other treatments is the main motivation behind this study.

Study objective

On the basis of morphology, experimentally, to analyse and assess the efficiency of the auto-haemotherapy method in comparison with conventional treatment sequences for curing erosive lesions in the oral cavity.

Methods and materials

The experimental part of the study was carried out at the premises of the Laboratory of Pathologic Modeling of the City of Volgograd Medical Research Centre. In order to complete the tasks of the study, we chose, as the subjects of the experiment, 32 dogs, 2 – 16 kilos in weight, kept in a vivarium (t° = 22-24 centigrade, relative air humidity 40-50%), under normal conditions and with a standard diet (GOST R 50258-92 Standard), in compliance with the rules and norms of laboratory work for pre-clinical studies in the Russian Federation (GOST 3 51000.3-96 and 1000.4-96 Standards), and in compliance with animal research regulations (Report of the AVMA Panel on Euthanasia JAVMA, 2001), along with the norms suggested by the International Guidelines of oral mucosa. Materials of this paper may be useful in dental practice for healing various inflammatory disorders in the facial maxillary area.
of the European Convention on the protection of vertebrate animals involved in experimental studies (1997). This experimental study was approved of by the Commission on Study Ethics of the Volgograd State Medical University (Protocol No. 214 – 2015 of April 29th 2015).

The experimental modeling of a pathologic process was done by way of generating a prolaps on the surface of mucosa (not beyond the epithelium) without penetrating connective tissue on the upper jaw, on both sides, sized 1x1 cm in the area of the first premolar in the oral cavity’s vestibule.

All the animals were divided into two groups: Group I – the subjects who received injections of platelet auto plasma, Group II (reference) – conventional therapy, the lesion did heal without any extra cure. To eliminate other factors affecting the final results of the experiment, which may be linked to individual characteristics of the subjects, observation groups were formed in such a way that they included the same animal.

All proceedings with the animals were carried out when dogs were under semi narcosis. Premedication with Rometar 2% solution – 0.05 ml per kilo of weight. In 15 minutes – a solution of Zoletile 100 – 0.02 ml per kilo of weight intravenously, maintaining the anaesthetic sleep for 30 minutes on average, for the whole period of the experimental proceedings. From each of the dogs, dark blood samples were taken in the amount of 2-7 ml, using a tourniquet, alcohol wipes, a needle, adapter, tube holder, adhesive strap and special vacuum tubes. Dark blood samples were drawn from the Cephalic vein of the forearm. Preliminarily, hair was cut out along the vein, skin sterilised with alcohol wipes (until a clear wipe was obtained). After removal of the tourniquet and needle, the puncture site was taped.

After sampling, a tube was placed in “Eva-20” centrifuge, and the centrifugation mode of 3200 r/min was used for five minutes. The use of special tubes allowed us to obtain 1.5±0.5 ml of platelet auto plasma.

To compare the effectiveness of PRP therapy, the following was done. In Group I, platelet plasma was injected to the erosion area on the left side along the transitory fold, in the amount of 2.0 ml. On the right-hand side, the lesion was left for the purposes of control and comparison with the standard treatment regimen:

- Application of anaesthesia (gels e.g. “Camistad”, “Holisaal”, “Lidochlor”, 5% pyromecainum ointment, etc.).
- Application of proteolytic ferments (0.1% solution of trypsin or chymotrypsin).
- Antiseptic treatment (0.05% solution of chlorhexidine, 1% solution of iodonium, medicinal herb decoctions, etc.).
- Application of keratoplasty (oily solution of Vitamin A), salve dressings (Celestoderm + Solcoseryl 1:1 for 30 min).

Examination of the dogs and control for epithelium regeneration in the lesions was undertaken daily for two weeks. The study results were recorded in writing and by photography. The results were fixed on 1st, 7th and 14th day of the experiment and recorded in the form of data tables for further analysis. To confirm clinical change in all the dog-subjects of the study, bioptic samples of connective tissue were taken and analysed. The materials under investigation were fixed in a 10% solution of neutral buffered formalin (pH 7.4) for 24 hours, after which a standard histological diagnosis was made using ascending alcohols, and finally enveloped in paraffin. A rotor microtome was used for making slices of 5–6 µm that were subsequently placed on the mounting slide. In order to reveal general pathologic manifestations (inflammatory, degenerative and dystrophic processes, reparations), the microslides were coloured with haematoxilin – eosine, Mallory Trichrome according to the generally adopted histological methods.

Photo recording of microscopic change was done using the Axio Scope equipment (Carl Zeiss, Germany) and a digital camera Power Shot (Canon, Japan). A morphometric study was done relying on the “Video TestMorpho-4” software package (Russia). The following were determined: the specific quantity of inflammatory infiltrate cells (%), the sclerotic or fibrotic area (%), and the blood vessel volume ratio (%), by standardized methods of morphometry used in morphological studies.

The study data were analyzed using variation
statistics, on the IBM PC/AT «Pentium-IV» in the Windows 2000 environment and application software packages Statistica 6 (Statsoft-Russia, 1999) and Microsoft Excel Windows 2000. Statistical analysis relied on variation statistics with determination of the mean (M), its average error (±m), and assessment of statistical significance by the group using Student’s t-test (t). Difference between parameters being compared was regarded to be significant at \( p < 0.01, t \geq 2 \).

**Results**

If injections are used for platelet auto plasma therapy and relying on a standard regimen, no complications or side effects were observed during the whole period of observation of the animals. In both groups of subjects, a positive outcome of therapy was revealed. That said, follow-up result assessment and comparison revealed certain differences.

At the beginning of the study, the morphological pattern of biopsy specimen in all groups was characterized by symptoms of active inflammation. A manifest polymorphcellular infiltration was revealed both in the epithelial layer and in the proper mucous plate; the infiltration was manifestly diffuse with neutrophilic dominance. The mucosa, in some areas, was covered by layers of unevenly thinned non-cornifying epithelial tissue. An expressed patchy acanthosis was observed. In the underlying tissues, fields of granulation tissues with neovascularity were found.

When the morphology of the biopsy specimen from animals receiving auto-haemotherapy were analysed, it was revealed that by the 7th day of the experiment the inflammatory reaction became “stationary”: the mucosa was covered with flat layers of unevenly thinned non-cornifying epithelial tissue, with areas of moderate acantosis. The inflammatory infiltrate was unapparent, positioned in a perivascular direction and lymphohistiocytic. Patchy hyperplasia of granulation tissues was noted.

By the 14th day of therapy the end to any inflammatory processes was observed. The mucosa was patchily covered with flat layers of unevenly thinned non-cornifying epithelial tissue, with isolated acantotic cords. In the underlying tissue there was focal sclerosis and patchy hyperplasia of granulation tissues.

The specific quantity of inflammatory infiltrate cells reliably dropped by day 7 of the therapy: from 16.2±0.29 percent to 2.1±0.51 percent; by day 14 no infiltration was observed.

By day 14, the zones of sclerosis/fibrosis gradually reduced from 14.2±1.2 percent down to 5.2±0.77 percent and was by that time significantly different. The volume ratio of blood vessels reliably declined, by day 7, from 9.21±0.25 percent down to 5.42±0.29 percent simultaneously with the abatement of hyperaemia symptoms; later, by day 14, it fell to 4.1±0.24 percent.

Analysis of the morphological pattern of biopsy samples from animals whose therapy was based on the conventional regimen revealed that by day 7 of the therapy the mucosa was patchily covered with flat layers of non-cornifying epithelial tissue with areas of uneven enlargements (hyperplasia) and acantosis. In the underlying tissue, a moderate diffuse leucocytic infiltration impure with segmented neutrophils was revealed.

By the 14th day of treatment, despite some clinical improvement, zones of non-specific chronic inflammation became visible in the underlying tissues with moderate focal lymphohysteocytic infiltration, some amount of fibroblasts and appearing patches of granulomatous inflammation with isolated gigantic cells of the foreign body type. The mucosa was patchily covered with flat layers of non-cornifying epithelial tissue with areas of uneven enlargements (hyperplasia) and acantosis. Patchy hyperplasia of granulation tissues was revealed with perifocal proliferation.

The specific quantity of inflammatory infiltrate cells reliably dropped by day 7 of the therapy: from 15.9±0.37 percent to 13.5±0.34 percent; by day 14 it slightly fell again down to 12.9±0.35 percent.

By day 14, the zones of sclerosis/fibrosis gradually reduced from 13.9±1.0 percent down to 9.7±0.72 percent and was by that time significantly different. The volume ratio of blood vessels did not reliably decline. The manifest reduction in the area of sclerosis/fibrosis by day 14 of observation, at the background of non-specific chronic inflammation with patchy lymphohysteocytic infiltration and
Persisting high volume ratio of blood vessels, is considered as the prevalence of another inflammation component that is characteristic of a transition to the chronic phase of inflammation.

A comparison of bioptic sample morphology, depending on the therapy being administered, revealed the following. At the moment of treatment onset, the bioptic morphology in all groups was similar and characterized by manifestations of an expressed inflammation with massive diffuse infiltration with prevalence of neutrophil leucocytes; focal acantosis was also very conspicuous. In terms of morphometrics, no significant differences were revealed at the beginning of the therapy.

On day 7 of the therapy, qualitative changes became apparent in bioptic samples, depending on the treatment being administered. In the group of animals receiving autohaemotherapy, moderate acantosis was observed; the infiltrate was mild and lymphohysteocytic, localized solely in a perivascular way.

In the reference group of animals, we revealed epithelial hyperplasia and expressed acantosis; leucocytic infiltration was diffuse and impure with segmented neutrophils. Thus, we can state that by day 7, in morphological terms, the best aspect of disease was observed in the group of dogs receiving autohaemotherapy, where no traces of inflammation symptoms were found. In the reference group, during this observation period, morphologically there were signs of an active inflammation.

On day 14 of the therapy, qualitative changes became apparent in bioptic samples, depending on the treatment being administered. In the group of dogs receiving platelet-rich plasma no inflammation whatsoever was revealed. There were mild isolated acantholytic cords and focal sclerosis in the underlying tissue. In the bioptic samples from the reference group of animals, zones of nonspecific chronic inflammation became visible with moderate focal lymphohysteocytic infiltration, some amount of fibroblasts and appearing patches of granulomatous inflammation with isolated gigantic cells of the foreign body type, along with hyperplasia of the mucosa with some patches of acantosis.

Thus, it can be stated that by day 14, in morphological terms, the best aspect of disease was observed in the group of dogs receiving autohaemotherapy, where no traces of inflammation symptoms were found. In the reference group, during this observation period, there were signs of non-specific non-chronic inflammation.

Discussion

Platelet auto plasma has been actively used for curing periodontal inflammations. Since in the pathogeny of chronic periodontal disease there prevail manifestations of an alternative inflammation leading to the destruction of the periodontal complex with a pathological activation of osteoclasts and bone tissue resorption, researchers have been striving to develop novel methods of cure that would facilitate periodontal tissue regeneration.

One of these techniques is platelet-rich plasma therapy – an injectable preparation of trombocytic plasma developed and proposed by Dr.Med. R.R. Akhmetov and Dr.Med. R.F.Zarudy in 2004; it has been used ever since for curing various inflammatory and atrophic conditions in the maxillofacial area.

Plasma rich in platelets contains large amounts of their alpha-granules that are reservoirs of bioactive proteins important for initiation and speeding up of tissue recovery and regeneration.

If the concentration of platelets goes up, it is followed by concentration of growth factors, such as: platelet-derived growth factor (PDGF-aa, PDGF-bb, PDGF-ab), transforming growth factor (TGF-bl, TGF-b2), vascular endothelium growth factor (VEGF). Animal tests and clinical studies have demonstrated that bone regeneration is enhanced if bone material is combined with PDGF, TGF-p and other growth factors.

Thrombocytes release about 70 percent of growth factors in the first ten minutes. Complete release takes place in an hour, after which more growth factors are synthesized in the course of eight days; then the thrombocytes die (R. Marx, E. Carlson, R. Eichstaedt et al., 1998).

Growth factors are released in exact proportions and in a defined sequence progressively as platelets
do activate themselves; their activity is local, they draw undifferentiated cells to the lesion area and trigger the process of these cells' mitosis, stimulate neoangiogenesis, which leads to active regeneration and maturation of both bones and soft tissues.15

Gfatter et al. demonstrated the effectiveness of activated thrombocytes, bound by fibrinogen, in fibroblast mitosis. Baeyens W., Glineur R., Evrard L. note that platelet-rich fibrin and plasma have been successfully used in different areas of medicine, particularly so in oral and maxillofacial surgery. In addition to the above factors, thrombocytes contain insulin-like growth factor (IGF) and epithelial growth factor (EGF). Their use leads to a better density of bone transplants and a quicker regeneration in maxillofacial surgery.4 They are a basis for bioactive membranes; on the other hand, membrane-based methods are a real challenge in practical healthcare.16

It has been established that in the presence of secondary and tertiary growth factors, the functions of the primary ones are modulated and regulated, which sets the use of platelet-rich plasma apart from recombinant growth factors that have proved to be less functional if compared to natural ones.17 Sanchez A.R., Sheridan P.J., Kupp L.I. (2003) showed that in addition to growth factors, platelets release a variety of other bioactive compounds (for instance, fibronectin, vitronectin and sphingosine 1-phosphate) that play a decisive role in wound healing.

It has been noted that injections of platelet-rich auto plasma improves tissue oxygenation that, in turn, enhances phagocytic and germicidal capabilities of immune cells, and supports collagenation and synthesis of other proteins.18 Additionally, the use of platelet-rich auto plasma demonstrates significant advantages: it is a simple procedure requiring no subsequent rehabilitation period, it is non-toxic and most natural carrying minimal risks of side effect, it can be combined with other therapies, and the risk of infection is fully eliminated.13

In order to ensure the presence of a platelet concentrate facilitating recovery, it is important to make the process of its preparation most effective in terms of the amount of thrombocytes coming from one blood sample since it is the former that release the necessary growth factors. Scientific evidence has been provided of better bone/soft tissue repair if a platelet concentrate is applied with the thrombocyte content of 300–600 percent against the initial level. The more growth factors are delivered to the affected area, the higher its healing potential. It has been proven that the stimulating effect of plasma therapy comes to bear in with platelet concentrations of min 1.000.000/ml.18

Clinical studies of platelet-rich auto plasma and its efficiency have demonstrated that this approach is more than promising.

In a clinical study,2 bone defects were successfully restored using porous bone materials and their combination with platelet-rich plasma, in cases of severe periodontal disease. L.V. Chudova (2009) provides a clinical and experimental justification of a combined use of enamel matrix proteins and auto-platelet concentrates for operative therapy of periodontal tissue recession.16 An experimental clinical study was done7 utilizing platelet-rich plasma for reparative surgery operations on jaws with the aim of quickening regeneration of extraction sites, and when installing intra-bone dental implants; the study has proven the effectiveness of local administration of platelet-rich plasma for bone regeneration.

Kaushick B.T., Jayakumar N.D. et al. (2011), for the purposes of gum disease surgery, successfully used a combination of platelet-rich plasma, hydroxyapatite and TCP ceramics. It was convincingly demonstrated that platelet-rich auto plasma is beneficial as part of the SFE procedure, which has manifested itself in shorter bone maturation times.5

As a result of platelet-rich auto plasma application, researchers were able to halt periodontal inflammations, prevent bone tissue shrinkage, improve local immunity and repair microflora imbalance in the oral cavity.19 Also interesting are studies on PRP therapy in veterinary science,
for pets (dogs and cats). The authors convincingly proved the effectiveness of this method in cases of chronic gum disease.6

Application of PRP therapy for curing catarhal gingivitis of I-II classes of severity allowed reducing the incidence of exacerbations and prolonging remission in periodontal diseases.12

Currently, there are several approaches to obtaining PR plasma. Prakash S., Thakur A. in their review outlined the history of clinical use of 1st and 2nd generation platelet-rich concentrates; at the same time, in respect of many an existing method and technique, they note that further studies are lacking on its effectiveness and combination with other therapies. Recently, researchers have been showing interest in the import phase-out method of PRP for curing periodontal conditions.10,19

The problem of administering platelet-rich auto plasma in cases of erosive lesions in the oral cavity have not been analysed in a satisfactory manner till the present day. The characteristics of this method are exactly what predetermines its wide implementation in clinical dentistry as an effective way of stimulating tissue regeneration.

As a result of the present study, for the first time experimental data have been obtained on the optimal use of PRP injections for curative therapy of erosive lesions in the oral cavity.

For the first time, proceeding from the experimental results of the study, the effectiveness of PRP injections has been justified as part of a combined therapy of dental illnesses. From our experiments it transpires that application of PRP therapy is safe and effective.

**Conclusion**

The results of the study have proven that PRP therapy is effective for curing erosive lesions in experimental animals. This method allows restoring tissue regenerative processes in shorter periods if compared with the conventional therapy designs, without any complications and side effects, to which testifies the morphological pattern of the total arrest of inflammations in a damaged tissue by day 14.

Thus, this experimental study confirms that PRP therapy is safe and effective as part of a combined therapy of erosive lesions in the oral cavity.

**Recommendations**

Based on the results of the study, we have developed and proposed an algorithm for using PRP injections to cure erosive-ulcerous oral lesions. It is carried out in several stages:

- **Step One:** specifying indications and counter indications.
- **Step Two:** preparing a patient for the therapy.
- **Step Three:** oral cavity sanitation.
- **Step Four:** preparing platelet-rich auto plasma.
- **Step Five:** injecting PRP to the affected area, per se.

The total duration of the course – 14-21 days. After the treatment, case follow-up must ensue with the frequency of two examinations in the first twelve months. After that, check-up examinations have to be done once annually. During the whole time of using this algorithm, no complications or side effects were observed.

If the proposed algorithm of using PRP method in a combined therapy of erosive-ulcerous lesions in the oral cavity is applied, then by day 21 of the treatment the quality of life of the patient demonstrates significantly better values and remains such for the whole period of observation, along with retaining reliable advantage. The proposed sequence of using PRP therapy is an effective and safe method of a combined dental treatment of oral diseases.

**Thus**

In order to better the outcomes of curing erosive-ulcerous lesions in the oral cavity, we recommend supplementing a combined therapy with PRP injections having anti-inflammatory effects and high regenerative properties.

To ensure a stable and prolonged therapeutic effect, we recommend administration of 1-3 injections of PRP during one course of treatment of 14-21 days.

During remission, with the aim of preventing exacerbation, we recommend minimum two additional injections.
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