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COMPARATIVE STUDY OF REPARATIVE REGENERATION OF THE ORAL MUCOSA IN EXPERIMENT

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ABSTRACT

At present, treatment of inflammatory-destructive diseases of the oral cavity is still a relevant problem. Despite the fact that any dentist disposes a wide range of medical preparations, it is difficult to choose one with the least amount of complications. While managing elderly patients with dental problems the goal is not only to facilitate healing of the oral mucosa, but also to prevent the recurrence of pathology. Injections of platelet autoplasma may be useful in treatment of inflammatory-destructive diseases of the maxillofacial region. Injections of platelet autoplasma for inflammatory-destructive diseases of the oral mucosa, effect of synergism of this method in combination with other therapeutic means justify the need for further study of this issue. In this work, in order to study the regenerative potential, the simulated wound defect in the oral cavity of 30 dogs was treated experimentally. Regeneration control, treatment and supervision lasted 14 days alongside with application of various pharmacotherapeutical methods. To exclude additional factors which might affect the final result of the experiment associated with individual physiological features, study of experimental local treatment methods was carried out within one body: traditional methods of treatment; application of Tysol composition with L-arginine; injections of platelet autoplasma were included in the treatment plan as well as a combined method of treatment. Platelet autoplasma injections combined with Tizol applications with L – arginine was proved to help restore and heal the oral mucosa much faster comparing to other treatment methods.

Keywords: regeneration, experiment, dogs, platelet autoplasma, Tizol, pain, hyperemia, wound, treatment.

Introduction. Works on modeling and research into regeneration potential of oral cavity wounds can be seen both in domestic and foreign sources being reflection of the fundamental aims of the research. [11]. Experiments provide the possibility of a deeper research into pathogenetic mechanisms of oral cavity diseases, substantiation of efficacy and directivity of new approaches to stimulation of the regeneration potential in the oral cavity without affecting the patient [12]. At present, there are some publications on experimental works to study regeneration potential of oral cavity tissues. This trend became popular mainly in surgical practice. Works on predictability of the oral mucosa healing (OMH) are associated with the principle of guided tissue regeneration. So, K.A.Aksenov et al. (2011) carried out an experiment on mini-pigs of Svetlogorsk breed making a model of wound process to study the process of surgical wounds regeneration in the oral cavity after their suturing and to increase efficacy of treatment in surgical dentistry clinics. This article reflects necessity to change the approach to the final stage of surgical interference - suturing for positive result of treatment. [1].

To substantiate experimentally application of recombinant epidermal growth factor, an experiment on white Wistar rats was performed. On the hard palate of laboratory animals wounds of 5 mm in diameter were made as deep as to the bone. Healing progress under immunodeficiency conditions against the background of recombinant growth factor therapy was evaluated planimetrically, pathomorphologically and histologically. The research proved a favourable effect of the carried out therapy on the wound healing against the background of immunodeficiency [2].

In therapeutic dentistry application of electrochemically activated solutions for comprehensive treatment of erosive ulcerous OMH lesions in patients with lichen ruber planus was experimentally explored to study the character of epithelization. Experiments were performed on chinchilla rabbits. The authors studied effect of electrochemically activated solutions on activity of antioxidant defense enzymes in the rabbit's isolated erythrocytes *in vitro*. Laboratory data of the rabbit's blood proved the effect of electrochemically activated solutions on the activity of antioxidant defense which depends on oxidation-reduction potential of the solution possessing electron-donor properties [3].

The problem of developing new highly effective methods of OM diseases is still pressing and important in therapeutic dentistry. Therefore, these are experimental studies that help obtain necessary data on efficiency of pathogenetic impact of new medical compositions and methods of therapy directed to stimulate reparative regeneration in the oral cavity prior to their clinical testing.

As alternative inflammation [6] prevail in the pathogenesis of inflammatory-destructive geriatric diseases and lead to destruction of the oral mucosa [5], at present researches are done into modern and effective methods of therapy restoring reparative regeneration of the damaged tissue [7].

In spite of the fact that medical science advances with seven-league strides and dentists possess accumulated experience, the issue of research into reparative process of the oral mucosa remains pressing [8]. This is the reason for further advanced researches into peculiarities of reparative regeneration of oral mucosa in order to enhance clinical efficiency of treatment and patients' quality of life.

These are platelets that release different growth factors which enhance healing of regenerative potential. Fibroblasts, in turn, produce collagen, hyaluronic acid and elastin. This process results in formation of young connective tissue. Growth factors also block osteoclasts and stimulate osteoblasts proliferation, thus suppressing bone loss and promoting its regeneration [9].

The data obtained with clinical researches substantiate therapeutic effect of PRP – therapy. Including injections of platelet autoplasma into the treatment plan, the researchers managed to reduce or eliminate inflammatory parodontal diseases, prevent and arrest bone destruction, enhance local immunity of the oral cavity and normalize oral microflora [4,10]

At present, PRP – therapy is performed either with injections or applications. Following centrifugal separation regime is

an important integral part of the method [4]. At the same time application of this method for inflammatory-destructive diseases of the oral mucosa, efficiency of PRP-therapy sinergism in combination with other therapeutic methods prove the necessity of further studies of the issue.

Objective of the study: Experimental evaluation of wound defect healing process in dogs' oral cavity in conditions of application of a new combined method and traditional plans of treatment.

Material and methods of the research. In the experiment, clinical evaluation of a standard wound defect in dogs' oral cavity was performed which helped qualitatively evaluate dynamics of the wound healing against the background of therapy. The study was performed in the laboratory of pathology modeling of SBI "Volgograd Center of Medical Sciences". The experiment was performed on 30 male dogs weighing from 2 to 16 kg, living in a vivarium (t° = 22-24 C°, relative air humidity 40-50%), with a natural regime and standard diet (GOST P 50258-92), following rules of laboratory practice while carrying out pre-clinical researches in the Russian Federation (GOST 3 51000.3-96 и 1000.4-96) as well as rules of humane treatment of animals (Report of the AVMA Panel on Euthanasia JAVMA, 2001) and International recommendations of European convention for Protection of Vertebrate Animals in experimental researches (1997). Experiments were approved by the Ethical Review Committee of the Volgograd State Medical University (Proceedings No 2036).

The animals were premedicated by 0.1% atropine injected in the left gastrocnemius muscle in the amount corresponding to the animal's weight (under the producer's instructions). Anesthesia was performed with "Zolitil-100" injected intramuscularly in the amount of 8 mg/kg (under the producer's instruction). When the animal stopped reacting to stimulation, it was immobilized with a device used for immobilization of the experimental animal for sampling bioptic material from the oral cavity (Patent No 62527 of 10.06.2016).

Teeth of the immobilized animals were cleaned with ultrasound, dental deposits were removed manually, oral mucosa was treated with antiseptic solution of 0.05% chlorhexidine bigluconate to eliminate a bacterial factor which could affect the course of experiment.

Experimental modeling of the pathological process was performed by making lesions on the oral mucosa of the upper and lower jaw, left and right from premolar 1 in the vestibule of the oral cavity. Four wounds 1x1 cm each were made on the mucosa.

The operation was performed under intravenous anesthesia by Zoletil and wounds made with a scalpel and pincers.

To exclude additional factors associated with individual physiological characteristics which could affect the final result of the experiment, research into experimental methods of local treatment was performed on one body.

Group 1 (control) – On the right lower jaw the dogs' OM wound was treated with a traditional method (application of Celestoderm: Solcoseril= 1:1).

Group 2 (experimental) – On the right upper jaw applications of Tizol with L-arginine were use for the complex treatment of OM would defect;

Group 3 (experimental) – On the left upper jaw the treatment plan included injections of platelet autoplasma;

Group 4 (experimental) – On the left lower jaw 2.0 ml of platelet autoplasma was administered into the OM wound defect by infiltration along the mucobuccal fold alongside with application of Tizol with L- arginine composition using sandwich-technique.

Platelet plasma was made by taking 7 ml of venous blood from the laboratory animals with a butterfly syringe into a vacuum test-tube. The test-tube was put into the centrifuge 80-2S (China) at 3 500 rpm for 5 min. With this method 3.5 ml of platelet autoplasma was obtained.

Subsequently, the wound defect was treated by injecting platelet autoplasma into the lesion.

The wound defect condition in the animals was clinically monitored daily for 2 weeks.

Hyperemia level was evaluated using clinical evaluation scale: 0 - no hyperemia, 1 point– insignificant hyperemia, 2 points – moderate hyperemia, 3 points– marked hyperemia. Edema of the wound edges was also evaluated using 4-point scale: 0 – no edema, 1 point – insignificant edema, 2 points – moderate edema, 3 points marked edema. Area of the wound defect, OM regeneration index and OM regeneration intensity were determined using the technique developed by L.N. Dedova and I.N. Fedorova (2005), with the help of our device to measure the lesion area in the oral cavity (RF Patent for useful model No 66417 of 07.11.2016). The results were considered on the 3d, 7th and 14th day of treatment and monitoring. Photo documentation Dynamics of OM wound defects treatment in experimental animals was photo documented with a Sony digital camera (8 megapixel). During experiment 480 photos were analyzed.

The data obtained were processed using the variational –statistical method, a PC and Microsoft Excel program for MS Windows XP /Microsoft Corp., USA, as well as Stat Soft Statistica v6.0. application package (A.P. Kulaichev, 2006) in accordance with standard methods of medical statistics. Statistical analysis was done using the method of variational statistics by determining mean (M), its mean error (±m), evaluation of significance of differences by group with Student's test (t). Difference between compared indicators was considered to be significant with $p< 0,05$, $t\geq 2$ (V.I.Sabanov, E.R.Komina, 1996, I.F.Sprace et al. , 2006).

Results and discussion. Morphometric examination of the wound defect in the oral cavity against the background of treatment showed neither complications nor side effects in all groups, positive results were obtained in all groups, though healing dynamics was different depending on therapy conducted. So, edema decreased more rapidly in the 4th group of dogs whose treatment plan included a combined method of treatment (platelet autoplasma injections in combination with Tizol and L-arginine applications). On the 3d day edema indicators in the 4th group were 1,5±0,09 points which was 1.8 times less comparing with group 1 (2,7±0,08 points); 1.5 times less comparing with group 2 (Tizol with L-arginine applications) and 1.4 times less comparing with group 3. This difference was statistically significant ($p<0,05$). Significant difference was also obtained on determining this indicator in groups 2 and 3 relative to the group with conventional treatment ($p<0,05$). The difference between group 2 and 3 was statistically insignificant ($p>0,05$). A week after the experiment began a slight edema was noted in the first three groups: group 1 – 1,8±0,13 points; group 2 – 1,3±0,07 points; group 3 – 1,1±0,11 points. In group 4 edema was estimated as 0,3±0,08 points, that indicates absence of edema in the dogs' oral cavity.

Differences between experimental groups relative to group 4 were also statistically significant ($p<0,01$), so, it can be noted that applying a combined method of wound treatment there is a clear tendency towards marked decrease of edema against the background of the treatment conducted. On the 14th day of the experiment all dogs on all four sides showed no signs of edema in the oral cavity.

Significant difference was also obtained on evaluation of hyperemia in the oral cavity. On the 3d day, hyperemia was 2,53±0,19 points in the group with conventional method of treatment which was 10% higher than in group 2 and 3 (2,3±0,05 points with p>0,05) and 58% significantly higher comparing with the group with treatment of platelet autoplasma injections combined with Tizol and L-arginine applications (1,6±0,09 points, p<0,05).

On the 7th day signs of hyperemia decreases in all groups. Significant difference was noted in group 4 comparing to other experimental groups (p<0,05). Hyperemia in group 4 was 0,5±0,09 points which was significantly 3 times lower comparing with group 1 (1,5±0,09 points), 2.6 times lower comparing with the group whose treatment plan included applications of Tizol with L-arginine, and 2.4 times less comparing with group 3. Between groups 1,2 and 3 no significant difference was noted (p>0,05) on evaluation of indicators of hyperemia in the oral cavity. On the 14th day hyperemia in the dogs' oral cavity was not noted.

On the 3d day significant decrease of the would defect diameter was noted, though in the group with combined method of treatment the diameter was 4,6±0,12 mm which was 34.7% lower comparing with group 1 with diameter of 6,2±0,18 mm which was 13% less comparing with group 2 (5,2±0,14 mm and 15% less comparing with the group whose treatment plan included platelet autoplasma injections (5,3±0,15 mm). Groups 2 and 3 showed significant difference relative to group 1 (p<0,05). The area of the wound defect also significantly decreased in all groups comparing with previously obtained data. It must be stressed that regeneration of the oral mucosa in the first three groups correspond to the 2nd degree of regeneration, and in the group with combined method of treatment the course of reparative processes was much more effective, degree III of the regenerative potential being the evidence. .

A week after dynamic monitoring and treatment of the dogs the area of the would defect significantly decreased in all groups, though in group 4 the postoperative would was not visually revealed, its area was 0,8 mm^2. In the group with conventional method of treatment the area of the wound defect was 8.7 mm^2, that was 2.4 times significantly higher comparing with group 2 (3.59 mm^2) and 3.3 times higher comparing group 3 (2.57 mm^2), with p<0,05.

After 14 days the wound defect disappeared in all four groups, no signs of inflammation were noted, the oral mucosa of all dogs fully regenerated.

Conclusions.

Thus our experiment revealed that Tizol with L – arginine applications arrests inflammation much quicker in comparison with conventional therapy that is proved by significantly obtained difference on determining quantitative criteria. There was no significant difference between this group and the group where injections of platelet autoplasma were applied which proves the advantage of these two methods comparing with the conventional therapy and efficacy of both methods to enhance regeneration of the oral mucosa.

In spite of the fact that after 2 weeks full regeneration of the connective tissue in the oral cavity was noted in all dogs, in the group with combined method of treatment there is a tendency towards more marked and earlier epithelization, which is proved by significant difference in all periods of the experiment. One can suppose that injections of platelet autoplasma alongside with applications of Tizol with L-arginine more effectively influence the process and healing comparing with other methods of therapy.

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EXPERIMENTAL PHYTOCORRECTION OF ACUTE D-GALACTOSAMINE HEPATITIS IN WHITE RATS

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ABSTRACT

The aim of our research was in defining hepatoprotective properties of new complex plant supplement on a rat model of D-galactosamine hepatitis. The new complex drug supplement is a dry extract consisting of Hypecoum erectus L., Hedysarum dauricum, Glycyrhhiza uralensis Fisch., Calendula officinalis and Scutellaria baicalensis. Intensity of the main pathogenetic syndromes was evaluated by biochemical tests, lipid peroxidation grade and morphological research. The use for the complex extract resulted in correction of functional state of the liver, inhibition of cytolysis and cholestasis, delay of LPO and enhancing synthetic function of liver manifested in albumin and fibrinogen increase.

Keywords: acute experimental hepatitis, hepatoprotective drugs, complex drug of medicinal plants.

Introduction. A necessity in finding new hepatoprotective drugs and supplements is dictated by the growing demand: increasingly widespread liver pathologies tending to chronic forms being caused by viruses, toxic agents including some medication [8, 9, 10]. Under these conditions, medicinal plants are of interest considering the wide range of their therapeutic effect, low toxicity and the possibility of gaining amplified effect by combining active components of complex plant supplements [1- 4].

The aim was in determining pharmacotherapeutical effectiveness of new complex medicinal supplement on a rat model of acute D-galactosamine toxic hepatitits.

Materials and methods of research. The researched plant supplement is a dry extract consisting of dry extracts of *Hypecoum erectum* L.; Papaveraceae grass, dry extract from *Hedysarum alpinum* L.; Leguminosae grass, dry extract of *Glycyrrhiza uralensis* Fisch.; Leguminosae roots, dried flower extract of *Calendula officinalis* L.; Compositae and dry root extract of *Scutellaria baicalensis* Georgi; Lamiaceae in proportion of 5:5:4:4:2.

Compounded preparations were performed using HPLC with UV-detector on MiLiChrom A-02 by ECONOVA (Милихром А-02, Эконова), Novosibirsk, Russia with column ProntoSIL-120-5-C18 AQ (2 × 75 mm, ∅ 5 μm; Metrohm AG, Herisau, Switzerland); mobile phase: 0.2 M $LiClO_4$ with 0.006 M $HClO_4$ (A), MeCN (B). During the separation process profile of gradient elution was set to 0–40` 5–100% B, 40–43` 100% B with speed of 100 μl/min, temperature set to 35°C with UV-detection at a wavelength of 210 nm. Concentration of substances was measured with commercially available samples (Sigma-Aldrich). Detected components included: glycyrrhizic acid 2.06 ± 0.04%, baikalin 1.85 ± 0.04%, protopin 1.09 ± 0.03%, mangipyrin 0.68 ± 0.02%, typhaneoside and narcissin combined 0.27 ± 0.01%.

The pharmacotherapeutical effective-