EXPERIMENTAL IN VITRO STUDY OF THE PENETRATION OF GEL COMPOSITION INTO THE BIOLOGICAL ENVIRONMENT BY ELECTROPHORESIS

Introduction. The number of undiagnosed dentoalveolar anomalies (DA) increases with age, and their flow is significantly burdened by the addition of periodontal tissue pathology [1]. Fixed orthodontic appliances used in the active period of orthodontic treatment of malocclusion can cause inflammation in the tissues of the periodontal complex. As a result, a violation of the oxidation-antioxidant balance leads to the development of oxidative stress with increased lipid peroxidation (LPO) and accumulation of free radicals [2].

The development of pathogenetically targeted agents and methods to reduce the level of local stress-modulated metabolic disorders in periodontal tissues before and during the active period of orthodontic treatment is relevant. It is flavonoids that are promising drugs for the prevention and reduction of periodontal disease signs under local oxidative stress [3].

In the present study, experimental electrophoresis was performed with the patented periodontal gel composition «Benzidaflaziverdin» (GCB), consisting of two active
components – «Proteflazid®» (LLC NKV «ECOPHARM», Ukraine) – drops (flavonoids) and benzidamine hydrochloride («T-Sept®» ICN, Polfa, Poland), tablet form, which is a topical non-steroidal anti-inflammatory drug) on a gel basis [4].

**Aim:** to study the effectiveness of penetration of a gel composition based on a flavonoid complex and benzidamine hydrochloride (GCB) into the medium from mammalian and human cells in semi-liquid agar by electrophoresis.

**Materials and methods.** The effectiveness of penetration of GCB and comparison drugs (positive control – «Cholisal» (Elfa, Poland) and «Gengigel» (RICERFARMA s.r.l, Italy); negative control – Doxorubicin (Kyivmedpreparat, Ukraine)) into the simulated periodontal tissue medium in an in vitro experimental model consisting of BALB-3T3 mouse fibroblasts and pseudonormal human keratinocytes of the HaCaT line. To mimic the density of the intercellular medium of biological tissues, 0.66% agar (Agar-Agar, Low Melting Agar, SERVA Feinbiochemica, Heidelberg, Germany) was added to the cell culture. A «Potik-1» device (SMEP) was used for electrophoresis.

In 12-well plastic multicups, cells of a specific cell line were grown and aluminium foil electrodes were used (Mini Bin™ aluminium foil Z691569 Heathrow Scientific HS23534A for MERK). The test samples were applied to the (+) electrode in one well and to the (-) electrode in the other. The third well was a control (no drug) to study the passage of electric current. The duration of the procedure (exposure) was from 10 to 20 seconds at a current of 0.2-0.4 mA. The effect of GCB, comparison drugs and different electrophoresis modes on the viability of cell lines was assessed using the MTT assay (Sigma, USA) [5].

**Results.** It was found that our patented GCB best promoted the proliferative activity of both types of experimental cells (keratinocytes, fibroblasts) and significantly outperformed the effect of the comparison drugs «Cholisal» and «Gengigel». Electrophoresis of cellular systems potentiated the cytoprotective and protective effects of GCB when applied to the «+» electrode.

For HaCat keratinocytes, the electrophoresis procedure, regardless of which drugs were used, did not significantly affect the number of cells in the tested population. At the later stages of the experiment (7 days), the addition of GCB also had no significant effect compared to the effect of the zero control and comparison drugs. At the same time, when electrophoresis was combined with the effect of comparison drugs, direct electric current levelled the suppressive effect of «Cholisal» and «Gengigel». Additional studies are needed to explain the possible mechanisms of such cytoprotective effects.

In the case of NIH 3T3 fibroblasts, as well as in the case of HaCat keratinocytes, the effect of levelling the suppressive effect of the comparison drug «Cholisal» by direct electric current was observed from 77.3% to 97.2%. For NIH 3T3 fibroblasts, the electrophoresis procedure, depending on how the GCB was applied (application to «+» or «-» electrodes) on day 3 of the experiment, negatively affected the number of cells in the population: 79.6% when applied to the «-» electrode versus 106.8% when applied to the «+» electrode, and 58.7% when applied GCB to the «-» electrode versus 85.8% when applied to the «+» electrode on day 5. At the same time, on day 7, no significant decrease in the number of cells was detected in the wells. A decrease in the number of cells was detected in the presence of the comparison drug «Cholisal» – the effect was noticeable on the 3rd (77.3%) and 7th (61.9%) day. This phenomenon was especially pronounced on day 5 (decrease to 51.2%). Electrophoresis partially eliminated the negative effect in the form of a decrease in the number of cells in the population under the influence of the comparison drug «Cholisal» on day 3 (99.2%), and this effect was enhanced on day 5 (38.1%). On the 7th day of the experiment, the negative effect of Holisal was fully compensated (101.8%). For unknown reasons, on the 5th day of the experiment, the negative effect was even more pronounced than without electrophoresis. It should be noted that under the influence of direct electric current, cells reacted more strongly to the presence of negatively charged molecules.
Thus, the patented GCB showed a better ability to promote the proliferation of all types of cells tested than the comparison drugs «Cholisal» and «Gengigel». The electrophoresis procedure potentiated the cytostimulatory and protective effect of GCB when the drug was applied to the «+» electrode. A study using an experimental model of cell cultures in a simulated environment demonstrated the significant potential of the electrophoretic procedure to enhance the penetration of GCB components deep into the periodontal tissue model. This is evidence that the duration of the electrophoresis procedure in the clinic can be reduced from the standard 15-20 minutes per jaw to 15-50 seconds. At the same time, the prolongation of GCB action and targeted local delivery of its active components (flavonoid complex and benzidamine hydrochloride) to periodontal tissues were maintained.

Conclusions. *In vitro* studies have established that the application of GCB on the anode («+» electrode) is optimal and experimentally proved the effectiveness of exposure of this gel composition in the oral cavity during electrophoresis for 15-50 seconds (current 0.2-0.4 mA), as opposed to the standard duration of the classical clinical procedure of 15-20 minutes. It has been shown that the patented GCB best promotes the proliferation of all types of test cells (fibroblasts, keratinocytes) and has a significant advantage over the comparison drugs («Cholisal», «Gengigel»). GCB modulates the ability of cells to withstand stress due to its antioxidant properties.

References:


