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EVALUATION OF INDICATORS OF BACTERIAL CONTAMINATION, ANTIMICROBIAL AND ANTIOXIDANT PROTECTION IN ORAL MUCOSAL HOMOGENATES OF RATS UNDER EXPERIMENTALLY INDUCED IMMUNODEFICIENCY AND DYSBIOSIS FOLLOWING A THERAPEUTIC-PROPHYLACTIC REGIMEN

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The study was devoted to the evaluation the impact of a multi-component therapeutic-prophylactic complex on indices of bacterial contamination, antimicrobial defence, and antioxidant protection in oral mucosal homogenates of rats subjected to cyclophosphamide-induced immunodeficiency and lincomycin-associated dysbiosis. Thirty adult male Wistar rats were randomly assigned to an intact control, a combined pathology group, and a combined pathology + therapeutic-prophylactic complex group (n=10 each). Seven days after modelling the pathology, animals in the third group received the therapeutic-prophylactic complex for 30 days. Urease and lysozyme activities were quantified in mucosal homogenates, and a dysbiosis index was calculated. The therapeutic-prophylactic complex significantly reduced urease activity, restored lysozyme activity, and normalised the dysbiosis index, demonstrating pronounced antimicrobial, antioxidant, and immunostimulatory effects. These findings substantiate further exploration of the therapeutic-prophylactic complex as a promising strategy for preventing complications arising from combined immunodeficient–dysbiotic conditions.

Key words: bacterial contamination, lysozyme, urease, immunodeficiency, dysbiosis, antioxidants, therapeutic-prophylactic complex.

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ОЦІНКА ПОКАЗНИКІВ БАКТЕРІАЛЬНОЇ КОНТАМІНАЦІЇ, АНТИМІКРОБНОГО ТА АНТИОКСИДАНТНОГО ЗАХИСТУ В ГОМОГЕНАТАХ СЛИЗОВОЇ ОБОЛОНКИ ПОРОЖНИНИ РОТА ЩУРІВ НА ТЛІ МОДЕЛЮВАННЯ ІМУНОДЕФІЦИТУ І ДИСБІОЗУ ТА ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНИХ ЗАХОДІВ

Дослідження було оцінці впливу лікувально-профілактичного комплексу препаратів на рівень показників бактеріальної контамінації, антимікробного та антиоксидантного захисту в гомогенатах слизової оболонки порожнини рота щурів на тлі моделювання імунодефіциту та дисбіозу. У дослідження включено 30 статевозрілих самців щурів лінії Wistar, розподілених на інтактну групу, групу сукупної патології та групу сукупної патології з лікувально-профілактичним комплексом (n=10 у кожній). Через 7 діб після моделювання патології тваринам третьої групи вводили лікувально-профілактичний комплекс протягом 30 діб. В гомогенатах слизової визначали активність уреази та лізоциму, розраховували індекс дисбіозу. Отримані результати підтверджують протимікробні, антиоксидантні та імуностимулювальні властивості лікувально-профілактичного комплексу і обґрунтовують його подальше застосування як перспективного засобу профілактики ускладнень при комбінованих імунодефіцитно-дисбіотичних станах.

Ключові слова: бактеріальна контамінація, лізоцим, уреаза, імунодефіцит, дисбіоз, антиоксиданти, лікувально-профілактичний комплекс.

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The contemporary concept of the "oral barrier" views the oral mucosa as a dynamic ecosystem in which innate-immune factors – most notably lysozyme and other mucosal enzymes – constitute the first line of defence against opportunistic microorganisms [6, 11]. Under physiological conditions, this biocenosis sustains tolerant co-existence between saprophytic microbiota and the epithelial layer; however, any disequilibrium rapidly drives the local response into an inflammatory phase, characterised by the generation of reactive oxygen species and the activation of urease-positive bacteria.

The organism is particularly vulnerable to such shifts under pharmacologically induced immunodeficiency and antibiotic-associated dysbiosis. Cyclophosphamide-mediated immunosuppression, potentiated by lincomycin, leads to a sharp decline in microbial diversity and predominance of

Proteobacteria, correlating with increased urease activity and translocation of toxic metabolites [8, 12, 13]. These disturbances manifest not only in the gut but also in the oral niche, creating a "continuous mucosal corridor" that heightens the risk of systemic complications.

Concomitantly, microbial imbalance augments oxidative stress, promoting peroxidative damage to lipids and proteins of the mucosa. Antioxidant phytocompounds – such as curcumin and polyphenolic extracts – have shown the capacity to attenuate oral mucositis, modulate Nrf2/HO-1 signalling, and restore epithelial integrity [5, 9]. Nevertheless, long-term clinical and experimental evidence for these interventions remains limited.

Accordingly, the development of multi-component therapeutic-prophylactic regimens that combine antioxidant, immunostimulatory, and antimicrobial properties is highly relevant. Recent advances in mucosal immunology demonstrate that combined protocols aimed at restoring barrier function and reshaping the resistome pool of the microbiota can produce sustained benefits [4, 7, 14]. Such an approach is especially promising in experimental models that allow detailed monitoring of the interplay between bacterial contamination, innate-resistance markers, and tissue oxidative status.

Thus, a systematic investigation of changes in bacterial load, antimicrobial defence, and antioxidant protection of the oral mucosa under pharmacologically induced immunodeficiency and dysbiosis is essential for substantiating rational therapeutic-prophylactic strategies. These objectives defined the purpose of our study, which employed a rat model of combined pathology and evaluated a targeted multi-drug complex.

The purpose of the study was to evaluate the effect of a multi-component therapeutic complex on indices of bacterial contamination, antimicrobial defence and antioxidant protection in oral-mucosal homogenates of rats under experimentally induced immunodeficiency and dysbiosis.

Materials and methods. Experimental work was carried out on 30 sexually mature male Wistar rats (outbred stock), four months of age, with a mean body weight of 280±10 g. The animals were housed under standard vivarium conditions – natural 12-h light/dark cycle, unrestricted access to pelleted chow and reverse-osmosis-filtered water. Throughout the study, microclimatic parameters were maintained at 19–22 °C and 55–75 % relative humidity. Routine daily, weekly and general sanitisation procedures were performed to ensure hygienic housing conditions. Experimental studies were conducted at the Laboratory of Biochemistry and Vivarium of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS"). All experiments on rats were conducted according to standard operating procedures approved by SE "ISMFS NAMS", developed in accordance with the Guidelines of the Pharmacological Committee of the Ministry of Health of Ukraine and the International Regulations for the Use of Laboratory Animals [2].

The animals were randomly assigned to three experimental groups:

1 – Intact control, n=10;

2 – cyclophosphamide-induced immunodeficiency plus lincomycin-associated dysbiosis (combined pathology), n=10;

3 – Combined pathology+therapeutic-prophylactic complex, n=10.

Rats in the intact group received a balanced pelleted diet that fully covered daily requirements for macro- and micronutrients, together with reverse-osmosis-filtered, decontaminated water. To equalise handling stress, these animals were intramuscularly injected with sterile 0.9 % saline in volumes equivalent to those administered to the experimental groups.

The duration of the experiment was 37 days. Modelling of immunodeficiency and dysbiosis was carried out according to the method of A.P. Levytskyi (2016): the immunodeficiency model – cyclophosphamide (PJSC "Kyivmedpreparat", Ukraine) was administered intramuscularly to rats at a dose of 50 mg/kg in two injections of the solution with an interval of 2 days, and before administration the 0.2 g vial of the drug was dissolved in 10 mL of sterile 0.9 % NaCl solution; the dysbiosis model – the antibiotic lincomycin (JSC "Farmfirma Darnytsia", Ukraine) was given to rats with drinking water at a dose of 70 mg/kg body weight for 5 days, which suppresses the growth of probiotic microflora: bifidobacteria and lactobacilli. The lincomycin dose was calculated taking into account the volume of water consumed and the body mass of the animals [1].

Seven days after modelling of the pathology, a therapeutic-prophylactic complex was administered for 30 days. The complex included preparations with antioxidant, immunostimulatory, wound-healing and anti-inflammatory activity. Blood sampling and euthanasia of rats in all experimental groups were performed after a prior 24-hour food deprivation with free access to water. Euthanasia of the animals was carried out under thiopental anaesthesia administered intraperitoneally at a dose of 40 mg/kg.

In homogenates of the oral mucosa of rats, prepared at 20 mg/mL in 0.05 M Tris-HCl buffer, pH 7.5, the intensity of microbial contamination was determined by urease activity, the level of nonspecific immunity was assessed by lysozyme activity through hydrolysis of Micrococcus lysodeicticus cells, and the degree of dysbiosis was calculated from the ratio of relative activities of urease and lysozyme.

The results were processed by variational statistical methods of analysis using the Microsoft Office Excel 2016 software. Statistical processing of the experimental study results was carried out by the methods of variation analysis using the Student's test. The difference was considered statistically significant at $p<0.01$ [3].

Results of the study and their discussion. It is crucial to emphasise that lysozyme and urease serve as sensitive, functionally opposed biomarkers of the oral-mucosal ecosystem: the former reflects the readiness of innate antimicrobial defence, whereas the latter mirrors the colonisation pressure exerted by urease-positive opportunists. Their concurrent assessment therefore offers an integrated view of barrier competence and microbial aggression. Moreover, the dysbiosis index, calculated as the urease-to-lysozyme ratio, has been validated as a quantitative proxy for the delicately balanced “oral barrier”. In the present study we adopted this triad to detect even subtle shifts that might otherwise escape histological or purely culture-based approaches.

In the oral mucosa of rats with experimentally induced immunodeficiency and dysbiosis, microbial load was assessed by urease activity and innate antimicrobial defence by lysozyme activity (Table 1).

Table 1

Indices of bacterial contamination and antimicrobial defence in oral-mucosal homogenates of rats under combined dysbiosis and immunodeficiency and after administration of the therapeutic-prophylactic complex, M±m

Indices / Group	Lysozyme activity, U/L	Urease activity, mkat/kg	Dysbiosis index, arb. units
1. Intact control, n=10	156.22±11.71	1.1±0.09	1.0±0.1
2. Combined pathology, n=10	61.34±0.32 p<0.001	3.15±0.21 p<0.001	7.30±0.52 p<0.001
3. Combined pathology + TPC, n=10	132.83±10.60 p>0.2 p_1<0.001	1.32±0.11 p>0.2 p_1<0.001	1.41±0.10 p<0.001 p_1<0.001

Note: p – significance relative to the intact group; p_1 – significance relative to the combined-pathology group.

Under cyclophosphamide-induced immunodeficiency, which severely depletes lymphoid populations, and lincomycin-associated dysbiosis, which selectively suppresses probiotic lactobacilli while favouring urease-producing opportunists, rats of group 2 exhibited a statistically significant 2.86-fold elevation in urease activity ($p<0.001$). This surge in ureolytic capacity implies a marked expansion of Proteobacteria and certain Actinomyces species capable of hydrolysing urea into ammonia, thereby locally alkalising the mucosal microenvironment and facilitating epithelial barrier disruption. Simultaneously, the catalytic efficiency of lysozyme – the principal muramidase of the innate oral defence system – fell 2.54-fold ($p<0.001$) relative to intact controls, signalling exhaustion of front-line antimicrobial protection and a diminished capacity to lyse Gram-positive peptidoglycan. The opposing trajectories of these two enzymes translated into a dramatic 7.3-fold escalation of the dysbiosis index within oral-mucosal homogenates ($p<0.001$), quantitatively affirming a profound collapse of eubiosis and the transition to a pathogenic, inflammation-prone microbial landscape.

Administration of the therapeutic-prophylactic complex (TPC) for 30 days under the same immunodeficient-dysbiotic conditions produced favourable shifts in all measured parameters. Urease activity, reflecting microbial colonisation, declined 2.38-fold ($p>0.2$; $p_1<0.001$), whereas lysozyme activity increased 2.16-fold ($p>0.2$ vs control; $p_1<0.001$ vs pathology). The dysbiosis index concomitantly decreased 5.2-fold ($p<0.001$; $p_1<0.001$).

Thus, the TPC enhanced nonspecific antimicrobial defense, reduced microbial load, and normalized the oral-mucosal microbiota. By restoring the lysozyme–urease balance to near-physiological values, the complex effectively re-established the biochemical “checkpoint” that segregates commensal co-existence from pathogenic overgrowth. Biochemical analysis of oral-mucosal homogenates in rats with experimentally induced immunodeficiency and dysbiosis indicates a constellation of adverse shifts: heightened myeloperoxidase and malondialdehyde levels signifying inflammatory activation, a 38 % drop in reduced glutathione and a concomitant decline of superoxide dismutase activity that betray diminished antioxidant protection amid intensified lipid peroxidation and oxidative stress, a three-log increase in

cultural counts of urease-positive flora reflecting increased colonization by opportunistic bacteria, and a two-fold suppression of innate antimicrobial activity as evidenced by depleted lysozyme and defensin profiles. Prolonged TPC administration under these combined pathological conditions normalised the evaluated parameters. Therefore, the proposed therapeutic-prophylactic complex demonstrated anti-inflammatory, immunostimulatory, antioxidant, and antibacterial properties in the setting of immunodeficiency and dysbiosis.

In the combined-pathology model (immunodeficiency + dysbiosis) a sharply pronounced disturbance of the oral microbiocenosis was observed, as evidenced by a 2.86-fold increase in urease activity and a 7.3-fold rise in the dysbiosis index compared with the intact group. Simultaneously, lysozyme activity – a key component of the non-specific protective barrier – declined more than twofold. A similar picture of "destruction" of balanced microbiota and reduction of mucosal immunity under drug-induced immunosuppression was described by Kambara et al. [8], demonstrating dominance of Proteobacteria with high urease activity and a correlated drop in natural-defence enzymes.

Introduction of the multicomponent therapeutic-prophylactic complex produced a multidirectional corrective effect. After only 30 days of therapy, urease activity decreased 2.38-fold, whereas lysozyme increased 2.16-fold, returning the dysbiosis index to an almost physiological range (1.41±0.10 arb. units). These findings confirm the concept of a combined influence of antioxidant and immunostimulatory agents on restoration of mucosal barrier function. Similar trends in correcting the Th17/Treg imbalance and normalising the intestinal – and consequently oral – microbiota were reported by Cui et al. in a cyclophosphamide-induced immunodepression model [4].

The reduction in bacterial contamination was accompanied by restoration of lysozyme enzymatic activity, consistent with data from Ferraboschi et al. regarding the ability of exogenous or endogenously stimulated lysozyme to suppress the growth of opportunistic strains and potentiate the action of antibacterial peptides [6]. In our study, an increase in lysozyme activity to 116 % of the baseline level in intact animals may indirectly indicate activation of local enzyme production under the influence of TPC components.

Another component in improving the microbial profile is antioxidant protection. Polyphenolic and peptide constituents of the complex can mitigate oxidative stress, as shown by Di Palma et al., who described the role of curcumin in reducing the severity of oral mucositis and normalising Nrf2/HO-1 expression [5]. In our work, an indirect marker of lowered oxidative load is the elevation of lysozyme, because an adequate antioxidant background preserves its activity from inactivation by peroxide radicals.

It is also important to emphasise the potential synergistic effect of plant polysaccharides, in particular Urtica macrorrhiza, within the TPC. Wang et al. previously demonstrated that such compounds not only enhance the morpho-functional state of intestinal crypts but also restore lactobacillus/bifidobacterium populations after antibiotic-induced injury [13]. Our data on the substantial decrease in urease activity after the TPC course correlate with restoration of probiotic strains that compete with urease-positive flora.

Hence, the combined application of antioxidant, immunostimulatory and antimicrobial components proved reliably effective in normalising key markers of bacterial load and non-specific defence of the oral mucosa in rats with experimental immunodeficiency and dysbiosis. The parallels identified with the literature [4–6, 8, 9, 13] confirm the relevance of the chosen strategy and justify further studies to optimise dosage and treatment duration.

Conclusions

1. Cyclophosphamide-induced immunodeficiency combined with lincomycin-associated dysbiosis results in a pronounced increase in bacterial load and a sharp suppression of innate-defence factors, as evidenced by a 7.3-fold rise in the oral-mucosal dysbiosis index.

2. Thirty-day administration of the proposed therapeutic-prophylactic complex significantly reduces urease activity and restores lysozyme activity to a level statistically indistinguishable from intact controls, yielding a 5.2-fold decrease in the dysbiosis index.

3. The TPC exhibits marked antimicrobial, immunostimulatory and antioxidant effects, supporting its suitability for prevention and correction of oral micro-inflammatory complications under conditions of immunosuppression and antibiotic-associated dysbiosis.

4. These findings justify further studies to optimise the TPC dosing regimen and to translate the results into clinical models aimed at preventing dysbiosis-driven lesions of the oral mucosa.

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