STRUCTURAL CHANGES OF THE ORAL MUCOSA IN RATS WITH INSULIN RESISTANCE, IODINE DEFICIENCY AND UNDER CONDITIONS OF THEIR COMBINATION

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Relevance. The relationship between endocrinopathies and the condition of the oral cavity is associated with disorders of metabolism, hemodynamics, immunological and neuroregulatory changes. Since the first line of contact with the environment of the oral cavity is the mucous membrane, changes in its properties adversely affect the functional state of other periodontal tissues.

Objective: to examine the structural changes of the oral mucosa in rats with combined endocrinopathy.

Materials and methods. The studies were carried on 120 rats, which were divided into three experimental (animals with iodine deficiency – ID, insulin resistance – IR, IR on the background of ID) and a control groups of animals. Histological examination of oral mucosa, computer morphometry and densitometry of objects were performed.

Results. In rats with ID, the epithelium of the mucous membrane was characterized by the development of acanthosis, expansion of the granular layer, enhanced keratin formation. The development of IR caused hyperplastic changes in the epithelium with the formation of unexpressed cords of acanthosis, epithelial basal hypercellular infiltration, which was confirmed by morphometric data. Under conditions of combined endocrinopathy, more pronounced changes in the histological structure of oral mucosa were observed. In particular, the development of mucosal edema in connective tissue, with predominantly macrophage hypercellularity at the periphery, was noted. Densitometric examination of the samples indicated a decrease in the optical density of connective tissue. At the same time, hyperplastic changes in most layers of the epithelium and pronounced changes in the vascular wall were observed.

Conclusions. Combined endocrinopathy leads to pronounced changes of oral mucosa, which can lead to disruption of the structure of periodontal tissues and the entire tooth-alveolar complex.

Key words: insulin resistance, iodine deficiency, oral mucosa, histological changes.

RELEVANCE

Pathology of endocrine glands is one of the most common non-infectious diseases of the modern world. The combination of several endocrine nosologies is especially dangerous in the prognostic aspect. The relationship between endocrinopathies and the state of oral cavity is quite close and it is associated with the disorders of metabolism, hemodynamics, immunological and neuroregulatory changes [2]. Since the first line of contact with the oral cavity environment is the mucous membrane, the changes of its protective, barrier and sensory properties adversely affect the functional state of other periodontal tissues. In particular, the violation of tolerance to glucose is a trigger for the microcirculatory changes in the oral mucosa, due to the accumulation of end products of glycation, damage of connective tissue proteins, formation of inflammatory cytokines and adipokines [3]. These changes lead to plasma hyperfiltration from the vascular net, hemorrhages, hypoxia, metabolic acidosis, followed by the development of structural changes in oral mucosa [6]. In turn, under conditions of thyroid hormones deficiency, the course of all metabolic processes is slowed down, what is manifested by the deposition of fibronectin and hydrophilic glycosaminoglycans in the tissues in the form of mucinous edema [4, 5].

Objective: to examine the structural changes of oral mucosa in rats with combined endocrinopathy.

MATERIALS AND METHODS

The studies were conducted on 90 female rats weighing 150-180 g, which were divided into three experimental groups (30 animals in each group). The 1st group included rats with iodine deficiency (ID), which was reproduced by keeping animals on a diet with limited iodine intake during two months [8]; to the 2nd - rats with insulin resistance (IR), which was modeled by adding to the drinking water of animals 10 % fructose solution during 8 weeks [7]; to the 3rd - rats with IR on the background of ID. The control group (n=30) included intact animals, which were kept on a standard diet, normal temperature and light regime of the vivarium.

Thyroid status was determined by the content of free triiodothyronine (fT3), thyroxine (fT4), thyroid-stimulating hormone of adenohypophysis (TSH) in the blood serum by enzyme-linked immunosorbent assay. The state of iodine supply of rats was studied by the examination of iodine concentration in the single urine portions, collected by metabolic cages method. The hydrocarbon metabolism was assessed by the level of immunoreactive insulin (IRI) in the blood serum, the content of glycosylated hemoglobin and the concentration of glucose in the blood.

For general and special histological examinations the fragments of the mucous membrane were fixed in a 10 % solution of neutral formalin (Ph-7.0). The fixation time was 24 hours. Formation of serial paraffin sections of oral mucosa with a thickness of 4-6 μm was performed on a sledge microtome. Histological sections of the mucosa were stained with hematoxylin and eosin, alcian blue according to Sidman and PAS staining (Periodic Acid Schiff Reaction) was made [1]. The studies were performed in a Leica DME optical microscope. In order to objectify the quantitative data, the computer morphometry and densitometry of objects in histological specimens were performed. Digital copies of the image were analyzed by using the computer program Image Tool 3.0 for Windows.

The morphometric analysis of oral mucosa was performed taking into account the following indicators: epithelial thickness; depth and width of acanthosis; thickness of basal, prickle, granular, keratinized layers of epithelium; perimeter and area of the cells nucleus of all epithelium layers; perimeter and area of cells of all epithelial layers; optical density of the ground substance of the connective tissue.

Keeping, feeding and euthanasia of animals were carried out following the generally accepted bioethical standards of humane treatment of experimental animals of international and national regulations for animal experiments: «European Convention for the Protection of Vertebrate Animals for Research and Other Scientific Purposes» (Strasbourg, 1986); «General ethical principles of animal experiments» (Ukraine, 2001); The Law of Ukraine «On protection of animals from cruel treatment» №2447-IV (Ukraine, 2006). Quantitative research results were analyzed using mathematical software package Statistic Soft 7.0. Statistically significant difference was considered at $p < 0.05$.

RESULTS AND THEIR DISCUSSION

The changes of thyroid homeostasis were observed in animals with ID compared to control data. It should be noted that the lack of iodine supply led to morphological changes of oral mucosa. Thus, the squamous epithelium, which covered the oral mucosa, was characterized by the development of acanthosis (Fig. 1).

It should be noted that keratohyalin granules were traced in some deeper epithelial cells, which indicated the expansion of the granular layer as a prerequisite for enhanced keratin formation (Fig. 2). Such changes were confirmed by the data of morphometric examination of the stratum corneum, the thickness of which in rats with ID by 20.9 % ($p < 0.02$) exceeded the similar indicators of animals in the control group. The lumen of vessels of the microcirculatory net as well as the small arteries and veins, was opened, which was clearly seen in PAS-staining due to the visualization of glycoproteins of the vascular walls.

Fig. 1. Epithelium of the oral mucosa under conditions of iodine deficiency Staining: hematoxylin and eosin $\times 200$

1 – acanthosis of the epithelium, 2 – basal layer of epithelium, 3 – prickle layer of epithelium, 4 – granular layer of epithelium, 5 – keratinized layer, 6 – subepithelial connective tissue

Fig. 2. Epithelium of the oral mucosa under conditions of iodine deficiency Staining: hematoxylin and eosin $\times 200$

1 – keratohyalin granules in the cells of prickle layer, 2 – acanthosis of the integumentary epithelium

The development of IR in animals of the 2nd experimental group was confirmed by changes of hydrocarbon metabolism. Violation of tolerance to glucose was accompanied by the structural rearrangement of oral mucosa. Thus, the epithelium thickness is uneven due to hyperplastic changes with the formation of unexpressed cords of acanthosis, caused by epithelial basal hypercellularity, which was confirmed by morphometric data. In particular, the thickness of the basal layer of the epithelium of rats of the 2nd experimental group increased by 53.4 % ($p < 0.05$) relative to the correspondent indicators in intact animals. It should be noted that the morphometric parameters of epitheliocytes of most layers of oral mucosa were decreased. In particular, the area of the cells of prickle and granular layers decreased

by 11.3 % ($p < 0.05$) and by 20.5 % ($p < 0.05$) according to the control values. Similar changes were observed in the cell nuclei. Thus, the perimeter and area of the nuclei of the cells of prickle and granular layers decreased by 11.0-37.0 % ($p < 0.05$) relative to similar indexes in intact animals. At the same time, in capillaries and arterioles narrowing of a lumen was noticed. The reason for these changes can be considered the plasma impregnation with loosening of the vessel wall with simultaneous endotheliocyte hyperplasia. The subepithelial basement membrane after the hematoxylin and eosin staining was seen indistinctly due to fusion with connective tissue fibers. Its clearer visualization was observed in areas of less pronounced acanthosis. After PAS-staining the state of a membrane visualized more accurately. In small arteries the tortuous internal elastic membrane with folds, areas of uneven width and depth was accurately traced. After PAS-staining the inner elastic membranes was PAS-positive. In the areas of pronounced tortuosity the increment accumulation of glycoproteins was found (Fig. 3).

FIG. 3. The oral mucosa under conditions of insulin resistance Staining: PAS $\times 400$
1 – PAS-positive basal membrane, 2 – epithelium,
3 – subepithelial connective tissue with PAS-positive glycoproteins

Under the conditions of IR on the background of ID the more pronounced changes of thyroid status and hydrocarbon metabolism was observed, which was accompanied by the violation of the histological structure of oral mucosa. In particular, the development of mucosal edema in the connective tissue was noticed, which could be traced by staining with hematoxylin and eosin in the form of blue areas, devoid of connective tissue fibers, due to their displacement. Mostly on the periphery of the edema areas a slight hypercellularity was found, mainly due to macrophages (Fig. 4).

In animals of the 3rd experimental group the densimetric examination of samples, staining by alcyan blue, showed a decrease in the optical density

Fig. 4. Oral mucosa under conditions of insulin resistance on the background of iodine deficit
Staining: hematoxylin and eosin $\times 400$
1 – mucous edema of the connective tissue of the mucous membrane, 2 – macrophage-leukocyte infiltration of connective tissue, 3 – intraepithelial macrophage-leukocyte infiltration

of connective tissue, in which the content of unsulfated glycosaminoglycans exceeded the analogical indicators in animals of the control group by 5.5 % ($p < 0.05$). The connective tissue was covered with multilayered squamous epithelium with hyperplastic changes in most layers. Such changes were confirmed by morphometric analysis, which indicated an increase in the thickness of the granular layer and stratum corneum of the epithelium of oral mucosa in rats with combined endocrinopathy by 26.8-60.7 % ($p < 0.05$) compared with the corresponding indexes in animals with isolated endocrine disease. Epitheliocytes were covered with a layer of keratin, the thickness of which was by 6.1 % ($p < 0.05$) higher than correspondent index in animals with isolated ID, which was by 28.3 % ($p < 0.05$) more than in rats of the control group. The changes in a vascular net of oral mucosa were noticed also. Thus, in some capillaries, small veins and arteries an increase in glycoproteins and more pronounced endothelial hypercellularity was found. The nuclei of such endothelial cells acquired an irregular circular shape and prolapsed into the lumen (Fig. 5).

CONCLUSIONS

The development of IR and ID is accompanied by the changes in the histological structure of oral mucosa, which can lead to disruption of the structure of periodontal tissues and the integral tooth-alveolar complex. The combined endocrinopathy leads to more pronounced changes in the mucous membrane, which should be considered in the complex treatment of patients with diabetes, hypothyroid dysfunction, and especially under conditions of their combination.

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Fig. 5. The structure of vessels under conditions of insulin resistance on the background of iodine deficit. Staining: PAS $\times 400$

- 1 – increase in glycoproteins in the venule wall,
2 – endothelium of venule, 3 – uneven accumulation
of glycoproteins along the inner elastic membrane
of the small artery, 4 – narrowing of the lumen
of the small artery

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СТРУКТУРНІ ЗМІНИ СЛИЗОВОЇ ОБОЛОНКИ ПОРОЖНИНИ РОТА У ЩУРІВ ІЗ ІНСУЛІНОРЕЗИСТЕНТНІСТЮ, ЙОДОДЕФІЦИТОМ ТА ЗА УМОВИ ЇХ ПОЄДНАННЯ

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Актуальність. Взаємозв'язок між ендокринопатіями та станом органів ротової порожнини пов'язаний із розладами метаболізму, гемодинаміки, імунологічними й нейрорегуляторними порушеннями. Оскільки першою лінією контакту з середовищем порожнини рота є слизова оболонка, зміни її властивостей негативно впливають на функціональний стан інших тканин пародонту.

Ціль: вивчити структурні зміни слизової оболонки порожнини рота (СОПР) у щурів із комбінованою ендокринопатією.

Матеріали та методи. Дослідження проведені на 120 щурах, які були розділені на три дослідні (тварини із йододефіцитом – ЙД, інсулінорезистентністю – ІР, ІР на тлі ЙД) та контрольну групу тварин. Здійснювали гістологічне дослідження СОПР, комп'ютерну морфометрію та денситометрію об'єктів.

Результати. У щурів із ЙД епітелій слизової оболонки характеризувався розвитком акантозу, розширенням зернистого шару, посиленням кератиноутворення. Розвиток ІР зумовив гіперпластичні зміни епітелію з формуванням невиражених акантотичних тяжів, епітеліальною базальною гіперклітинністю, що підтверджувалося морфометричними даними. За умов комбінованої ендокринопатії спостерігали більш виражені зміни гістологічної будови СОПР. Зокрема, відмічали розвиток слизового набряку у сполучній тканині, із переважно макрофагальною гіперклітинністю по периферії. Денситометричне дослідження препаратів вказувало на зменшення оптичної щільності сполучної тканини. Разом із тим спостерігали гіперпластичні зміни більшості шарів епітелію та виражені зміни судинної стінки.

Висновки. Комбінована ендокринопатія призводить до виражених змін СОПР, що може зумовити порушення структури тканин пародонта та цілісного зубо-альвеолярного комплексу.

Ключові слова: інсулінорезистентність, йододефіцит, слизова оболонка порожнини рота, гістологічні зміни.

ТСТРУКТУРНЫЕ ИЗМЕНЕНИЯ СЛИЗИСТОЙ ОБОЛОЧКИ ПОЛОСТИ РТА У КРЫС С ИНСУЛИНОРЕЗИСТЕНТНОСТЬЮ, ЙОДОДЕФИЦИТОМ И ПРИ ИХ СОЧЕТАНИИ

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Актуальность. Взаимосвязь между эндокринопатиями и состоянием органов ротовой полости обусловлена расстройствами метаболизма, гемодинамики, иммунологическими и нейрорегуляторными нарушениями. Поскольку первой линией контакта со средой полости рта является слизистая оболочка, изменения ее свойств негативно влияют на функциональное состояние других тканей пародонта.

Цель: изучить структурные изменения слизистой оболочки полости рта (СОПР) у крыс с комбинированной эндокринопатией.

Материалы и методы. Исследования проведены на 120 крысах, которые были разделены на три опытных (животные с йододефицитом – ЙД, инсулинорезистентностью – ИР, ИР на фоне ЙД) и контрольную группу животных. Проводили гистологическое исследование СОПР, компьютерную морфометрию и денситометрию объектов.

Результаты. У крыс с ЙД эпителий слизистой оболочки характеризовался развитием акантоза, расширением зернистого слоя, усиленным кератинообразованием. Развитие ИР обусловило гиперпластические изменения эпителия с формированием невыраженных акантотичных тяжей, эпителиальной базальной гиперклеточной инфильтрацией, что подтверждалось морфометрическими данными. В условиях комбинированной эндокринопатии наблюдали более выраженные изменения гистологического строения СОПР. В частности, отмечали развитие слизистого отека в соединительной ткани, с преимущественно макрофагами по периферии. Денситометрическое исследование препаратов указывало на уменьшение оптической плотности соединительной ткани. Вместе с тем наблюдали гиперпластические изменения большинства слоев эпителия и выраженные изменения сосудистой стенки.

Выводы. Комбинированная эндокринопатия приводит к выраженным изменениям СОПР, что может обусловить нарушение структуры тканей пародонта и целостного зубо-альвеолярного комплекса.

Ключевые слова: инсулинорезистентность, йододефицит, слизистая оболочка полости рта, гистологические изменения.