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MORPHOLOGICAL AND MORPHOMETRIC CHANGES IN MAJOR SALIVARY GLANDS AFTER EXPERIMENTAL CHEMOTHERAPY

Khaydarova Nargiza Mukhiddinovna
haydarova.nargiza@bsmi.uz

Assistant of the Department of Surgical Dentistry,
Bukhara State Medical Institute

Bukhara State Medical Institute named after Abu Ali ibn Sina, Uzbekistan, Bukhara, st. A. Navoi.
1 Tel: +998 (65) 223-00-50 e-mail: info@bsmi.uz

Resume: This study analyzed the morphological and morphometric changes occurring in the major salivary glands (parotid and submandibular glands) under the influence of experimental chemotherapy. A chemotherapy model was developed using cisplatin in six-month-old white outbred rats, and the resulting changes were assessed using histological and morphometric methods. The findings demonstrated a significant disruption in the secretory function and tissue structure of the salivary glands following chemotherapy. Chemotherapy is a chemical treatment method that involves the use of drugs with cytotoxic effects against malignant tumors. The drugs used in chemotherapy can induce various changes in the salivary glands. Therefore, this study investigated the morphological alterations in the tissue of major salivary glands resulting from chemotherapy for breast cancer.
Keywords: chemotherapy, salivary gland, morphology, morphometry, cisplatin

Introduction.

The number of people alive five years after a cancer diagnosis in the EU is estimated at 53.5 million. Researchers estimate that about one in five people will develop cancer in their lifetime. About one in nine men and one in twelve women will die from the disease. By 2024, lung cancer will be the most common cancer worldwide, with 2.5 million new cases (12.4% of the total). Breast cancer is the second most common cancer with 2.3 million cases (11.6%), followed by colon cancer with 1.9 million cases (9.6%), prostate cancer with 1.5 million cases (7.3%) and stomach cancer with 970,000 cases (4.9%). Exposure to certain drugs and environmental chemicals can lead to oxidation of cellular lipids and proteins, as well as cell death due to degradation of cellular components and DNA damage [2,11,12]. Antineoplastic drugs (e.g., cyclophosphamide and 5-fluorouracil), antibiotics (e.g., metronidazole), and analgesics (e.g., tramadol) are known to cause damage at the salivary gland level.

Animal studies provide strong indirect evidence that antioxidants can reverse drug- or chemical-induced changes in the salivary glands. However, definitive evidence of antioxidant effects in human salivary glands remains elusive [13,14].

Most studies have used tissue homogenates for biochemical analysis of oxidative and antioxidant markers, and hematoxylin and eosin (HE) staining and immunohistochemistry for histopathological and immunohistochemical diagnosis. Although some studies have evaluated changes in salivary biomarkers, only a few have also evaluated histopathological changes in salivary glands after antioxidant therapy in animal models [12,15,16]. Nevertheless, a number of experimental observations have shown significant changes in gland morphology during the immune response.

The aim and objectives of the study are to analyze cytomorphological changes in the major glands under the influence of chemotherapy in experimental breast cancer and to introduce a method for its biocorrection.

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Objective of the study: To determine the morphological and morphometric changes that occur in the major salivary gland tissue as a result of chemotherapy for breast cancer.

Materials and methods

Morphometric examinations were carried out in the laboratory of the Bukhara State Medical Institute based on regulatory and methodological documents. The studies were carried out in accordance with the requirements of the National Guidelines for the Care and Use of Laboratory Animals and the European Convention for the Protection of Vertebrate Animals Used for Experimental Research or Other Scientific Purposes (ETS No. 123,

Strasbourg, 1986) based on legal and methodological documents in our republic. Rats were divided into 3 large main groups (n=30): Group 1 - healthy experimental animals in standard vivarium conditions, white non-breed rats in the control group (n=10); Group 2: The group in which mammary cancer was induced by administering the carcinogen 7,12-dimethylbenzanthracene (n=10); Group 3: The group in which rats with cancer were intragastrically injected with 0.7 ml of distilled water via a gastric metal probe for 21 days, and in the following days, cisplatin was administered intravenously at a dose of 0.4 mg/kg (n=10);

In our experiment, we used the carcinogenic substance 7,12-dimethylbenzanthracene (DMBA), an oncogenic polycyclic aromatic hydrocarbon (C20N16), to induce cancer. It is a colorless crystal with a yellowish tinge, soluble in fats, and practically insoluble in water. It is not found in nature.

A single subcutaneous injection of 0.1 mg of dimethylbenzanthracene into the chest area of group 2 white outbred rats stimulated the formation of sarcomas, and application to the skin led to the formation of papillomas and skin cancer in rats. The use of dimethylbenzanthracene caused the appearance of local and distant tumors more often than other substances in this group.

During the research process, observations were made on the dynamics of growth and development of rats, their general condition and behavior. It was found that there were no changes in the general condition and behavior of the animals. After that, the experimental animals were weighed at the appropriate time in the morning, decapitated under ether anesthesia on an empty stomach and taken for experiments.

Animals were killed in accordance with international recommendations for medical and biological research using laboratory animals. The main objects of the research were 0.5 × 5 cm sections taken from the salivary glands of white outbred rats, and micropreparations were examined using the hematoxylin and eosin, Van Gieson method. Hematoxylin-eosin staining was performed as follows:

A section prepared from organ tissue was placed in distilled water and stained with hematoxylin solution for nuclei. Then the tube was washed in running water, differentiated in 0.3% hydrochloric acid solution. (i.e., excess color was reduced.) Then the tube was washed in running water, washed in Scott's aqueous solution. Then it was rinsed in running water, stained in Eosin solution for 2 minutes. Dehydrated, cleared, and observed.

After the staining steps, we can see that the cell components: collagen - white - are stained in pink, acidophilic cytoplasm - red, muscles - dark pink, basophilic cytoplasm - purple, erythrocytes - cherry red, and the nucleus - blue.

In histology, Van Gieson staining of micropreparations was used to differentiate connective tissue from tissues, to distinguish smooth muscle from tumors, and to determine the increased amount of collagen in tissues and organs as a result of various diseases.

Micropreparations were stained in the following order:

To remove paraffin from the section, they were placed in a xylene solution and placed in a decreasing solution of ethyl alcohol, for example, 2 portions were placed in orthoxylene for 2-5 minutes, and in alcohol solution 96%, 90, 80% solutions were placed alternately for 3 minutes.

Iron hematoxylin was stained with Weigert's stain for 3-16 minutes.

Washed in running water for several minutes. Then washed in distilled water.

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Stained with Van Gieson's stain for 5 minutes. Washed in distilled water for 5-15 seconds. Immersed in 96% ethanol solution for 1-2 minutes.

Neutral balm is dripped onto the slide and covered with a coverslip. It was immersed in 96% ethanol for 1-2 minutes.

According to the results obtained, we can see that the cell nucleus is stained black, collagen is red, tissue elements such as muscle tissue and erythrocytes are stained yellow.

Statistical processing of the obtained material was carried out using traditional variational statistical methods using the Excel program on a personal computer based on the Pentium IV processor using a software package for medical and biological research. The principles of evidence-based medicine were used in the organization and conduct of the studies.

Results and analysis.

(In the control group, the normal structure of the salivary glands was preserved, and serous and mucous acini, ducts and connective tissue components were observed in a physiological state. In our experiment, the morphological indicators of the submandibular salivary gland of 5-month-old white outbred rats were studied. Macroscopically, the submandibular salivary gland in rats is a paired organ, located on the medial side of the lower jaw, in the neck area. This gland has a soft, elastic structure, and is light pink in color. Its shape is medium-sized, slightly elongated or ovoid, and is well supplied with arteries, veins and lymphatic vessels. Macroscopically, the structure of the submandibular salivary gland consists of the main glandular tissue and the capsule surrounding it. The blood vessels leading to the gland and the salivary ducts leading out are also clearly visible. The salivary gland is mixed (seromucinous) in terms of secretion, containing serous and mucous cells, but serous cells predominate.

When examined microscopically, the gland is covered with a thin connective tissue capsule, which is divided into segments by tissue trabeculae. Each segment consists of an acinus and the beginning of a secretory duct. This gland consists of two types of pure protein and mixed secretion. There are many pure protein-producing acini, and their structure consists of excretory ducts, like the final secretory part of the parotid gland. The acini consist of two types of cells: pyramidal cells with secretory properties and myoepithelial cells located between the acinus and the basement membrane. The upper part of the pyramidal cells contains small oxyphilic secretory granules on the nuclear surface, while the wider basal part is basophilic. There are microvilli at the tip of the secretory cell, and a large number of secretory granules are located in the apical cytoplasm. Their amount varies depending on the functional state of the cell. Among the cells involved in protein secretion, there is an intercellular secretory duct, through which the cell product enters the acinus cavity. Contractile fibrils located in the cytoplasm of myoepithelial cells squeeze the acinar epithelium of this cell, ensuring the release of cell secretion into the acinar cavity.

The following changes were noted in the experimental group:

1.Acinuses underwent atrophy and vacuolization;
2. Nuclei decreased in size ($p<0.01$);
3 The stromal component significantly increased (up to 30–35%);
4. The number of tubules decreased, and signs of destruction were detected in some.

Acini are the main secretory structures of the salivary gland, which produce secretions.

After experimental chemotherapy, acini atrophied, that is, their size decreased, their cells became sparse, and their cytoplasm shrank. Vacuolization is the formation of vacuoles inside the cell, which is a sign of degenerative changes. This condition indicates a decrease in the metabolic activity of the cells and indicates a slowdown in their functional activity.

A significant decrease in the size of the cell nucleus was statistically significant ($p < 0.01$). This indicates a decrease in genetic activity and protein synthesis in the nucleus.

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Such changes lead to cellular stress, apoptosis (programmed death), or restriction of cellular activity.
Due to the reduction of the parenchymal (functional) tissues of the salivary glands, the stromal (connective) tissue increases in relative and absolute terms. This is often accompanied by a process of fibrosis, that is, an increase in the number of collagen fibers and fibroblasts is observed. An increase in the stroma to 30–35% indicates a reorganization of the internal structure of the gland, tissue replacement, and a reduction in functional parts. The salivary glands have excretory ducts that drain the secretion. Experiments have revealed a decrease in the number of these ducts, signs of destruction (damage, disintegration) around their periphery - cell wall disruption, nuclear pyknosis, and cytoplasmic fragmentation. This indicates a decrease in the drainage (excretory) activity of the gland, and as a result, the production and excretion of saliva is disrupted. Chemotherapy (especially cisplatin) dramatically disrupts the structural integrity of the salivary glands. Degenerative and dystrophic processes (atrophy, vacuolization, nuclear regression) are observed in the secretory cells, tissue structure reorganization (increase in stroma), and damage to the excretory structures (decrease in the number of tubules and destruction). These changes limit the overall functional capabilities of the gland and can be clinically manifested in conditions such as xerostomia (dry mouth).
From this table we can see that in the experimental chemotherapy group:
The diameter of the acini was significantly reduced compared to the control group (21.5 ± 2.1 μm → 15.2
± 1.8 μm; $p < 0.01$), indicating atrophy and reduction in volume of the secretory cells.
The diameter of the nucleus was also significantly reduced, indicating dystrophic changes in the participating cells (6.8 ± 0.5 μm → 4.9 ± 0.6 μm; $p < 0.01$).
The percentage of the stromal component more than doubled (14.2% → 32.6%; $p < 0.01$), indicating an increase in connective tissue and the possibility of fibrosis of the glandular tissue.
The number of tubules was significantly reduced (6.4 → 3.2; $p < 0.05$), indicating a shortening or destruction of the secretory pathways.
Morphometric parameters:

Parameter	Control group	Experimental group	p-value
Acinus diameter (μm)	21.5 ± 2.1	15.2 ± 1.8	<0.01
Nucleus diameter (μm)	6.8 ± 0.5	4.9 ± 0.6	<0.01
Stromal component (%)	14.2 ± 2.3	32.6 ± 3.1	<0.01
Number of tubules (n/ob.)	6.4 ± 1.0	3.2 ± 0.8	<0.05

Conclusion

The results showed that cisplatin significantly negatively affects the structure of the major salivary glands. Mainly, degeneration of secretory cells, reactive growth of the stroma, and disruption of the ducts were noted. This can lead to clinical symptoms such as decreased salivary secretion and dryness in the oral cavity (xerostomia). Morphometric parameters revealed a decrease in secretory activity, parenchymal shrinkage, and an increase in connective tissue components. This suggests that this may lead to functional insufficiency of the salivary glands.
These morphological changes indicate the need to maintain oral health in patients during clinical chemotherapy.

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