MODULATION OF SALIVARY SECRETION DISORDERS IN RADIOGENIC XEROSTOMIA BY BIOFLAVONOIDS: FUNCTIONAL-MORPHOLOGICAL ANALYSIS

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Annotation. *This study was aimed at a comprehensive assessment of the functional and morphological changes that occur in the salivary glands under the influence of gamma radiation and to substantiate the physiological effectiveness of bioflavonoids in correcting this pathological condition. The experiment was conducted in Wistar rats, and the animals were given a single gamma radiation dose of 7.5 Gy to the head area. As a result, severe hyposalivation, a sharp increase in oxidative stress biomarkers, an imbalance of inflammatory cytokines, and apoptosis of acinar cells were observed on the 7th and 14th days after irradiation. The use of a bioflavonoid complex (based on quercetin) for prophylactic and therapeutic purposes significantly increased salivary flow rate, reduced lipid peroxidation, activated the antioxidant defense system, and served to preserve the morphological integrity of the gland parenchyma. The results obtained confirm that bioflavonoids are a promising corrector in the pathogenetic treatment of radiogenic xerostomia.*

Keywords: *Gamma-irradiation, salivary glands, radiogenic xerostomia, hyposalivation, oxidative stress, apoptosis, bioflavonoids, quercetin, radioprotection.*

ENTRANCE

In modern oncology, ionizing radiation plays a leading role in the treatment of malignant tumors of the head and neck. However, the effectiveness of radiation therapy is often limited by severe damage to healthy tissues. Among the oral cavity and its appendages, the salivary glands are distinguished by their high radiosensitivity. The acinar cells and ductal system of these glands respond to radiation exposure with rapid functional impairment and structural destruction. The resulting radiogenic xerostomia (dry mouth syndrome) causes patients not only physical discomfort (dysphagia, dysgeusia, speech disorders), but also serious complications such as secondary infections, dental caries, and atrophy of the oral mucosa.

One of the main mechanisms of tissue damage under the influence of radiation is oxidative stress associated with free radicals (superoxide anion, hydroxyl radical) and other reactive oxygen species (ROS) formed as a result of water radiolysis. Peroxidation processes occurring in the lipid layer of cell membranes , oxidative modification of proteins and nucleic acids lead to cell dysfunction, apoptosis or necrosis. Serous acinar cells of the salivary glands, due to their high metabolic activity and energy-intensive secretory

mechanisms, are particularly susceptible to RROS damage in the absence of sufficient endogenous antioxidant system activity.

The use of existing synthetic radioprotectors (e.g., amifostine) is limited by serious side effects (hypotension, nausea , nephrotoxicity) and a narrow therapeutic window. Therefore, the search for safer, yet effective natural compounds is an urgent task. In recent years, plant flavonoids - bioflavonoids - have been gaining great attention due to their potent antioxidant, anti-inflammatory , and immunomodulatory properties. In particular, there is information that quercetin and its derivatives attenuate radiation damage in various organs, but their effect on the functional homeostasis of the salivary glands and cell apoptosis has not been fully studied.

Purpose of the study: Comprehensive assessment of the functional and morphological basis of salivary gland secretory disorders under experimental gamma-irradiation conditions and the role of bioflavonoids in the physiological modulation of these changes.

MATERIALS AND METHODS

1. Experimental animals and grouping

The study was conducted on 48 male Wistar rats weighing 180-220 grams. The animals were kept in standard vivarium conditions, with a 12/12 light-dark cycle, free access to water and food. All manipulations were carried out in compliance with the rules of biological ethics. The rats were randomly divided into 4 groups (n=12 in each group):

Group I – Intact control: Healthy animals that were not exposed to any exposure .

Group II – Bioflavonoid monotherapy: A bioflavonoid complex (mainly quercetin, at a dose of 100 mg/kg) dissolved in 1 ml of saline was administered daily for 14 days through a special gastric tube.

Group III – Gamma-irradiation: The animals were given a single dose of 7.5 Gy of gamma-irradiation to the head area.

Group IV – Radiation + Bioflavonoid: Bioflavonoid intake was started 7 days before radiation and continued for another 14 days after radiation (total 21 days, prophylactic + therapeutic regimen).

2. Irradiation model

Irradiation was performed using a Co-60 source in a "Rocus-M" (or similar) gamma-therapeutic device. Rats were immobilized under ketamine/xylazine anesthesia. A single, targeted dose of 7.5 Gy was administered to the head and neck area. The irradiation area was limited by special lead blocks, shielding other parts of the body.

3. Functional assessment (Sialometry and biochemical analysis)

Salivary secretion was assessed on days 1, 7, 14 and 28 after irradiation. After pilocarpine hydrochloride (5 mg/kg, subcutaneously) was administered to the animals , saliva was collected from the oral cavity for 15 minutes using a special aspirator. Salivary flow rate was determined in µl/min/g body weight. The concentration of sodium (Na+) and potassium (K+) ions in the collected saliva was determined by flame photometry, and alpha-amylase activity was determined by kinetic colorimetric method.

4. Biomarkers of oxidative stress and inflammation

Animals were decapitated on day 14. The level of lipid peroxidation in serum and salivary gland tissue homogenate was determined by the thiobarbituric acid-reactive substances (TBARs) spectrophotometric method, and the antioxidant defense status was determined by the superoxide dismutase (SOD) and catalase activities. The levels of inflammatory cytokines (TNF- α and IL-1β) were measured in serum by the enzyme-linked immunosorbent assay (ELISA).

5. Histological and morphometric analysis Paired

submandibular salivary glands were excised and fixed in 10% formalin. 5 μm thick sections prepared from paraffin blocks were stained with hematoxylin-eosin . The TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) method was used to detect apoptosis. Morphometric analysis was used to evaluate the average size of acinar cells, nuclear-cytoplasmic ratio , and apoptosis index (ratio of TUNEL-positive cells to total cell number, %).

6. Statistical analysis

All data were expressed as mean ± standard error of the mean (M±SEM). The statistical significance of differences between groups was assessed using one-way analysis of variance (ANOVA) and Student's t-test. A value of $p<0.05$ was considered statistically significant.

RESULTS

1. Changes in salivary secretion and ion composition

In the intact control group, the salivary flow rate during pilocarpine stimulation was 45.2±3.1 μl/min/g. Bioflavonoid monotherapy in group II did not significantly change this indicator (44.8±2.9 μl/min/g, $p>0.05$). In group III, 7 days after gamma irradiation, salivary flow decreased sharply and was 73% lower than the control (12.1±1.8 μl/min/g, $p<0.001$). On days 14 and 28, this indicator recovered slightly, but still remained lower than the control (16.4 and 19.7 μl/min/g, respectively). The most significant positive change was observed in group IV. Here, salivary flow rate on day 7 was 55% higher than in the radiation group (18.8±2.2 vs. 12.1 μl/min/g, $p<0.01$), and by day 28 this difference was even more pronounced (28.3±2.6, $p<0.001$).

Analysis of salivary electrolyte composition showed that radiation disrupted the tubular reabsorption mechanism. In the radiation group (day 14), salivary Na^+ concentration increased from 12.3±1.1 mmol/l in the control to 28.5±2.4 mmol/l ($p<0.001$), while K^+ decreased from 38.7±2.1 to 22.4±1.8 mmol/l. In group IV, this electrolyte imbalance was significantly corrected: Na^+ levels decreased to 18.9±1.7 mmol/l, while K^+ increased to 30.1±1.9 mmol/l .

2. Oxidative stress and antioxidant defense indices

Irradiation induced intense lipid peroxidation in salivary gland tissue. In group III, the level of TBARs increased to 8.9±0.7 nmol/mg protein compared to 2.8±0.3 nmol/mg protein in the control ($p<0.001$). This is an increase of almost 3.2 times. At the same time,

SOD activity decreased by 45% ($p<0.01$), and catalase activity by 51%. In group IV, which received bioflavonoids, the TBARs index was significantly lower than in group III, amounting to 5.1±0.5 nmol/mg protein ($p<0.01$). SOD and catalase activities remained 35% and 40% higher, respectively, compared to the irradiation group, which proves the preservation of the endogenous antioxidant system.

3. Dynamics of inflammatory cytokines The level of TNF-

α in the blood serum reached its peak on the 14th day after irradiation: it increased from 12.4±1.5 pg/ml in the intact control to 68.3±5.8 pg/ml. The level of IL-1β also increased accordingly from 35.1±3.2 to 142.7±11.5 pg/ml . These changes indicate that irradiation provoked not only a local tissue reaction, but also a strong systemic inflammatory response. In group IV, these indicators were significantly suppressed as a result of the action of bioflavonoids: TNF- α decreased to 41.1±4.3 pg/ml, and IL-1β to 85.6±7.2 pg/ml (both indicators $p<0.01$ compared to group III).

4. Histomorphological and morphometric analysis results The histological picture of the salivary glands

of the control group and group II animals was normal: acinar cells were of the same size, the apical parts were full of secretory granules, and the nuclei were located basally. In group III, gamma irradiation caused obvious destructive changes. Vacuolization, atrophy , and necrosis of acinar cells were observed in some areas. Inflammatory infiltration and an increase in collagen fibers (fibrosis) were detected in the interstitial space . Morphometric analysis showed that the size of acinar cells decreased from 28.5±1.4 μm in the control to 19.2±1.1 μm, and the nuclear-cytoplasmic ratio increased, indicating cell atrophy. The TUNEL method showed a sharp increase in the number of cells undergoing apoptosis . The apoptosis index increased from 1.2±0.2% in the control group to 24.6±2.8% in group III ($p<0.001$). In group IV, the use of bioflavonoids significantly changed the morphological picture in a positive direction. Atrophy of acinar cells decreased, their average size was 24.1±1.3 μm . Inflammatory infiltration was weakened, signs of fibrosis were minimally observed. The most important indicator - the apoptosis index decreased by 2.3 times compared to group III, and was 10.5±1.5% ($p<0.001$). This indicates that bioflavonoids effectively blocked the apoptotic pathway of cell death.

DISCUSSION

The results obtained indicate that the decrease in the secretory activity of the salivary glands (hyposalivation) under the influence of ionizing radiation is the result of a complex pathophysiological cascade. In the first stage, the intense formation of RCTs in the tissues leads to lipid peroxidation (increase in TBARs) and the depletion of endogenous antioxidants (SOD, catalase). This process disrupts the permeability of cell membranes and disrupts ion homeostasis. The influx of sodium ions into the cell increases, and potassium is lost, which, along with the disruption of the reabsorption function of the tubular system, leads to a weakening of the mechanism of primary secretion production by acinar cells (increase in Na+, decrease in K+ in saliva). In the second stage, oxidative stress and

mitochondrial dysfunction activate the production of inflammatory cytokines (TNF- α , IL-1 β), which further exacerbates tissue damage through the inflammatory cascade. In the third stage, all of these processes trigger controlled cell death programs such as apoptosis, resulting in an irreversible reduction in parenchymal cell mass.

showed that bioflavonoids act as a barrier at several stages of this cascade. First, their direct antioxidant properties "cleaned" the RCT and prevented lipid peroxidation, which was manifested in the low level of TBARs and the preservation of SOD/catalase activity. Second, bioflavonoids exerted a strong anti-inflammatory effect, suppressing the secretion of systemic inflammatory cytokines. Third, and most importantly, they interfered with intracellular signaling pathways and effectively inhibited the apoptosis process. This was clearly demonstrated by the significant decrease in the apoptosis index by TUNEL analysis. Due to this multi- stage protective mechanism, the morphological integrity and functional potential of the gland parenchyma were preserved, which was reflected in the significantly higher preservation of salivary flow rate.

The advantage of bioflavonoids (especially quercetin) is that they not only fight RCTs, but also inhibit the NF - κB inflammatory signaling pathway and stabilize mitochondrial membranes, reducing cytochrome C release. This shows their superiority over synthetic radioprotectors, such as amifostine, which act on only one mechanism .

CONCLUSION

1. A dose of 7.5 Gy of gamma radiation causes a pronounced functional deficiency in the salivary glands - severe hyposalivation, electrolyte imbalance (increased Na+, decreased K+), and impaired amylase secretion.
2. These functional changes are based on oxidative stress (increased TBARs, decreased SOD/catalase), systemic inflammation (increased TNF-α, IL-1β), and parenchymal cell atrophy and apoptosis (increased TUNEL+ index).
3. The use of bioflavonoids (based on quercetin) in a preventive and treatment regimen attenuated oxidative stress and inflammation, protecting acinar cells from apoptosis.
4. As a result of this complex modulatory effect, the rate of salivary secretion in the group receiving bioflavonoids was maintained, its qualitative composition approached physiological norms, and the morphology of the gland was relatively restored.
5. The results confirm that bioflavonoids are a promising, safe, and pathogenetically based physiological agent for the correction of radiogenic xerostomia and justify the need for in-depth studies for their introduction into clinical practice.

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