Evaluation of morphological changes of the pancreas in the conditions of experimental action of sodium glutamate

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Sodium glutamate, also known as monosodium glutamate (MSG), is frequently used as a flavor enhancer in the food industry. Excessive consumption of sodium glutamate can be harmful to human health. The aim of this study was to investigate the morphological features of the exocrine part of the pancreatic gland under experimental conditions with prolonged exposure to sodium glutamate in the diet. In the experimental study on rats after modeling the action of monosodium glutamate (sodium glutamate) at a dose of 70 mg/kg body weight, the exocrine part of the pancreatic gland was examined using light and electron microscopy after 2, 3, and 5-7 weeks. The experimental study was conducted on white male laboratory rats of reproductive age with an average weight of 168.0±5.0 g. The animals were randomized into two groups: group 1 (n=6) included intact rats and group 2 (n=15) consisted of animals receiving sodium glutamate in their diet. Histological specimens were stained with hematoxylin, eosin, and azan, and electron microscopy samples were stained using the Reynolds method. The results were statistically analyzed using ANOVA analysis with Statistics 20.0.0.2 software. At the early stage of the experiment, changes were observed in the acini with the formation of small clusters comprised of 2-3 acinar cells exhibiting increased accumulation of zymogen, which is an early important sign of pancreatitis. Swelling and replacement of the pancreatic gland with connective and adipose tissue progressed over the study period and were accompanied by structural alterations in the pancreatic gland. Round-cell infiltrates appeared in the areas where ducts and vascular bundles were located starting from the 5th week of observation, indicating the development of an inflammatory process. Histopathological changes at the 6th and 7th weeks following prolonged administration of sodium glutamate were similar to the pattern of pancreatitis in humans. Atrophy, degenerative changes, and inflammation were observed in the exocrine part of the pancreatic gland after 6-7 weeks of prolonged oral sodium glutamate intake. Thus, prolonged inclusion of sodium glutamate at a dose of 70 mg/kg body weight in the diet leads to irreversible destructive, degenerative, and inflammatory changes in the pancreatic gland.

Keywords: histology, experiment, rats, sodium glutamate, pancreas.

Introduction

Sodium glutamate, monosodium glutamate, is used as a flavor enhancer in the food industry [15, 16, 17]. Glutamate is one of the 20 amino acids involved in nitrogen and energy metabolism and is necessary for supporting many aspects of metabolism. In glutamate metabolism, reactions can have anabolic or catabolic nature depending on the tissue, glutamate dehydrogenase, which is located in mitochondria, and transaminases [3, 4]. The Food and Drug Administration (FDA) has stated that sodium glutamate is safe as a flavor enhancer, but its safety as a food additive remains a subject of debate, considering the significant number of articles discussing its potential negative impact on the body. Despite the widespread use of sodium glutamate in the food industry, some questions regarding its influence on the body remain unanswered.

Currently, over 2500 additives are deliberately added to food products to preserve their properties and extend their shelf life. One of the most widely used additives in Ukraine and worldwide is sodium glutamate [8, 9, 11], which enhances appetite and intensifies the taste of products, leading to increased daily food consumption. Excessive energy in the body can disrupt metabolism, contribute to
overweight, and ultimately lead to obesity and organ system disorders [2, 4, 6, 18]. Therefore, studying the effects of sodium glutamate on various systems and organs of the body, particularly in association with the state of the pancreas, is an important medical and social issue. Consequently, one approach to expanding our understanding of the effects of sodium glutamate is to investigate its pathogenesis using experimental animal models [5, 6, 9, 11]. This approach is an integral part of modern medicine as it allows for the creation of models and provides unlimited possibilities for studying causal factors that contribute to pathogenesis depending on the research objectives. However, in recent years, the use of monosodium glutamate (sodium glutamate) as a food additive to improve taste and prolong shelf life has become widespread worldwide.

The aim of the study is to determine the morphological characteristics of the exocrine part of the pancreas under experimental conditions during prolonged inclusion of sodium glutamate into the diet.

Materials and methods

The article is a fragment of the research work 28A-2019 "Morphological characteristics of internal organs and vascular channel in normal ontogenesis and regularities of their restructuring in obesity and the influence of physical factors on the body" № state registration 0119U102059, 2019-2023.

Animals. The white laboratory rats were randomized into two groups: group 1 (n=6) involved the intact rats; group 2 (n=15) included animals that received MSG. The rats were maintained in the vivarium of Danylo Halytovsky Lviv National Medical University. Animals were killed by decapitation under anesthesia 2, 3, 5, 6 and 7 weeks after the start of the experiment, which was necessary for blood collection for biochemical studies. The experiment was carried out according to the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 86/609/ EEC (1986) and the Law of Ukraine № 3447-IV "On the protection of animals from cruelty".

Monosodium Glutamate Administration. Rats of the experimental group received the food supplement at a dose of 70 mg/kg rat weight daily for 7 weeks [4]. MSG was administered orally through a pipette. The dose of 70 mg chosen by us for a rat corresponds to 700 or 816 mg for a human weighing 60 or 70 kg, respectively [1]. The control group of animals received a standard diet without the addition of sodium glutamate.

Histological study. The pancreatic tissue of rats was fixed in a 10 % neutral formalin solution, dehydrated in ascending concentrations of alcohols (ethanols), cleared in xylene, and embedded in paraffin.

Serial 7-10 microns thick sections were obtained using the Reichert microtome (Austria). The deparaffinised sections were stained with hematoxylin and eosin (combination) and azan. Photomicrographs of the specimens were obtained using a research microscope Olympus BX63 (Germany) with "CellSens Dimension 1.8.1" software.

The focus of the study was the exocrine part of the pancreas. For objectivization, histological slices were analyzed semi-quantitatively (in points) by three parameters: "acini structure", "fat tissue replacement", and "connective tissue replacement".

The following scale was used to estimate pancreatic acini: 0 - normal structure, 1 point - the presence of single small acini, which included 2-4 acinar cells, the main mass made up of large acini (more than 9 cells); 2 points - the presence of 30-50 % small acini, solitary large acini, destructive changes in acinar cells are observed; 3 points - the presence of more than 50 % small acini, blurred cell membrane contour, destruction in acinar cells is observed.

The growth of connective tissue in the pancreas area was evaluated: 1 point - formation around single ducts; 2 points - around 50 % of ducts; 3 points - formation around ducts and between acini.

Replacement of the pancreatic tissue by adipose tissue: 1 point - scattered individual adipocytes; 2 points - approximately 30 % between lobules; 3 points - between lobules and acini.

Electron-microscopic study. Pancreatic fragments were fixed in a buffered 4 % glutaraldehyde solution. Post-fixation was performed in a 1 % solution of osmium tetroxide (OsO4) followed by dehydration in ethyl alcohol (from 50° to 100°) and acetone. Then, the samples were impregnated with a mixture of epoxy resin and Araldite. Ultrathin sections (50 to 100 nm) were prepared using the LKB 2188 Ultratome NOVA ultramicrotome (Ukraine), and they were contrasted with uranyl acetate and lead citrate using the Reynolds method. The analysis of the sections was performed using a Tesla BS-500 transmission electron microscope (Sweden).

Statistical analysis. The average values of the obtained digital indicators were presented as M±σ (M - average value, σ - standard deviation). To determine the differences between the developmental weeks in pancreatic gland disorders, the Kruskal-Wallis test was used. A p-value of less than 0.05 was considered significant.

Results

Study of the effect of sodium glutamate on the pancreatic gland (2 weeks). The exocrine part of the pancreatic gland mainly exhibited normal morphology. In the majority of acinar cells, nuclei were polarly located, uniform in shape and size, and featured 2-4 nucleoli. Electron microscopy revealed increased functional activity of acinar cells, with an elevated content of zymogen granules in the cytoplasm, displacing the nucleus towards the cell membrane. Some mitochondria exhibited elongated shape, with disrupted cristae organization. The Golgi complex showed a reduction
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regions of the organ. In certain acini, acinar cells with signs of necrosis were identified.

The intercalated ducts were dilated due to swelling. Ultrastructural analysis of the centroacinar cells revealed isolated cells with pyknotic nuclei, and their mitochondria exhibited a low density of cristae with a disrupted arrangement.

In the interlobular ducts, homogeneous fluid and large peripherally located vacuolar inclusions were observed. Empty intercalated vessels and congested interlobular vessels were also observed.

Study of the effect of sodium glutamate on the pancreas (5 weeks). During this period the increase of destructive changes in the pancreas was revealed. The thickening of its capsule and expansion of connective tissue partitions between lobules and acini, separating them from each other, were revealed. The connective tissue also extended between the acini, separating them from each other.

In addition, the adipose tissue areas were found around the vessels and interlobular ducts (Fig. 2). Structural disorders were also found in acini. Significantly enlarged acini were observed, which consisted of up to 15 acinar cells, as well as small-sized acini with 2-4 cells showing disrupted polarity.

Acini were unevenly distributed throughout the territory of the pancreas. Some acinar cells contained pyknotic nuclei. Swelling of connective tissue between acini was observed throughout the territory, and dilated and congested intercalated blood capillaries were present.

Sometimes, near dilated interlobular ducts, signs of acinar destruction, desquamation, and necrosis of acinar cells were observed, along with foci of fibroblast proliferation. Fragments of acini were located within the loose connective tissue, where round-cell infiltrates (Fig. 3) were identified, indicating the development of an

Fig. 1. Two weeks after the start of the experiment. Acinar cell. Nucleus with festooned shape and invaginations of the nuclear membrane. Cytoplasm containing zymogen granules of varying sizes. Vacuoles. Contrasted using Reynolds’ method. x10000.

Fig. 2. Fragment of rat pancreas: the proliferation of adipose and connective tissue, acini of different sizes. Five weeks after the start of the experiment. Azan. x100.

Fig. 3. Fragment of rat pancreas: signs of acinar destruction, desquamation, and necrosis of exocrine pancreatic cells. Foci of round-cell infiltration ( ). Interlobular ducts with granular pink-proteinaceous precipitate ( ). Five weeks after the start of the experiment. Hematoxylin-eosin. x100.
inflammatory process. Interlobular vessels were dilated, with flattened and elongated endothelium, containing fluid with occasional blood cells. The lumen of interlobular ducts showed a granular pink proteinaceous precipitate.

Study of the effect of sodium glutamate on the pancreas (6 weeks). The histological picture of the pancreatic gland at this stage of the experiment did not differ from that observed after 5 weeks. Its exocrine part consisted of acini of varying sizes and shapes with moderately pronounced signs of acinar cell degeneration. Connective tissue proliferation was detected in interlobular and interacinar areas. Increased areas of adipose tissue were observed around blood vessels and interlobular ducts.

Study of the effect of sodium glutamate on the pancreas (7 weeks). During this period, there was an intensification of degenerative changes in the exocrine portion of the pancreatic gland. Small-sized acini of various shapes were predominantly present, and clear boundaries were not defined between some of them. Acinar cells were disorganized, and pyknotic nuclei were observed in some cells. These cells exhibited mitochondria of varying sizes, with many showing signs of swelling, matrix clarification, and disrupted cristae organization. Some cells exhibited lysed nuclear membranes, reduced components of the Golgi complex, and lysed membranes of the granular endoplasmic reticulum, along with decreased mitochondrial density and cristae disruption. Centroacinar cells also showed signs of degeneration, with a significant portion of their mitochondria being swollen and exhibiting low cristae density.

Connective tissue proliferated between acini, lobules, and in the interstitial spaces. Some acini were separated by layers of connective and adipose tissue (Fig. 4). Marked swelling was observed throughout the organ.

Perivascular edema and signs of hemostasis were observed. Some ducts were dilated.

During the application of the statistical method using the Kruskal-Wallis test (Table 1), a significant difference was found among the study periods of 2, 3, 5-7 weeks in terms of histological indicators: connective tissue (H=30.47, p=0.0001), adipose tissue (H=40.08, p=0.0001), and acini (H=30.92, p=0.0001).

Degenerative changes in the structure of the pancreatic gland increased with the duration of the experiment.

Discussion

The focus of our work was to study the changes in the pancreatic gland of rats after prolonged administration of sodium glutamate. According to the available data [11], in industrially developed countries (Europe and the USA), the average daily consumption of sodium glutamate per person is estimated to be 300-1000 mg/day. In the UK, it ranges from 600-2000 mg/day, and in Nigeria, it is 560-1000 mg/day. However, these values depend on the sodium glutamate content in food products and individual taste preferences. For our study, we chose a dosage of 70 mg/kg of sodium glutamate (equivalent to 600 or 816 mg per day for an average-weight person of 60 or 70 kg) based on preclinical animal studies that demonstrated toxic effects on the heart, liver, kidneys, and other systems at high concentrations of sodium glutamate (0.5-6 g) [6]. To draw accurate conclusions regarding the use of lower concentrations of sodium glutamate, further research is necessary.

Using histological methods in rat experiments, it was found that the administration of sodium glutamate at doses of 40 and 80 mg/kg for 28 days resulted in damage to neurons in the brain, hippocampus, and cerebellum [12], administration for 42 days led to degenerative and atrophic changes in the liver [4], while at a dosage of 15 and 30 mg/kg, the structure of the pancreatic gland in rats was disrupted after 30 days [8]. This may be due to the temporary high concentration of sodium glutamate in the plasma, which exhibits its toxic effects, particularly with chronic use [13]. The localization of lysosomal hydrolases with digestive enzymes also leads to necrosis and apoptosis of acinar cells, which may be a mechanism for zymogen activation and its detrimental effects on cells. Another mechanism could be the damage to acinar cell organelles, accompanied by the activation of redox signaling (reactive oxygen species), which is an important early event in the pathogenesis of pancreatitis [13]. Localization of lysosomal

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<th>Term, week</th>
<th>Histological parameters (points, n=21)</th>
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<tr>
<td></td>
<td>Connective tissue</td>
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<tr>
<td>2</td>
<td>1.189±0.091</td>
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<tr>
<td>3</td>
<td>1.292±0.133</td>
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<tr>
<td>5</td>
<td>1.948±0.110</td>
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<td>6</td>
<td>2.101±0.102</td>
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Fig. 4. Fragment of the rat pancreas. Disruption of acinar structure. Connective tissue. Seven weeks after the start of the experiment. Hematoxylin-eosin. x200.
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hydrolases with digestive enzymes also leads to necrosis and apoptosis of acinar cells, which can be a mechanism for the activation of zymogen and its destructive impact on cells. Another mechanism can be the damage to acinar cell organelles accompanied by the activation of redox signaling (reactive oxygen species), which is an important early sign in the pathogenesis of pancreatitis [13]. Additionally, during the early stage of pancreatitis, acinar cells synthesize and release cytokines and chemokines, which attract and activate inflammatory cells [13, 14]. In our study, with increasing duration of the experimental period, we observed progressive edema, replacement of pancreatic parenchyma with connective and adipose tissues, and deviations in the histological structure of the acini in the pancreatic gland. Our findings are consistent with other studies where obesity in animals was induced by high-fat diets, resulting in pancreatic steatosis, inflammation, and fibrosis [1, 7, 9, 10].

Thus, the experimental modeling of sodium glutamate and subsequent investigation of the structure of the pancreatic gland allowed us to identify the main pathomorphological changes. It was established that the damage to acinar cells triggers a complex mechanism that disrupts the exocrine component of the pancreatic gland.

In the future, we plan to study the metabolic changes in the organism and their correlation with histological alterations during the modeling of pancreatic gland obesity in animals using sodium glutamate.

Conclusion
1. Electron microscopy examination after 2 weeks from the beginning of the experiment revealed early disruptions in the organization of acinar cells, increased presence of zymogen granules, and mild changes in their membranous organelles.
2. During the 5-7 week study period, the exocrine part of the pancreatic gland exhibited the appearance of infiltrates, indicating the development of a local inflammatory process, along with the formation of connective and adipose tissue in the interstitial space and parenchyma. These changes can be classified as pancreatitis.

References
ОЦІНКА МОРФОЛОГІЧНИХ ЗМІН ПІДШЛУНКОВОЇ ЗАЛОЗИ В УМОВАХ ЕКСПЕРИМЕНТАЛЬНОЇ ДІЇ ГЛУТАМАТУ НАТРІЮ

Літвак Ю. В.

В якості підсилювачу смаку в харчовій промисловості часто використовують натрієву сіль глутамінової кислоти або глутамат натрію. Надмірне споживання глутамату натрію може завдати шкоди здоров'ю людини.

Мета роботи: встановити морфологічні особливості екзокринної частини підшлункової залози в умовах експерименту при тривалому введенні в раціон глутамату натрію.

В експериментальному дослідженні на щурах після моделювання дії мононатрієвої солі глутамінової кислоти (глутамату натрію) 70 мг/кг маси тіла досліджували екзокринну частину підшлункової залози через 2, 3, 5-7 тижнів методами світлової та електронної мікроскопії. Експериментальне дослідження проводили на білих лабораторних щурах-самцях репродуктивного віку із середньою масою 168,0±5,0 г. Тварини були рандомізовані на дві групи: група 1 (n=6) включала інтактних щурів; до групи 2 (n=15) увійшли тварини, які отримували глутамат натрію у раціон.

Отримані гістологічні препарати зафарбовували гематоксиліном, еозином та азаном, електронно-мікроскопічні - за Рейнольдсом. Результати оброблені статистично програмою Statistics 20.0.0.2 з використанням аналізу ANOVA. На ранній стадії розвитку експерименту були виявлені зміни в ацинусах з утворенням маленьких форм з 2-3 ацинарними клітинами з підвищеним в них накопиченням зимогену, що є ранньою важливою ознакою панкреатиту. Набряк, заміщення підшлункової залози сполучною і жировою тканиною прогресує з термінами дослідження і супроводжується порушенням будови підшлункової залози.

Ми спостерігали появу круглоклітинних інфільтратів в ділянках розташування проток і судинних пучків, починаючи з 5-го тижня спостереження, що відображає розвиток запального процесу. Гістопатологічні зміни на 6-7-му тижні після тривалого введення глутамату натрію аналогічні картині панкреатиту у людини. Через 6-7 тижнів після тривалого введення глутамату натрію регенерація екзокринної частини підшлункової залози спостерігалася атрофічна, дегенеративна та запальна зміни. Таким чином, тривалий прийом у раціон глутамату натрію у дозі 70 мг/кг маси тіла приводить до незворотніх деструктивних, дегенеративних та запальних змін підшлункової залози.

Ключові слова: гістопатологія, експеримент, щури, глутамат натрію, підшлункова залоза.