Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

• Փորձարարական և տեսական հոդվածներ • Экспериментальные и теоретические статьи •
• Experimental and theoretical articles •

Biolog. Journal of Armenia, 2-3 (75), 2023

DOI: 10.54503/0366-5119-2023.75.2-3-79

INVESTIGATION OF HISTO-MORPHOMETRIC INDICATORS OF THE PANCREAS STRUCTURE IN RATS AFTER ADMINISTRATION OF L-TRYPTOPHAN

R.V. YANKO

Bogomoletz Institute of Physiology National Academy of Sciences of Ukraine, Kyiv
biolag@ukr.net

The aim of the work was to study the histo-morphometric changes in the pancreas tissue in rats after the administration of L-tryptophan. The study was conducted on 24 male Wistar rats of 3 months of age. The animals were kept in uniform conditions and on a standard diet. The rats of the experimental group received L-tryptophan (France) at a dose of 80 mg/kg orally every day. The duration of the experiment was 28 days. Histological preparations of pancreas were prepared according to standard methods. Histo-morphometry of digital images was carried out using the computer program "ImageJ". Histo-morphometric signs of a decrease in the activity of the exocrine part of the pancreas were revealed in healthy young rats treated with L-tryptophan. This is evidenced by the smaller size of acinus, the epithelium height and the smaller area of exocrinocytes. Endocrine activity of the rat's pancreas, after exposure to this amino acid, did not undergo significant changes. It was shown that administration of L-tryptophan probably reduced the amount of connective tissue in the pancreas. This is relevant for practical medicine, regarding the use of this amino acid in the prevention and complex treatment of pancreas fibrosis.

Tryptophan – pancreas – rats

Աշխատանքի նպատակն էր ուսումնասիրել առնետների ենթաստամոքսային գեղձի հյուսվածքի հիստոմորֆոմետրիկ փոփոխությունները L-տրիպտոֆանի ընդունումից հետո: Հետազոտությունն իրականացվել է Wistar 3 ամսական 24 արու առնետների վրա: Կենդանիներին պահում էին ստանդարտացված պայմաններում և ստանդարտ սննդակարգով: Փորձարարական խմբի առնետներն օրական ստացել են L-տրիպտոֆան (Ֆրանսիա) 80 մգ/կգ դոզայով բերանային եղանակով: Փորձի տևողությունը 28 օր էր: Ենթաստամոքսային գեղձի հյուսվածքաբանական պատրաստուկները կատարվել են ստանդարտ մեթոդով: «Image J» համակարգչային ծրագրի միջոցով կատարվել է պատրաստուկների թվային պատկերների հիստոմորֆոմետրիա: Պարզվել է, որ L-տրիպտոֆանով բուժված առողջ երիտասարդ առնետների ենթաստամոքսային գեղձում նկատվել են նրա էկզոկրին հատվածի ակտիվության նվազման հիստոմորֆոմետրիկ նշաններ: Դրա մասին են վկայում ացինուսի ավելի փոքր չափը, էպիթելի բարձրությունը և էկզոկրինոցիտների ավելի փոքր տարածքը: Առնետների ենթաստամոքսային գեղձի էնդոկրին ակտիվությունն, այս ամինաթթվի հետ շփումից հետո էական փոփոխություններ չի կրել: Ցույց է տրվել, որ L-տրիպտոֆանի ընդունումը զգալիորեն նվազեցրել է ենթաստամոքսային գեղձի միացնող հյուսվածքի քանակը: Սա տեղին է պրակտիկ բժշկության համար՝ կապված գեղձի ֆիբրոզի կանխարգելման և համալիր բուժման մեջ այս ամինաթթվի օգտագործման հետ:

Տրիպտոֆան – ենթաստամոքսային գեղձ – առնետներ

Целью работы было исследование гисто-морфометрических изменений ткани поджелудочной железы крыс после введения L-триптофана. Исследование проведено на 24 крысах-самцах линии Вистар 3-месячного возраста. Животные находились в унифицированных условиях и стандартном рационе питания. Крысы подопытной группы ежедневно получали перорально L-триптофан (Франция) в дозе 80 мг/кг. Продолжительность эксперимента составляла 28 суток. Гистологические препараты поджелудочной железы производили по стандартной методике. Гистоморфометрию цифровых изображений препаратов проводили с помощью компьютерной программы "ImageJ". У поджелудочной железе здоровых молодых крыс, получавших L-триптофан, наблюдали гисто-морфометрические признаки снижения активности её экзокринной части. Об этом свидетельствовали меньшие размеры ацинусов, высота эпителия и меньшая площадь экзокриноцитов. Эндокринная активность поджелудочной железы крыс, после воздействия этой аминокислоты, не претерпевала существенных изменений. Показано, что введение L-триптофана существенно снижало количество соединительной ткани в поджелудочной железе. Это актуально для практической медицины, относительно использования этой аминокислоты в профилактике и комплексном лечении фиброза железы.

Триптофан – поджелудочная железа – крысы

Tryptophan is an essential amino acid that plays a role in many metabolic functions. It is a unique building material for proteins [13]. Tryptophan serves as a precursor to important endogenous indolamines such as serotonin and melatonin, which act as neurotransmitters, neuromodulators, and neurohormones [16]. Increased synthesis of these signaling molecules can improve health, quality of life and well-being [11]. Tryptophan is used in the treatment of depression and sleep disorders, to solve cognitive disorders, anxiety or neurodegenerative diseases [7]. Decreased secretion of tryptophan is associated with obesity, anorexia, bulimia nervosa, and other diseases [15].

However, despite the well-studied role of tryptophan in the body, its influence on the histo-morphometric changes of the pancreas remain little studied. The effect of tryptophan in pancreas pathology, primarily pancreatitis, oncology, fatty degeneration, etc. was studied [5, 8, 15]. At the same time, the obtained data are often ambiguous, which may be related to the use of different dosages of L-tryptophan in experiments, the duration of research, etc. Currently, the question of how pronounced is the expediency of using tryptophan to improve the functioning of healthy pancreas remains open. All this requires a more detailed study of the role and mechanisms of the effect of this amino acid on the gland.

The aim of the work was to investigate the histo-morphometric changes of the rats pancreas tissue after the administration of L-tryptophan.

Materials and methods. The study was conducted on 24 male Wistar rats of 3 months of age, which were kept in uniform conditions and on a standard diet. The animals were divided into 2 groups. The control group included intact animals, and the experimental group included rats that received daily oral L-tryptophan (France) at a dose of 80 mg/kg. The duration of the experiment was 28 days. Rats were removed from the experiment by decapitation under light ether anesthesia the day after the last dose of L-tryptophan. Research was conducted in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985).

For morphological and morphometric studies, tissue samples were randomly selected from the central part (body) of the pancreas, from which histological preparations were made according to the standard method: fixed in Bouin's liquid, dehydrated in alcohols of increasing concentration and dioxane. The obtained samples were embedded in paraffin. Paraffin sections (6 μm thick) were made on a sled microtome. Staining of the obtained sections was carried out according to the Van Gieson method [12]. With the use of a digital camera, micropreparations were photographed on a microscope "Nikon Eclipse E100" (Japan).

Histo-morphometry of exo- and endocrine part was performed on the histological sections of the pancreas tissue. In the exocrine part of the gland, the diameter and cross-sectional area of acinus, the height and area of exocrinocytes, their nucleus and cytoplasm were measured, the number of nucleolus in the nucleus of exocrinocytes and the average number of cells in the acinus were counted. In the endocrine part of the gland, the average number of Langerhans islets per unit area (0.25 mm^2) and the number of endocrinocytes were calculated, the cross-sectional area and diameter of the islets were measured, and the density of the cells was determined. The relative area of the exo- and endocrine part, as well as the connective tissue in the gland was determined by the method of superimposing point morphometric grids [1, 17]. Morphometry was performed using the computer program "Image J 1.34p". Glucose concentration was determined in blood serum.

The obtained results were processed by the methods of variation statistics using the software "Statistica 6.0 for Windows" ("StatSoft", USA) and "Excel 2010" ("Microsoft", USA). The normality of the distribution of digital arrays was checked using the Pearson test. If the distribution is normal, the Student's t-test was used to estimate the coefficient of difference reliability of the difference between the control and experimental groups. Differences were considered probable at $p < 0,05$.

Results and Discussion. It was found that the rat's pancreas of the experimental group had a preserved physiological structure, which is divided into exo- and endocrine parts. The exocrine part accounts for about 95% of the pancreas mass. The structural and functional unit of the exocrine part of the pancreas is the acinus, which is the secretory section and the interstitial section, which is the initial section of the excretory ducts. Acinus look like sacs surrounded by reticulin fibers, vessels and nerve fibers. The secretory section consists of a basement membrane, on which epithelial cells of a conical shape lie – exocrinocytes in the amount of 7-11. The base of these cells is expanded, the apex is narrowed. Each cell has one round or oval nucleus, which divides the cell into two zones: homogeneous and zymogenic. The zymogenic (apical) part of the cell contains secretory granules of the immature (enzyme) secretion zymogen, which stain oxyphilically. In the homogeneous (basal) part of the cells, there is an abundance of ribosomes, which determines the basophilia of this zone (fig. 1).

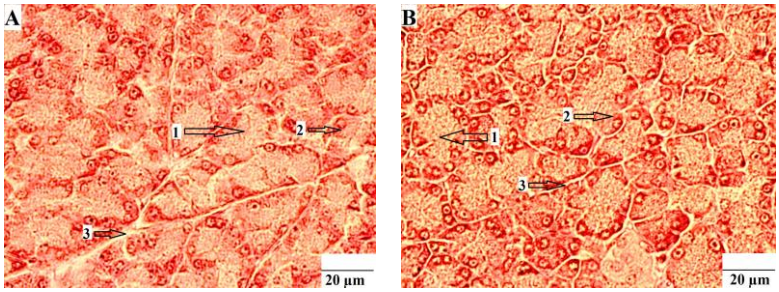

Fig. 1. Photomicrograph of the pancreas section of a control (A) and a rat treated with L-tryptophan (B). 1 – acinus, 2 – exocrinocyte, 3 – interacinus connective tissue. Van Gieson staining. $^{X}800$.

In the exocrine part of the rat's pancreas, treated with L-tryptophan, the mean diameter and area of acinus were significantly smaller by 15 and 26 %, respectively, compared to the control. The exocrinocytes area, their nucleus and cytoplasm placed in them was also probably smaller by 12, 13 and 12 %, respectively. The epithelium height of the acinus had a tendency to decrease by 11%. However, the number of nucleolus in the nucleus of exocrinocytes, on the contrary, was probably 24 % higher than in the control (Table, Fig. 1). Hyperplasia of nucleolus can be one of the signs of activation of

the protein synthetic function of cells, or (to a lesser extent) the growth of physiological regeneration at the intracellular level [2]. Therefore, according to most histo-morphometric indicators, L-tryptophan slightly reduces the activity of the exocrine part of the pancreas.

The endocrine part occupies a much smaller area of the pancreas tissue. It is formed by Langerhans islets, which are scattered throughout the gland. The islets are separated from the acinus by a thin connective tissue layer and are round clusters of endocrinocytes permeated by a dense network of capillaries. In the endocrine part of the rat's gland treated with L-tryptophan, a significantly lower number of Langerhans islets by 13 % compared to the control was observed. However, their sizes were probably larger (area – by 14 %, diameter – by 12 %) than in the control. In addition, a trend towards an increase in the number of endocrinocytes in the islet was found (Table 2). In general, according to these indicators, we can conclude that tryptophan does not have a pronounced effect on the state of the endocrine function of the pancreas. This is confirmed by the unchanged concentration of glucose in the blood serum of experimental animals.

Table 1. Morphometric indicators of the pancreas structure (M ± m)

Indicators	Control	L-tryptophan
Exocrine part		
Relative area, %	77,7 ± 1,5	84,4 ± 2,1
Acinus diameter, μm	30,6 ± 0,8	26,0 ± 0,6*
Acinus area, μm^2	933 ± 14	693 ± 22*
Area, μm^2 exocrinocyte nucleus cytoplasm	 123,9 ± 4,4 18,8 ± 0,5 105,1 ± 4,0	 109,0 ± 4,2* 16,3 ± 0,4* 92,7 ± 1,8*
Nuclear-cytoplasmic relationship	0,180 ± 0,004	0,180 ± 0,005
Number of exocrinocytes in the acinus	7,5 ± 0,1	6,8 ± 0,1
Number of nucleolus in the nucleus	1,56 ± 0,04	1,94 ± 0,09*
Epithelium height of the acinus, μm	12,2 ± 0,2	10,9 ± 0,3
Endocrine part / Langerhans islets		
Relative area, %	5,1 ± 0,4	5,2 ± 0,4
Number of islets / 0.25 mm^2	1,12 ± 0,10	0,98 ± 0,09*
Islets area, μm^2	14653 ± 153	16642 ± 190*
Islets diameter, μm	111,2 ± 9,1	124,5 ± 12,9*
Number of endocrinocytes in the islet	189,2 ± 18,1	205,1 ± 10,6
Density of endocrinocytes placement in the islets / 1000 μm^2	12,9 ± 0,5	12,3 ± 0,4

*$p < 0,05$ significant differences compared to the control.

We found that the administration of L-tryptophan significantly reduces the amount of connective tissue in the pancreas. This is evidenced by the probably smaller relative area of the connective tissue (by 38 %), the stromal-parenchymal index (by 42 %), the smaller width of the interlobular (by 33 %) and interacinus (by 12 %) connective tissue layers in the gland of the experimental rats, compared to the control (fig. 2). The connective tissue is the most important component of the histo-hematic barrier, and a decrease in its number and thickness of layers contributes to better oxygen transport to the parenchymal elements of the gland, improves the conditions for the flow of metabolic processes and the penetration of hormones through the histo-hematic barrier into the blood [9]. That is, the use of L-tryptophan is a promising direction in the prevention and treatment of fibrosis in the pathology of the pancreas. However, this requires a more detailed study.

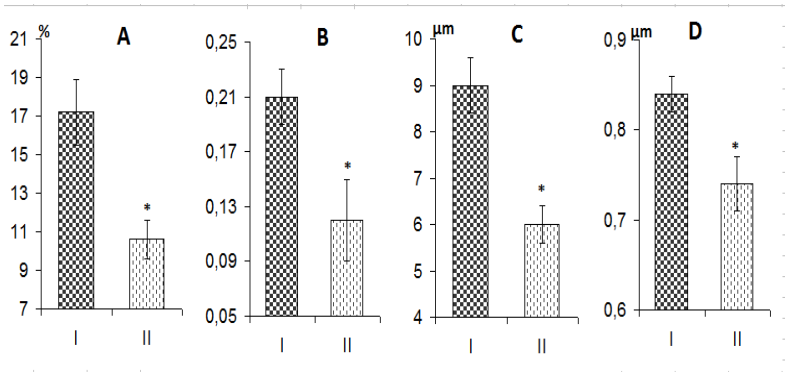

Fig. 2. Relative area of connective tissue (A), stromal-parenchymal index (B), width of interlobular (C) and interacinus (D) connective tissue layers. Group I – control; group II – rats that received L-tryptophan. *p < 0,05 significant differences compared to group I.

Most of the data in the literature are devoted to clinical and experimental studies of the influence of tryptophan on the morpho-functional state of the pancreas in this or that pathology and the effectiveness of its use for the correction of already existing disorders in various diseases [3]. The beneficial effect of tryptophan can be associated with: (1) strengthening of the antioxidant protection of the pancreas tissue by direct removal of toxic radical oxygen and nitrogen [10], (2) preserving the activity of antioxidant enzymes such as: superoxide dismutase, catalase or glutathione peroxidase [4], (3) a decrease in the production of the pro-inflammatory cytokine tumor necrosis factor α, which is accompanied by the stimulation of anti-inflammatory IL-10 [18], (4) an improvement in the blood flow of the pancreas and a decrease in neutrophil infiltration [5], (5) a reduction of apoptosis and necrosis in the inflamed tissue of the pancreas [6], and (6) promoting the regenerative process in the gland [14].

Conclusions

1. Histo-morphometric signs of a decrease in the activity of the exocrine pancreas in healthy young rats treated with L-tryptophan (at a dose of 80 mg/kg) for 28 days were revealed. This is evidenced by the smaller size of acinus, the epithelium height and the smaller exocrinocytes area.

2. Endocrine activity of the rat's pancreas, after administration of L-tryptophan, did not undergo significant changes.

3. Administration of L-tryptophan probably reduced the amount of connective tissue in the pancreas. This is relevant for practical medicine, regarding the use of this amino acid in the prevention and complex treatment of pancreas fibrosis.

REFERENCES

1. *Adeyemi D.O., Komolafe O.A., Adewole O.S., Obuotor E.M., Abiodun A.A., Adenowo T.K.* Histomorphological and morphometric studies of the pancreatic islet cells of diabetic rats treated with extracts of Annona muricata. Folia Morphol (Warsz), *69*, 2, 92-100, 2010.
2. *Dubois M.L., Boisvert F.M.* The Nucleolus: Structure and function. The Functional Nucleus, *23*, 29-49, 2016. doi: 10.1007/978-3-319-38882-3_2.

3. *Góralska M., Ceranowicz P., Warzecha Z., Dembinski A., Bonior J.* Effects of melatonin and its analogues on pancreatic inflammation, enzyme secretion, and tumorigenesis. Int. J. Mol. Sci., *18*,10-14, 2017. doi: 10.3390/ijms18051014.
4. *Hoseini S.M., Yousefi M., Mirghaed A.T., Paray B.A., Hoseinifar S.H., Doan H.* Effects of rearing density and dietary tryptophan supplementation on intestinal immune and antioxidant responses in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, *528*, 735537, 2020.
5. *Jaworek J., Leja-Szpak A., Nawrot-Porąbka K., Szklarczyk J., Kot M., Pierzchalski P., Góralska M., Ceranowicz P., Warzecha Z., Dembinski A., Bonior J.* Effects of melatonin and its analogues on pancreatic inflammation, enzyme secretion, and tumorigenesis. Int. J. Mol. Sci., *18*, 10-14, 2017. doi: 10.3390/ijms18051014.
6. *Jaworek J., Szklarczyk J., Jaworek A.K., Nawrot-Porąbka K., Leja-Szpak A., Bonior J., Kot M.* Protective effect of melatonin on acute pancreatitis. Int J Inflam., *2012*, 173675, 2012. doi: 10.1155/2012/173675.
7. *Kałużna-Czaplińska J., Gątarek P., Chirumbolo S., Chartrand M.S., Bjørklund G.* How important is tryptophan in human health? Crit Rev Food Sci Nutr., *59*, 1, 72-88, 2019. doi: 10.1080/10408398.2017.1357534.
8. *Leja-Szpak A., Jaworek J., Nawrot-Porabka K., Palonek M., Mitis-Musioł M., Dembiński A., Konturek S.J., Pawlik W.W.* Modulation of pancreatic enzyme secretion by melatonin and its precursor; L-tryptophan. Role of CCK and afferent nerves. J Physiol Pharmacol., *55*, 2, 33-46. 2004.
9. *Longnecker D.S.* Anatomy and histology of the pancreas. Pancreapedia: Exocrine Pancreas Knowledge Base, 2021. doi: 10.3998/panc.2021.01.
10. *Nayak B.N., Buttar H.S.* Evaluation of the antioxidant properties of tryptophan and its metabolites in in vitro assay. J Complement Integr Med., *13*, 2, 129-36, 2016. doi: 10.1515/jcim-2015-0051.
11. *Poeqqeler B., Singh S.K., Pappolla M.A.* Tryptophan in nutrition and health. Int J Mol Sci., *23*, 10, 5455, 2022. doi: 10.3390/ijms23105455.
12. *Rehfeld A., Nylander M., Karnov K.* Histological Methods. In: Compendium of Histology. Springer, Cham., 2017. doi: 10.1007/978-3-319-41873-5_2.
13. *Richard D.M., Dawes M.A., Mathias C.W., Acheson A., Hill-Kapturczak N., Dougherty D.M.* L-tryptophan: basic metabolic functions, behavioral research and therapeutic indications. Int J Tryptophan Res., *23*, 2, 45-60, 2009. doi: 10.4137/ijtr.s2129.
14. *Saponara E., Grabliauskaite K., Bombardo M., Buzzi R., Silva A.B., Malagola E., Tian Y., Hehl A.B., Schraner E.M., Seleznik G.M., Zabel A., Reding T., Sonda S., Graf R.* Serotonin promotes acinar dedifferentiation following pancreatitis-induced regeneration in the adult pancreas. J Pathol., *237*, 4, 495-507, 2015. doi: 10.1002/path.4595.
15. *Shipelin V.A., Trusov N.V., Apryatin S.A., Shumakova A.A., Balakina A.S., Riger N.A., Gmoshinski I.V., Nikityuk D.B.* Effects of tyrosine and tryptophan in rats with diet-induced obesity. Int. J. Mol. Sci., *22*, 24-29, 2021. doi: 10.3390/ijms22052429.
16. *Tordjman S., Chokron S., Delorme R., Charrier A., Bellissant E., Jaafari N., Fougerou C.* Melatonin: pharmacology, functions and therapeutic benefits. Curr Neuropharmacol., *15*, 3, 434-43, 2017. doi: 10.2174/1570159X14666161228122115.
17. *Yanko R., Levashov M., Chaka O.* Morpho-functional features of the pancreas in different ages rats with alimentary obesity. Acta Scientific Gastrointestinal Disorders, *5*, 11, 03-09, 2022.
18. *Yuhas Y., Holtmann H.,Shemer-Avni Y., Sarov I., Wallach D.* Inhibition of tumor necrosis factor-induced cell killing by tryptophan and indole. Eur Cytokine Netw., *1*, 1, 35-40, 1990.

Received on 28.03.2023