Hnatiuk V. V., Kononenko N. M. The study of the relationship between the levels of melatonin in the blood serum and melatonin-positive-labeled cells in ulcerative lesions of the stomach in male rats of different age = Вивчення взаємозв'язку між рівнями мелатоніну в сироватці крові та мелатонін-позитивно-міченими клітинами при виразковому ураженні шлунка у щурів-самців різного віку. Journal of Education, Health and Sport. 2016;6(9):524-530. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.154731
http://ojs.ukw.edu.pl/index.php/johs/article/view/3874

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 755 (23.12.2015).
755 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 05.08.2016. Revised 25.08.2016. Accepted: 21.09.2016.

UDC 616.33-002.44-055.1: 616.155.32:612.325

THE STUDY OF THE RELATIONSHIP BETWEEN THE LEVELS OF MELATONIN IN THE BLOOD SERUM AND MELATONIN-POSITIVE-LABELED CELLS IN ULCERATIVE LESIONS OF THE STOMACH IN MALE RATS OF DIFFERENT AGE

V. V. Hnatiuk, N. M. Kononenko

National University of Pharmacy, Kharkiv, Ukraine

Melatonin is a universal adaptogen that regulates the function of many body systems. The main source of the melatonin synthesis is the epiphysis where it is produced from the amino acid tryptophan under the control of suprachiasmatic nuclei of the hypothalamus depending on the intensity of the signal from photoreceptors of the retina. Moreover, the content of melatonin in the body is not only associated with the pineal gland secretion by pinealocytes, but with the extrapineal sources of its synthesis – the mucous membrane of the gastrointestinal tract, thymus, placenta, etc.

In recent years the attention of researchers is attracted to the study of the role of melatonin in the pathogenesis of erosive and ulcerative lesions of the stomach. Therefore, the **aim** of our research was to study the relationship between the levels of melatonin in the blood serum and melatonin-positive-labeled cells in ulcerative lesions of the stomach in male rats of different age of 9, 15 and 20 months corresponding to the human age of 29–30, 43–44, 55–56 years, respectively. In the course of the study conducted it was found that on the background of ulcerative lesion there was a significant reduction of both the level of melatonin in the blood and the number of melatonin-positive-labeled cells in gastro mucosa. Under the influence of ulcerative lesion the number of melatonin-positive-labeled cells significantly decreased in all age groups; moreover, significant differences between the number of melatonin-positive-labeled cells in animals of different age in the experimental groups were absent. The level of melatonin in the blood serum decreased more in

rats at the age of 9 and 20 months. In this time the number of melatonin-positive-labeled cells significantly decreased in rats at the age of 9 and 15 months. The greatest decrease of parameters is observed in rats at the age of 9 months, and it corresponds to the human age of 29-30 years.

Keywords: melatonin, age, sex, ulcer, stomach, immunohistochemical.

ВИВЧЕННЯ ВЗАЄМОЗВ'ЯЗКУ МІЖ РІВНЯМИ МЕЛАТОНІНУ В СИРОВАТЦІ КРОВІ ТА МЕЛАТОНІН-ПОЗИТИВНО-МІЧЕНИМИ КЛІТИНАМИ ПРИ ВИРАЗКОВОМУ УРАЖЕННІ ШЛУНКА У ЩУРІВ-САМЦІВ РІЗНОГО ВІКУ

В. В. Гнатюк, Н. М. Кононенко

Національний фармацевтичний університет, Харків, Україна

Проведено дослідження рівнів мелатоніну в сироватці крові та кількості мелатонін-позитивно-мічених клітин при виразковому ураженні шлунка у щурів-самців різного віку 9, 15 та 20 міс., що відповідає віку людини 29-30, 43-44 та 55-56 років. Встановлено, що на тлі виразкового пошкодження відбувається достовірне зниження як рівня мелатоніну в крові, так і кількості мелатонін-позитивно-мічених клітин в слизовій оболонці шлунка. Під впливом виразкового ураження кількість мелатонін-позитивно-мічених клітин достовірно знижується у всіх вікових групах, при цьому достовірні відмінності між кількістю мелатонін-позитивно-мічених клітин у тварин різного віку експериментальних груп відсутні. Мелатонін в сироватці крові найбільше знижується у щурів віком 9 та 20 міс., в той час як кількість мелатонін-позитивно-мічених клітин значно зменшена у щурів віком 9 та 15 міс.. Таким чином, найбільші зниження показників спостерігаються у щурів віком 9 міс., що відповідає віку людини 29-30 років.

Ключові слова: мелатонін, вік, стать, виразка, шлунок, імунногістохімія.

ИЗУЧЕНИЕ ВЗАИМОСВЯЗИ МЕЖДУ УРОВНЯМИ МЕЛАТОНИНА В СЫВОРОТКЕ КРОВИ И МЕЛАТОНИН-ПОЛОЖИТЕЛЬНО-МЕЧЕНЫМИ КЛЕТКАМИ ПРИ ЯЗВЕННОМ ПОРАЖЕНИИ ЖЕЛУДКА У КРЫС САМЦОВ РАЗНОГО ВОЗРАСТА

В. В. Гнатюк, Н. М. Кононенко

Национальный фармацевтический университет, Харьков, Украина

Проведено исследование уровней мелатонина в сыворотке крови и количества мелатонин-положительно-меченых клеток при язвенном поражении желудка у крыс-самцов разного возраста 9, 15 и 20 мес., что соответствует возрасту человека 29-30, 43-44 и 55-56 лет. Установлено, что на фоне язвенного повреждения происходит достоверное снижение как уровней мелатонина в крови, так и количества мелатонин-положительно-меченых клеток в слизистой оболочке желудка. Под влиянием язвенного поражения количество мелатонин-положительно-меченых клеток достоверно снижается во всех возрастных группах, при этом достоверные различия между количеством мелатонин-положительно-меченых клеток у животных разного возраста экспериментальных групп отсутствуют. Мелатонин в сыворотке крови больше снижается у крыс в возрасте 9 и 20 мес., в то время как количество мелатонин-положительно-меченых клеток более снижено у крыс в возрасте 9 и 15 мес. Таким образом, наибольшее снижение показателей наблюдаются у крыс в возрасте 9 мес., что соответствует возрасту человека 29-30 лет.

Ключевые слова: мелатонин, возраст, пол, язва, желудок, имунногистохимия.

Introduction. Melatonin is a universal adaptogen that regulates the function of many body systems. Being a neurotrasmitter it supports the sleep-wake cycle, the conduction velocity, the body temperature; as a hormone it affects proliferation – inhibits mitoses of almost all cells at the metaphase level, has an impact on the synthesis of regulatory peptides of the hypothalamus; as an immunomodulator it activates mitoses of stem cells in the red bone marrow, functions of the thymus and lymphocytes [1]. The main source of the melatonin synthesis is the epiphysis where it is produced from the amino acid tryptophan under the control of suprachiasmatic nuclei of the hypothalamus depending on the intensity of the signal from photoreceptors of the retina [2]. Moreover, the content of melatonin in the body is not only associated with the pineal gland secretion by pinealocytes, but with the extrapineal sources of its synthesis – the mucous membrane of the gastrointestinal tract, thymus, placenta, etc. [3]. In addition, the concentration of melatonin in the alimentary tract is 10-100 times higher than in the blood, and in 400 times higher than in the epiphysis itself [4]. The main source of the extrapineal melatonin secretion in the gastric mucosa

(GM) is enterochromaffin cells. They are part of the gastroenteropancreatic neuroendocrine diffuse system – it is a universal system of adaptation and the body balance control in general and GM in particular [5]. In recent years the attention of researchers is attracted to the study of the role of melatonin in the pathogenesis of erosive and ulcerative lesions of the stomach [6]. However, there are no works studying the relationship between the amount of melatonin in the blood serum and melatonin-producing cells GM in ulcerative lesion of the stomach.

Therefore, the **aim** of our research was to study the relationship between the levels of melatonin in the blood serum and melatonin-positive-labeled cells (MPLC) in ulcerative lesions of the stomach in male rats of different age.

Materials and methods. The study was performed in 36 male rats at the age of 9, 15 and 20 months corresponding to the human age of 29–30, 43–44, 55–56 years, respectively; they were divided into 6 groups: groups 1–3 were control male rats of the corresponding age, groups 4–6 – experimental rats of the corresponding age with ulcerative lesion of the stomach. Animals of control and experimental groups were kept on a standard diet and temperature conditions under natural light without the influence of artificial light sources for 2 weeks. On day 15 of the experiment the animals of control groups were removed from the experiment with taking the blood and samples of GM, and gastric ulcers were simulated to the rats of experimental groups by the method of alcohol-prednisolone damage. Prednisolone was introduced intragastrically in the dose of 20 mg/kg dissolved in 80 % ethyl alcohol in the dose of 6 ml/kg [7]. Blood sampling and samples of GM in animals of groups 4–6 were taken on day 18 of the experiment. The level of melatonin in the blood serum of rats was determined by enzyme-linked immunosorbent assay using a set of Melatonin ELISA (“IBL-International”, Germany). The study of GM samples was performed by the method of immunohistochemical staining with the primary antibodies to melatonin (Biorbyt, UK) and the secondary Alexa Fluor 488 conjugated antibodies (Abcam, UK). All interventions and euthanasia of animals were carried out in accordance with the requirements of the Commission on Bioethics of the National University of Pharmacy (NUPh) and "General ethical principles of experiments on animals" agreed with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the first National Bioethics Congress (Kyiv, 2001). The statistical confidence was assessed by one-way ANOVA test, the difference at $p<0.05$ was considered to be significant [8]. “Statistica V.8.0” and Excel software was used.

Results and Discussion. In the course of the study conducted it was found that on the background of ulcerative lesion there was a significant ($p\leq0.05$) reduction of both the level of melatonin in the blood (Fig. 1) and the number of MPLC in GM (Fig. 2).

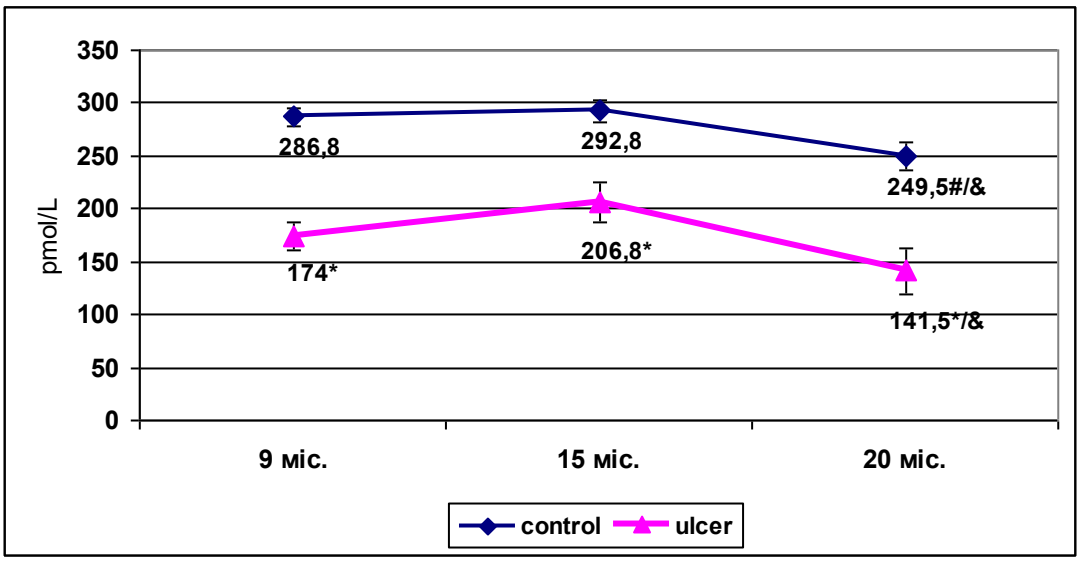

Fig. 1. The level of melatonin in male rats of different age on the background of ulcerative lesion of the stomach

Note: * p≤0.05 in relation to the control, # p≤0.05 in relation to rats aged 9 months,
& p≤0.05 in relation to rats aged 15 months.

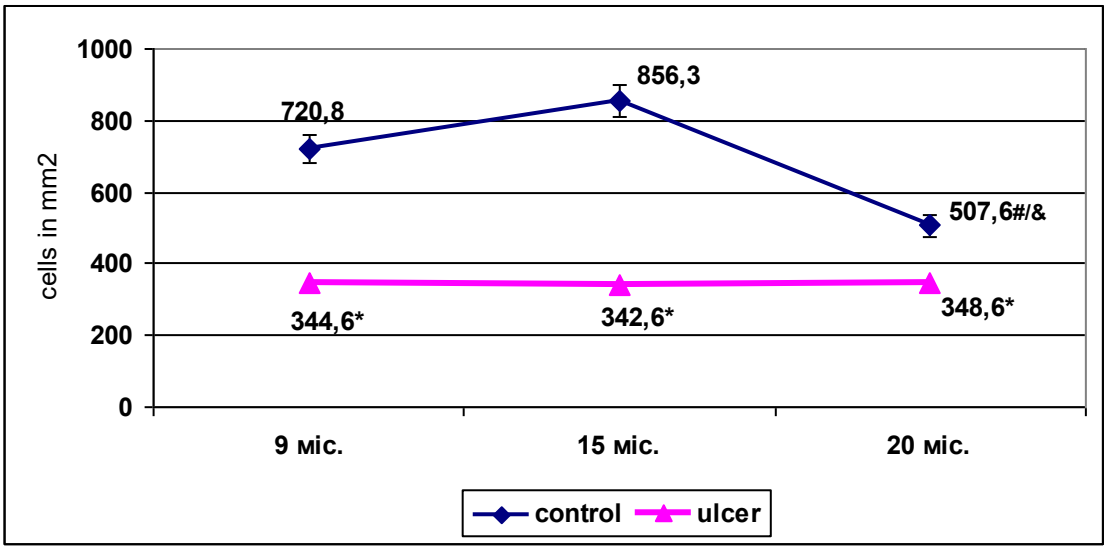

Fig. 2. The number of MPLC in male rats of different age on the background of ulcerative lesion of the stomach

Note: * p≤0.05 in relation to the control, # p≤0.05 in relation to rats aged 9 months,
& p≤0.05 in relation to rats aged 15 months.

It was determined that the level of melatonin decrease in the blood serum and the number of MPLC in different age groups reduced in a different way. Thus, the greatest decrease of melatonin in the blood serum was in rats at the age of 20 and 9 months – by 43% and 39%, respectively (p≤0.05), while the number of MPLC significantly reduced in rats aged 15 months – by 60% and aged 9 months – by 52%.

The data obtained indicate that melatonin, which is produced in MPLC, is secreted not only exocrinely, performing a protective function of GM, and interstitially as well with formation of a definite part of the total melatonin circulating in the body [9]. Under the influence of ulcerative lesion the number of MPLC significantly ($p \leq 0.05$) decreased in all age groups; moreover, significant differences between the number of MPLC in animals of different age in the experimental groups were absent. The different percentage reduction in the number of MPLC in rats of different age in relation to the control – by 52% in young, by 60% in mature and the least decrease in old rats – by 31% can be explained by the presence of the existing atrophic processes of GM in rats at the age of 20 months confirmed by the literature data [10] and the significant difference between the number of MPLC in rats aged 20 months in relation to rats aged 9 and 15 months in the animals of control groups in our experiment.

Furthermore, the level of melatonin in the blood serum decreased more in rats at the age of 9 and 20 months. In addition to ulcerative lesion of the extrapineal source the age involution of the pineal gland observed in human and animals with age affects the existing degree of reduction of the amount of melatonin in the blood serum in rats at the age of 20 months [11]. A significant decrease of melatonin in the blood serum in rats at the age of 9 months corresponding to the human age of 29-30 years on the background of ulcerative lesions indicates creation of a new neurohormonally relationships formed with the deficiency in extrapineal melatonin and, as a result, in the total melatonin in the blood serum. It is likely to be associated with gender peculiarities of ulcer disease [12].

Conclusions

1. The ulcerative lesion of the stomach leads to a significant decrease in the level of melatonin in the blood serum and the number of melatonin-positive-labeled cells in the gastric mucosa.

2. The greatest decrease of parameters is observed in rats at the age of 9 months, and it corresponds to the human age of 29-30 years.

References

1. Anisimov, V.N., 2007. Melatonin role in the body, the use in the clinic. Sistema, Sankt-Peterburg. (in Russian)

2. Perreau-Lentz, S.,. Kalsbeek, A, Garidou, M.L., Wortel, J., van der Vliet, J., van Heijningen, C., Simonneaux, V., Pevet, P., Buijs, R.M., 2003. Suprachiasmatic control of melatonin in rats: Inhibitory and stimulatory mechanisms. Eur. J. Neurosci. 17, 221–228.

3. Kvetnoy, I.M., 2012. Ekstrapinealnyiy melatonin: rol v hronobiologii i hronomeditsine. Vestnik RUDN [Bulletin of Peoples' Friendship University of Russia]. 7, 126. (in Russian)

4. Phadeenko, G.D., Haponova, O.G., 2008. Rol melatoninu u patogenezi funkcionalnikh pozladiv travnogo kanalu. Liki ludini. 4 (120), 98–100. (in Ukraine)

5. Bubenik, G.A., 2002. Gastrointestinal melatonin: localization, function and clinical relevance. Digestive Diseases and Sciences. 47(10), 2336–2348.

6. Oparin, A.A., Shapovalova, O.E., Dvoyashkina, Yu.I., Lavrova, N.V., 2010. Melatonin i zabolevaniya zheludochno-kishechnogo trakta [Melatonin and gastrointestinal diseases], Mezhdunarodnyiy meditsinskiy zhurnal. 16 (4), 68–72. (in Russian)

7. Stefanov, O.V., 2001. Doklinichni doslidzhennya likarskih zasobiv. Metod. rekomendatsiyi. Vidavnichiy dIm «Avitsena», Kyiv. (in Ukraine)

8. Kochetov, A.G., Liang, O.V., Masenko, V.P., Zhirov, I.V., Nakonechnikov, S.N., Tereshchenko, S.N., 2012. Statistical methods of medical data. RKNPK, Moscow. (in Russian)

9. Danilova, R.K. (ed), 2011. Rukovodsto po histologii. T.2. SpetsLit, Sankt-Peterdurg. (in Russian)

10. Iarigina, V.N., Melenteva, A.S (ed.), 2010. Rukovodsto po gerontologii I geriatrii. T. 3. Klinicha geriatria. Razdel 2.3. Bolezni organov pishchevarenia. Glava 1. Osobenosti I semiotika morfofunktsionalnikh ixmenenii sistemi organov pishchevarenia stareiushchego cheloveka. GEOTAR-Media, Moscow. (in Russian)

11. Bondarenko, L.A., 2015. Nekotoryie gormonalne mehanizmyi uskorennogo stareniya pri gipopinealizme. Mat. nauk.-prak. konf. “ChotirnadtsyatI DanilevskI chitannya. Dosyagnennya ta perspektivi eksperimentalnoyi i klinIchnoyi endokrinologii”. HarkIv, pp. 14–20. (in Russian)

12. Islamova, E.A., 2009. Vozrastnyie osobennosti yazvennoy bolezni zheludka i dvenadtsatiperstnoy kishki [Age features of peptic and duodenal ulcer disease]. Saratovskiy nauchno-meditsinskiy zhurnal [Saratov Journal of Medical Scientific Research]. 5(4), 569–571. (in Russian)