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[1]Danylo Halytskyi Lviv National Medical University,
[2]Taras Shevchenko National University of Kyiv, Ukraine

YA. SAVYTSKYY[1], D. GOLYSHKIN[2], T. FALALYEYEVA[2]

Influence of melanin on the lesion in the gastric mucosa of rats caused by neuro-muscular tension according to Selye

Wpływ melaniny na organiczne zmiany chorobowe w śluzówce żołądka szczurów spowodowane przez napięcie neuromięśniowe wg Selye

INTRODUCTION

Although up today enough effective pharmaceutical substances have been developed and they are widely used for the treatment of ulcer disease of stomach and duodenum, the amount of drugs for the prevention of development and recurrence of ulcer disease is limited. Considering that stress, which continually accompanies people in the present society, is the starting factor of the mechanism of ulcer disease pathogenesis, efforts of many scientists are devoted to the study of the mechanism of action of stress on gastric and duodenal mucosa and generation of prophylactic drugs from stress action on it.

Results of many studies point that the natural adaptogenes include melanin [3,6]. Antiradical and antioxidative effects of melanin [3,9] and its cytoprotective effects on ulceration in stomach evoked by serotonin [7], ethanol [7], immobilizational stress in modification by Groisman and Karevina [1] make melanin perspective as an alternative way for the prevention of development of stress injuries in gastric mucosa (GM). But the data about the influence of melanin on the development of damages in GM evoked by the method of neuromuscular tension by Selye are absent.

The aim of this work was to investigate the effect of melanin on the development of damages in GM caused by the method of neuromuscular tension according to Selye and oxidant and antioxidant status in GM of rats.

MATERIAL AND METHODS

The study was carried out on 30 white rats maintained in accordance with the guidelines of Animal Ethical Research Committee of Taras Shevchenko National University of Kyiv. The animals were divided into 3 groups. The animals were deprived of food for 24 hrs prior to the experiments with free access to water. To the rats of I and II groups we injected 0.5 ml water *per os*. The animals of III groups were injected melanin in a dose of 5 mg/kg *per os* 15 min before stress. The rats of II and III

group were subjected to stress by the method of neuromuscular tension by Selye [8]. We used a lethal dose of urethane (3 g/kg, intraperitoneally) for rats. We cut up their stomachs along the small curvature and carefully washed out. Then on gastroscope at transillumination by means of a magnifier (x4) we performed careful examination. We counted up the area of ulcers and the length of erosions.

Oxidant and antioxidant status in GM of rats was estimated by the content of conjugated dienes, thiobarbituric acid reactive substances (TBARS), Shiff bases [4] and the activity of antioxidat enzymes superoxide dismutase (SOD) [2] and catalase [5] in homogenate of GM.

Melanin which we used in our experiments is the product of life activity of yeast-like fungi *Nadsoniella nigra sp. X-1*. Microorganisms were seeds from samples of vertical rocks of island Galindez (Academic Vernadskyy Ukrainian Antarctic station). Melanin of the present origin consists of polyphenolcarboxylic complex in 95%.

Our data by test Shapiro-Wilks' W test were normally distributed. All results are expressed as the M±m of n values. Statistical comparisons between groups were conducted using the Student's t-test for unpaired data. Statistical significance was set at $p<0.05$.

RESULTS

It was established that GM in rats of I group was healthy. Two hours after the end of stress in the GM of II group ulcers and erosion developed, the area and length were respectively 7.03 ± 1.29 mm^2 and 3.50 ± 1.28 mm per stomach. Stress also led to a significant intensification of lipid peroxidation processes. The concentration of conjugated dienes, TBARS, Shiff bases were increased by 63% ($p<0.05$), 99% ($p<0.05$) and 82% ($p<0.05$), accordingly. The activity of SOD and catalase was enhanced by 29% ($p<0.05$) and 37% ($p<0.05$), accordingly.

Prophylactic injection of melanin decreased the area of ulcers by 75% ($p<0.05$). But the decrease of the length of erosions was statistically unreliable. Reduction of gastric lesions was accompanied by normalization of the content of lipid peroxidation products in GM. Melanin increased the activity of SOD and decreased the activity of catalase compared with the activity of these enzymes in the group of rats susceptible to stress (Table 1).

DISCUSSION

Development of ulcerative-erosive lesions in the GM of rats after stress action is accompanied by an increase in the GM of content of lipid peroxidation products that corresponds to numerous literature data [10]. Simultaneously in these rats the activity of SOD and catalase in homogenate of GM was enhanced. Prophylactic use of melanin before the application of stress leads to a decrease of ulcer formation and normalization of lipid peroxidation in GM: the content of conjugated dienes, TBARS and Shiff bases did not differ from control levels. The activity of SOD continued to grow but a statistically significant decrease was found in the activity of catalase, compared to the stress group. This indicates that the generation of hydrogen peroxide is faster. Because hydrogen peroxide is a substrate for catalase activity which is under the influence of melanin decreases, we concluded that melanin has the ability to decompose hydrogen peroxide to water and oxygen. The fact that melanin does not prevent the formation of erosions is evidence of incomplete decomposition of hydrogen peroxide.

Table 1. Influence of melanin on oxidant and antioxidant status in gastric mucosa in rats after causing stress (M±m)

Parameter	Control (n=10)	Stress (n=10)	Stress + Melanin (n=10)
Conjugated dienes, nMol x mg of protein^{-1}	211.97 ± 17.84	345.68 ± 24.56*	209.88 ± 12.37$^{\#\#\#}$
Thiobarbituric acid reactive substances, nMol x mg of protein^{-1}	102.71 ± 7.53	203.87 ± 13.62*	114.74 ± 7.71$^{\#\#\#}$
Shiff bases, U. x mg of protein^{-1}	5.80 ± 0.36	10.56 ± 0.61*	5.99 ± 0.31$^{\#\#\#}$
Superoxide dismutase, U. x min^{-1} x mg of protein^{-1}	0.41 ± 0,03	0.53 ± 0.04*	0.68 ± 0.05$^{*/\#}$
Catalase, μMolx min^{-1} x mg of protein^{-1}	9.01 ± 0.51	12.37 ± 0.98*	10.21 ± 0.62$^{\#}$

* $p < 0.05$ compared with control group $^{\#}$ $p < 0.05$ $^{\#\#\#}$ $p < 0.001$ compared with stress group

CONCLUSIONS

Melanin at least partially prevented the development of oxidative stress, which manifested itself in the protection of gastric mucosa from ulceration after stress according to Selye.

REFERENCES

1. Beregova T. et al.: The involvement of peroxisome proliferators-activated receptors in the antiulcer action of melanin. Abstracts of 3rd International Symposium on Ulcer Healing and Regenerative Medicine. San Diego, USA, 8, 2008.
2. Chevari S. et al.: Rol' superoxiddismutasi v okislitelnyh processah kletki i metod opredeleniya ee v biologicheskih materialah. Labor. Delo, 11, 678, 1985.
3. Constantinescu C.S.: Melanin, melatonin, melanocyte-stimulating hormone, and the susceptibility to autoimmune demyelination: a rationale for light therapy in multiple sclerosis. Med. Hypotheses, 45, 5, 455, 1995.
4. Kolesova O.E. et al.: Perekisnoe okislinie lipidov i metody opredeleniya produktov lipoperoksidacii v biologicheskih sredah. Labor. Delo, 9, 540, 1984.
5. Koroluk M.A. et al.: Metod aktivnosti katalazi. Labor. Delo, 1, 16, 1998.

6. Meredith P. et al.: The physical and chemical properties of eumelanin. Pigment Cell Res., 19, 6, 572, 2006.
7. Savytskyy Ya.M. et al.: The influence of melanin on cytoprotective processes in gastric mucosa. Exper. Clin. Physiol. Med., 1, 29, 2001.
8. Selye H.: Stress dependent changes in the gastrointestinal tract. Verh. Dtsch. Ges. Inn. Med., 75, 213, 1969.
9. Stepien K. et al.: Reduction of 13-hydroperoxy-9,11-octadecadienoic acid by dopamine-melanin. Biochim. Biophys. Res. Commun., 244, 3, 781, 1998.
10. Tandon R. et al.: Oxidative stress and antioxidants status in peptic ulcer and gastric carcinoma. Indian J. Physiol. Pharmacol., 48, 1, 115, 2004.

SUMMARY

In acute experiments on 30 white rats it was established that melanin decreased the area of ulcers in gastric mucosa evoked by stress according to Selye. But the decrease of length of erosions was statistically unreliable. Reduction of gastric lesions was accompanied by normalization of the content of lipid peroxidation products in gastric mucosa. Melanin increased the activity of superoxide dismutase and decreased the activity of catalase compared with the activity of these enzymes in the group of rats susceptible to stress. The obtained data allow to consider melanin as a promising means of prophylaxis against stress lesions of the gastric mucosa.
Key words: melanin, stress, gastric mucosa

STRESZCZENIE

W eksperymencie przeprowadzonym na 30 białych szczurach wykazano, że melanina zmniejsza powierzchnię wrzodów w śluzówce żołądka wywołanych przez stres wg Selye. Jednakże spadek długości erozji był statystycznie nieistotny. Redukcji zmian chorobowych żołądka towarzyszyła normalizacja zawartości produktów peroksydacji lipidów w jego śluzówce. Melanina zwiększała aktywność dysmutazy ponadtlenkowej i zmniejszała aktywność katalazy w porównaniu z aktywnością tych enzymów w grupie szczurów wrażliwych na stres. Uzyskane dane pozwalają uważać melaninę za obiecujący środek w profilaktyce chorób śluzówki żołądka związanych ze stresem.

Słowa kluczowe: melanina, stres, śluzówka żołądka