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ABOUT INVOLVEMENT OF PEROXISOME-PROLIFERATOR-ACTIVATED RECEPTOR GAMMA IN MECHANISM OF ANTIULCER ACTION OF MELANIN ISOLATED FROM ANTARCTIC YEASTS

V.M. Kukharskyy[1], N.V. Chyizhanska[2], O.I. Tsyryuk[1]

[1] Department of Pharmaco-physiology of Institute of physiology of Biological faculty of Kiev National Shevchenko University, academician Glushkov av., 2, bd 12, Kiev, Ukraine, tfalalyeyeva@mail.ru
[2] Poltava State Agriculture Academy, Skovoroda street, 1/3, Poltava, 36003, Ukraine, pdaa@agroak.poltava.ua

The involvement of peroxisome proliferator-activated receptors gamma in antiulcer action of melanin.
V.M. Kukharskyy, N.V. Chyizhanska, O.I. Tsyryuk
Abstract. In acute experiments on 55 white rats we investigated one of the possible mechanisms of cytoprotective action of melanin via activation of peroxisome proliferator-activated receptors gamma. It was established that immobilization stress by Groisman and Karevina, evoked in gastric mucosa of ulcers, erosions and hemorrhages. Melanin in dose 5 mg/kg defended gastric mucosa against development of injuries evoked by stress. Irreversible selective antagonist of peroxisome proliferator-activated receptors gamma GW9662 in dose 1 mg/kg removed the cytoprotective action of melanin on formation of stress injuries. We concluded that antistress action of melanin on gastric mucosa in rats completely or partially is governed by activation of peroxisome proliferator-activated receptors gamma.
Key words: melanin, stress, peroxisome proliferator-activated receptors gamma.

1. Introduction

The epidemiological and experimental studies convincingly show anti-inflammatory activity of polyphenols - natural compounds, some of which are representatives of melanin pigments. Previously we have shown that melanin significantly reduces the number of lesions in gastric mucosa of rats, caused by the action of stress factors [1, 2]. In further research we found that implementation of the protective properties of melanin in stressful influences associated with increasing levels of nitric oxide, which has an important role in cytoprotection [3]. In the search of possible mechanisms of biologically-active action of various natural polyphenol compounds several other groups of researchers [4, 5] concluded that one of the possible targets of these compounds can be peroxisome proliferator-activated receptors gamma (PPARγ).

Synthesis and regulation of the overwhelming number of pro-inflammatory cytokines is regulated at the level of transcription, which leads to increasing, or suspension of inflammatory processes. Peroxisome proliferator-activated receptors (PPARs) is considered one of the molecular links between inflammatory cytokines and transcription factors. PPARs belong to a group of nuclear hormone receptors, which are activated by specific internal and external factors [6]. Currently, the researchers identified three isoforms of these receptors that are encoded by separate genes (PPARα, PPAR β/δ and PPARγ). In turn, the best characterized isoform, PPARγ divided into three subtypes (PPARγ1, γ2 and γ3) [7]. All PPAR isoforms form heterodimeric complexes with retinoic X receptors (RXR), this complexes joining the so-called Peroxisome Proliferator Response Element (PPRE). PPRE functioning as central regulators of cell differentiation, apoptosis, inflammatory reactions and energy metabolism [8]. Activation of PPARγ leads to supression of inflammatory reactions of

different origin. Such protection is possible, both from the improvement of glucose metabolism, reduce insulin resistance and reduce level of pro-inflammatory cytokines. Signaling pathways including supression of activation of NF-κB, together with a decreased expression and/or activity of proteins AP-1, TGF-β1, MCP-1, ICAM-1 and iNOS [9]. It was also shown that PPARγ ligands lead to increased levels of nitric oxide by enhancing activity of eNOS, and the reducing expression of pathogenic forms of nitric oxide synthase iNOS, which corresponds to our previously obtained data for the study of mechanisms of action of melanin [3]. Therefore in this work was determined the possible involvement of PPARγ in cytoprotection of melanin in the gastric mucosa of rats after exposure to stress.

2. Object and methods of investigations

The investigation was carried out on 55 white rats, 180-200g, males, according to international principles of the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes [10]. Anti-stress effects of melanin was evaluated for its protective effect on gastric mucosal lesions in rats caused by combined cold stress and immobilisation stress in modification by Groisman and Karevina, named by the "social" stress. Rats were divided into four groups of animals, 11 to 16 individuals. Rats first (control) group before applying stress was given water in quantity 0.5 ml, orally. Second group of rats before applying stress was given aqueous solution of melanin in a dose of 5 mg/kg, orally in volume 0.5 ml. Third group of rats before applying stress entered orally in the 0.5 ml of water and 0.5 ml solution of antagonist PPARγ GW9662 at a dose 1mg/kg, i.p. in 0.5 ml of water. Fourth group of rats before applying stress entered oral aqueous solution of melanin in a dose of 5 mg/kg, in volume 0.5 ml and the antagonist GW9662 at a dose 1mg/kg, i.p. in 0.5 ml of water.

To obtain neurodystrophic gastric lesions rats placed in a perforated cylindrical metal container with a transparent eye opposite the head of a rat, rat's tail through a small hole was taken out. After immobilization of rats in the containers, their tails was placed on ice and left in this state. Two hours later tails released from ice and place the containers with rats in a colony of free rats, which created conditions for their natural existence (lighting, water, food). 24 hours after the beginning of the experiment rats was removed from containers and euthanased by cervical dislocation. After removal of the stomach, it was cutted for small curvature and thoroughly washed by water. Gastric injuries was divided on: 1) ulcers - in a deep round lesions with fibrotic mucosa touch the bottom and inflammatory seam around, 2) erosion - in the form of cracks mucosa and 3) hemorrhage - brown or black spots different forms, and counted in gastroscopic apparatus. Calculated total area of each type of lesions for each stomach and the average area of lesions for the group.

Our date by test Shapiro-Wilks' W test were normally distributed. All results are expressed as the M±SD of n values. Statistical comparisons between groups were conducted using the Student's t-test for unpaired data. Statistical significance was set at $p<0.05$. Processing results performed in the statistical software package “Statistica 6”.

3. Results and discussion

In our research was shown, that stress evokes wide-spread effect and leads to various injuries on gastric mucosa: ulcers, erosions and haemorrhages. Melanin given in dose 5mg/kg markedly protected gastric mucosa from injuries, evoked by immobilization stress and decrease of ulcers area on 84%, number of haemorrhages on 56% and length of erosions on 61% (table). Inhibitor of PPARγ GW9662 and melanin, given together did not reduce stress-induced injuries in gastric mucosa, This means, that protective effect of melanin was markedly eliminated by the treatment with selective PPARγ inhibitor.

Table

The influence of melanin (5 mg/kg, i.g.) and antagonist of PPARγ GW9662 (1 mg/kg, i.p.) on stress-induced acute gastric mucosal injuries, M±SD

Group	n	Ulcers, mm^2	Erosions, mm	Haemorrhages, $мм^2$
1. Control	15	4,86±7,44	2,41±1,61	0,5±0,52
2. Melanin	16	0,78±1,14*	0,94±2,24***	0,22±0,87*
3. GW9662	11	5,17±6,84	5,13±5,11##	0,53±1,07
4. Melanin+ GW9662	13	2,51±3,55	10,35±6,83###	0,76±2,27

* - p<0,05; ## - p<0,01: ###, *** - p<0,001;
n – number of animals, * - comparison to control, # - comparison to melanin.

It is well known that pigment melanin, as well as many others natural polyphenols, protect gastric mucosa from injuries evoked by stress, serotonin, aspirin, etc. Heretofore researches connect cytoprotective action of melanin to its antioxidant activity. The present study suggests that potent gastroprotective activity of melanin against stress-induced lesions realizes by different ways, not only its antioxidant activity. Analysis of literature shows that PPARγ agonists play a protective role in gastric lesion caused by various factors, both chemical and stressful nature. Based on our results we suggests that protective effect of melanin in gastric ulcerogenesis fully or partially caused by the mechanism of activation of PPARγ . Growth of NO level in the blood after melanin treatment apparently realized via activation of PPARγ . It remains an open question as to whether a direct interaction with melanin receptors PPARγ, or other agent is involved.

4. Conclusion

The obtained data allow to assert that anti-stress effect of melanin in the gastric mucose in rats completely or partially realise due to activation of PPARγ.

References

1. **Chyizhanska N., Tsyryuk O., Beregova T.** About the participation of nitric oxide in cytoprotection of melanin in the mucous membrane of the stomach. // Bulletin of the problems of biology and medicine. – 2005. – Vol.3. – P.56-59.
2. **Savitskiy Ya., Kimakovich V., Beregova T.** New data on the action of melanin on gastric acid secretion and gastric mucosa // Programme and Abstracts 4th Parnas Conference "Molecular Mechanisms on Cell Activation: Biological Signals and Their Target Ensymes. – Wroclaw, 2002. – P.108.
3. **Chyizhanska N., Kukharskyy V., Tzyryuk O., Beregova T.** The Influence of melanin on the expression of epithelial isoforms of nitric oxide synthase (eNOS) in the gastric mucosa of rats. // Problems of ecological and medical genetics and clinical immunology. – 2005. – Vol.5. – P.52-59.
4. **Inoue H.** [Endogenous ligands for PPARs] // Nippon Rinsho.-2005.-Vol.63, № 4.-P.578-583.
5. **Kim C.S., Park W.H., Park J.Y.** et al. Capsaicin, a spicy component of hot pepper, induces apoptosis by activation of the peroxisome proliferator-activated receptor gamma in HT-29 human colon cancer cells // J.Med.Food.-2004.-Vol.7, № 3.-P.267-273.
6. **Feige J.N., Gelman L., Michalik L.** et al. From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions // Prog.Lipid Res.-2006.-Vol.45, № 2.-P.120-159.
7. **Fajas L., Auboeuf D., Raspe E.** et al. The organization, promoter analysis, and expression of the human PPARgamma gene // J.Biol.Chem.-1997.-Vol.272, № 30.-P.18779-18789.
8. **Escher P., Wahli W.** Peroxisome proliferator-activated receptors: insight into multiple cellular functions // Mutat.Res.-2000.-Vol.448, № 2.-P.121-138.
9. **Michalik L., Wahli W.** Involvement of PPAR nuclear receptors in tissue injury and wound repair // J.Clin.Invest.-2006.-Vol.116, № 3.-P.598-606.
10. **Hollands C.** The Animals (scientific procedures) Act 1986. // Lancet. - 1986. Vol.2, № 8497. - P.32-33.