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The role of cyclooxygenase isoforms in the mechanisms of cytoprotection of gastric mucosa under the influence of hexapeptide Arg-α-Asp-Lys-Val-Tyr-Arg

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ABSTRACT

In experimental epinephrine-induced gastric lesions in rats it was shown that olygopeptide Arg-α-Asp-Lys-Val-Tyr-Arg exerts cytoprotective effect, evaluated by the decrease of the area and severity of the damage of gastric mucosa as well as decrease of NOS activity, mainly due to the decrease of iNOS and NO production in gastric mucosa. Selective COX-2 blockade enhanced the cytoprotective action of AALVTA. Nonselective blockade by COX-1 caused significant decrease of the gastroprotective properties of AALVTA, showing involvement of COX-1 isoform in the mechanisms of cytoprotection of GM under the action of this hexapeptide.

Keywords: cyclooxygenase, isoforms, cytoprotection, gastric mucosa

INTRODUCTION

Recent studies showed the high cytoprotective activity of some olygopeptides, in particular glyprolines, Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val known as BPC 157, Arg-α-Asp-Lys-Val-Tyr-Arg (AALVTA), able to reduce the damage of gastric mucosa (GM) [5, 8, 10]. These effects might be explained by their multidirectional influence on homeostasis, intercell communication, although the interactions of these substances with the cyclooxygenases (COX)/prostaglandins system remains unclear.

MATERIAL AND METHODS

The aim of research was to study the gastroprotective action of AALVTA in interactions with the COX-1/COX-2/prostaglandins system.

The studies were conducted in 42 white Albino rats in keeping with the international regulars on experimental procedures on laboratory animals. The rats were fed on a standard rat chow and water ad libitum.

Gastric lesions (GL) were induced by intraperitoneal injection of epinephrine (2 mg/kg) [14].

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The rats were divided into 7 groups. The first group included the intact animals and in the other groups we investigated the action of the following medications: [2] epinephrine, [3] AALVTA (1μkg/100g), [4] selective COX-2 blocker celecoxib (10 mg/kg); [5] combined action of AALVTA and celecoxib; [6] nonselective COX-1 blocker indomethacin (10 mg/kg); [7] combined action of AALVTA and indomethacin. All animals, except of the intact ones, 15 minutes before the application of the investigated medications were pretreated with epinephrine.

After 24 hours, the animals were anaesthetized by urethane (1.1 mg/kg) were sacrificed by decapitation and gastric mucosa and blood were taken for investigations.

The area and severity of GL were evaluated using the method of planiometry and 12-grade scale.

With the purpose to investigate the NO-synthase (NOS) system status, NOS activity [11] and nitrite anion content [3] in GM and L-arginine level in plasma [1] were measured. All results were processed by the method of variation statistics.

RESULTS AND DISCUSSION

The rats introduced to epinephrine developed severe structural and hemorrhagic lesions of GM – in 90% animals the ulcer of the stomach was evaluated and the other 10% had deep erosions and hemorhages. The mean dam-

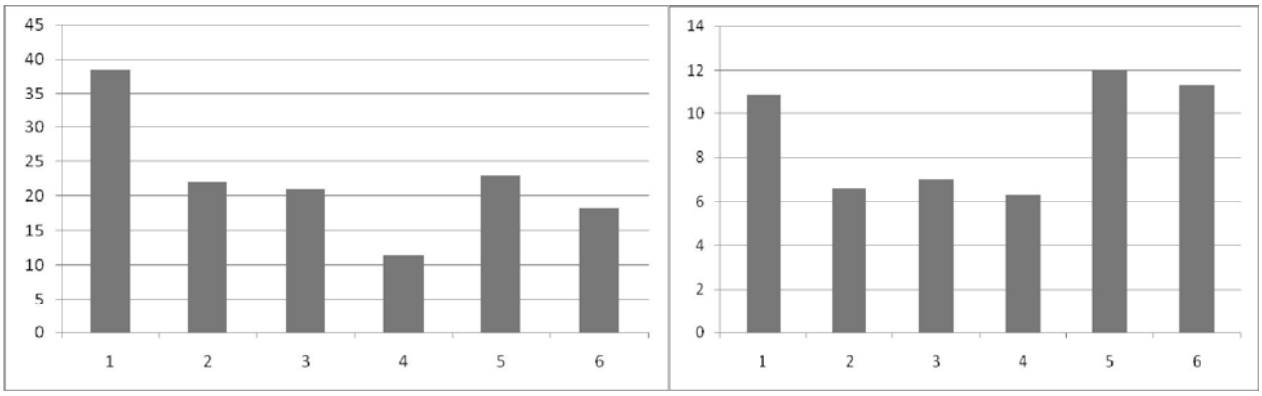

Fig. 1. A – the area (mm2) and B – severity (grades) of GL under conditions of isolated action of AALVTA and on the background of nonselective COX-1 blockade with indomethacin and selective COX-2 blockade with celecoxib, where the effects of the following drugs are: 1 – epinephrine; 2 – AALVTA and epinephrine; 3 – celecoxib and epinephrine; 4 – AALVTA, celecoxib and epinephrine; 5 – indomethacin and epinephrine; 6 – AALVTA and indomethacin

aged area made up 38.5±5 mm^2 and 10.9±2 grades (Fig. 1). In rats, administered AALVTA in epinephrine-induced stress, the damaged area of GM was 42% smaller and the severity of GL decreased by 39.44%. The ulcer of the stomach developed in 45% animals. The action of celecoxib decreased the area of GL by 45% and index of damage by 37% respectively whereas the ulcer of the stomach was seen in 37.5% animals. Combined action of AALVTA and celecoxib led to more significant decrease of the damaged area of GM (by 70%) in epinephrine-induced stress, compared to the isolated administration of each drug and only 25% of the enrolled animals developed the ulcer of the stomach. Indomethacin introduction induced 40% decrease of the area of GL, although the gastric damage was more severe compared to isolated action of epinephrine – 100% of the investigated animals had ulcer of the stomach. The simultaneous action of AALVTA and indomethacin enhanced the ulcerative effect of indomethacin – the damaged area almost did not change in comparison to the isolated action of this hexapeptide on the background of epinephrine, but 75% animals developed stomach ulceration.

Under physiologic conditions cNOS is supposed to be dominating in GM while iNOS, producing large amounts of high active nitrogen oxide is being upregulated in inflammation, trauma, ulceration [7]. In our studies the proportion of eNOS/iNOS in intact animals made up 2.8, responding with the data provided by other authors. In experimental ulceration of the stomach NOS activity acutely increased by 68%, mainly due to the 9 times increase of iNOS activity, while ĺNOS activity did not change significantly in GM. The nitrite-anion content increased by 39.7%, in GM, L-arginine concentration in plasma decreased by 42.0% (Table 1).

Introduction of AALVTA in epinephrine-induced stress decreased total NOS activity by 29%, iNOS activity – by 47.3% ($p<0.05$), iNOS activity remained almost unchanged, nitrite-anion content decreased by 31.8% in GM and L-arginine concentration in plasma increased by 30%.

The action of celecoxib on the background of epinephrine decreased NOS activity by 21%, łNOS – by 20% and the tendency to decrease of ĺNOS activity and NO in GM as well as L-arginine in plasma was also evaluated.

The combined action of AALVTA and celecoxib in epinephrine-induced stress enhanced the decrease of total NOS activity by 44.3%, iNOS – by 52% and nitrite-anion content – by 32% in GM, In plasma L-arginine concentration increased almost 2 times.

The indomethacin action in epinephrine-induced stress also decreased total NOS activity by 39%, iNOS – by 56% and there was tendency to increase of iNOS activity in GM. NO content in GM and L-arginine concentration in plasma remained almost unchanged.

Under conditions of the action of AALVTA on the background of indomethacin in epinephrine-induced stress the indices of NOS, iNOS activity, NO content in GM did not differ much from those obtained in animals, introduced to isolated action of epinephrine.

Table 1. NOS activity, NO content in GM and L-arginine concentration in plasma under conditions of isolated action of Arg-α-Asp-Lys-Val-Tyr-Arg and on the background of nonselective COX-1 blockade with indomethacin and selective COX-2 blockade by celecoxib

Series of investigations	NOS (nmol/min·g of protein)	eNOS (nmol/min·g of protein)	iNOS (nmol/min·g of protein)	NO (μmol/g)	L-arginine (μmol/ml)
Intact animals	0.699±0.42	0.516±0.32	0.184±0.11	16.1±2.55	43.0±2.09
Epinephrine	2.19±0.33*	0.48±0.225	1.71±0.28**	26.7±3.11*	24.9±4.39*
AALVTA + epinephrine	1.56±0.26#	0.658±0.10	0.901±0.17#	18.2±2.93#	35.4±9.40
Celecoxib + epinephrine	1.74±0.18#	0.46±0.23	1.27±0.15#	15.6±2.64#	27.2±3.52
AALVTA + celecoxib + epinephrine	1.22±0.34#	0.402±0.31	0.818±0.22#	18.1±3.0	40±4.24
Indomethacin+_epinephrine	1.332±0.19#	0.58±0.25	0.75±0.36#	23±2.54	26±4.12
AALVTA + indomethacin + epinephrine	2.26±0.26	0.44±0.43	1.82±0.61	25±2.72	23±3.46

Epinephrine-induced GL are accompanied by acute activation of NOS, which causes the decrease of L-arginine-concentration in plasma due to its utilization by iNOS. Introduction of Arg-α-Asp-Lys-Val-Tyr-Arg inhibits NOS activity in GM.

AALVTA caused enhancement of cytoprotection processes of the stomach, which could be explained by its degradation into di- and tripeptides, containing arginine, able to inhibit NOS.

Prostaglandins are hormone-like mediators, playing crucial role in such important mechanisms of GM defense as decrease of acid secretion, stimulation of mucous production, blood flow bicarbonathe secretion, phospholipids production [6]. Prostaglandins, providing GM integrity are being produced by the methabolism of arachidonic acid by constitutive COX-1 isoform while COX in mammals is being upregulated by inflammatory mediators, growth factors, mitogenic stimuli and the role of this isoform in gastroprotection and ulcer healing is not yet clearly understood. Assuming these data we can suggest that AALVTA enhances the cytoprotection of the stomach through stimulation of the production of the defensive prostaglandins. The decrease of the gastroprotective action of AALVTA under conditions of nonselective COX-1 blockade with indomethacin might be explained by the stimulating effect of this hexapeptide on polymorphinuclear leukocytes, as it is known that the severity of experimental gastropathy, caused by nonstheroidal anti-inflammatory drugs, is significantly lower in rats with induced neutropenia [4, 9].

It is also known that AALVTA decreases COX-2 activity [9], thus it can be hypothesized that under conditions of selective COX-2 blockade with celecoxib the inflammatory action of AALVTA is being increased, having the positive influence on the mechanisms of cytoprotection of the stomach [8]. The isolated action of celecoxib in epinephrine-induced stress also showed some cytoprotective effect but it was less significant than in the case when combined with AALVTA.

CONCLUSIONS

Hexapeptide AALVTA exerted gastroprotective effect on GM in epinephrine-induced experimental GL in rats through the decrease of NOS activity.

Selective COX-2 blockade enhanced the cytoprotective action of AALVTA.

Nonselective blockade by COX-1 caused significant decrease of the gastroprotective properties of AALVTA, showing involvement of COX-1 isoform in the mechanisms of cytoprotection of GM under the action of this hexapeptide.

REFERENCES

1. Aleynikowa T.L. et al. Rukovodstvo k prakticheskim zaniatijam po biohimii. *Medicyna*, 128, 2000.
2. Belostotskiy N.I. Yazveobrazovanie v slizistoy obolochke zheludka krys pod vliyaniyem katekholaminov. *Patologicheskaya fiziologia i eksperimentalnaya medicyna* 1, 24, 1988.
3. Green L. C., David A. W. Analysis of nitrate, nitrite and (1515) nitrate in biological fluids. *Anal. Biochem*, 126, 131, 1982.
4. Halter, Schmassmann, Peskar, et al. Cyclooxygenase 2—implications on maintenance of gastric mucosal integrity and ulcer healing: controversial issues and perspectives. *Gut*, 49(3), 443, 2001.
5. Ilic S. et al. Stable gastric pentadecapeptide BPC 157 and insulin induced gastric lesions in rats. *J. Phys. Pharm.*, 2 (60), 40, 2009.
6. Konturek PC, Kania J, Burnat G, Hahn EG, Konturek SJ. Prostaglandins as mediators of COX-2 derived carcinogenesis in gastrointestinal tract. *J Physiol Pharmacol,* 56, 5, 57, 2005.
7. Li P., Yin Y.L., Li D.F. et al. Amino acids and immune function. *Br. J.Nutr.,* 98, 237, 2007.
8. Orlovsky O.V. Klitynnyj imunitet u hvoryh na vyrazku dvanadciatypaloi kyshky, uskladnenu shlunkovo-kishkovoju krovotecheju ta jogo korekcia imunofanom. *Gastroenterologia*, 37, 539, 2006.
9. *Problemy patogeneza i terapii imunnyh rasstrojstv,* pod red. Lebedeva V.V., Moskva, 141, 2002.
10. Samonina G.E. et al. Tripeptid Pro-Gly-Pro i gomeostaz slizistoj obolochki zeludka. *Neurochimia*, 1 (25), 128, 2008.
11. Sumbaev V.V., Yasinskaya I.M. Vliyanie DDT na aktivnost sintazy oksida azota v pecheni, legkich i golovnom mozge. *Sovrem. Probl. Toksikol*, 3, 3, 2000.