Metabolism of PGP Peptide after Administration via Different Routes

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Main pathways of degradation of PGP peptide possessing antiulcer and antithrombotic activities were studied after its intraperitoneal, intragastric, and intraintestinal administration. In experiments on rabbits we showed by HPLC that unmodified PGP is released into the blood after administration by all three routes and is detected in the plasma over 3-5 h. PG dipeptide is a more stable PGP metabolite presumably determining (together with tripeptide) its pharmacological properties.

Key Words: *oligopeptides; glyprolines; metabolites*

Numerous studies performed during the last decade validate the necessity of preclinical studies of PGP peptide as a new antiulcer and antithrombotic agent. Animal experiments demonstrated its pronounced protective effects on the gastric mucosa in various models of ulcer formation [1,2,6,12]. PGP improves blood rheology by inhibiting some elements of blood clotting and thrombosis [4,5]. Its antiulcer activity is determined by inhibition of acid production in the stomach [2], reduction of visceral pain sensitivity [2], and normalization of gastric bloodflow reduced by ulcerogenic agents [7].

In animal experiments PGP is usually injected intraperitoneally [1,2,6]. In humans oral treatment is more preferable for long-term therapy. We investigated the possibility of intact PGP peptide penetration into the blood and the main pathways of its degradation after intraperitoneal, intragastric, and intraintestinal administration.

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MATERIALS AND METHODS

Experiments were carried out on female rabbits (2 kg). [^{3}H]PGP (molar radioactivity 80 Ci/ml) was injected in a dose of 200 μCi per animal together with unlabeled peptide (1 mg/kg) via three routes: intraperitoneally and intragastrically (as an aqueous solution) and orally (dry preparation in capsules insoluble in the stomach). Blood (2.5 ml) was collected from the ear vein into a tube with heparin (25 U/ml blood) 15, 30, 60, 180, and 300 min after peptide administration. Plasma was separated by 15-min centrifugation at 2000 rpm, 1 M HCl was added (1:10 v/v) for inactivation of peptidases, and the mixture was immediately frozen at -20°C.

PGP, PG, and GP were extracted from the plasma with 80% acetonitrile (10 ml solvent/ml plasma). The mixture was centrifuged at 4°C and 5000 rpm for 15 min. The supernatant was collected and dried in a rotor evaporator. Dry residue was dissolved in methanol (1 ml initial plasma/10 ml methanol). The resultant solution was centrifuged under the same conditions, the supernatant was again dried in a rotor evaporator, and the dry residue was dissolved in 1 ml distilled water.

[^{3}H]PGP and its metabolites [^{3}H]PG and [^{3}H]GP were isolated by HPLC on Kromasil columns (5 μ, 4×150 mm) at 20°C using a Beckman 165 spectropho-

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tometer at λ=220 and λ=254. The resultant samples in a mixture with PGP, PG, and GP (10 μg of each peptide) were subjected to gradient elution in a mixture of eluents A (0.1% trifluoroacetic acid) and B (80% methanol in eluent A), after which PGP, PG, and GP peaks were distinguished. A 0-13% gradient of eluent B for 22 min was used. The retention times for PG, GP, and PGP at a flow rate of 1 ml/min under these chromatographic conditions were 4.09, 5.9, and 15.4 min, respectively. The content of PGP, PG, and GP in plasma extracts was estimated from the radioactivity under each peak. For evaluation of the amount of radioactive label bound to amino acids formed after degradation of injected PGP and incorporated into plasma proteins, summary radioactivity of protein fractions precipitated by centrifugation was measured.

RESULTS

Study of PGP metabolism showed that the concentration of tripeptide in the plasma peaked 30 min after its intraperitoneal injection. Plasma concentration of PGP did not exceed 4.5% of the total dose of injected peptide. However, 2% of the administered peptide persisted in the plasma for 5 h after injection (Fig. 1, *a*), which far surpassed the life time in the blood for the majority of natural oligopeptides (several minutes) [11,14]. Long-term persistence of intact PGP can be determined by the presence of protease-resistant proline residues in the molecule [3,8,9]. The main PGP metabolite is PG dipeptide, *i.e.* the peptide degradation proceeds via cleavage of C-terminal proline. The content of GP is 4-fold lover than of PG (Fig. 1, *a*).

After intragastric administration the maximum PGP concentration in the blood was recorded after 15-30 and was 2.5 times lower than after intraperitoneal injection (Fig. 1, *b*), which was most likely caused by PGP destruction by gastrointestinal peptidases. After intraperitoneal injection the ratio of the studied peptides at the peak of their blood concentrations was 1:1.1:0.3 for PGP, PG, and GP, respectively, while

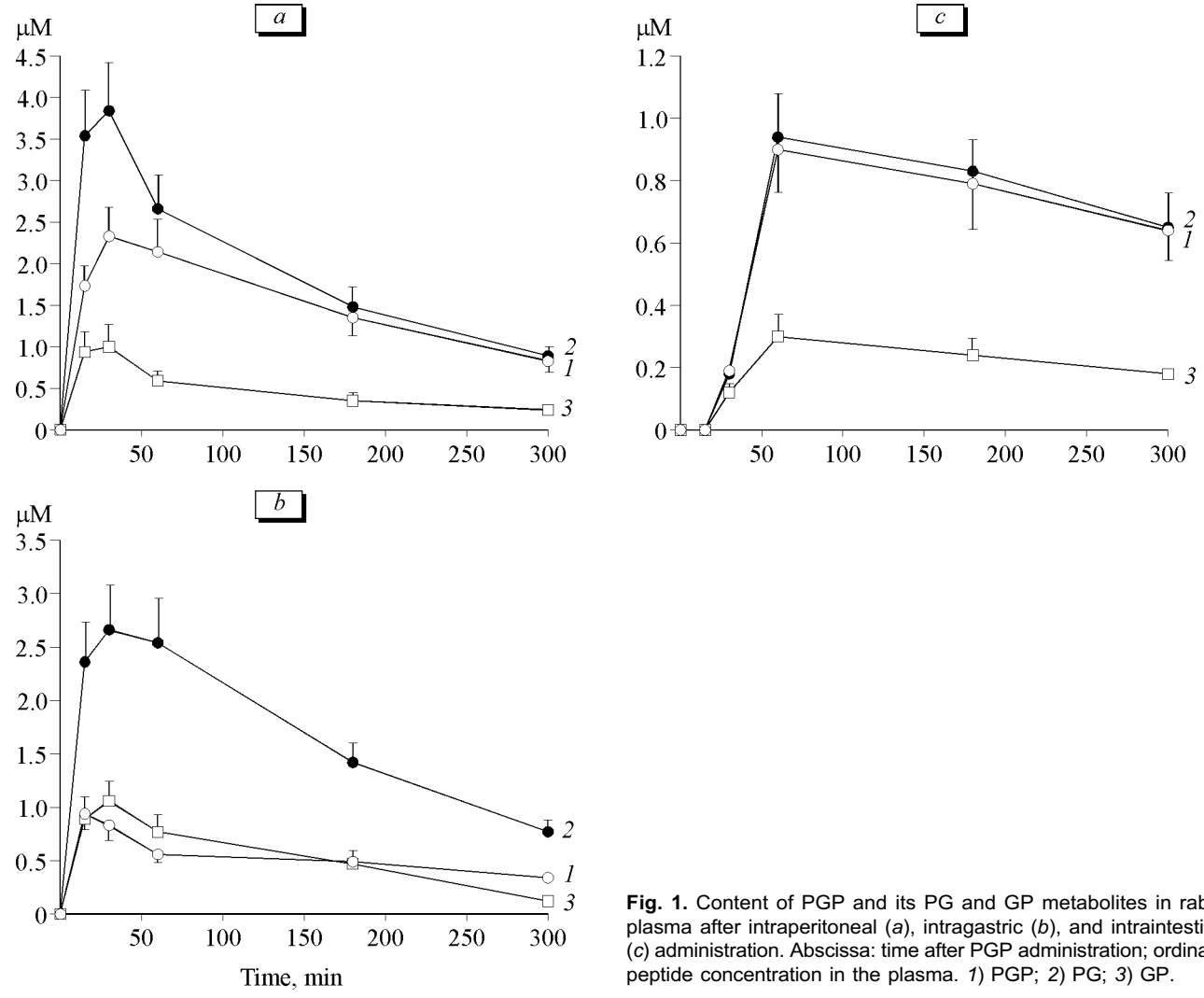

Fig. 1. Content of PGP and its PG and GP metabolites in rabbit plasma after intraperitoneal (*a*), intragastric (*b*), and intraintestinal (*c*) administration. Abscissa: time after PGP administration; ordinate: peptide concentration in the plasma. *1*) PGP; *2*) PG; *3*) GP.

TABLE 1. Distribution of Radioactive Label in the Plasma between PGP, PG, and GP and Proteins Precipitated by Centrifugation

Time after administration, min	Route of administration					
	intraperitoneal		intragastric		intraintestinal	
	1	2	1	2	1	2
15	8.5	8.5	5.6	5.4	0	0
30	10.1	9.1	6.0	5.7	0.7	0.7
60	7.8	5.7	5.0	5.9	3.2	1.8
180	4.7	5.8	3.2	8.1	2.7	4.3
300	2.9	5.3	1.7	10.2	2.2	6.1

Note. *1*) PGP, PG, and GP radioactivity; *2*) radioactivity of proteins precipitated by centrifugation. The data are presented in percent of injected radioactivity.

after intragastric administration this ratio was shifted towards PG and GP (1:3.2:1.3).

Administration of PGP in capsules insoluble in the stomach decelerated absorption of the peptide and its metabolites in the blood: they were not detected in the plasma 15 min after administration, while the peak concentrations were recorded after 60 min (Fig. 1, *c*). This delay was most likely due to the fact that the transport and dissolution of the capsule in the intestine took some time. Surprisingly, the maximum PGP concentration in the plasma after intraintestinal administration was almost the same as after intragastric administration, though the peptide was not exposed to gastric proteinases. It is possible that blood plasma peptidases play the key role in tripeptide proteolysis. However the maximum concentrations of PG and GP markedly decreased after intraintestinal administration, in comparison with intragastric one. These data suggest that partial absorption of the dipeptides starts in the stomach and/or they are more liable to destruction by gastric enzymes than PGP. The PGP: PG:GP ratio at the peak of their plasma concentration after intraintestinal administration was 1:1.1:0.3.

After administration by all three routes the major part of PGP was rapidly hydrolyzed to amino acids by plasma enzymes. During the first 60 min after PGP administration radioactivity of plasma proteins precipitated by centrifugation was comparable with radioactivity of PGP and its metabolites PG and GP. Later radioactivity of precipitated proteins increased, while the level of label bound to the studied oligopeptides decreased, *i.e.* PGP and dipeptides degraded further to amino acids and are incorporated into blood proteins (Table 1).

Starting from the 1950s, it was considered that proteins and short peptides are completely hydrolyzed to amino acids, which were then transferred through the apical membrane of intestinal epitheliocytes. Recent studies demonstrated absorption of some partially or completely uncleaved minor peptides (glycylglycine, dipeptides consisting of proline and hydroxyproline residues, and some other poorly hydrolyzed or hydrolysis-resistant di- and tripeptides) [8]. Transport of these molecules can be realized by enterocytes through pino- and phagocytosis, united by the term "endocytosis". The data obtained during the last 3-4 decades indicate that minor peptides can be carried through the apical membrane by specific transporting systems [8,13].

However, the percentage of peptides absorbed from the gastrointestinal tract into the blood in intact form was not determined. According to published data, 0-30% peptides are absorbed in intact from (this parameter depends on their size and amino acid composition) [8,10]. In our study the maximum concentration of PGP in the plasma after oral intake did not exceed 2% of the administered dose of the peptide. The peptide concentration in the blood is a complex variable depending on the rate of gastrointestinal absorption and degradation by gastrointestinal and blood plasma peptidases and on its retention in organs and tissues. Therefore our data can just approximately characterize the total amount of the peptide absorbed from the gastrointestinal tract in intact form. However the fact of PGP absorption and long-term presence in the blood as a tripeptide opens perspectives for therapeutic use of PGP in tablets and capsules convenient for oral treatment.

Intragastric and intraintestinal administrations provide different ratios of the tripeptide and its metabolites. After intragastric administration the blood concentration of PG 3-fold surpassed that of GP and PGP, while after intraperitoneal injection (60 min postinjection) and intragastric administration the concentrations of PGP and PG in the blood are virtually the same, while that of GP 3-4-fold lower. Differences in the metabolism of the studied compound at various routes of administration can be explained as follows: after

parenteral administration the drug is hydrolyzed primarily by plasma peptidases, while the formation of metabolites after oral intake is determined by drug cleavage by gastrointestinal peptidases and by the effect of the first passage through the liver.

Since the effects of PG and GP on the gastrointestinal mucosa are different (PG is more effective as an antiulcer agent in ethanol model of ulcer formation and GP is more effective in stress-induced ulcer [4]), our results can be useful in the creation of the most effective dosage forms adapted to special forms of gastrointestinal mucosa injuries.

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REFERENCES

1. M. A. Abramova, G. E. Samonina, and I. P. Ashmarin, *Neirokhimiya,* **13**, No. 3, 209-214 (1996).
2. M. A. Abramova, G. E. Samonina, Ch. V. Mamedov, *et al.*, *Vestn. Moskovsk. Universiteta*, Ser. Biology, No. 2, 7-10 (1997).
3. I. P. Ashmarin, A. A. Kamenskii, L. A. Lyapina, *et al.*, *Vopr. Biol. Med. Farmatsevtich. Khim.*, No. 1, 24-27 (2002).
4. I. P. Ashmarin, E. P. Karazeeva, L. A. Lyapina, *et al.*, *Biokhimiya*, **63**, No. 2, 149-155 (1998).
5. I. P. Ashmarin, V. E. Pastorova, and L. A. Lyapina, *Byull. Eksp. Biol. Med.*, **125**, No. 5, 496-499 (1998).
6. S. E. Zhuikova, L. A. Smirnova, Z. V. Bakaeva, *et al.*, *Ibid.*, **129**, No. 10, 300-302 (2000).
7. G. E. Samonina, G. N. Kopylova, V. I. Sergeev, *et al.*, **87**, No. 11, 1488-1492 (2001).
8. N. M. Timofeeva, N. N. Iezuitova, and L. V. Gromova, *Uspekhi Fiziol. Nauk*, **31**, No. 4, 24-37 (2000).
9. D. F. Cunningham and B. O'Connor, *Biochim. Biophys. Acta*, **1343**, No. 2, 160-186 (1997).
10. M. L. Gardner, *Annu. Rev. Nutr.*, **8**, 329-350 (1988).
11. S. Muller, R. Dunker, and G. Hochhaus, *Pharmazie*, **51**, No. 8, 581-585 (1996).
12. G. Samonina, L. Lyapina, G. Kopylova, *et al.*, *Pathophysiology*, **7**, No. 1, 69-73 (2000).
13. D. Walker, D. T. Thwaites, N. L. Simmons, *et al.*, *J. Physiol.*, **507**, Pt. 3, 697-706 (1998).
14. Y. Yamamoto, Y. Tsutsumi, and T. Mayumi, *Curr. Drug Targets*, **3**, No. 2, 123-130 (2002).