Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 6 pp. 1049–1053, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

THE INFLUENCE OF SELECTED CARDIOVASCULAR AND ANTIDIABETIC DRUGS ON PEPSIN ACTIVITY *IN VITRO* DIGESTION

JOANNA SULIBURSKA[1]*, PAWEŁ BOGDAŃSKI[2] and BARBARA CHINIEWICZ

[1]Department of Human Nutrition and Hygiene, Poznan University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland
[2]Department of Internal Medicine, Metabolic Disorders and Hypertension, Poznan University of Medical Sciences, Szamarzewskiego 84, 60-569 Poznań, Poland

Abstract: The aim of this study was to assess the influence of selected cardiovascular and antidiabetic drugs on *in vitro* pepsin activity. A total of 13 drugs were analyzed at one concentration (one tablet of drug in 50 mL of solution with deionized water). The enzyme activity was determined by the Folin method based on the reaction of tyrosine with the Folin reagent. It was found that ACE inhibitors (apo-perindox, prinvil, gopten) caused a significant decrease in the activity of pepsin, while indapen (a diuretic) and glucophage (an antidiabetic drug) increased the activity of this enzyme. It can be concluded that indapamide, metformin and angiotensin converting enzyme inhibitors influenced pepsin activity and these drugs can affect the digestion process in the patients undergoing treatment.

Keywords: drugs, pepsin, digestion

Abbreviations: ACE-I – angiotensin converting enzyme inhibitors, PAU – proteolytic activity units

It is known that a high percentage of the human population worldwide suffers from cardiovascular diseases and diabetes. Frequently, people with these conditions also have accompanying illnesses and patients diagnosed with these diseases must be treated with large amounts of prescription drugs (1).

In the treatment of hypertension a combination therapy is very common. A range of blood pressure-lowering agents includes antihypertensive drugs: ACE inhibitors, diuretics, Ca-antagonists and β-blockers (2, 3). Patients with cardiovascular diseases are also very often treated with antihyperlipidemic drugs and anticoagulant drugs (e.g., aspirin) (4). Metformin is widely used for treating patients with type 2 diabetes mellitus (5). In addition to their therapeutic effect, these drugs can also cause side effects and interactions with other drugs and food, which frequently result in the discontinuation of treatment by patients (6, 7).

Cardiovascular and antidiabetic drugs can affect the gastrointestinal tract and hinder the adsorption of nutrients (7). Some drugs may influence the metabolism and extraction of vitamins and minerals (8, 9). Loop diuretics increase the excretion of potassium, calcium, sodium and magnesium (10). Treatment with various medications may cause nutritional disorders. It was observed that metformin causes vitamin B_{12} deficiency and captopril influences zinc deficiency in the organism (11, 12).

It is supposed that due to their chemical structure some drugs can change the conditions of the enzymatic digestion process, especially pH in the gastrointestinal tract, and thus they affect the digestion of nutrients and the nutritional status of the organism.

The aim of this study was to assess the effect of antihypertensive, antihyperlipidemic, antidiabetic as well as antiaggregant drugs on pepsin activity and *in vitro* digestion.

EXPERIMENTAL

A total of 13 drugs were analyzed: hypotensive drugs (metocard, lokren, cardilopin, apo-perindox, prinvil, gopten, indapen, tialorid and lorista), an antihyperlipidemic drug (lipanthyl supra), an antiaggregant drug (polocard) and antidiabetic drugs

* Corresponding author: e-mail: jsulibur@up.poznan.pl; phone: 48 61 8487334

Table 1. Characteristic of the drugs.

Drugs	Active substance [mg/1 tablet]	Pharmaceutical additives in drugs	Drug class
Metocard	Metoprolol (47.5)	microcrystalline cellulose, methylcellulose, corn starch, glycerol, ethylcellulose, magnesium stearate, hypromellose, stearic acid, titanium dioxide	β-blocker
Lokren	Betaksolol (20.0)	lactose monohydrate, sodium starch glycolate, microcrystalline cellulose, colloidal anhydrous silica, magnesium stearate, hypromellose, macrogol 400, titanium dioxide, iron oxide (E172)	
Cardilopin	Amlodipine (10.0)	colloidal anhydrous silica, magnesium stearate, sodium starch glycolate, microcrystalline cellulose	Ca-antagonist
Apo-perindox	Perindopril (3.34)	anhydrous lactose, magnesium stearate	ACE inhibitor
Prinvil	Lisinopril (20.0)	mannitol, calcium hydrogen phosphate, maize starch, gelatinized magnesium stearate, iron oxide	
Gopten	Trandolapril (2.0)	corn starch, lactose monohydrate, povidone, sodium stearyl fumarate, gelatin, titanium dioxide, iron oxide yellow, erythrosine, sodium lauryl sulfate	
Indapen	Indapamide (1.5)	lactose monohydrate, carbomer, hydroxypropylcellulose, magnesium stearate,colloidal anhydrous silica, talc, hypromellose, titanium dioxide, lactose monohydrate, macrogol 3000, glycerol triacetate, iron oxide yellow (E172), iron oxide red (E172), black iron oxide (E172)	Diuretic
Tialorid	Amiloride+ hydrochlorothiazide (55.0)	lactose monohydrate, maize starch, povidone, talc, magnesium stearate	
Lorista	Losartan (50.0)	corn starch, corn starch gel, microcrystalline cellulose, colloidal anhydrous silica, magnesium stearate, lactose monohydrate, cellulose, hypromellose, talc, propylene glycol, titanium dioxide (E171)	Angiotensin II receptor antagonist
Lipanthyl supra	Fenofibrate (160.0)	sodium lauryl sulfate, lactose monohydrate, povidone K25, crospovidone, microcrystalline cellulose, colloidal anhydrous silica, sodium stearyl, polyvinyl alcohol, titanium dioxide (E171), talc, soy lecithin, xanthan gum, sunset yellow S (E110), allula red AC (E129), indigo carmine (E132)	Antihyperlipidemic
Polocard	Acetylsalicylic acid (150.0)	corn starch, cellulose powder, sodium carboxymethyl starch tablet coating: hypromellose, methacrylic acid copolymer, triethylcitrate, titanium dioxide, talc, sodium lauryl sulfate, with red cochineal carmine (E-124), colloidal silica, sodium bicarbonate	Antiaggregant
Amaryl	Glimepirid (4.0)	lactose monohydrate, sodium carboxymethyl starch, magnesium stearate, microcrystalline cellulose, povidone, indigo	Antidiabetic
Glucophage	Metformin (150)	magnesium stearate, carmellose sodium, hypromellose.	

(amaryl, glucophage). Characteristics of the drugs are shown in Table 1. One concentration of each drug was prepared – one tablet of the drug was dissolved in deionized water to form 50 mL of the solution. The concentrations of active substance of the drugs are shown in Table 2.

The studied materials included also pepsin (porcine gastric mucosa, Sigma-Aldrich), albumin (from hen eggs, GR, POCH) and the Folin-Ciocalteu reagent (Merck). The activity of the enzyme was determined using the colorimetric method based on the reaction of the albumin degradation product complexed with the Folin-Ciocalteu reagent. The reaction solutions were composed of the following sequence: 1 mL of albumin solution (1%) in deionized water, 1 mL of deionized water, 1 mL of drug solution (with the concentration of the active substance depending on the drug) and 1 mL of pepsin solution in 1 M HCl (16 g in 100 mL). The mixtures were incubated at 40°C for 5 min, then the reaction was discontinued by an addition of 2 mL of 5% trichloroacetic acid (TCA) and the reaction system was incubated for 20 min. Subsequently, the solutions were centrifuged at 3000 rpm, then 1 mL of the supernatant was incubated at 40°C for 20 min with 5 mL of 0.2 mol Na_2CO_3/L of aqueous solution, and 1 mL of the Folin-Ciocalteu reagent (diluted with deionized water at the proportion of 1:5, v/v). Successively, absorbance was measured at $\lambda = 625$ nm with the use of a Specol spectrometer (Zeiss, Germany). Pepsin activity was expressed in Proteolytic Activity Units (PAU) and calculated from the equation (13):

$$PAU/mL = A \times V/A_1 \times t = 4.938 \times 10^{-4} \times A$$

where A – absorbance of sample, A_1 – absorbance of 1 mEq of tyrosine (1620), V – sample volume (4 mL), t – reaction time (5 min).

The control sample contained 1 mL of deionized water instead of the drug solution. In order to eliminate the influence of the reaction between the drugs and the Folin-Ciocalteu reagent, a reference sample was prepared, containing the solution of the drug and the Folin-Ciocalteu reagent without pepsin.

Each experiment was performed in 10 replications.

Statistical analysis

The experimental results were given as the means ± SD of three parallel measurements. The statistical analysis was carried out employing the STATISTICA 7.0 software and using the ANOVA and the Tukey test at the significance level of $\alpha = 0.05$.

RESULTS

The results obtained in this study are shown in Table 2. It was found that ACE inhibitors (apoperindox, prinvil, gopten) caused a significant

Table 2. Activity of pepsin according to drugs.

Drugs	Active substance [mg%]	PAU [$\times 10^{-6}$/mL]	% activity (+) or inhibition (–)
Control sample	0	68.6 ± 2.4^{b}	-
Metocard	95	73.7 ± 3.0^{b}	(+) 7.4
Lokren	40	73.6 ± 4.8^{b}	(+) 7.3
Cardilopin	20	64.2 ± 5.6^{ab}	(–) 6.4
Apo-perindox	6.68	58.5 ± 0.6^{a}	(–) 14.7
Prinvil	40	60.7 ± 0.5^{a}	(–) 11.5
Gopten	4	58.5 ± 1.3^{a}	(–) 14.7
Indapen	3	$81.0 \pm 2.9^{c,A}$	(+) 18.1
Tialorid	110	$72.3 \pm 1.3^{cb,B}$	(+) 5.4
Lorista	100	62.2 ± 2.1^{ab}	(–) 9.3
Lipanthyl supra	320	69.4 ± 1.3^{b}	(+) 1.2
Polocard	300	62.2 ± 1.3^{ab}	(–) 9.3
Amaryl	8	$61.4 \pm 0.6^{ab,C}$	(–) 10.5
Glucophage	300	$82.0 \pm 3.1^{c,D}$	(+) 19.5

PAU – proteolytic activity units; abc – significant differences between drugs; A, B – significant differences among diuretics; C, D – significant differences among antidiabetic drugs.

reduction of pepsin activity (above 10%). Moreover, indapen (a diuretic) and glucophage (an antidiabetic drug) increased the activity of the enzyme by almost 20%. No marked effect of the drugs on PAU was observed. However, it was found that β-blockers and tialorid (a diuretic) slightly reduced pepsin activity, while cardilopin (a Ca-antagonist), lorista (an angiotensin II receptor antagonist), polocard (an antiaggregant drug) and amaryl (an antidiabetic drug) insufficiently inhibited this enzyme.

In addition, the effect of drugs from the same class was analyzed. Significant differences were found among diuretics and among antidiabetic drugs. The value of PAU in the samples with indapen was much higher than in the samples with tialorid. In turn, amaryl inhibited pepsin, while glucophage definitely increased its activity. Within the group of β-blokers and ACE inhibitors the drugs similarly affected pepsin activity.

DISCUSSION AND CONCLUSION

A review of the literature shows that there are few studies assessing the effect of drugs on digestive processes.

Others authors observed that some cardiovascular drugs affect cellular enzymes (14). It was found that metformin activated AMP kinase through an inhibition of AMP deaminase (15). A study by Iakubov and Usmanova (16) needs to be cited when discussing the effect of drugs on the gastrointestinal tract. The above mentioned authors observed that ACE inhibitors had a gastroprotective effect in patients treated with nonsteroidal anti-inflammatory drugs.

Practically, no research papers describing the effect of analyzed drugs on digestion and bioavailability of nutrients have been found in the available literature.

In this study, we observed that ACE inhibitors, indapen and glucophage, change the *in vitro* activity of pepsin. The mechanism of this interaction may be related to the influence of drugs on the binding of the substrate with the enzyme. It may be assumed that in the case of metformin and indapamide the molecules of drugs are bound to the enzyme or the substrate, or both, and this may lead to the formation of active complexes easily degraded to final products. An opposite effect can be caused by molecules of ACE inhibitors due to their different chemical structure. Moreover, the acidic medium of the reaction can affect the charge of the drug molecules (especially ACE-I, indapamide and metformin), which may influence the interface between the substrate and the enzyme and this interaction can affect the proteolytic activity of pepsin.

Adverse effects in the gastrointestinal tract of patients, caused by the analyzed drugs, do not occur very often. A relatively small percentage of patients treated with indapamide reported constipation, diarrhea or abdominal pain (17). Amlodipine and other Ca-inhibitors caused vomiting and nausea in some patients (18, 19). ACE inhibitors can induce intestinal angioedema and also these drugs are associated with slight vomiting, nausea, diarrhea and constipation (20, 21). Digestive disorders (diarrhea, vomiting) represent the most common metformin side-effect (around 30% of patients) (22). Different pathophysiological hypotheses have been proposed to explain the metformin-induced diarrhea and vomiting,

According to Bouchoucha et al. (23), there is a lack of experimental data to explain these highly patient-dependent adverse effects of metformin. It is supposed that this data may partly explain this problem.

Our study has some limitations; first of all, it should be noted that a model system was used, which does not take into account the factors in the digestive system in the living organism. The model adopted in this experiment cannot clearly explain the mechanism of the interaction between pepsin and the analyzed drugs.

Based on the results obtained in this study, it can be concluded that indapamide, metformin and angiotensin converting enzyme inhibitors influenced pepsin activity. It can therefore be assumed that selected hypotensive and antidiabetic drugs can affect the digestion process in patients.

REFERENCES

1. Cohn J.N.: Cardiovasc. Drugs Ther. 24, 297 (2010).
2. Destro M., Cagnoni F., D'Ospina A., Ricci A.R., Zaninelli A., Preti P.: Cardiovasc. Drug Discov. 5, 69 (2010).
3. Gonzalez-Juanatey J.R., Mazon R.P.: Rev. Esp. Cardiol. 61, 861 (2008).
4. Giliarov M.I.: Kardiologiia 47, 90 (2007).
5. Jermendy G.: Orv. Hetil. 151, 2025 (2010).
6. Aman S.F., Hassan F., Naqvi B.S., Hasan S.M.: Pak. J. Pharm. Sci. 23, 313 (2010).
7. Handbook of Food-Drug Interactions. McCabe B.J., Frankel E.H., Wolfe J.J. Eds., CRC Press, Boca Raton FL 2003.
8. McBain A.M., Brown I.R., Menzies D.G., Campbell I.W.: J. Clin. Pathol. 41, 933 (1988).
9. Galli F., Iuliano L.: Med. Hypotheses 74, 707 (2010).

10. Law P.H., Sun Y., Bhattacharya S.K., Chhokar V.S., Weber K.T.: J. Am. Coll. Cardiol. 46, 142 (2005).
11. Jager J., Kooy A., Lehert P., Wulffele M.G., van der Kolk J., Bets D. Verburg J., Donker A.J.M., Stehouwer C.D.A.: BMJ 340, c2181 (2010).
12. Golik A., Zaidenstein R., Dishi V., Blatt A., Cohen N., Cotter G., Berman S., Weissgarten J.: J. Am. Coll. Nutr. 17, 75 (1998).
13. Krejpcio Z., Wojciak R.W.: Pol. J. Environ. Stud. 11, 251 (2002).
14. Jiang Q.J., Xu G., Mao F.F., Zhu Y.F.: J. Zhejiang. Univ. Sci. B. 7, 228 (2006).
15. Ouyang J., Parakhia R.A., Ochs R.S.: J. Biol. Chem. 286, 1 (2011).
16. Iakubov A.V., Usmanova Sh.E:. Lik. Sprava 2, 47 (2004).
17. Akram J., Sheikh U.E., Mahmood M., Donnelly R.: Curr. Med. Res. Opin. 23, 2929 (2007).
18. Opie L.H.: Cardiovasc. Drugs Ther. 2, 177 (1988).
19. Fletcher A., Bulpitt C.: Drugs 44, 135 (1992).
20. Weingartner O., Weingartner N., Bohm M., Laufs U.: BMJ Case Reports 2009. pii: bcr09.2008.0868 (2009).
21. Ol'binskaia L.I., Zheleznykh E.A.: Ter. Arkh. 73, 22 (2001).
22. Strack T.: Drugs Today 44, 303 (2008).
23. Bouchoucha M., Uzzan B., Cohen R.: Diabetes Metab. 37, 90 (2011).

Received: 23. 08. 2011