9. *IN VITRO* INVESTIGATION OF THE EFFECT OF CHITOSAN PREPARATIONS ON DIGESTIVE ENZYMES

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1. Introduction

Many drugs containing natural macromolecular compounds with primarily supportive action are used in the clinical practice in the treatment of obesity. Chitosans manufactured by various producers in the form of capsules were used in the trial: Vitana®, Hitec Nutrition®, Chromdiet® as well as pure chitosan manufactured by Vanson®. Xenical® preparation was used as reference (as the only slimming drug which does not exert central effect), which was treated as a selective inhibitor of gastric and pancreatic lipase. The measurements were performed by means of a kinetic method according to Bio Merieux (63 108 application), in which the time in which the chinonoimine derivative appeared is proportional to pancreatic activity [1]. Specificity of the test was obtained using co-lipase and deoxycholane as activators. Moreover, the remaining pancreatic enzymes – amylase and tripsin were also determined.

The aim of the study was to investigate the effect of products containing chitosan and adjuvant substances on the inhibition of digestive enzymes.

2. Materials and method

The measurements were performed by means of a kinetic method according to Bio Merieux (63-108 application), in which the time in which the chinonoimine derivative appeared is proportional to pancreatic activity. Specificity of the test was obtained using co-lipase and deoxycholane as activators. Moreover, the remaining pancreatic enzymes – amylase and tripsin were also determined.

Enzymes inhibition was investigated by means of a dynamic method in a biopharmaceutical model imitating in vitro conditions [2 - 4].

The investigation was performed in a water bath with a shaker, taking into consideration to maintain conditions imitating those in the human digestive tract.

Amplitude of shaking as well as rate and temperature (37 °C) were determined. Next 120 mg of the mixture was introduced to centrifuge vials and 3 ml of aqua purificata were added (to obtain solutions containing 1 mg in 25 μl). Next 0.0125; 0.025; 0.250; 2.5 ml (what corresponded to 0.5; 1; 10; 100 mg of the sample) were collected to separate tubes completed with 0.05 M HCl to 0.2 ml.(imitation of gastric environment). The mixture was mixed in a shaker (amplitude - 300 rpm) at 37 °C until the mixture was dissolved and a uniform liquid was obtained. The liquid was then shaken (amplitude - 300 rpm) for 2 hrs. pH 6.4 was obtained with 0.2 M Na_2CO_3 (imitation of duodenal environment). Next solution containing pancreatic enzymes (lipase, amylase, protease) with a known activity were added. Aqua purificata was added to obtain final volume of 1 ml in every case [5].

The samples were analyzed using a colorimetric method (LIPC) in Bio Merieux 63 108 apparatus in order to determine quantitatively catalytic activity of lipase. 1,2-diglyceride was used as a substrate for lipase. The staining intensity of the formed chinonoimine derivative is directly proportional to lipase activity U/L .

α - pancreatic amylase was determined according to Cobas Integra application (α - Amylase EPS Pancreatic), in which p-nitrophenol release rate is directly proportional to catalytic activity of the enzyme in the sample [6 - 9].

Tripsin was determined according to H. Bartelheimer and H. Maring's application based on degradation of 1% casein solution.

The measurements were taken 30, 45, 60 min after the samples were combines with pancreatic enzymes in the conditions of intestinal passage. Control trial was also taken (without mixture) [10 - 13].

3. Results and discussion

Table 1 presents the results of measurements of the activity of pancreatic lipase in the presence of applied products used as dietary supplements in general slimming therapies. As indicated by the investigations presented in Table 1, inhibition of lipase activity by the addition of chitosan in the biopharmaceutical model depends on the dose of the product and time of exposure. Depending on the applied product and its amount, the activity of lipase decreases from 16.2 to 21.4 % of the total activity after administration of a minimum dose of 0.5 mg/ml for 30 min exposure. The decrease of the activity is maintained at constant level during the whole intestinal passage regardless of the applied chitosan sample. The use of surface active compounds (adjuvant substances in the drug form) results in additional decrease of the enzymatic activity due to an inhibitory effect of the surfactant which decreases the surface tension. Adjuvant substances present in

Table 1. *Investigation of the effect of inhibitor in mixtures containing chitosan and adjuvant substances and Xenical® as reference on the activity of pancreatic lipase 30, 45, 60 min at 37 °C, after addition of 0,5 mg, 1 mg, 10 mg, 100 mg of inhibitor. (mean values from 3 trials).*

	Enzymatic activity of lipase U/L											
	30 min				45 min				60 min			
Amount of inhibitor in mg/ml	0.5	1.0	10	100	0.5	1.0	10	100	0.5	1.0	10	100
Control (0 mg/ml of inhibitor)	173	173	173	173	173	173	173	173	173	173	173	173
Chitosan A (Vitana® Capsules)	145	128	115	86	136	103	98	78	128	92	77	74
Chitosan B (Hitec Nutrition Capsules®)	137	106	98	79	128	80	72	68	115	68	62	58
Chitosan C (Chromdiet® Capsules)	136	126	87	74	116	107	93	77	111	94	86	74
Chitosan D (pure chitosan)	144	128	114	86	135	104	98	78	128	91	77	74
Chitosan D + surfactant	62	57	19	16	54	52	15	14	49	47	14	14
Chitosan D + surfactant + kolidon 25®)	59	56	18	16	53	51	14	12	48	46	15	15
Xenical® (Capsules)	30	22	0	0	21	14	0	0	16	7	0	0

Standard deviation S - from 0,13 U/l to 2,54 U/l Relativity index Wz - 0,084 to 2,28%.

the capsule mass have little effect on the decrease of the enzyme activity. Standard deviation calculated for lipase ranged from 0.13 U/L to 2.54 U/L, while the relativity index W_z was from 0.084 to 2.28%, what proves high precision of the findings.

The measurements presented in Tables 2 and 3 (Figure 1) confirm the hypothesis of low effect of chitosan products on such enzymes as α – amylase and tripsin. Only the use of pure chitosan in a capsule decreases the activity of amylase by 9.38%, while the use of commercial products decreases the activity of this enzyme by to 7.25 %. On the other hand, the activity of tripsin remains unchanged, what proves low effect of the products on this enzyme.

Table 2. *The effect of inhibitor in mixtures containing chitosan and auxiliary substances on the activity of pancreatic amylase 30, 45, 60 minutes at. 37 °C, after addition of 0.5 mg, 1 mg, 10 mg, 100 mg of the inhibitor (mean values from three trials).*

	Enzymatic activity of amylase U/L											
	30 min				45 min				60 min			
Amount of inhibitor in mg/ml	0.5	1.0	10	100	0.5	1.0	10	100	0.5	1.0	10	100
Control (0 mg/ml of inhibitor)	32	32	32	32	32	32	32	32	32	32	32	32
Chitosan A (Vitana® Capsules)	31	31	32	31	31	31	31	31	31	31	31	31
Chitosan B (Hitec Nutrition Capsules®)	30	29	29	29	30	30	30	30	31	31	31	31
Chitosan C (Chromdiet® Capsules)	30	30	30	30	30	30	30	30	30	30	30	30
Chitosan D (pure chitosan)	29	29	31	30	30	30	31	31	31	31	31	31
Chitosan D + surfactant	30	30	30	30	31	31	31	31	31	31	31	31
Chitosan D + surfactant + kolidon 25®)	31	31	31	31	31	31	31	31	31	31	31	31

Standard deviation S - from 0.47 U/l to 1.54 U/l Relativity index Wz - 0.064 to 1.28%.

Table 3. *The effect of inhibitor in mixtures containing chitosan and auxiliary substances on tripsin activity after 30, 45, 60 minutes at 37 °C, after use of 0.5 mg, 1mg, 10 mg, 100 mg of the inhibitor.(mean from three trials).*

	Enzymatic activity of amylase U/L											
	30 min				45 min				60 min			
Amount of inhibitor in mg/ml	0.5	1.0	10	100	0.5	1.0	10	100	0.5	1.0	10	100
Control (0 mg/ml of inhibitor)	138	138	138	138	138	138	138	138	138	138	138	138
Chitosan A (Vitana® Capsules)	138	138	138	138	137	137	138	138	137	138	137	138
Chitosan B (Hitec Nutrition Capsules®)	136	138	138	138	138	137	137	138	137	138	137	138
Chitosan C (Chromdiet® Capsules)	135	137	137	137	137	137	137	137	137	138	137	138
Chitosan D (pure chitosan)	137	137	138	137	137	137	137	137	137	137	137	137
Chitosan D + surfactant	138	137	137	137	137	137	137	137	137	137	137	137
Chitosan D + surfactant + kolidon 25®)	138	138	138	138	138	138	138	138	138	138	138	138

Standard deviation S - from 0.37 Ph Eur U to 1.92 Ph Eur U Relativity index Wz - 0.26 to 1.39%.

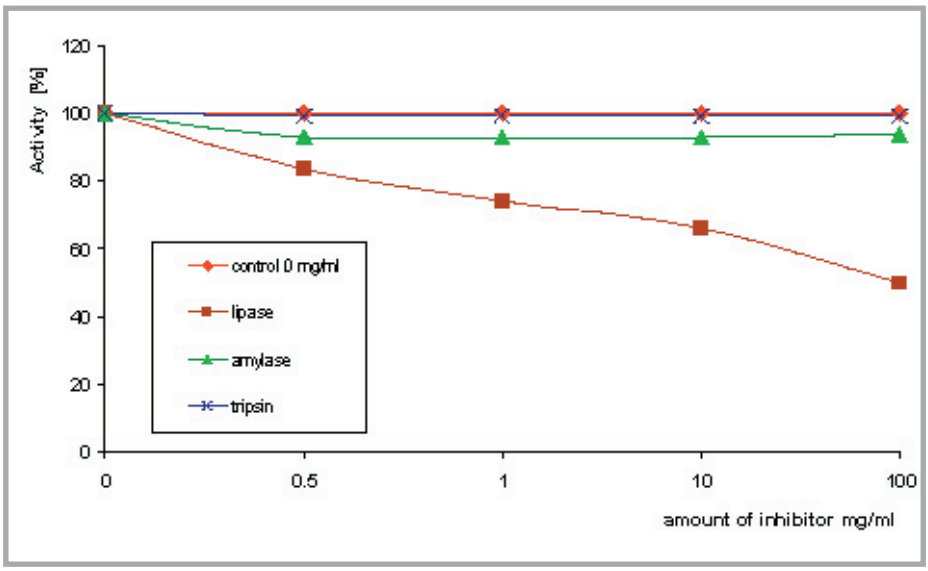

Figure 1. *The effect of pancreatic enzymes: pancreatic lipase, a-amylase and tripsin inhibition by chitosan (in relation to control sample with 0 mg/ml of the inhibitor) in vitro in 30, 45, 60 min.*

4. CONCLUSION

In view of obtained results, preparations containing chitosan affect the activity of lipase and amylase (decrease the activity less than 10%), while their effect on tripsin is statistically insignificant.

The investigations enable determination of the inhibitory effect of chitosan and chitosan with adjuvant substances on pancreatic lipase. The decrease of activity by about 26% is observed after administration of about 1 mg of the extract after 30 min, and by 47% after 60 min of exposure to the inhibitor. The effect of chitosan on pancreatic α−amylase (4% to 10% decreased activity) and tripsin (0% decrease) is very low. There is a higher possibility of interaction between chitosan and digestive enzymes in compound preparations.

5. References

1. **Leybold A., Junge W.:** Importance of collipase for the measurement of serum lipase activity. Adv. Clin. Enzymol. 4, 1986, 60 - 67.
2. **Meler J., Pluta J., Ulanski P., Krotkiewski M.:** Fat-binding capacity of non- modified and modified chitosans. In: Progress on Chemistry and Application of Chitin and its Derivatives. Vol.IX (ed.: H. Struszczyk), Polish Chitin Society, Łódź 2003, pp. 129 - 136,
3. **Meler J., Pluta J.:** The effect of auxiliary substances the activity of lipase pancreatic biopharmaceutical patternel of digestive tract. In: Progress on Chemistry and Application of Chitin and its Derivatives. Vol. X (ed.: H. Struszczyk), Polish Chitin Society, Łódź 2004, pp. 131 - 137.
4. **Roussel A. Canaan S., Egloff M. P., et al.:** Crystal structure ofhuman gastric lipase and model of lyposomal acid lipase ,two lipolytic enzymes of medical interest . J. Biol. Chem., 274, 1999, 16995 - 17002.
5. **Pietrzak A. Chodorowska G., Lecewicz-Toruń B., et al.:** Activity of serum lipase EC 3. 1.1.3. in psoriatics suffering from psoriasis. JEADV. October 1996, 7 (suppl. 2), 192.
6. **Junge W, Waldenström J, Boumann A, et al.:** Evaluation of the Assays for Total and Pancreatic α - Amylaze based on 100% Cleavage of Et –G7 – PNP AT 6 European Clinical Centres (poster Medlab 97). Basel, Switzerland: 12th IFCC European Congress of Clinical Chemistry, August 17 – 22, 1997.
7. **Salt WB, Schenker S.:** Amylase - its clinical significance: A review of the literature. Medicine. 1976, 55, 269-289.
8. **Robyt J. F, French D.:** Multiple attack and polarity of action of porcine pancreatic α-amylase. Arch Biochem Biophys. 1970, 138, 662-670.
9. **Rauscher E, von Bülow S, Hägele EO, Neumann U, Schaich E.:** Ethylidene protected substrate for the assay of human α-amylase. Fresenius Z Anal Chem.1986, 324, 304-305.
10. **Davidson N. O.:** Intestinal lipid absorption. W: Textbook of Gastroenterology. Third Edition. Lippincott W. and Wilkins E., Philadelphia, New York, Baltimore, 1999, 428 - 456
11. **Pluta J. Meler J.:** Studies on the influence of auxiliary substances on the physico-chemical characteristics ofophtalmic drugs. Acta Poloniae Pharmaceutica 59, 2002, 247 - 252.
12. **Guerciolini R.**: Mode of action of orlistat. Int. J. Obesity 21, 1997, 12 - 23.
13. **Zhi J., Melia A., Funk C., et al.:** Metabolic profiles of minimally aboserbet orlistat in obese/ overweight volunteers. J. Clin. Pharmacol, 36, 1996, 1006 - 1011.