The effect of various types of chitosan used as dietary supplements on bioavailability of certain H-2 histamine receptor inhibitors

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Abstrakt
The clinical practice of obesity treatment uses many natural macromolecular compounds which assist in slimming. Chitosan is used in the treatment of obesity due to its lipid, cholesterol, fatty acids, triglycerides and bile acids binding capacity. It is indigestible; it dissolves in the acid environment in the stomach, where it binds many water molecules, forming stable adsorptive gel. A molecule of cationic polymer, such as chitosan, is capable of binding acid drugs. The aim of the study was to determine the capability of binding H-2 receptors antagonists by chitosans present in slimming drugs. The phenomenon of H-2 antagonist receptors absorption was investigated by means of a dynamic method on a biopharmaceutical model which imitated the conditions in vitro. The amount of drug absorbed by chitosan was calculated from the difference in concentrations of the investigated drug before and after sorption. The results of measurements of the bounded drug were used to calculate the mean percentage of the absorbed dose. The obtained findings demonstrate that drugs such as Famotidine and Ranitidine are absorbed by chitosans at applied pH ranges, and the binding capability depends on environmental pH, concentration of the applied drug and the kind of chitosan. Mean absorption of drugs by chitosans ranged from 0.866 g to 2.449 g per 1 g of chitosan. The highest absorption was observed at pH above 7.

Key words: H-2 receptors antagonists; chitosans; Ranitidine hydrochloride; Famotidine hydrochloride; adsorption
1. Introduction

The clinical practice of obesity treatment uses many natural macromolecular compounds which assist in slimming. Chitosan is used in the treatment of obesity due to its lipid, cholesterol, fatty acids, triglycerides and bile acids binding capacity. It is an effective source of soluble dietary fibres. It is indigestible; it dissolves in the acid environment in the stomach, where it binds many water molecules, forming stable adsorptive gel. A molecule of cationic polymer, such as chitosan, is capable of binding acid drugs [1].

The aim of the study was to determine the capability of binding H-2 receptors antagonists by chitosans present in slimming drugs. H-2 receptors antagonists strongly affect basal secretion, what is manifested, among others, by very strong inhibition of nocturnal secretion. They stimulate the secretion of gastrin, but they do not affect gastric emptying, the tonus of the cardiac sphincter, or pancreatic secretion. The drugs are well absorbed from the gastrointestinal tract, while neutralizing drugs and food delay absorption [2 - 4].

2. Materials and method

High purity natural krill chitosans with deacetylation of 85% to 95%, not degraded and degraded by 5 to 30 kGy radiation dose were used in the study.

The investigations were performed in an imitated gastrointestinal tract, which met the standards of Polish Pharmacopoeia VIII [5].

The studies were carried out at 37 °C. 0.03 g portions of chitosan were weighed and put to 5 ml glass centrifuge vials. Next 2 ml of 0.05 M HCl were added to achieve pH 2 of the solution, what corresponds to natural fasting gastric pH.

Next 0.1 M Na₂CO₃ were added to the vials to achieve pH 6.4 (pH in the duodenum) and stirred for 0.5 hour (300 rpm). The mixture was then alkalinized with sodium carbonate to achieve pH 7.0 - 7.6, corresponding to pH of the intestinal juice. The samples were incubated at 37 °C in a shaker (300 r.p.m.) for 2.5 hours.

Next the samples were brought to room temperature and centrifuged (2100 x g) for 20 minutes. The vials were then left for 30 minutes to stabilize and next, depending on the kind of investigated drug, certain amount of the solution was collected from over the sediment and determined spectrophotometric analysis.

- In case of ranitidine hydrochloride, 0.01 ml of the solution was transferred to a 10 ml test tube and completed to 10 ml with 0.1 M HCl.
- In case of famotidine, 0.05 ml of the solution was collected, transferred to a test tube and completed to 5 ml with 0.1 M HCl.

The investigations were performed with the use of generally accepted doses of drugs (0.1 mg/10 ml; 0.15 mg/10 ml; 0.2 mg/10 ml – for Ranitidine (1), and 0.02 mg/5 ml; 0.04 mg/5 ml ; 0.05 mg/5 ml – for Famotidine (2)).
3. Results and discussion

3.1. The effect of radiation degradation rate on intrinsic viscosity of chitosans

The analysis of the effect of radiation degradation rate on intrinsic viscosity shows that a decrease in mean molecular weight of chitosan causes a decrease of this parameter (Table 1).
The highest decrease in intrinsic viscosity under the effect of an increased dose of degradation radiation (0 - 30 kGy) was observed in case of Huasu chitosan and it was 0.4451 dm$^3$g$^{-1}$.

The lowest change in intrinsic viscosity was observed for chitosan 352, and it was 0.062 dm$^3$g$^{-1}$. The remaining chitosans demonstrated a moderate decrease in viscosity with increased dose of degradation radiation 0 - 30 kGy, which in case of chitosan Chito Clear TM 1015 was 0.2550 dm$^3$g$^{-1}$, for chitosan 652 - 0.1517 dm$^3$g$^{-1}$ and for chitosan 343 - 0.3702 dm$^3$g$^{-1}$ (Figure 1).

3.2. Investigation of Ranitidine and Famotidine binding by degraded and non-degraded chitosans

The investigation of ranitidine and famotidine binding by radiation degraded and non-degraded chitosan confirmed the hypothesis that it depends on the radiation dose and the origin of the polymer.

The analysis of the effect of intrinsic viscosity on absorption capability of the investigated drugs by chitosans demonstrates nonlinearity of the drug binding. In case of ra-
nitidine hydrochloride, an increased quantity of absorbed drug with an increase in intrinsic viscosity was observed for chitosan Chito Clear and chitosan Huasu. On the other hand, the amount of bounded drug decreases with an increased intrinsic viscosity for chitosan 652 and chitosan 352. Chitosan 343 with increasing viscosity absorbs initially increased and then decreased amounts of nitidine hydrochloride. The relation was observed for all investigated doses of nitidine hydrochloride: 0.1 g; 0.15 g and 0.2 g (Figure 2 - 6).

**Figure 1.** Dependence from intrinsic viscosity in $dm^3g^{-1}$ chitosans from degradation rate in kGy.

**Figure 2.** The relation between Ranitidine binding by 1 gram chitosans Chito Clear and viscosity average molecular weight M in kDa.
Figure 3. The relation between Ranitidine binding by 1 gram chitosans 652 and viscosity average molecular weight M in kDa.

Figure 4. The relation between Ranitidine binding by 1 gram chitosans 343 and viscosity average molecular weight M in kDa.

Figure 5. The relation between Ranitidine binding by 1 gram chitosans 352 and viscosity average molecular weight M in kDa.
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Figure 6. The relation between Ranitidine binding by 1 gram chitosans Huasu and viscosity average molecular weight M in kDa.

Standard deviation for Ranitidine ranged from 0.006 to 0.042. Determined relativity coefficient ranged form 0.53% to 4.82%, what confirms high accuracy of measurements.

In case of famotidine, the amount of bounded drug increases with an increased viscosity for chitosans 343 and Huasu, and decreases for chitosans Chito Clear and 652. Chitosan 352 reveals a significant differentiation of the findings in relation to the dose; the absorption increases for doses 0.02 g and 0.05 g, and falls down slightly with 0.04 g (Figure 7-11).

Figure 7. The relation between Famotidine binding by 1 gram chitosans Chito Clear and viscosity average molecular weight M in kDa.
Figure 8. The relation between Famotidine binding by 1 gram chitosans 652 and viscosity average molecular weight $M$ in kDa.

Figure 9. The relation between Famotidine binding by 1 gram chitosans 343 and viscosity average molecular weight $M$ in kDa.

Figure 10. The relation between Famotidine binding by 1 gram chitosans 352 and viscosity average molecular weight $M$ in kDa.
The significante of relation between the total percentage of mean adsorption investigate drugs on chitosans evaluated statistically by means of a uni-factorial Anova?Manova analysis, post hoc Nir test, confirmed statistically significant differences in the percentage of value adsorption of drugs on chitosans. Standard deviations of mean adsorbance levels for Famotidine were in the limits from 0.006 to 0.52 and variation coefficients were from 0.46% to 4.39%.

3.3. Investigation of Ranitidine and Famotidine binding by chitosans present in dietary supplements

The binding of ranitidine and famotidine by chitosans present in dietary supplements confirms the hypothesis of aggregative character of chitosans in relation to these drugs. The chitosans used in the study present in dietary supplements available on the slimming products market are capable of binding on an average of 1.26 g per 1 g of chitosan. The obtained results of absorption of the investigated drugs by chitosans from dietary supplements were presented in a graph.

The least amounts of ranitidine hydrochloride are absorbed by Chromadiet®, while Vitana® demonstrated the highest absorption. Bio-Active® absorbs much more ranitidine hydrochloride than Nutrisearch® (Figure 12).

Famotidine is best absorbed by Nutrisearch®, while the remaining investigated preparations reveal a similar level of famotidine absorption (Figure 13).
Figure 12. The amount of ranitidine hydrochloride bound by 1 g of chitosans present in dietary supplements.

Figure 13. The amount of Famotidine hydrochloride bound by 1 g of chitosans present in dietary supplements.
Standard deviation ranged from 0.006 to 0.042. Determined relativity coefficient ranged from 0.53% to 4.89%, what confirms high accuracy of measurements [7]. Mean absorption of drugs by chitosans ranged from 0.866 g to 2.449 g per 1 g of chitosan. The highest absorption was observed at pH above 7.

4. Conclusion

A interaction between famotidine, ranitidine and chitosan can be confirmed, what affects the bioavailability of the drugs.

5. References
