STUDIES OF NORFLOXACIN ADSORPTION ON CHITOSAN

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Abstract
In clinical practice a lot of natural macromolecular compounds which operation is based on assisting weight loss are used in the obesity treatment. These measures swell in the digestive tract and form the polymer gel system, which has the ability to adsorb up to 5 times more lipids relative to its own weight. When using dietary supplements containing chitosan, sometimes it comes to illness and in the therapy other agents such as chemotherapeutics are used. The aim of our study was to determine the binding capacity of chemotherapeutic norfloxacin present in the digestive tract model by chitosans found in slimming medicines, depending on variable physico-chemical factors. Phenomenon of norrfloxacin adsorption was studied by a dynamic pharmaceutical model simulating in vitro conditions. The amount of adsorbed chemotherapeutic agent by chitosan was calculated by the difference in concentrations of study drug before and after sorption. The results of measurements of quantities bounded norfloxacin were used to calculate the average percentage of adsorbed dose. The results show that norfloxacin is adsorbed by the chitosans in the applicable pH ranges, and the binding capacity depends on the pH, viscosity and concentration of chemotherapeutics, as well as the type of chitosan and additional substances in the gastrointestinal tract. The average amount of chemotherapeutic adsorption in the system chitosan-nutrients, depending on the pH ranged from 80 to 98%. The highest number of adsorption points above pH 7. In conclusion, the addition of dietary supplement such as chitosan reduces the amount of administered uniformly chemotherapeutic and simultaneously has a large impact on the bioavailability. The observed dependence may require changes in therapeutic process.

Key words: *chitosan, norfloxacin, adsorption.*

1. Introduction

In clinical practice a lot of natural macromolecular compounds which operation is based on assisting weight loss are used in the obesity treatment. These measures swell in the digestive tract and form the polymer gel system, which has the ability to adsorb up to 5 times more lipids relative to its own weight.

When using dietary supplements containing chitosan, sometimes it comes to illness and in the therapy other agents such as chemotherapeutics are used. The aim of our study was to determine the binding capacity of chemotherapeutic norfloxacin present in the digestive tract model by chitosans found in slimming medicines, depending on variable physico-chemical factors.

Norfloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV [1], enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. This mechanism can also affect mammalian cell replication. In particular, some congeners of this drug family (for example those that contain the C-8 fluorine)[2], display high activity not only against bacterial topoisomerases, but also against eukaryotic topoisomerases and are toxic to cultured mammalian cells and in vivo tumor models[3-5].

2. Materials and method

2.1. Materials

It takes advantage in work about degree from deacetylation 85 for 95% natural chitosans; from 5 for 30 kGy degrade dose radiation (***Table 1***). Norfloxacin (1-ethyl 6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid).

2.2. Method

Phenomenon of norfloxacin adsorption was studied by a dynamic pharmaceutical model simulating in vitro conditions. The amount of adsorbed chemotherapeutic agent by chitosan was calculated by the difference in concentrations of study drug before and after sorption. The results of measurements of quantities bounded norfloxacin were used to calculate the average percentage of adsorbed dose.

Chromatographic analyses were carried out using a Gilson® liquid chromatograph, with an a UV/Vis detector set at 278 nm. The choice of the conditions and the kind of column was carried out using several methods described by the US 26 Pharmacopeia . A Lichrosorb® C8 Chromatographic column (10 mm, 20 cm_4.6 mm) was used.using a buffer solution [PO_4H_3:PO_4HNa_2] adjusted to pH 3.0–acetonitrile (85:15, v:v) as the eluent, pumped at a flow rate of 1,4 ml/min. The eluent was filtered (pore size, 0.45 mm) before use and then degassed by sonication in a ultrasound bath.

The study was a trial to evaluate the effect of a chitosan solution (Chitosan type 352, 20 kGy) on the solubility of the investigated Norfloxacin. The trial was performed in gastric

environment (pH 2) with the use of two samples: A and B. Sample A contained only active substances, while sample B contained active substances in the presence of a polymer.

Sample A: weighed portions of active substances – 150 mg of Norfloxacin (the amounts present in generally available drugs) were added and reduced to pH 2 with 0.05 n HCl.

Sample B: 300 mg of chitosan were added and shaken until dissolved; next the sample was reduced to pH 2 with 0.05n HCl and weighed portions of active substances were added.

The mixtures were shaken (300 rpm) for 2 hours at 37ºC, what imitates the conditions in the stomach. Next they were cooled to room temperature, centrifuged (2100xg) for 20 minutes and left to stabilize for 0.5 hours. 1.5 ml samples were collected from above the sediment, transferred to Eppendorf's tubes and repeatedly subjected to centrifugation (15000xg) for 10 minutes.

After stirring, the test tubes contents were evaluated spectrofotometrically.

2.3. Examining the adsorption of Norfloxacin

Adsorption of Norfloxacin was investigated by means of a dynamic method in the range of concentrations in a generally administered single dose using a pharmaceutical model of the alimentary tract on the basis of a modification of the test according to Polish Pharmacopoeia for such preparations [5 - 8]. The investigation was performed in water bath with a shaker, maintaining the conditions maximally resembling those in the alimentary tract. Shaking amplitude was set at 300 rpm and the temperature at 37 ºC.

2 ml solutions of chitosans were measured to 5 ml shaker vials and reduced to pH 2, what corresponds to fasting gastric pH. The applied volume of the solution was equivalent to 0.03 g of chitosan. Next amounts of active substances corresponding to 150 mg of the substance (amount of the active substance in a therapeutic dose) were added and shaken (300 r.p.m.) for 2 hours. Next 0.2 n Na_2CO_3 was added to the vial contents to reduce it to pH 7.0 – 7.6, what corresponds to the intestinal juice and colon. The samples were incubated at 37 ºC, shaking (300 r.p.m.) for 2.5 hours.

The investigated sample was brought to room temperature and centrifuged (2100 × g) for 20 minutes, and next left for 0.5 hours to stabilize. Next a definite amount of the sample from above the sediment was collected to empty test tubes and a definite amount of determination references was added.

After stirring, the test tubes contents were evaluated spectrophotometrically.

2.4. Measurement of viscosity and determination of average molecular weight

Measurements were led at constant temperature 25 °C with Ubbelohde viscometer [Polish Pharmacopoeia VI]. Water solution of 0.1 M acetic acids was employed and it filter solution for separating insoluble fraction 0.2 M sodium chloride. For all solutions and time

Table 1. *Intrinsic viscosity [η] and viscosity - average molecular weight $M_{[\eta]}$ of the investigated chitosans (* - deacetylation degree).*

Chitosan	Dose of degrading radiation, kGy	Intrinsic viscosity [η], dm^3g^{-1}	Viscosity-average molecular weight $M_{[\eta]}$, kDa
PRIMEX (85)*	0	0.2852	348
	5	0.2545	343
	10	0.2282	293
	15	0.2057	270
	20	0.1872	242
	30	0.1576	205
CHITO CLEAR TM 1015 (95)*	0	0.5100	725
	5	0.4172	584
	10	0.3440	453
	15	0.2910	396
	20	0.2580	348
	30	0.2550	344
Chitosan HUASU (92)*	0	0.7437	1087
	5	0.5843	839
	10	0.5185	738
	15	0.3717	612
	20	0.3303	454
	30	0.2986	407
CHITOZAN 352 (95)*	0	0.2117	282
	5	0.1949	258
	10	0.1696	222
	15	0.1639	214
	20	0.1575	177
	30	0.1497	194
Chromdiet®	0	0.1872	242
Bio–active®	0	0.1576	205
Witana®	0	0.1774	229

of outflow gauge them three with solutions of viscometer. At least five measurements were executed for each concentration. Since the Mark-Houwink parameters used to recalculate intrinsic viscosity into viscosity-average molecular weight are known for chitosan in this solvent composition ($K = 1.81 \times 10^{-6}$ dm3 g^{-1}, $\alpha = 0.93$) [9].

3. Results and discusion

3.1. The effect of chitosan on Norfloxacin solubility

The applied concentrations of chitosan were equivalent to those commonly used in medical preparations. The investigation was performed in strongly acid environment of the stomach, and in these conditions drugs which are weak acids are weakly dissociated and hardly soluble (***Figure 1***). In the experiment imitating the natural gastric environment, chitosan occurs in the form of gel and its enhancing effect on Norfloxacin solubility (round 10%) cannot be excluded, as this possible property of the polymer may be masked by more pronounced adsorption. Thus it may be assumed that in the investigated concentration rang-

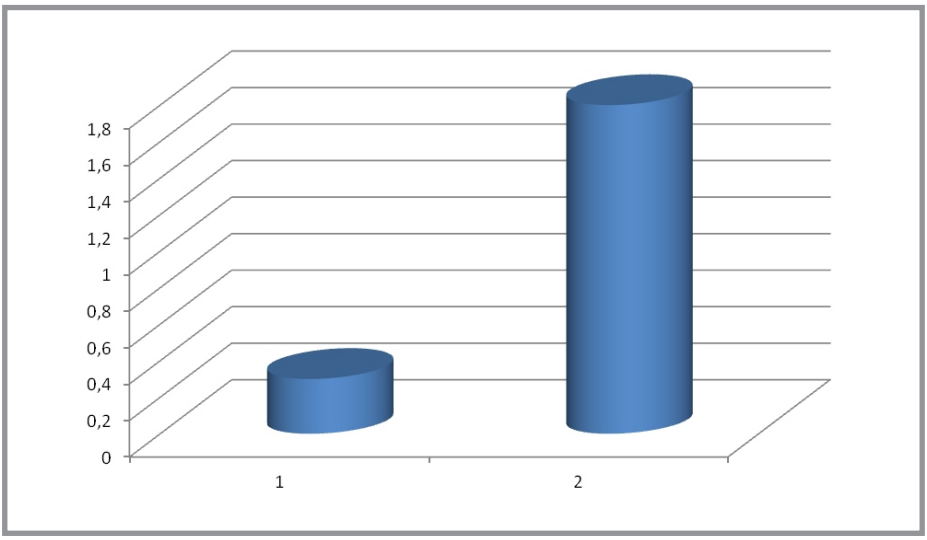

Figure 1. *Changes in Norfloxacin solubility without the addition of a polymer (1) and with polymer addition (2) on the basis of changes in their concentration (C%, mg%).*

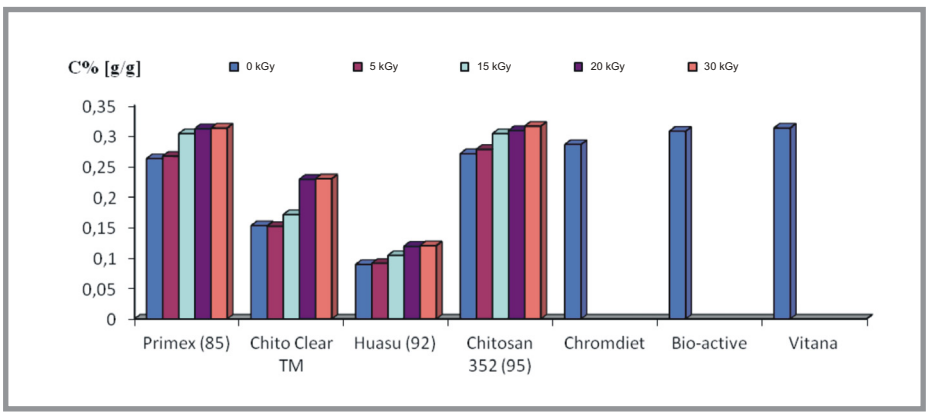

Figure 2. *Norfloxacin binding by various kinds of chitosans [g/g] in relation to degradation radiation rate in kGy.*

es chitosan no longer affects Norfloxacin solubility, and the process of Norfloxacin adsorption in the gastric environment mitigates the harmful effect of the drugs on gastric mucous membrane.

3.2. The effect of degradation radiation on Norfloxacin absorption by chitosans

Analysis of the effect of degradation radiation rate on the capability of chitosans to absorb Norfloxacin reveals certain regularity, in which a decrease in chitosan intrinsic viscosity is associated with increased volume of bound drug (***Figure 2***).

Analysis of mean viscosity molecular mass measurements revealed that the values for chitosans change in relation to the polymer radiation degradation rate. The findings prove that norfloxacin is absorbed on chitosan at applied pH ranges, and the binding capability depends on the kind of chitosan and its degradation.

The findings of measurements of norfloxacin absorption by chitosan contained in simultaneously administered OTC preparations confirmed the hypothesis that the absorption varies significantly depending on preparation. It is the highest in case of Vitana®, and the weakest in case of Chromdiet® preparation.

The binding of norfloxacin by individual preparations available on the market reveals similar rates, but they are significantly higher in comparison to absorption of this drug by chitosans from various manufacturers. Chitosan contained in medicinal preparations is capable of binding almost 100% of the administered drug dose, thus is affects markedly the bioavailability of simultaneously administered norfloxacin (***Figure 2***).

Mean absorption rate was observed to range from 80 to 98% depending on the kind of chitosan.

The fact of lowest absorption at pH 6.4 may be attributed to chemical properties of chitosan, which reveals the charge only at pH < 6.7 and then it may reveal electrostatic absorption in relation to active substances with weak acid pH [9].

At pH above 7.6, corresponding to the intestinal contents environment, mean absorption for the highest dose of the drug on chitosan ranged from 97% to 100%.

4. Conclusion

Norfloxacin interacts with a number of other drugs, as well as a number of herbal and natural supplements. Such interactions increase the risk of anticoagulation and the formation of non-absorbable complexes, as well as increasing the risk of toxicity [3 - 4].

An increase in norfloxacin absorption on a polymer at increasing pH from 7.6 to 8.0 may be explained by the swelling properties of chitosan, which forms a conglomeration in the form of emulsion system.

Basing on the above considerations, it may be assumed that an antagonistic interaction occurs between the investigated drug and the polymer, which consists in absorption of the drug on a polymer such as chitosan. In conclusion, the addition of dietary supplement such as chitosan reduces the amount of administered uniformly chemotherapeutic and simultaneously has a large impact on the bioavailability. The observed dependence may require changes in therapeutic process.

5. References

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