STUDIES ON ADSORPTION CLARITHROMYCIN ON CHITOSANS DEGRADED RADIATION IN PHARMACEUTICAL "*IN VITRO*" MODEL

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Abstract
Connections of polymers and biopolymers with biologically active compounds are recently the subject of intensive research. Low molecular weight active ingredient combined with a polymer has, in many cases, the modified action. Therefore the objective of testing was to clarify the mechanism of antibiotic drug clarithromycin interactions effects with dietary supplements containing chitosan. The phenomenon of adsorption of the antibiotic has been studied by the static method in the concentration range generally taken single dose using a pharmaceutical gastrointestinal tract model. It was observed that the average sorption depending on the type of chitosan was within the limit from 43.2% to 77.0%. At pH above 7.6 corresponding to gut environment filled with food contents, the average amount of sorption for the highest dose of chitosan were within 58% to 100%. Based on the above considerations can be stated that between study drug and the polymer an antagonistic interaction exist by involving the adsorption of drugs from this group on chitosan.

Key words: *chitosan, clarithromycin, interaction, sorption.*

1. Introduction

Connections of polymers and biopolymers with biologically active compounds are recently the subject of intensive research. Low molecular weight active ingredient combined with a polymer has, in many cases, the modified action. On the other hand, the use of inappropriate polymers can result in incompatibilities drug-polymer. Particularly important are interactions involving mainly the occurrence of adsorption phenomena and the production of the complex connections that reduce the drug effect. Clarithromycin((3*R*,4*S*,5*S*,6*R*,7*R*, 9*R*,11*S*,12*R*,13*S*,14*S*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-ethyl-12,13-dihydroxy-4-{[(2*R*,4*S*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-7-methoxy-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione. Clarithromycin is used to treat certain bacterial infections, such as pneumonia, bronchitis (infection of the tubes leading to the lungs), and infections of the ears, sinuses, skin, and throat. It also is used to treat and prevent disseminated *Mycobacterium avium* complex (MAC) infection [a type of lung infection that often affects people with human immunodeficiency virus (HIV)]. It is used in combination with other medications to eliminate *H. pylori*, a bacterium that causes ulcers. Clarithromycin is in a class of medications called macrolide antibiotics. It works by stopping the growth of bacteria. Antibiotics will not kill viruses that can cause colds, flu, or other infections.

With purpose our of work was epithet abilities bonds Clarithromycin depending on of variables of factors physico-chemicals, appearing in of model of digestive tract through chitosans appearing in cures aiding slimming down.

2. Materials and method

2.1. Materials

It takes advantage in work about degree from deacetylation 92 for 95% natural chitosans; from 5 for 30 kGy degrade dose radiation (Primex™, Chito Clear™, Huasu™, Chitosan 352 – Chitine France). Fromilid 250 (Clarithromycin), compressi coate 250 mg – KRKA (N 75225), Novo mesto, Slovenia

2.2. Method

The phenomenon of the adsorption of the cure was being examined with dynamic method in the pharmaceutical model imitating conditions in vitro. Amount absorptions of cure through chitosans they calculated from the difference of checked concentrations of preparations before and after sorption.

The examinations were performed spectrophotometrically at wavelength of 192 nm (the regression line determined for Clarithromycin was $y = 0.364x + 0.0261$, $R^2 = 0.9984$), using Aqua purified solution.

Clarithromycin are hardly soluble in water. In literature there are reports on the effect of microcrystalline chitosan (MCCh) on drugs solubility in water. As high as a 10-fold increase in active substance solubility at MCCh concentration levels from 0.00 to 0.15% was demonstrated in case of. Further increase in the amount of MCCh did not have any effect on the solubility of drugs in water [2].

The study was a trial to evaluate the effect of a chitosan solution (Chitosan type Huasu, sample) on the solubility of the investigated Clarithromycin. The trial was performed in gastric environment (pH 2) with the use of two samples: I and II. Sample I contained only active substances, while sample II contained active substances in the presence of a polymer.

Sample I: weighed portions of active substances – 25 mg of clarithromycin (the amounts present in generally available drugs) were added and reduced to pH 2 with 0.05 M HCl.

Sample II: 100, 200 and 300 mg of chitosan were added and shaken until dissolved; next the sample was reduced to pH 2 with 0.05 M HCl and weighed portions of active substances were added.

The mixtures were shaken (300 r.p.m.) for 2 hours at 37 ºC, what imitates the conditions in the stomach. Next they were cooled to room temperature, centrifuged (2,100×g) 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 (15,000×g) for 10 minutes. Next, a definite amount of the sample from above the sediment was transferred to empty test tubes and a definite amount of solvent was added to determine the sample:

- 1.5 ml of Clarithromycin sample was transferred to a test tube and 8.5 ml of reference were added;

After stirring, the test tubes contents were evaluated spectrophotometrically (wavelength of 192 nm).

2.3. Examining the adsorption of Clarytromycine

Adsorption of Clarithromycin 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 [3 - 7]. 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 25 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 0.1 M 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 (2,100×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 [8]. 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 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 ($K = 1.81 \times 10^{-6}$ dm^3g^{-1}, $\alpha = 0.93$) [4].

3. Results and discusion

3.1. The effect of chitosan on Clarytromycin 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 Clarithromycin 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 ranges chitosan no longer affects Clarithromycin solubility, and the process of Clarithromycin

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

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

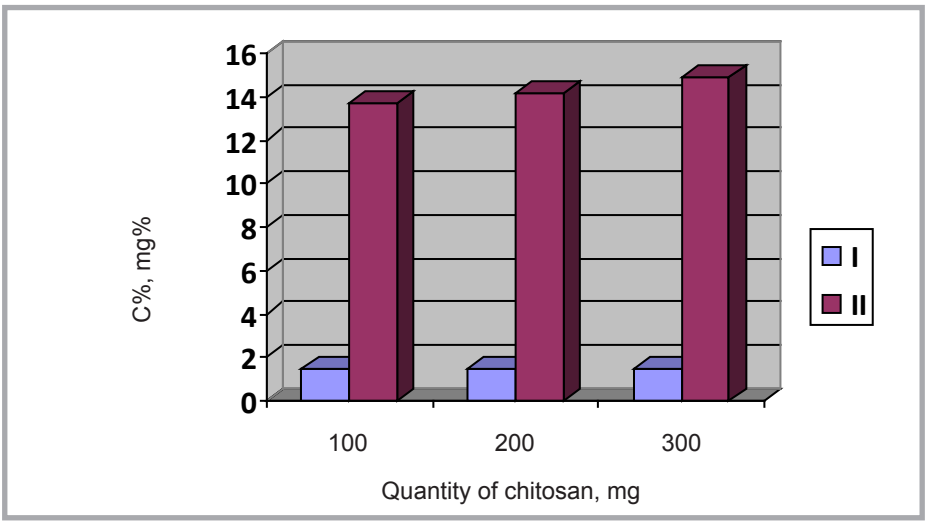

Figure 1. *Changes solubility of the Clarithromycin without the addition of a polymer (I) and with polymer addition (II) on the basis of changes in their concentration (C% in mg%, temp. 37 °C and pH 2.0).*

adsorption in the gastric environment mitigates the harmful effect of the drugs on gastric mucous membrane.

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

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

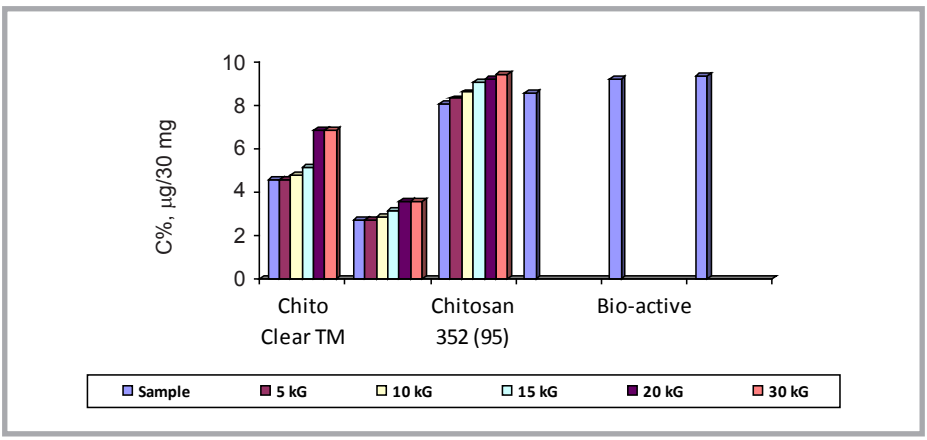

Figure 2. *Clarithromycin binding by various kinds of chitosans in relation to degradation radiation rate in kG (temp. 37 °C and pH 7.6).*

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 Clarithromycin 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 Clarithromycin 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 Clarithromycin 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 clarithromycin (***Figure 2***).

Mean absorption rate was observed to range from 43.2% to 77% 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 [6.7]. In this case, the force conditioning of the chemisorption bonds are also covalent forces with more or less the participation of ionic strength. Poor solubility of clarithromycin, confirms that the adsorption of undissolved system is greater. Adsorbent layer which is chitosan shows active sites of the acidic and alkaline character therefore the molecules are more localized at these sites.

At pH above 7.6, corresponding to the intestinal contents environment, mean absorption for the highest dose of the drug on chitosan ranged from 58% to 100%.

4. Conclusion

An increase in sulfasalazine 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.

5. References

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