RESEARCH OF INTERACTION BETWEEN METRONIDAZOLE TABLETS AND METAL SALTS “IN VITRO”

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ABSTRACT

INTRODUCTION: Along with the efficacy and safety of drugs, the interaction of drugs with each other and with other accompanying substances is important, too. There are no data about the interaction or influence of antacids and other drugs with polyvalent cations on the metronidazole bioavailability. The purpose of this research was the studying of the metronidazole release kinetics from the tablets in an environment that simulates stomach conditions with the addition of metal salts, which are part of the widespread drugs. The research was conducted to assess the impact of possible interactions between the active substance and polyvalent metal cations on their bioavailability and efficacy.

MATERIAL AND METHODS: Metronidazole tablets were chosen as research object. 0.1 N HCl solution with addition of metal salts was chosen as medium dissolution. The “PharmaTest-DT70” Device with basket, the “Evolution 60S” Spectrophotometer as well as the “AB 204 S/A METTLER TOLEDO” analytical balances were used in the study.

RESULTS: Research of chemical interaction between metronidazole and metal salts, which are part of the widespread drugs, in the experiment “in vitro” was carried out. Metal salts don’t influence the metronidazole release from the tablets, as evidenced by dissolution profiles and similarity factors for each of the cases.

CONCLUSIONS: The chemical interactions between the chosen medicines were not observed in the “in vitro” experiment. Thus, separate intake of metronidazole with other drugs, containing metal cations, is important for further research in the “in vivo” experiment.

Keywords: Metronidazole tablets, test “Dissolution”, metal salts, interaction

INTRODUCTION

Along with the efficacy and safety of drugs, the interaction of drugs with each other and with other accompanying substances, such as components of food, mineral water, other drinks etc. is no less important. Medicines containing polyvalent metal cations, such as antacids, iron supplements, de-nol, etc. constitute a significant group of drugs for medical use. Common recommendations for use are present-
ed in the guidelines such as separate intake of medicines with metal salts with other drugs because of the possibility of insoluble complexes formation. But patients do not always follow the common rules of taking drugs, referring to the need of immediate elimination of symptoms of heartburn and pain in the stomach (19).

With regard to some drugs such as pantoprazole (16), ketoprofen, amoxicillin (18) it was noted that antacids in therapeutic concentrations do not influence the bioavailability whereas the interaction during simultaneous application has not been established (16). Thus, the general recommendations in the given case need not be taken into account.

There are no data on the problem of interaction or influence of antacids and other drugs with polyvalent cations on the bioavailability as far as some other drugs such as metronidazole are concerned (8, 17). In this case doctors and pharmacists advise being guided by the general recommendations for using drugs containing metal cations. But these recommendations are not always confirmed experimentally.

As for metronidazole and a number of drugs, used for the treatment of gastrointestinal pathology, obtaining reliable data on the interaction of drugs with polyvalent cations is important since the probability of their combined use due to a number of different causes is very high. Therefore, in our opinion, research in this direction is important for practical medicine and pharmacy.

As a result of previous works (3-5,7,9-10) the probability of interaction between metronidazole and some metal salts, which are part of different pharmacological drugs groups, was established. It should be noted that research of interaction was carried out without complying with specific conditions of the reaction medium that simulates stomach conditions and reactants were used in molar ratios according to valence metal cations.

The aim of this study was to investigate the kinetics of metronidazole release from the tablets in an environment that simulates stomach conditions, (pH 1.2; t° (37±0.5) °C) with the addition of metal salts, which are part of the common drugs containing metal cations, to assess the impact of possible interactions between the active substance and polyvalent metal cations upon the bioavailability and pharmacological efficacy of both drugs.

**MATERIAL AND METHODS**

The object of study is metronidazole tablets “Metronidazole-Zdorovya” with the content of the active substance constituting 250 mg (manufactured by ”Zdorovya” Pharmaceutical company Ltd., Kharkiv, Ukraine, Series: 40415).

The metal salts such as anhydrous calcium chloride, magnesium sulphate heptahydrate, aluminium sulphate oktadekahydrate, iron (II) sulphate heptahydrate, iron (III) chloride hexahydrate, bis-muth subnitrate were of analytical purity grade and used without further purification. They were added to the dissolution medium as components of pharmaceuticals of inorganic nature.

The working standard sample (WSS) of metronidazole (manufactured by Hubei Hongyuan Pharmaceutical Co., Ltd, China, Series: 20130541) was used to prepare the metronidazole reference solution.

The assaying of active ingredient was conducted by spectrophotometric method in the ultraviolet and visible spectrum by the standard method.

The “PharmaTest-DT70” Device with basket (Germany), the “Evolution 60S” Spectrophotometer (USA), the ”AB 204 S/A METTLER TOLEDO” analytical balances (Poland) as well as class A measuring vessel and reagents that conform to the State Pharmacopoeia of Ukraine (SPhU) (12-14) which are harmonized with the European ones (1) were used in the study.

The release kinetics was studied according to the SPhU monographs, Supplement 2 “2.9.3. Test “Dissolution” for solid dosage forms”, “5.N.2. Research of bioavailability and bioequivalence of generic medicines” (13), the guidelines “Drugs. Investigation of bioequivalence” (6), the monograph USP 26 Test “Dissolution” (18), the SPhU monograph, Supplement 4 “Metronidazole tablets” (14).

Statistical analysis of the results was performed according to the SPhU monograph, Supplement 1 “5.3.N. Statistical analysis of the results of chemical experiments” (12). Microsoft Office Excel was used for calculations and statistical analysis of obtained data.
To simulate the environment of the stomach, 0.1 M solution of hydrochloric acid of pH 1.2 was used. The calculated amounts of salts were dissolved in it separately. The amounts of metal salts were calculated in terms of quantitative content of metal cations in the medium therapeutic doses of appropriate drugs.

Conditions of the "Dissolution" test were as follows: the dissolution medium volume was 1000 mL, the environment temperature was (37±0.5) °C; the basket’s rotational speed – 100 rev/min. The overall time of the study constituted 95 min. The samples were taken every 5 minutes, after the point of 15 min – every 15 min., after the point of 45 min – every 10 min. Samples were taken manually with the Mohr pipette with a capacity of 10.0 mL of the middle area between the surface of the dissolution medium and the top of the basket, no closer than 1 cm from the surface vessel. The volume of elongated aliquot was not compensated by pure solvent, which was taken into account in the calculation formula. Investigated samples were filtered through the filter “Blue Ribbon” immediately after extraction.

**Test solution.** 2.0 ml of investigated sample filtrate was adjusted to the volume of 25.0 ml with 0.1 M hydrochloric acid solution.

**Reference solution.**

The amount of metronidazole WSS about 0.0250 g is dissolved with 0.1 M hydrochloric acid solution and adjusted to the mark of 100.0 ml with the same solvent. The aliquot of 2.0 ml of the resulting solution is taken and placed in the 25.0 ml volumetric flask and adjusted to the mark with the same solvent.

**Compensation solution.**

To measure the absorbance of the researched samples, the compensation solution was prepared in the following way: 2.0 ml of dissolution medium was placed in a 25.0 ml volumetric flask and the volume adjusted to the mark with 0.1 M hydrochloric acid solution.

For measuring the reference solution as compensation solution, 0.1 M hydrochloric acid solution was used.

The measurement of the absorbance of the test solutions and the reference solution was carried out on a spectrophotometer at 277 nm wavelength in a ditch with a layer thickness of 10 mm.

The assaying of metronidazole in the taken samples was calculated using the formula

\[
x, \% = \frac{A \cdot m_0 \cdot P \cdot (1000 - V_n) \cdot 2.0 \cdot 25.0}{A_0 \cdot m \cdot 100.0 \cdot 2.0 \cdot 25.0},
\]

where:
- \( A \) – absorbance of the test solution;
- \( A_0 \) – absorbance of the reference solution;
- \( m \) – study drug sample weight amount;
- \( m_0 \) – standard sample weight amount;
- \( P \) – quantitative content of metronidazole in WSS, in percentage;
- \( V_n \) – volume of taken aliquot.

Rationing: at least 80% of the nominal metronidazole content.

The similarity of dissolution profiles was established by calculating the similarity factor \((6,11,13)\) where:

\[
f_2 = 50 \cdot \log \left[ \frac{100}{1 + \frac{1}{n} \cdot \sum_{i=1}^{n} \left( \frac{R_{(i)} - T_{(i)}}{R_{(i)}} \right)^2} \right]
\]

number of control points;
- \( R_{(i)} \) – average of the active substance amount, which turned into solution at each control point for reference preparation (as a percentage of the value specified on the label);
- \( T_{(i)} \) – average of the active substance amount, which turned into solution at each control point for test preparation (as a percentage of the value specified on the label) \((6,11,13)\).

Also, for each of the control points the relative standard deviation of the mean result was calculated, which must satisfy the following criteria: for the first control point it must be less than 20%, and for the rest of control points it must be less than 10% \((6,11,13)\).

**RESULTS AND DISCUSSION**

According to the literature metronidazole is absorbed well and rapidly taken orally, with the bioavailability constituting approximately 100%; the concentration maximum is achieved in 1-2 hours depending on the dose – from 6 to 40 mg/l. It binds to plasma proteins up to 20%. Metronidazole pene-
Artem Myhal, Olga Golovchenko, Victorlya Georgiyants

trates the tissues and body fluids, passes through the histohematogenous barriers, provides therapeutic concentrations in cerebrospinal fluid and brain tissue, and penetrates into breast milk, actively secreted with saliva and gastric juice. The half-life is 6-10 hours. About 30-60% of metronidazole is biotransformed in the liver. Up to 60-80% of metronidazole is excreted mainly by renal excretion, the unchanged metronidazole amounts to about 20%; from 6% to 15% of it is excreted with the bile and feces (7, 5,19).

Thus, according to the literature (7,15,19) and experimental data for biopharmaceutical solubility and degree of penetration according to the Biopharmaceutics Classification System (BCS) (2,6,13,20) metronidazole can be attributed to the first class of solubility, which confirms the possibility of in vitro bioequivalence studies.

Dissolution profiles are shown in Fig. 1. Research results of the kinetics of metronidazole release from the tablets in dissolution medium, which contains dissolved metal salts in concentration, which were calculated from the average therapeutic applicable concentration, are presented in Table 1. Besides the concentration values of dissolved medical substance, these similarities factors and relative standard deviations for each of investigated samples are also presented.

As can be seen in Figure 1, the dissolution profiles are very similar to each other, deviation within the allowed norms, nature of charts are not significantly different between themselves and the control experiment. Disintegration of tablets and the dissolution of the metronidazole active substance in all cases in these conditions have been observed within five minutes from the start of the research, as evidenced by the dissolution profiles shown in Figure 1.

![Fig. 1. Dissolution profiles of metronidazole in dissolution mediums with the addition of metal salts](image)

**Table 1. Results of in vitro research into release kinetics of metronidazole from tablets in the mediums with the addition of metal salts**

<table>
<thead>
<tr>
<th>Time, min.</th>
<th>0,1M HCl</th>
<th>0,1M HCl with CaCl₂</th>
<th>0,1M HCl with MgSO₄</th>
<th>0,1M HCl with Al₂(SO₄)₃</th>
<th>0,1M HCl with FeSO₄</th>
<th>0,1M HCl with FeCl₃</th>
<th>0,1M HCl with Bi(OH)₂NO₃</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>100.44</td>
<td>102.03</td>
<td>101.41</td>
<td>100.06</td>
<td>104.24</td>
<td>98.70</td>
<td>101.31</td>
</tr>
<tr>
<td>10</td>
<td>98.92</td>
<td>100.37</td>
<td>98.99</td>
<td>99.46</td>
<td>103.78</td>
<td>97.49</td>
<td>99.50</td>
</tr>
<tr>
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<td>98.37</td>
<td>99.19</td>
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<td>99.08</td>
<td>102.24</td>
<td>96.64</td>
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<tr>
<td>30</td>
<td>97.87</td>
<td>99.18</td>
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<tr>
<td>45</td>
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<td>99.29</td>
<td>97.15</td>
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<td>55</td>
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<td>91.76</td>
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<td>94.81</td>
<td>90.48</td>
<td>90.21</td>
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<tr>
<td>95</td>
<td>92.03</td>
<td>92.94</td>
<td>91.39</td>
<td>90.81</td>
<td>94.44</td>
<td>89.61</td>
<td>88.79</td>
</tr>
<tr>
<td>similarity factor, f₂ ≥ 50</td>
<td>-</td>
<td>92.70</td>
<td>98.35</td>
<td>98.63</td>
<td>84.01</td>
<td>90.43</td>
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</tr>
<tr>
<td></td>
<td>1.06</td>
<td>1.79</td>
<td>0.53</td>
<td>0.70</td>
<td>1.01</td>
<td>1.11</td>
<td>0.84</td>
</tr>
</tbody>
</table>

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According to the regulatory documents (6,13,18), as the research was carried out regarding the reference preparation, the confirmation of equivalence relative to it, for all of the samples similarity factors $f_2$, were calculated, which should be $\geq 50$. In the case of bioequivalence studies of generic drugs relative to the original preparations, if the concentration of the active ingredient is more than 85% for 15 minutes, the similarity factors need not be taken into account (6,13). However, in the context of this study, we decided to calculate these indicators, as the concept of this experiment is different from the described documented procedures for determining drugs bioequivalence. As a result of the calculations, similarity factors for five conducted studies were more than 90%, only in the case of the dissolution medium with the addition of Fe (II) salts, the figure was 84%, but it also satisfies the requirements.

As a result of the statistical processing of the obtained data (12) it was found that they satisfied the given requirements (6,13) and constitute less than 2% for each of the studies.

Thus, the obtained data shows that the chemical interaction of metronidazole with metal salts, which are part of antacid drugs as well as drugs for the iron deficiency treatment, and some other preparations containing metal cations, has no significant effect on the bioavailability of metronidazole in the “in vitro” experiment. The separate intake of metronidazole with other drugs, containing metal cations, is important for further research in the “in vivo” experiment.

CONCLUSIONS

1. In the average therapeutic concentrations the chemical interaction between the metal salts, which are part of antacid and other drugs of inorganic nature, and metronidazole did not have any impact on the therapeutic concentration of the latter, and may have no significant impact on the bioavailability of metronidazole in the “in vitro” experiment.

2. Similarity factors of dissolution profiles of metronidazole tablets in various mediums are $\geq 90\%$, in the case study of solubility in the medium with Fe$^{3+}$ salt, the figure is 84% (dissolution profiles are considered similar if the similarity factor is $f_2 \geq 50\%$) (6,13).

3. The value of relative standard deviation also conforms with the given criteria and is no more than 2% for each of the samples.

4. The obtained results confirm the importance further research of interaction of metronidazole with the antacids and other metal salts containing drugs in the “in vivo” experiment.

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