IN VITRO INTERACTION BETWEEN TRIMETHOPRIM AND SOME ANTACIDS AND EDIBLE CLAY

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ABSTRACT
The in vitro interaction between trimethoprim and some adsorptive materials was investigated; the kinetics and extent of adsorption of the drug were determined. Adsorption studies with trimethoprim powder using three antacids viz; magnesium trisilicate, aluminium hydroxide, magnesium hydroxide as well as edible clay were carried out. Trimethoprim tablets were formulated and their physicochemical properties were evaluated. The effects of the antacids and edible clay on the tablet disintegration time and dissolution were determined. The physicochemical parameters of the tablets (mass, disintegration time, friability, hardness and content of active) met official BP specifications. The adsorption of trimethoprim onto the antacids and edible clay increased as the concentration of the adsorbents increased. Adsorption of the drug by the adsorbents followed the rank order; magnesium trisilicate > aluminium hydroxide > edible clay > magnesium hydroxide. There was no significant increase in the disintegration times of the tablets, and retardation of dissolution in the presence of the antacids and the edible clay followed the same rank order; magnesium trisilicate > aluminium hydroxide > magnesium hydroxide > edible clay. Co-administration of trimethoprim with adsorptive antacid formulations and concomitant consumption of edible clay with trimethoprim needs to be strongly discouraged.

Keywords: Trimethoprim, Adsorption, Adsorptive antacids, Edible clay, Dissolution

INTRODUCTION
Trimethoprim (5-(3,4,5-trimethoxybenzyl)pyrimidin-2,4-diyi-diamine) is a dihydrofolate reductase inhibitor. It acts in the same metabolic pathway as the sulphonamides by blocking the synthesis of tetrahydrofolic acid which is necessary for the synthesis of certain amino acids of microorganisms. It may be bacteriostatic or bactericidal depending on the growth conditions of the organisms. Trimethoprim is used for the treatment of infections due to sensitive organisms in gastro-enteritis, urinary tract infections and respiratory tract infections including sinusitis and otitis. It is formulated with sulphamethoxazole as co-trimoxazole which is a mixture of 1 part trimethoprim and 5 parts sulphamethoxazole. The synergistic antimicrobial activity of co-trimoxazole results from its inhibition of two sequential steps in the synthesis of tetrahydrofolic acid; sulphamethoxazole inhibits the incorporation of p-aminobenzoic acid (PABA) into folic acid and trimethoprim prevents reduction of dihydrofolate to tetrahydrofolate. Antacids are commonly used drugs which either directly neutralize acidity, increasing the pH, or reversibly reduce or block the secretion of acid by gastric cells to reduce acidity in the stomach (Zajac et al., 2013). They are also considered inert and free of pharmacological effect by many patients and physicians. When administered with other drugs, antacids can alter absorption or excretion of these agents, at times reducing their plasma level and therapeutic effect and in other cases causing toxicity. The effectiveness of these substances is influenced by the surface areas of the solids. Their administration as fine powders in the form of suspensions allows a large surface area to be available immediately for contact with
the gastric content. Although many antacid-drug interaction studies have been carried out in animals or in-vitro, several clinically important interactions and their mechanisms have been verified in man. Antacids which reduce drug dissolution or bind drugs in the gastrointestinal tract have been shown to inhibit absorption and reduce efficacy of ampicillin, ciprofloxacin, digoxin, temafloxacin, phenytoin and levotyroxine (Hurwitz et al., 1973; Brown and Juhl, 1976; Granneman et al., 1992; Mersebach et al., 1999; Landmark and Patsalos, 2010; Thapa, 2013).

Edible clays are eaten in Papua New Guinea for their nutritive value and its consumption is popular amongst Nigerian females who consume substantial amounts ostensibly for their ability to stop diarrhoea, prevent nausea and vomiting as well as the discomfort associated with hyperacidity (Aloko, 1992). Consequently, the aim of this work is to investigate any possible in vitro interaction of trimethoprim, with some commonly used adsorptive antacids and edible clay.

MATERIALS AND METHODS

Materials

Trimethoprim (Shouguang Fukang, Pharmaceutical Company Ltd, Shandong, China), magnesium stearate, magnesium trisilicate, aluminium hydroxide and magnesium hydroxide (BDH Chemicals Poole, U.K.), sodium hydroxide pellets (May and Baker, Nigeria), lactose monohydrate (Milkaut, Argentina), corn starch (African Product Ltd, South Africa), modified cellulose gum (FMC Corporations, USA), hydroxyl propyl methyl cellulose (HPMC), (NFXII, Shin-etsu, Japan) and talc (Halewood Chemicals Ltd, Staines, England) were used as received. Edible clay was locally purchased and processed; all water used was double distilled; all the sieves were BSS (Endecotts, UK).

Methods

Preparation of the edible clay powder

The clay lumps were broken into small pieces and soaked in water for about 24 h. The water was decanted several times to remove the salt content of the clay. The final product was sun dried, and the now dried clay was reduced to fine powder in an electric dry mill. The fine powder was then passed through a sieve of aperture size 0.125 mm.

Calibration curve of trimethoprim

Different concentrations of trimethoprim (0.5, 1.0, 1.5, 2.0 and 2.5 mg/100 mL) were prepared in 0.1N NaOH. The resultant concentrations were read in the spectrophotometer (Spectronic 21D, Milton Roy, USA) at 287 nm against a blank of 0.1N NaOH. The experiments were replicated and the mean values of the results were used for further computations.

Adsorption studies

One gram of antacid was weighed into each of five conical flasks. A known amount of trimethoprim was weighed and dissolved in sufficient water to give 100 mL solution. The solution was then added to the antacid in the conical flask. The suspension was shaken and then placed in the water bath at 37 ± 0.5 °C and equilibrated for 120 min; with intermittent shaking. Previous experimentation showed that complete equilibration occurred after 1 h. After equilibration, the suspension was filtered through a Whatman No. 1 filter paper, and a 2 mL portion of the filtrate was made up to 100 mL with 0.1N NaOH solution. The absorbance of the resultant solution was read in the spectrophotometer at 287 nm against a blank which was similarly prepared without drug. The various concentrations of the drug used in the study were 10, 20, 30, 40, 50, 60, 70 and 80 mg/100 mL. All the experiments were carried out in replicates.

Preparation of trimethoprim granules and tablets

The formula used in the preparation of the granules and tablets of trimethoprim is shown in Table I. The required amount of trimethoprim, lactose and half of the disintegrant were dry-mixed in the planetary mixer (Moulinex, France) for 5 min. The binder solution was added and intimately mixed with the dry mass until an even wet mass was obtained. The wet granules were passed through a 1.40 mm mesh screen and dried at 60°C for 4 h. The dry granules were passed through a 1.00mm mesh screen and further dried for 6 h at 50°C. The dried granules were dry mixed with 1 % w/w of magnesium stearate/talc mixture and the extra-granular disintegrant. The mixture was compressed into 500mg tablets in the tablet press (Manesty Machines, UK) at a fixed compression pressure of 30 units. The resulting tablets were stored in airtight container until use.

Table I: Formula for preparation of trimethoprim tablets

<table>
<thead>
<tr>
<th>Item</th>
<th>Ingredients</th>
<th>Quantities/tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Trimethoprim</td>
<td>200 mg</td>
</tr>
<tr>
<td>Filler</td>
<td>Lactose</td>
<td>250 mg</td>
</tr>
<tr>
<td>Binder</td>
<td>5 % w/v HPMC</td>
<td>Qs</td>
</tr>
<tr>
<td>Disintegrant</td>
<td>Cellulose gum/maize starch (10:1)</td>
<td>20 mg</td>
</tr>
<tr>
<td>Glidant</td>
<td>Magnesium stearate</td>
<td>1 % w/w</td>
</tr>
<tr>
<td>Lubricant</td>
<td>Talc</td>
<td>1 % w/w</td>
</tr>
</tbody>
</table>

Evaluation of tablets

Friability test

Twenty previously weighed tablets were subjected to
cascading and free fall shocks in a friabilator (Erweka Apparatebau, Germany). The friabilator was operated at 25 rpm for 4 min. The tablets were re-weighed after dusting off adherent particles if any. Friability was then calculated as the percentage loss in weight.

**Tablet dimensions**

A micrometer screw gauge (Griffin, Germany) was used to measure the thickness and diameter of each of twenty tablets. The mean of these values was calculated and recorded.

**Tablet hardness**

Hardness tester (Campbell Electronics, Model HT-30/50, India) was used to measure the hardness of each of ten tablets. The mean of these values was calculated and recorded.

**Tablet weight uniformity**

The weight of each of 20 tablets was determined using an electronic balance (College B154, Mettler Toledo, Switzerland). The mean weight and standard deviation were calculated.

**Content of active drug**

Twenty (20) tablets were randomly selected and crushed to fine powder. A quantity of the powdered tablets equivalent to 500 mg trimethoprim was weighed and dissolved in about 50 mL of 0.1N NaOH solution in a 100 mL volumetric flask. The volume was made up with more solution. Necessary dilution was carried out to obtain a final concentration of 100 μg/mL. The solution was thereafter filtered through a Whatman No 1 filter paper and the absorbance of the filtrate determined at 278 nm using 0.1N NaOH as blank.

**Disintegration test**

Ten tablets were individually subjected to BP disintegration test (Mk IV. Manesty Machines Ltd, UK) and the mean value of the disintegration time was noted. The disintegration medium was water at a temperature of 37 ± 0.5 °C. In the other study, 500 mg of each of the antacids (magnesium hydroxide, magnesium trisilicate, aluminium hydroxide, and edible clay) was added to 500 mL disintegration medium in order to evaluate the effect of these antacids on the disintegration time of the tablets. This was repeated with 1 g and 2.5 g of each of the antacids and the clay.

**Dissolution studies**

Dissolution profiles were obtained for the trimethoprim tablets in 1 L of distilled water maintained at 37 ± 0.5°C. The effect of the antacids and edible clay on dissolution was studied by adding 2.5 g of the adsorptive antacid to the dissolution medium and 5 mL samples were withdrawn from the dissolution medium at specific time intervals. The sample was filtered and suitably diluted with 0.1N NaOH solution and the amount of trimethoprim determined spectrophotometrically against a 0.1N NaOH blank at 287 nm. Equal volumes of fresh dissolution medium were used to replace those withdrawn for analysis. The procedure was carried out with different amounts of magnesium trisilicate. The experiments were carried out in duplicate.

**Statistical analysis**

Descriptive statistics was done for all data using Microsoft Excel (2007). Mean and standard deviations of triplicate determinations were computed and reported. Differences between mean was determined using ANOVA while p < 0.05 was considered significant.

**RESULTS**

The evaluation of the physicochemical properties of the tablets gave the following values: diameter (12.36 ± 0.004 mm), thickness (3.36 ± 0.29 mm), hardness (4 ± 0.67 Kg), friability (1.0 ± 0.7 %), weight uniformity (505 ± 0.5 mg), content of active (101 ± 1.24 %), disintegration time (117 ± 5.78 sec) and dissolution rate (90 % in 30 min). The adsorption studies showed significant adsorption of trimethoprim onto all the adsorptive antacids and the edible clay. Adsorption was most significant onto magnesium trisilicate followed by aluminium hydroxide, edible clay and magnesium hydroxide, in that order (Figure 1).

**Figure 1.** Adsorption of trimethoprim onto magnesium trisilicate (■), aluminium hydroxide (▲), edible clay (∗) and magnesium hydroxide (●).
The results in Table II, on disintegration time of trimethoprim tablets in the presence of some antacids, showed a rapid disintegration of the tablets with the adsorptive antacids and clay having no significant effect on the disintegration time in the various concentrations tested.

**Table II: Disintegration times of trimethoprim tablets in various media at 37 ± 0.5 °C**

<table>
<thead>
<tr>
<th>Disintegration medium</th>
<th>Concentration (% w/v)</th>
<th>Disintegration times (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-</td>
<td>117 (5.78)</td>
</tr>
<tr>
<td>Magnesium trisilicate</td>
<td>0.1</td>
<td>119 (4.07)</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>125 (3.55)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>125 (4.68)</td>
</tr>
<tr>
<td>Aluminium hydroxide</td>
<td>0.1</td>
<td>120 (3.23)</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>115 (4.08)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>119 (3.74)</td>
</tr>
<tr>
<td>Magnesium hydroxide</td>
<td>0.1</td>
<td>115 (3.12)</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>120 (3.45)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>123 (3.43)</td>
</tr>
<tr>
<td>Edible clay</td>
<td>0.2</td>
<td>126 (3.78)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>120 (4.08)</td>
</tr>
</tbody>
</table>

The results from the dissolution studies to determine the dissolution of trimethoprim from the tablets in the presence of antacids are shown in Figure 2. The antacids had a significant inhibitory effect on the dissolution of trimethoprim from the tablets. This inhibitory effect can be seen in the variation of the amounts of trimethoprim dissolved from the tablets in the presence of the antacids. The inhibitory effect or retardation on dissolution is of the rank order: magnesium trisilicate > aluminium hydroxide > magnesium hydroxide > edible clay. Figure 3 also shows that the concentration of the adsorptive antacids affects the amount of the drug adsorbed. The inhibitory effect of the antacid increases with increase in the concentration of the antacids.

**DISCUSSION**

The physicochemical parameters of the tablets would suggest that tablets of good quality were made and used in the study. The tablets met official British Pharmacopeia specifications (BP, 2007). None of the tablets deviated from the mean weight of the tablets by 5 % or by 10 % of the active content. Also, all the tablets disintegrated within 15 min. The rapid disintegration (< 3 min) of the tablets could be attributed to the disintegrant incorporated in the formulation. Although friability and hardness tests are not official test, they relate to the strength of the tablet. Ofoefule et al. (1998), suggested that friability values between 0.8 - 1.0 % of the tested tablets without capping, lamination or breaking up in the course of the test are ideal while tablet hardness of 4 kgf was considered to be the minimum for a satisfactory tablet (Rudnic and Schwartz, 2000).

On the adsorption studies, the significant adsorption of trimethoprim onto all the adsorptive antacids and the edible clay calls for concern in therapeutics because of the possible implication on the bioavailability of orally administered drugs (Iwuagwu and Aloko, 1992). Adediran et al. (2007) and Al-Bayati and Ahmed (2011) working on the interactions of chloroquine phosphate tablets with some antacids and the adsorption and
desorption of trimethoprim from aqueous solutions respectively, obtained similar results. The clinical implications of this in vitro interaction, if it also occurs in vivo, would mean that the bioavailability of orally administered trimethoprim could be reduced in the presence of these adsorptive antacids. The antacids had no effects on the disintegration times of the trimethoprim tablets although Iwuagwu and Aloko (1992) reported that delays in the disintegration of chloroquine phosphate tablets in the presence of some adsorptive antacids. This result could be because of the high quality super-disintegrant (HPMC) used in the study. The dissolution of trimethoprim from the tablets in the presence of antacids would indicate retardation on the dissolution of trimethoprim from the tablets. The variations in amount dissolved from the tablets in the presence of the various adsorbents were observed to be directly related to the different capacities and concentrations of the adsorbents to adsorb trimethoprim (Figures 2 and 3). It may be inferred therefore, that the apparent retardation of dissolution from the trimethoprim tablets in the presence of antacids may be due to the significant interaction which occurred between the drugs that dissolved out of the tablets and the antacids studied via relatively strong adsorptive forces. As dissolution follows disintegration of tablets prior to drug absorption, the therapeutic action of a drug could be compromised by these antacids when administered concurrently since adequate concentration of the drug reaching the site of action would not be achieved.

In concurrent administration of these antacids with drugs such as antibiotics, apart from the resultant drug failure from inability to attain therapeutic concentration in the system, drug resistance by microorganisms is another possible danger that could occur.

CONCLUSION

From the study, there was significant adsorption of trimethoprim onto the adsorptive antacids and edible clay. The adsorption will reduce the amount of the active drug available for absorption. This may in turn reduce the bioavailability of the drug with subsequent drug resistance and therapeutic failure. Therefore, the concomitant administration of these antacids with trimethoprim should be discouraged.

REFERENCES


