

Effects of Cadmium, Lead, Manganese, and Zinc at WHO Safe Limits in Drinking Water on the *in Vitro* Availability of Chloramphenicol

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ABSTRACT: In the present study, The *in vitro* availability of chloramphenicol was studied in the presence of lead (Pb), cadmium (Cd), manganese (Mn) and zinc (Zn) at 0.01, 0.003, 0.5 and 3 mg/L respectively corresponding to WHO safe limits in drinking water for each of the metals. The *in vitro* availability study was carried out in simulated gastric juice (0.1 M HCl) and intestinal pH (phosphate buffer pH 9.0) at 37°C using a dissolution apparatus. Samples were taken at 15 minutes intervals over a period of 3 hours and analyzed for concentration of chloramphenicol available using a Helios Zeta Model 164617 UV/VIS spectrophotometer at 278nm. In simulated gastric juice, a maximum availability of 99.48 % was achieved for chloramphenicol alone, while 89.48, 93.48, 105.88 and 93.60% of the drug was detected in the presence of Cd, Mn, Pb, and Zn respectively. The corresponding maximum availabilities in simulated intestinal pH were 64.12 for chloramphenicol alone, and 64.36, 83.76, 80.72 and 64.96 % in the presence of Cd, Mn, Pb, and Zn respectively. The presence of Cd did not affect the overall availability of chloramphenicol in both media; while the availability of the drug was observed to increase slightly in the presence of Pb in both media. In simulated gastric juice, Mn and Zn did not affect the availability of chloramphenicol. However, their presence slightly increased the availability of the drug in simulated intestinal pH. The results of this study indicate that the absorption and subsequent bioavailability of chloramphenicol may not be reduced by Cd, Pb, Mn, and Zn at concentrations corresponding to WHO safe limits in drinking water. Bioavailability may even be enhanced especially in the intestine, which may result in therapeutic effectiveness.

Key words: Chloramphenicol, metal cations, *in vitro* availability

INTRODUCTION

Chloramphenicol, 2,2-Dichloro-N-[(α R, β R)- β -hydroxy- α -hydroxymethyl-4-nitrophenethyl] acetamide, is a broad spectrum bacteriostatic antibiotic against a wide range of Gram-negative and Gram-positive organisms as well as other organisms, but at higher concentrations or against some very susceptible organisms it can be bactericidal (Sweetman, 2002). It is used in treatment of human infections with *Salmonella typhi*, and other forms of salmonellosis, and other life-threatening infections of central nervous system and respiratory tract (Parfitt, 1999). Its ability to cause aplastic anaemia and resistance has limited the use of the drug. However, it is still widely used in low-income countries (including Nigeria) because of its cheapness (Sweetman, 2002).

The availability of Chloramphenicol was reported to be significantly reduced when the drug was interacted with cadmium and lead in simulated gastric and intestinal pH at stoichiometric ratio of 2:1 (Musa *et al.*, 2010). Interaction studies of ofloxacin with metal ions results in decrease bioavailability of ofloxacin (Sultana *et al.*,

2007). Availability of levofloxacin was reported to significantly decrease when the drug was interacted with several metals including Cd, Mn and Zn (Sultana *et al.*, 2004). Some antacids have been reported to significantly retard the availability of glibenclamide (Aryane *et al.*, 2004).

As a result of such interactions, the absorption and consequent bioavailability of different drugs especially antibiotics may be significantly affected. Heavy metals commonly contaminate the environment and drinking water sources (Musa *et al.*, 2008). The WHO safe limits for Cd, Pb, Mn and Zn in drinking water are 0.003, 0.01, 0.5 and 3 mg/L respectively (WHO, 1993). These limits address the toxic effects of these metals. This research work was aimed at evaluating the effect of Cd, Pb, Mn and Zn at concentrations corresponding to the WHO limits on the availability of chloramphenicol in simulated gastric and intestinal pH.

MATERIALS AND METHODS

Equipment and reagents

All metal salts (i.e. Lead chloride, Manganese Chloride, Zinc Chloride, Cadmium chloride and Chromium trioxide) and chemicals used were of analytical grade. Standard chloramphenicol powder was obtained as gift from Doyin pharmacy Nigeria limited. Chloramphenicol capsules were products of Green field pharmacy (JIANGSU) Co. Ltd (Batch-04-5485). Potassium dihydrogen orthophosphate was BDH-Laboratory reagents. Dissolution studies were performed using an ERWEKA (GMBH) dissolution apparatus. A double scanning UV/VIS spectrophotometer (Helios Zeta, Model 164617) was used to monitor the drug content.

Identification and assay of Chloramphenicol

Both the Chloramphenicol reference standard and Chloramphenicol capsules were identified and assayed using official methods (BP, 2002).

Preparation of dissolution media

Dissolution was carried out in simulated gastric juice (containing 0.1M hydrochloric acid) and intestinal pH (phosphate buffer pH 9). Both media were prepared using official methods (BP 2002).

Preparation of calibration curve

Stock solutions (1 mg/ml) of Chloramphenicol reference standard were prepared in 0.1M hydrochloric acid and phosphate buffer pH 9. The absorbance of a 50 μ g/ml solution was measured in the range of 200 – 350 nm, which showed maxima at 278nm. Successive serial dilutions of the stock were done to obtain standard solutions of concentrations 10, 20, 30, 40 and 50 μ g/ml. The absorbance of each solution was measured at the maxima (278nm). A plot of concentration against the corresponding absorbance was done in the case of each media. A straight line was obtained which obeyed the Beer-Lambert's law in each case. The Chloramphenicol contents in the two media during the *in vitro* availability studies were extrapolated from the calibration curves.

Availability of Chloramphenicol

The *in vitro* availability of Chloramphenicol in simulated gastric juice and intestinal pH at 37°C was determined using dissolution apparatus as outlined in BP (2002) with slight modification to the top of the basket in order to prevent air entrapment during dissolution. A capsule of

chloramphenicol (250 mg) was placed in the dissolution basket and allowed into the dissolution medium. The dissolution process was monitored over a period of 180 minutes, with aliquots (10 ml) being withdrawn at 15 minutes intervals and assayed for drug content available. The volume of the dissolution medium was maintained after each withdrawal by an immediate replacement with 10 ml of the dissolution medium maintained at the same temperature in the same bath.

For the *in vitro* availability of chloramphenicol in the presence of the metals, the drug was interacted with amounts equivalent to 0.01, 0.003, 0.5 and 3.0 mg/L of lead, cadmium, manganese and zinc salts respectively in each dissolution media (1L) at concentrations corresponding to WHO safe limits for each of the metals in drinking water.. The process described above was then repeated to determine the drug content after each sampling.

RESULTS

The results of the identification test for both chloramphenicol reference standard and capsules revealed the presence of the active ingredient and the content assayed was within the official limits (98-105%). For the qualitative determination of drug content, it was observed that the calibration curves of chloramphenicol in both simulated gastric juice (0.1M hydrochloric acid) and intestinal pH (Phosphate buffer pH9) at 278nm obeyed the Beer-Lambert's Law. The linear relationship between absorbance (A) and concentration (C in % w/v) is given by the regression equation $A=0.030C + 0.003$ and $A=0.033C + 0.002$ for chloramphenicol in 0.1M hydrochloric acid and phosphate buffer pH9 respectively. The coefficient of correlation (r) in each case is 0.999. The calculated molar absorptivities were $1,061 \pm 0.0004 \text{ M}^{-1}\text{cm}^{-1}$ and $1,123 \pm 0.0003 \text{ M}^{-1}\text{cm}^{-1}$ for 0.1M hydrochloric acid and phosphate buffer pH9 respectively.

The results of the *in vitro* availability of chloramphenicol at different time intervals in simulated gastric juice and intestinal pH at 37°C alone and in the presence of Cd, Mn, Pb, and Zn in their concentration corresponding to WHO safe limits in drinking water are shown in Tables 1 and 2.

Table 1: Availability of Chloramphenicol at Different Time Intervals in Simulated Gastric Juice at 37°C

Time (min)	Cpc (% Conc)	Cpc + Cd (% Conc.)	Cpc + Mn (% Conc.)	Cpc + Pb (% Conc.)	Cpc + Zn (% Conc.)
15	70.28	74.52	76.92	95.20	78.12
30	87.20	85.88	89.60	95.08	88.12
45	88.80	89.48	91.20	97.32	92.80
60	91.60	88.80	93.60	94.28	93.60
75	90.52	88.68	91.48	105.88	92.80
90	90.00	89.48	90.68	97.20	92.52
105	90.52	88.40	92.00	91.08	91.72
120	90.12	87.60	90.52	93.32	91.20
135	88.52	85.72	90.92	95.08	91.60
150	99.48	87.32	88.48	90.92	90.40
165	90.52	85.60	89.60	89.48	88.28
180	87.88	85.48	93.48	90.00	89.48

Table 2: Availability of Chloramphenicol at Different Time Intervals in Simulated Intestinal pH at 37°C

Time (min)	Cpc (% Conc)	Cpc + Cd (% Conc.)	Cpc + Mn (% Conc.)	Cpc + Pb (% Conc.)	Cpc + Zn (% Conc.)
15	56.60	20.84	62.20	3.40	34.44
30	38.44	38.80	74.92	58.68	58.08
45	54.80	54.80	80.72	72.48	63.88
60	83.88	62.08	80.72	81.20	64.48
75	60.24	59.64	79.52	83.28	63.04
90	61.32	59.04	80.12	83.64	64.24
105	60.12	59.52	78.80	83.76	64.96
120	62.20	63.76	77.20	82.08	64.36
135	59.88	56.60	78.80	81.32	62.22
150	60.00	55.04	76.72	81.80	60.24
165	57.92	64.36	75.18	80.12	59.04
180	64.12	54.32	74.32	79.28	62.08

DISCUSSION

In simulated gastric juice at 37°C chloramphenicol achieved a maximum availability of 99.48% (248.70mg/l) alone and respective maximum availabilities of 89.48%, 93.60%, 105.88% and 93.60% (223.70, 234.00, 264.70 and 234.00 mg/l) in the presence of Cd, Mn, Pb and Zn. Presence of Cd, Mn and Zn did not affect the availability of chloramphenicol, while the availability of the drug was increased in the presence of Pb. The increase in concentration of the drug in this study is contrary to what was reported by Musa *et al.* (2010), also in the in vitro availability and interaction studies of ciprofloxacin, Levofloxacin and ofloxacin in the presence of some metals including Cd, Mn, Pb and Zn. The little reduction in drug content in the presence of Cd, Mn and Zn in

simulated gastric juice may be because the complexation of certain drugs by polyvalent cations is not favoured in acid medium (Sanchez *et al.*, 1994) and also the low concentrations of the metal cations used in this study.

In simulated intestinal pH, chloramphenicol alone achieved a maximum availability of 64.12 % (160.30mg/l) at 30°C. The maximum availabilities of 64.36%, 83.76%, 80.72% and 64.96% (160.90, 209.40, 201.80 and 162.40 mg/l) were obtained in the presence of Cd, Mn, Pb and Zn respectively. Presence of Cd did not affect the maximum availability of chloramphenicol. However, the presence of Mn, Pb and Zn increased the overall maximum availability of chloramphenicol.

It is evident from the results that Cd did not affect the overall observed maximum availability of chloramphenicol in both media whereas presence of Pb increased the availability of the drug in both media. In simulated gastric juice, Mn and Zn did not affect the availability of the drug however their presence increased the availability of the drug in simulated intestinal pH. The fact that there are reports suggesting dissolution rate of certain antibiotics is markedly reduced at high pH values (Sultana *et al.*, 1984), a similar trend was also reported where the availability of levofloxacin was markedly reduced in the presence of different metals (including Cd, Mn and Zn) in a medium of pH 9 at 37°C (Sultana *et al.*, 2004), in the present study a different pattern emerged as increase in the availability of Chloramphenicol was observed in the presence of Cd, Pb, Mn and Zn in both media which was more pronounced in intestinal pH. This may be attributed to the fact that WHO safe limits of the metals was used in this study while other studies that reported significant decrease in availability of drug used relatively higher concentrations of metals (Sultana *et al.*, 2004; Sultana *et al.*, 2007; Musa *et al.*, 2010).

CONCLUSION AND RECOMMENDATIONS

From the results obtained, it can be concluded that the absorption and subsequent bioavailability of chloramphenicol may not be reduced by Cd, Mn, Pb and Zn metals at concentrations corresponding to WHO safe limits for each of the metals in drinking water. Bioavailability of drug may even be enhanced and this effect was observed to be more pronounced in the simulated intestinal pH than in simulated gastric juice which may result in therapeutic effectiveness. Furthermore, the pH of a medium is an important factor in the dissolution and subsequent availability of drugs as observed in this study. Drinking water should be treated to ensure the concentrations of these heavy metals are within the WHO safe limits as these limits have been shown to be adequate with respect to interaction with chloramphenicol. Further studies on the effect of actual concentrations of these metals found as contaminant in drug and food should be evaluated.

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