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Original Research Article

Comparative study of oxytetracycline and doxycycline on calcium chelation: in-vitro assay

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ABSTRACT

Background: Tetracycline class of antibiotics differ in their pharmacokinetic profile and chelating property. Objective of present study is to assess the effect of oxytetracycline and doxycycline on calcium chelation

Methods: For estimation of calcium chelation of Oxytetracycline and Doxycycline, EDTA method (P. Trinder) and calcium binding assay was followed. Different doses of Oxytetracycline (25 mg, 50 mg and 100 mg) and Doxycycline (25 mg, 50 mg and 100 mg) were used in EDTA method and different concentrations of calcium were used in calcium binding assay. The procedure was done according to the standard methodology.

Results: The intensity of colour appear to be increased with increase in dose of the Oxytetracycline (25 mg, 50 mg, 100 mg) as the concentration of calcium binding increases. But in Doxycycline intensity of colour is more with 100 mg as compared with 25 mg and 50 mg The UV absorption spectrum of solution of Oxytetracycline (1mM) was changed after the addition of CaCl2 to provide different concentration of Ca^{2+} (0.1, 0.5 and 1.0 mM). With minor shift in the absorption coefficient and no shift in wavelength were observed for Doxycycline.

Conclusions: The study concludes that oxytetracycline has more calcium chelating property than doxycycline.

Keywords: Calcium chelation, Doxycycline, Oxytetracycline

INTRODUCTION

Chelation is a type of bonding of ions and molecules to metal ions. It involves the formation or presence of two or more separate coordinate bonds between a polydentate (multiple bonded) ligand and a single central atom.[1] Usually these ligands are organic compounds, and are called chelants, chelators, chelating agents, or sequestering agents.

Chelation is useful in applications such as providing nutritional supplements, in chelation therapy to remove toxic metals from the body, as contrast agents in MRI scanning, in manufacturing using homogeneous catalysts, and in fertilizers.

Chelation in the intestinal tract is a cause of numerous interactions between drugs and metal ions (also known as minerals in nutrition). As examples, antibiotic drugs of the tetracycline and quinolone families are chelators of Fe^{2+}, Ca^{2+} and Mg^{2+} ions.[2] Chelation of antibiotics with the ions interferes with their absorption from gastrointestinal tract.

Different classes of tetracycline antibitotics include oxytetracycline, tetracycline, chlortetracycline, minocycline and doxycycline. They differ in their pharmacokinetic profile and chelating property. There exists paucity in literature regarding the comparative calcium chelating property of doxycycline and oxytetracycline.

Therefore, this in-vitro study was designed to study and compare chelating property of oxytetracycline and doxycycline.

METHODS

Calcium chelation by Trender's method

When test drug is treated with calcium reagent, the naphtha hydroxamic acid present in the reagent precipitates calcium as calcium naphtha hydroxamate. This precipitate is then dissolved in EDTA and treated with ferric nitrate (colour reagent). A reddish brown coloured complex is formed. The absorbance of which is measured colorimetrically at 530 nm. The intensity of the colour is directly proportional to the concentration of the calcium present. A standard calcium carbonate is treated similarly and the colour intensities are compared.

Reagents

- Calcium reagent (Naphtal hydroxamic acid)
- EDTA solution
- Colour reagent (Ferric nitrate)
- Calcium standard ($CaCO_3$): Concentration=0.1 mg/ml

Procedure was done according to standard methodology by P. Trinder.[3]

Calcium binding assay

Spectrophotometer is an instrument used to measure absorbance at various wavelengths. It is similar to the absorptiometer except that it uses diffraction gratings or glass prism to produce mono chromatic light.

- In a 1 cm quartz cuvette 0.1 ml solution of Oxytetracycline (0.1mM) was mixed with 0.8 ml methanol and 0.1 ml of 0.05 Tris buffer (pH 7.4).
- The UV absorption spectrum of the solutions was obtained initially in the absence of Ca^{++}.
- Then (10 µl) of an aqueous solution containing CaCl2 was added to the solution and new absorption curves in the presence of Ca^{++}(0.1, 0.5 or 1 mM) were obtained.
- The changes in the UV profile in the range of 200-500 nm region and the shift of the maximum absorption after addition of $CaCl_2$ were recorded by using UV- spectrophotometer.
- Same procedure was done for doxycycline (0.1 mM). The UV absorption spectrum of the solutions was obtained initially in the absence of $Ca^{++}$and then in presence of different concentration of Ca^{++}(0.1, 0.5 or 1 mM).

RESULTS

Calcium estimation by Trinder's method

For estimation of calcium chelation, EDTA method was followed (P. Trinder). Different doses of oxytetracycline (25 mg, 50 mg and 100 mg) and doxycycline (25 mg, 50 mg and 100 mg) were used. The procedure was done according to the standard methodology.

Table 1: Effect of oxytetracycline and doxycycline on calcium estimation by Trinder's method.

Drug	Oxytetracycline			Doxycycline		
	OD	Ca conc. mg/dl	% Chelation	OD	Ca conc. mg/dl	% Chelation
25 mg	2.90	2.4	24	1.50	1.0	10
50 mg	3.34	2.8	28	2.24	1.7	17
100 mg	3.89	3.4	34	3.96	3.5	35

Oxytetracycline

The base line calcium concentration was found to be 10 mg/dl. On adding oxytetracycline at different doses of (25 mg, 50 mg, 100 mg) the % chelation of calcium was found to be 24 %, 28 % and 34 % respectively as compared to baseline (Table 1).

Doxycycline

Similarly, on adding doxycycline at different doses (25 mg, 50 mg, 100 mg) the % chelation of calcium was found to be 10 %, 17 % and 35 % respectively as compared to baseline (Table 1).

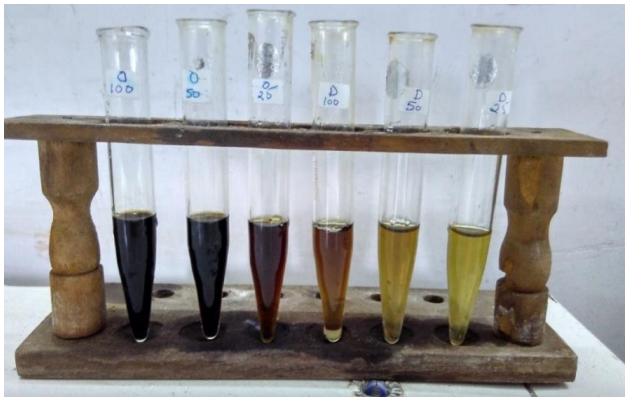

Figure 1: Effect of oxytetracycline and doxycycline on calcium estimation by Trinder's method.

The intensity of colour appear to be increased with increase in dose of the oxytetracycline (25 mg, 50 mg, 100 mg) as the concentration of calcium binding increases. But in doxycycline intensity of colour was more with 100 mg as compared with 25 mg and 50 mg as depicted in Figure 1.

Calcium chelation assay

The calcium binding activity of oxytetracycline and doxycycline was investigated and are depicted in Table 2.

Table: 2 Effect of oxytetracycline and doxycycline on calcium binding assay.

	Oxytetracycline (OD)	Doxycycline (OD)
Without calcium	1.459	0.534
0.1 mM Calcium	1.444	0.513
0.5 mM Calcium	1.369	0.533
1.0 mM Calcium	1.427	0.564

Calcium chelation assay was done using different concentration of calcium and in absence of calcium. The UV absorption spectrum of solution of oxytetracycline (1mM) was changed after the addition of $CaCl_2$ to provide different concentration of Ca^{2+} (0.1, 0.5 and 1.0 mM) (Figure 2).

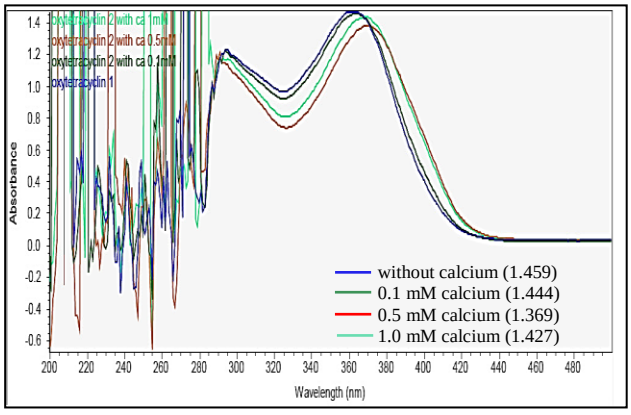

Figure 2: Ultraviolet spectrum of oxytetracycline in the absence and presence of increasing concentration of Ca++ (0.1, 0.5 and 1.0 mM).

Calcium chelation with oxytetracycline in absence of calcium was 1.459, with 0.1 mM calcium was 1.444, with 0.5 mM calcium was 1.369 and with 1.0 mM calcium was 1.427 (Figure 2). Calcium chelation with Doxycycline in absence of calcium was 0.534, with 0.1 mM calcium was 0.513, with 0.5 mM calcium was 0.533 and with 1.0 mM calcium was 0.564 (Figure 3 and 4). With minor shift in the absorption coefficient and no shift in wavelength were observed for doxycycline (Figure 3). Thus, oxytetracycline chelates more of calcium as compared to doxycycline. The onset of action of doxycycline is slow as compared to oxytetracycline.

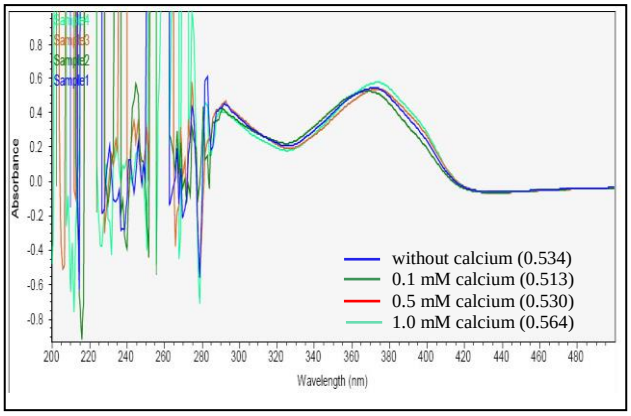

Figure 3: Ultraviolet spectrum of doxycycline in the absence and presence of increasing concentration of Ca^{2+} (0.1, 0.5 and 1.0 mM).

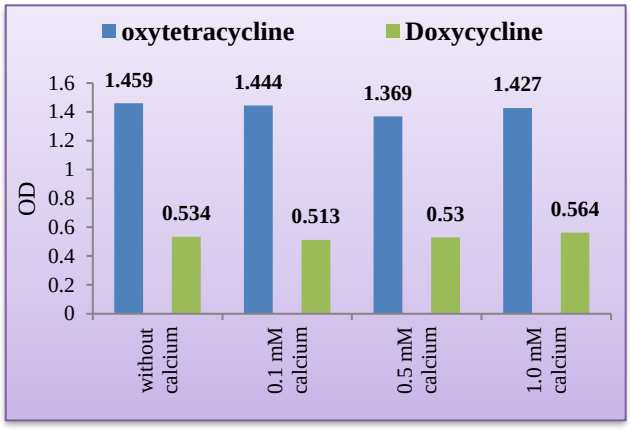

Figure 4: Effect of oxytetracycline and doxycycline on calcium binding assay.

DISCUSSION

Tetracyclines were developed as a result of the screening of soil samples for antibiotics. The first of these compounds, chlortetracycline, was introduced in 1947. Tetracyclines were found to be highly effective against various pathogens including rickettsiae, as well as both gram-positive and gram-negative bacteria, thus becoming the first class of broad-spectrum antibiotics. Many other interesting properties, unrelated to their antibiotic activity, have been identified for tetracyclines which have led to widely divergent experimental and clinical uses.[4]

Tetracyclines have a high affinity to form chelates with polyvalent metallic cations such as Fe^{+++}, Fe^{++}, Al^{+++}, Mg^{++} and Ca^{++}. Many of these tetracycline-metal complexes are either insoluble or otherwise poorly absorbable from the gastro-intestinal tract. Milk and other dairy products, antacids containing polyvalent cations, as well as various iron salts ingested simultaneously with tetracycline derivatives, might interfere with their absorption by 50 to 90% or even more.[5]

Tetracycline antibiotics in apolar solvents chelate calcium in a different conformation from that of the Mg chelate. Evidence for this different conformation is adduced from the fluorescence, absorption and circular dichroism spectra of the antibiotic bound to Ca and Mg respectively. The conformation of the antibiotic chelated to Ca is a high affinity form. Only those divalent cations of a size similar to or greater than that of Ca are able to induce this conformation.[6]

Leyden JJ in his study observed that absorption of both antibiotics was significantly decreased by administration with iron (77% inhibition with minocycline and 81% with tetracycline), milk (27% inhibition with minocycline, 65% with tetracycline), and food (13% inhibition with minocycline and 46% with tetracycline). The inhibitory effect on absorption with food and milk was significantly greater for tetracycline than for minocycline.[7]

In other study, conducted by Martin SS showed that metal ion form complexes with both the fully-deprotonated and mono-protonated forms of the tetracycline.[8]

Tetracycline antibitotics include oxytetracycline, tetracycline, chlortetracycline, minocycline and doxycycline. They differ in their pharmacokinetic profile. The older tetracyclines are incompletely absorbed from gastrointestinal tract. Doxycycline and minocycline are completely absorbed irrespective of food. Tetracyclines form complexes with calcium and other metals. Absorption of tetracycline is significantly decreased by administration with iron milk and food.[7] Tetracyclines are widely distributed in the body. The plasma protein binding of oxytetracycline is low where as that of Doxycycline is high. Most tetracyclines are excreted in urine except doxycycline, which is excreted in bile. Marked alteration of intestinal flora is seen with tetracycline as compared with doxycycline. Incidence of of photoxicity is on high side with doxycycline as compared with other tetracyclines. In study a conducted by Wessels et al the results obtained suggest that Ca^{2+} forms a 1:2 ligand: metal complex with TC.[9]

In current study, by both the methods we found that oxytetracycline chelates calcium and these findings are comparable with earlier studies. The base line calcium concentration was found to be 10 mg/dl. On adding oxytetracycline at different doses of (25 mg, 50 mg, 100 mg) the % chelation of calcium was found to be 24 %, 28 % and 34 % respectively as compared to baseline. (Table1). The UV absorption spectrum of solution of Oxytetracycline (1mM) was changed after the addition of $CaCl_2$ to provide different concentration of Ca^{2+} (0.1, 0.5 and 1.0 mM) Figure 2.

Similar findings were observed by Uris MR et al. They analysed calcium complexes of oxytetracycline by use of frog heart method with improved apparatus employing a vertical shutter, photoelectric transducer, bridge circuit, and recording system. This method was readily applicable to complex biologic solutions and produced results comparable to those in the preceding article obtained by spectrophotometry on pure solutions.[10]

Authors Button C and Mulders MS, studied the effect of oxytetracycline in propylene glycol on blood ionized calcium and plasma total calcium in sheep and found that ionized calcium concentrations in whole blood significantly depressed after oxytetracycline IV.

In the present study, by both the methods we noted that doxycycline chelates calcium and these findings are comparable. On adding doxycycline at different doses (25 mg, 50 mg, 100 mg) the % chelation of calcium was found to be 10 %, 17 % and 35 % respectively as compared to baseline (Table 1). With minor shift in the absorption coefficient and no shift in wavelength were observed for doxycycline (Figure 3).

Doxycycline has been reported to have less binding with ions such as calcium.[11] Schach von Wittenau noted that doxycycline levels 1 hour after drug administration were lower when the drug was given after full breakfast as compared to when it was given on an empty stomach.[11] Kshirsagar NA et al significant noted that there was difference in the plasma values determined 4, 6 and 8 hours after drug administration, AUC and C-max being also significantly lower after administration of doxycycline with food.[12]

In present study, we observed that oxytetracycline chelates more of calcium in comparision with doxycycline. The onset of action of doxycycline was slow as compared to oxytetracycline. The intensity of colour appear to be increased with increase in dose of the oxytetracycline (25 mg, 50 mg, 100 mg) as the concentration of calcium binding increases. But in doxycycline intensity of colour was more with 100 mg as compared with 25 mg and 50 mg as depicted in Figure 1.

CONCLUSION

This in-vitro study concludes that oxytetracycline has more calcium chelating property than doxycycline.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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