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In Vivo Investigation of the Interaction of Ampicillin (AMP), Oxytetracycline (OTC) and 2-Mercaptoimidazol (MI) with Calcium Chloride Salt

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Keywords

In Vivo, Stability Constant, Ampicillin, Oxytetracycline and 2-Mercaptoimidazol

The interaction of antibiotic Interaction of Ampicillin, Oxytetracycline and 2-Mercaptoimidazol with some Salts has been investigated by In vivo studies. It's found that there were no significant changes observed in the mean value of serum ionized calcium of the treated rats compared with the corresponding levels of control untreated rat group upon treatment with 1.4 mg/kg body weight. This is an interesting result because calcium complex ion in aqueous solutions with AMP, MI and OTC, but the same investigated compounds doesn't show significant decrease in serum ionized calcium and Sodium due to the ability of the compounds to form complexes with calcium and Sodium at physiological conditions i.e. (inside living organisms).So administration of those compounds has no side effect on serum ionized calcium on developing any hypocalcaemia related diseases.

Introduction

A stability constant (formation constant, binding constant) is an equilibrium constant for the formation of a complex in solution. It is a measure of the strength of the interaction between the reagents that come together to form the complex. There are two main kinds of complex compounds formed by the interaction of a metal ion with a ligand and supramolecular complexes [1-10], such as host-guest complexes and complexes of anions. The stability constant provide the information required to calculate the concentration of the complex in solution [1]. Oxytetracycline hydrochloride has many areas of application in chemistry, biology and medicine. It's a broad-spectrum antibiotic, active against a wide variety of bacteria. However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infections [2]. Calcium is the most abundant mineral element in the body with about 99 percent in the bones primarily as hydroxyapatite. The remaining calcium is distributed between the various tissues and the extracellular fluids where it performs a vital role for many life sustaining processes. Among the extra skeletal functions of calcium are involvement in blood coagulation, neuromuscular conduction, excitability of skeletal and cardiac muscle, enzyme activation, and the preservation of cell membrane integrity and permeability. Serum calcium levels and hence the body content are controlled by parathyroid hormone (PTH), calcitonin, and vitamin D. An imbalance in any of these modulators leads to alterations of the body and serum calcium levels. Increases in serum PTH or vitamin D are usually associated with hypercalcemia. Increased serum calcium levels may also be observed in multiple myeloma and other neoplastic diseases. Hypocalcemia may be observed e.g. in hypoparathyroidism, nephrosis, and pancreatitis [3-6]. In-vivo are those in which the effects of various biological entities are tested on whole living organisms usually animals including humans, and plants as opposed to a partial or dead organism. Effects of various biological entities are tested on whole living organisms usually animals including humans, and plants as opposed to a partial or dead organism [7]. However, in the research field it used to validate in vitro findings in vertebrates closest to humans. The most used animal models are mice, rats, and other rodents. It is

useful for the production of polyclonal antibodies applied in immunoassays and diagnostic immunology [8].

Experimental

Ampicillin (AMP), Oxytetracycline (OTC) and 2-Mercaptoimidazol (MI)were purchased from sigma Aldrich and Calcium chloride from Merck Germany .Double-distilled water was used throughout this study.

Preparation of Reagents Solutions

All experiments were performed using adult male albino rats, with an average body weight of 100 to 120 g purchased from Theodore Bilharz Research Institute, Giza, Egypt. The rats were housed in steel mesh cage and were provided with commercial standard diet and tap water. For the investigation of the effect of AMP, OTC and MI on serum calcium level, the compound was orally administered one dose for one day. At the end of treatment period, the rats deprived of food and were sacrificed by decapitation. Blood samples were collected by sacrificing the rats by decapitation under ether anesthesia. The collected blood samples were placed in dry clean centrifuge tubes and allowed to clot at room temperature for 30 minutes. Serum samples were then obtained by centrifugation at 3000 rpm for 10 minutes. These samples were kept in clean well-stopped glass vials at -20°C performing serum calcium was analyzed usually within the same day.

Results and Discussion

Spectrophotometric determination of stability constants

Job's method of continuous variation

The spectrophotometric method is especially well suited for the study of complexes not sufficiently stable to permit their isolation from solution. Work of this type has been done by Job who developed the method of continuous variation [9]. This Method makes use of any measurable additive property of two species [10, 11]. Any complex formed by the two species must give a value for the separate species. the simple application of the method involves an equilibrium of type A + nB = ABn where A represents a metal. B a coordinating group and ABn a complex. Solutions are prepared in which the mole fraction of the compounds are varied and the total number of moles of both, is kept constant.If there is no complexing the plot of extinction coefficient or absorbance against mole fraction or concentration is a straight line but if a complex is formed, the plot deviates from linearity. The Deviation is maximum at mole fraction corresponding to the composition of the complex. When the deviation is plotted against mole fraction, the maximum point gives the desired composition. The conclusion may be verified by repeating the process at other wavelengths. Since the position of the maximum is independent of wavelength.

A complex ion is formed in solution by general formula

$$M^{n+} + L \rightarrow [ML]^{n+}$$

This equilibrium can be represented as:

$$K_f = \frac{\alpha ML}{(\alpha M^{n+})(\alpha L)}$$

Where "α" is activity of each species present at equilibrium.

The activity of any compound A is the product of its concentration and an activity coefficient $\gamma_{\pm}$ as:

$$[\alpha] = [A]\gamma_{\pm}^{A}$$

For dilute solutions the above equilibrium will reduce to:

$$K_f = \frac{[ML]}{[M]^{n+}[L]}$$

If a number of ligands co-ordinate with a metal ion in successive steps are as follows:

$$M^{n+} + L \rightarrow [ML]^{n+} \qquad K_1 = \frac{[ML^{n+}]}{[M^{n+}][L]}$$

$$ML^{n+} + L \rightarrow [ML]_2^{n+} \qquad K_2 = \frac{[ML_2]}{[ML^{n+}]\,[L]}$$

And so on, the stepwise equilibrium constants K_1, K_2, ..., K_n are known as stability constants.

The overall equilibrium is as follows:

$$M^{n+} + XL \rightarrow [ML_x]^{n+}$$

And its expression can be written as:

$$B_x = \frac{[ML_x]}{[M^{n+}][L]^x}$$

B_x is known as the overall stability constant.

The stock solution of ligand ($2.5x10^{-2}$M) and metals solution ($2.5x10^{-2}$M) were prepared according to the requirement of Jobs method. In series of flasks the sum of the number of moles of ligand plus sum of the number of moles of metal were kept constant.

The stoichiometry of various complexes was determined by plotting absorbance against various 'concentration of metal to ligand. This graph was used to determine the stability constants of various complexes by using the following formula.

$$K_f = \frac{\frac{A}{A_m}}{n^n C^n \left(1-\frac{A}{A_m}\right) C^{n+1}}$$

Where *A* is absorbance at break point, $A_m$is theoretical absorbance *n* is number of coordinating ligand and *C* is concentration of metal or ligand

It is apparent from Fig.(1) at pH=2.6 formation of 1:1 M:L of calcium and OTC complex takes place. The stability of this complex were calculated and are listed in Table(1), this calcium complex show K_f value $88.88x10^{-4}$.

Table (1). *Spectrophotometric Studies of Complex in Solution (OTC) and Calcium chloride.* λ_{max}*= 360 A=1.118* A_m*=1.120*

Sr.No.	Metal ion ($1x10^2$M)ML	Ligand ($1x10^2$M)ML	Absorbance (nm) pH
1	10	0	1.098
2	9	1	1.098
3	8	2	1.103
4	7	3	1.109
5	6	4	1.113
6	5	5	1.118
7	4	6	1.115
8	3	7	1.110
9	2	8	1.105
10	1	9	1.10
11	0	10	1.09

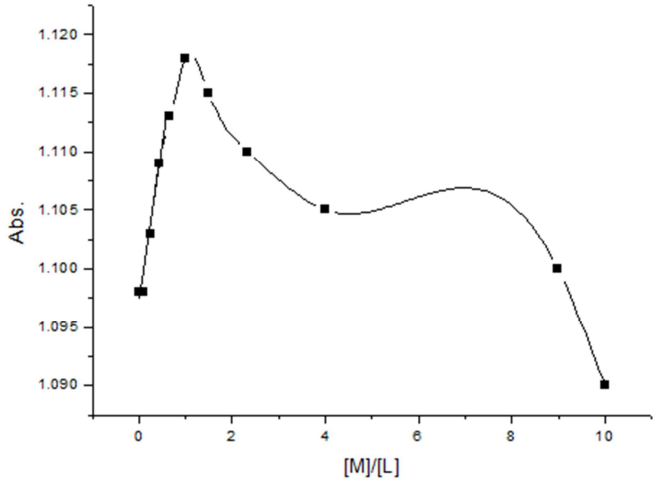

Fig. (1). *UV/Visible Spectra of OTC and Calcium Chloride solution in Methanol***.**

In order to test effect of AMP, OTC and MI on serum level of ionized calcium, 16 rats were used and the treated groups were classified as follows:

(1) Control group: Healthy rats treated with 1 ml saline using an intragastric tube.

(2) Ampicilline treated group: Healthy rats treated orally with AMP (1.4 mg/kg body weight) using an intragastric tube.

(3) 2-Mercaptoimidazole treated group: Healthy rats treated orally with MI (1.4 mg/kg body weight) using an intragastric tube.

(4) Oxytetracycline treated group: The rats were injected with OTC (1.4 mg/kg body weight) using an intragastric tube.

Serum ionized calcium was estimated in serum (table 1) by employing the method of Mclean and Hastings, 1935 [14] as adopted by Beeler and Catrou, 1983 [15] using the following formula (1).

$$\text{Ionized calcium}\,(\text{mg/dl}) = \frac{\text{SCa}\,(\text{mg/dl}) \times -\text{SPr}\,(\text{g/dl}) \times 0.33}{\text{SPr}\,(\text{g/dl}) + 6} \quad (1)$$

Where SCa = Serum calcium and SPr = Serum protein.

Table 2. *Statistical analysis of the mean serum levels of ionized calcium in normal and compounds-treated groups after oral administration of dose (1.4 mg/Kg).*

	Control	Ampicillin sodium salt	2-mercaptoimidazole	Oyxtetracycline hydrochloride
Mean	1.09	1.07	1.08	1.07
Std. Deviation	0.07	0.01	0.01	0.06
Std. Error	0.04	0.01	0.02	0.03

Table (2) illustrates that, there were no significant change observed in the mean value of serum ionized calcium of the treated rats compared with the corresponding levels of control untreated rat group upon treatment with 1.4 mg/kg body weight. This is an interesting result because calcium complex ion in aqueous solutions with AMP, OTC and MI but the same investigated compounds doesn't show significant decrease in serum ionized calcium due to the ability of the compounds to form complexes with calcium at physiological conditions i.e. (inside living organisms).So administration of those compounds has no side effect on serum ionized calcium on developing any hypocalcaemia related diseases.

Conclusion

In this paper we study the interaction of antibiotic Interaction of Ampicillin, Oxytetracycline and 2-Mercaptoimidazol with some Salts has been investigated by In Vivo Studies. It's found that there were no significant changes observed in the mean value of serum ionized calcium of the treated rats compared with the corresponding levels of control untreated rat group upon treatment with 1.4 mg/kg body weight. This is an interesting result because calcium complex ion in aqueous solutions with AMP, MI and OTC, Moreover Stability constant values are used as an eminent tool by biochemists because it helps to determine the properties of metal-ligand reactions in aqueous medium over and above the actual biological system. Extremely low stability constant values (log K_f) (ranging from negative to 1) indicate that the metal-ligand complex is not only soluble in water but also readily dissociates into metal ion and ligand. For stability constants values above 6, less metal ions are released .and these compounds are not significant in biological systems as they consume more stomach acids to dissociate the metal ion from the complex. For various drugs to remain in biologically active form, the stability constants values should be in the range of 3 to 5 [14] The stability constant values were found to be in biologically active range (log K_f less than 6) in our last works[13-37]. These stability constant values could be quite informative for a biochemist during drug-design or drug discovery, which is the major implication of present study.■

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