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Der Pharmacia Lettre, 2011, 3(3): 320-324
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Study of the effect of Ampicillin Trihydrate on protein binding of Oseltamivir Phosphate

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ABSTRACT

Protein bound drugs are pharmacokinetically inactive where unbound drugs are pharmacologically active.Highly protein bound drugs have reduced Pharmacological effect (invitro) with Albumin. Free drug concentration in the plasma is responsible for the observed pharmacological effect or therapeutic response. Oseltamivir phosphate is an antiviral drug used in the treatment and prophylaxis of both influenza virus A and influenza virus B. It is currently the drug of choice for the prevention of influenza virus infection . Invitro Protein binding study of oseltamivir phosphate was carried out for 200 mins and The percentage of bound drug was found to be 80% and percentage of free drug was found to be 20%.In co-administration of Oseltamivir phosphate with Ampicillin, The percentage of bound drug was found to be 68.6% and percentage of free drug was found to be 31.40% Increase in percentage of free drug was found to be 57%, shows beneficial drug interaction.

Keywords: Oseltamivir phosphate, Ampicillin trihydrate, protein binding, Co-administration

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INTRODUCTION

Oseltamivir phosphate is an antiviral drug used in the treatment and prophylaxis of both influenza virus A and influenza virus B. Fig 1

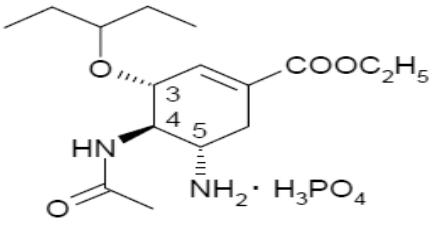


Fig 1 : Structure of Oseltamivir phosphate

Oseltamivir phosphate is readily absorbed and rapidly converted (half-life [$t_{1/2}$], 1 to 3 h) to its active carboxylate metabolite via hepatic esterases [1]. At least 75% of an oral dose reaches the systemic circulation as oseltamivir carboxylate, while less than 5% of an oral dose reaches the systemic circulation as oseltamivir phosphate. Once it is formed, oseltamivir carboxylate is minimally bound (3%) to human plasma proteins. It has a $t_{1/2}$ of 6 to 10 h and is eliminated by the kidney by a first-order process that includes glomerular filtration and tubular secretion by an anionic transporter system. [2]

Ampicillin is a beta-lactam antibiotic that has been used extensively to treat bacterial infections. [3] Fig 2.

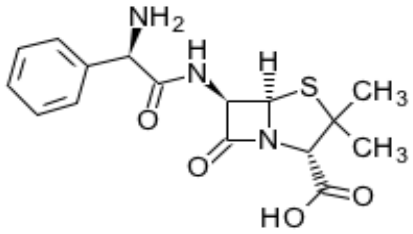


Fig 2: Structure of Ampicillin trihydrate

Co-administration of Oseltamivir phosphate with Ampicillin trihydrate shows beneficial drug interaction, thus increase bioavailability of oseltamivir phosphate.

MATERIALS AND METHODS

Equipment

Shimadzu UV Pharma Spectrophotometer 1700, Elico L1613 pH Meter Shimadzu (ELB 300) Electronic balance, Shimadzu (BL 22OH) Electronic balance

Reagents and solutions

All employed chemicals were of analytical grade and high-purified water was used throughout. Oseltamivir phosphate pure sample was obtained as a gift sample from Hetero drugs, Hyderabad, India. The gift sample of pure drug Ampicillin trihydrate was received from Parabolic drugs. Ltd.

Preparation of the standard graph of Oseltamivir Phosphate:

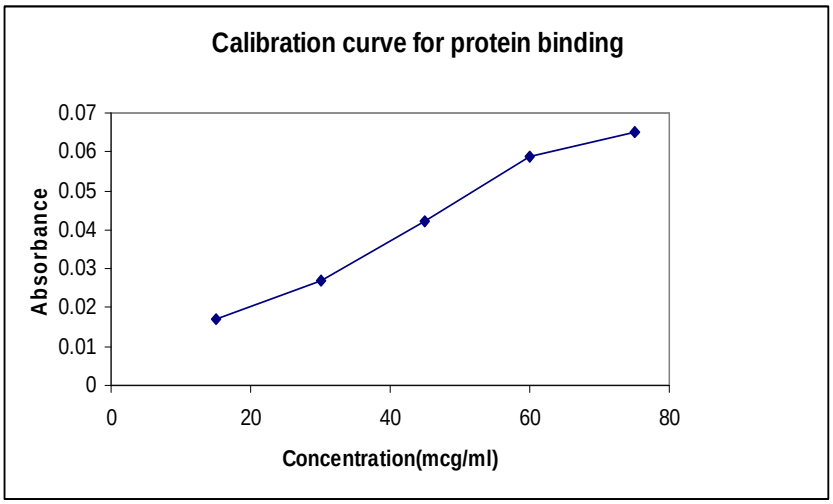
1) **Preparation of primary stock solution:**

10mg of oseltamivir phosphate taken in 100ml standard flask and make up the volume with 7.2 pH Phosphate buffer .This gives 100µg/ml.This is the primary stock solution.

2) Preparation of various concentrations of oseltamivir phosphate:

From the primary stock solution the concentrations ranging from 15 µg/ml to 75µg/ml was prepared by diluting with phosphate buffer. A Standard graph was plotted by taking Time on X-axis and Absorbance on Y-axis. Fig 3

Fig-3: Standard graph of Oseltamivir phosphate



Preparation of 2.8 X 10^{-4} M Solution of Egg Albumin

0.315 g of egg albumin flakes is dissolved in 25 ml of distilled water. It is shaken well and is kept aside.

Table-1: Protein binding studies of oseltamivir phosphate and ampicillin trihydrate

Time(mins)	Absorbance of 1.63×10^{-4} M Oseltamivir phosphate	Absorbance of the mixture of Oseltamivir phosphate and Ampicillin
0	0.019	0.004
20	0.023	0.017
40	0.042	0.023
60	0.026	0.245
80	0.022	0 .077
100	0.021	0.207
120	0.020	0.234
140	0.018	0.142
160	0.017	0.176
180	0.012	0.149
200	0.012	0.043

Studies on protein binding:

25 ml OF 1.63 X 10^{-4} M of oseltamivir phosphate is prepared using a buffer of pH 7.2. A boiling tube open on both sides is taken and a semipermeable membrane is tied on to the neck of the boiling tube. The egg albumin solution 10ml is taken inside the semipermeable membrane. The boiling tube is then immersed into the beaker containing the drug Oseltamivir phosphate 1.63 X 10^{-4} M. Immediately at zero time, 1ml of solution is pipetted out from the beaker and is replaced with 1ml of water. Readings are taken at 10, 20, 40, 60, 80, 100, 120 min and absorbance at 525 nm and is noted. Once equilibrium is reached there will be no further change in absorbance.So the constant value of absorbance is noted. From the absorbance, by using the standard graph,

determine the concentration in mcg/ml by extrapolation. From the concentration in mcg/ml determine the concentration in moles. [4] Table1

Preparation of 1.63×10^{-4} M solution of oseltamivir phosphate:
410.4 gms of drug dissolved in 1000ml of distilled water to obtain 1M .For 1.63×10^{-4} M solution 0.067gms of drug dissolved in 1000ml of distilled water.

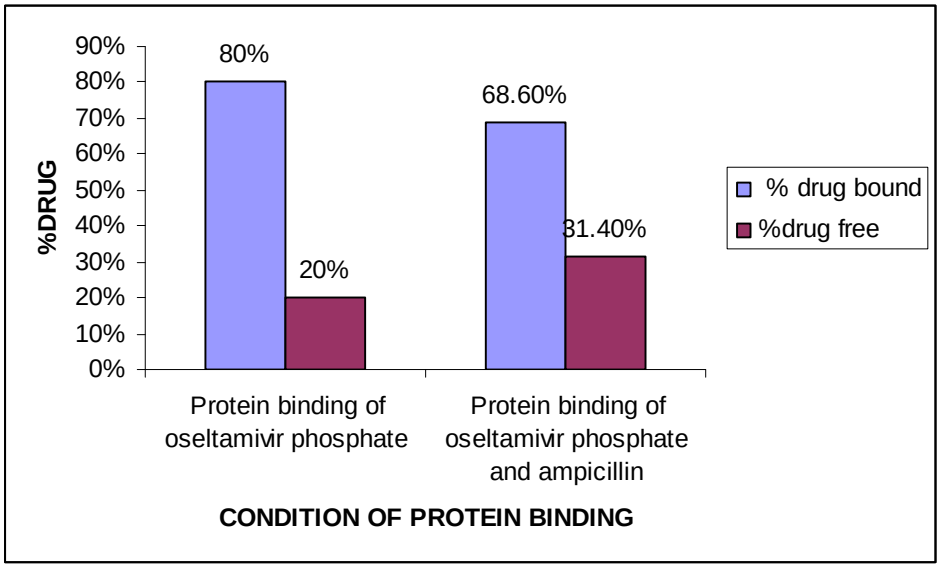
Procedure
From the prepared oseltamivir phosphate solution 15ml solution was taken in a beaker and 15ml of albumin solution in boiling test separated by membrane.1ml of drug solution was taken from beaker for every 20mins and replaced with buffer immediately.1ml of solution was diluted to 10ml of buffer and absorbance was noted until a constant value by using UV spectrophotometer.

Preparation of 32.68×10^{-4} M solution of Ampicillin trihydrate:
403.45 Gms of drug dissolved in 1000ml of distilled water to obtain 1M. For 32.68×10-4 M solution 1.3184gms of drug dissolved in 1000ml of distilled water.

Table 2: Percentage of free drug of oseltamivir phosphate in presence of ampicillin trihydrate

Drug	Protein binding of oseltamivir phosphate	Protein binding of oseltamivir phosphate and ampicillin	% increase in free drug
% drug bound	80%	68.6%	11.4%
%drug free	20%	31.4%	

Fig 4 protein binding study of oseltamivir phosphate



Procedure for preparation of mixture of Oseltamivir Phosphate and Ampicillin trihydrate:
The concentration of Oseltamivir phosphate and Ampicillin trihydrate in the mixture was chosen on the basis of the dose of Ampicillin that is co-administered with Oseltamivir Both the drug

solutions equal to 15 ml was taken into the beaker and 15ml of albumin solution in boiling test separated by membrane.1ml of drug solution was taken from beaker for every 20mins and replaced with buffer immediately.1ml of solution was diluted to 10ml of buffer and absorbance was noted until a constant value by using UV spectrophotometer.

From the protein binding studies of mixture of Oseltamivir phosphate and Ampicillin trihydrate the effect of Ampicillin trihydrate on the protein binding of Oseltamivir phosphate was determined. Table2, Fig 4

RESULTS

Protein binding study of oseltamivir phosphate was carried out for 200 mins. The percentage of bound drug was found to be 80% and percentage of free drug was found to be 20%. Protein binding of oseltamivir was determined in the presence of Ampicillin trihydrate for 200 mins. The percentage of bound was found to be 68.6%and percentage of free drug was found to be 31.40%.Thus co-administration of Oseltamivir phosphate with Ampicillin trihydrate shows 57% increase in free drug concentration.

CONCLUSION

Invitro protein binding studies of Oseltamvir phosphate shows increased bioavailability of Oseltamivir phosphate in co-administration with Ampicillin trihydrate, thus protein binding of Oseltamivir phosphate was found to be altered in presence of Ampicillin trihydrate

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