ORAL INTESTINAL PERMEABILITY AND PHARMACOKINETIC EVALUATION OF OLMESARTAN MEDOXOMIL COMPLEXES IN ALBINO MALE RABBITS

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ABSTRACT

For poorly soluble drugs, solubility is one of the rate limiting parameters to achieve their desired concentration in systemic circulation for pharmacological response. The solubility of these drugs can be solved by different technological approaches during the pharmaceutical product development such as: solid dispersion, Micronization, Salt formation, inclusion complexation etc. Olmesartan medoxomil (OLM) is an angiotensin II receptor antagonist used in the treatment of hypertension. The absolute bioavailability is about 26%, it is bound to plasma proteins to an extent of about 99%. So there is strong need to enhance aqueous solubility of hydrophobic drug by different methods like solid dispersion, inclusion complexation etc. Hence the study aimed to develop inclusion complexes of the model drug (olmesartan medoxomil) and evaluate the physicochemical parameters of the inclusion complexes, in-vitro intestinal permeability studies and in-vivo pharmacokinetic studies and revealing that naringin (P-glycoprotein inhibitor) can be used a pharmaceutical excipient for inclusion complex to increase in intestinal permeability and in-vivo pharmacokinetic performance of olmesartan. However, further studies are required for comprehensive, systematic, multi-disciplinary evaluation of various claims to make effective use of these products.

INTRODUCTION

Inclusion complex

An Inclusion complex is formed when a macrocyclic compound possessing an intramolecular cavity of molecular dimension, interacts with a small molecule that can enter the cavity.
The macrocyclic molecule is called the ‘host’ and the small included molecule is called the “guest”.

The complexes can be formed either in solution or in the solid state and water is typically the solvent of choice, inclusion complexation can be accomplished in co-solvent systems and with some non-aqueous solvents.

DRUG PROFILE
Olmesartan medoxomil (prodrug) is the latest angiotensin II receptor antagonist and P-glycoprotein substrate. Potential advantages of this drug include once-daily dosing, an absence of significant adverse reactions, a well-tolerated side-effect profile, and a cost-effective average wholesale price. Soluble in methanol, slightly soluble in ethanol, insoluble in water. Oral bioavailability average is 26% and no food interferes with absorption.

MATERIALS
CHEMICALS
1. DRUG: olmesartan medoxomil
2. POLYMERS: β-cyclodextrin
3. OTHER CHEMICALS: Naringin, HPLC grade Methanol, Double distilled water etc.,

EXPERIMENTAL ANIMALS
- Healthy, six male wistar rats weighing 200-220g, and Healthy six male albino rabbits, weighing 1.2-1.5kg were procured from the Teena Biolabs Pvt. Ltd. (Reg, no. 177/99 CPCSEA), Hyderabad, Andhra Pradesh.
- Animals were housed at CPCSEA approved animal house of Vaagdevi Institute of Pharmaceutical Sciences, (1533/PO/a/11/CPCSEA) Warangal.
- The animals were kept under standard laboratory condition (12 hr light and 12 hr dark cycle) and had free access to commercial pellet diet (Vyas labs Ltd, Hyderabad, India) with water ad libitum.
- The study was approved by the Institutional Animal Ethical Committee of University college of Pharmaceutical Sciences, dated (14/03/2012). Ethical norms were strictly followed during all experimental procedure.

METHODOLOGY
The proposed work was carried out in following stages:
A. Preformulation studies.
B. Drug-polymer inclusion complexes.
C. Characterization of prepared complexes.
D. Intestinal permeability studies using rat everted sac model.
E. Pharmacokinetic studies in rabbits.

**INTESTINAL PERMEABILITY STUDIES**

![Intestinal Permeability Studies Diagram]

**PHARMACOKINETIC EVALUATION**

- **Study Design:** crossover design with a washout period of 10 days between the doses.
- **Sample Size:** Six albino male Rabbits.
- **Route of administration:** Oral route
- **Treatment:** single dose (10mg/Kg)
  - Pure drug (Olmesartan medoxomil)
  - Solid dispersion without and with Naringin
  - Inclusion complex without and with Naringin
- **Sampling:** Blood samples will be collected pre-dose (0hr) and pre-determined post dose at various time points (0, 0.5, 1, 1.5,2,3,4,6,8 hrs) through a marginal ear vein into anticoagulant-treated polypropylene tubes after drug administration.
- Blood samples collected were centrifuged immediately at 10,500rpm to separate the plasma. The plasma samples collected were stored at –4°C.

**SAMPLE EXTRACTION**

- To 1000 μL of plasma samples in a borosilicate glass tube were added 5 mL of HPLC grade acetonitrile. After vortex mixing for 10 min at room temperature, the samples were centrifuged at 10,500 rpm for 10 min.
- The upper organic layer was transferred to a glass container and evaporated inside a vacuum oven at 40°C.
The dry residue was dissolved in 1 mL of the mobile phase. The mixture was sonicated well for 10 min, and 20 μL of this solution was injected into liquid chromatography.

**STANDARD SAMPLE PREPARATION**
- Standard samples were prepared by spiking blank plasma with known amounts of valsartan (internal standard 10 µg), olmesartan medoxomil, and range of 10-80 µg/mL used for construction of calibration curve.

**DATA ANALYSIS**

**Phase Solubility**
The solubility constant (Kc) was calculated from the slope of the linear plot of the phase solubility diagram according to equation,

\[ K = \frac{\text{slope}}{S_0(1 - \text{slope})} \]

Where, So is the solubility of the drug in absence of CD.

**Drug content**

\[ \text{Actual OLM content in weight} = \frac{\text{quantity of inclusion complex}}{\text{theoretical amount of OLM in inclusion complex}} \times 100 \]

**Calculation of the apparent permeability coefficients**
Apparent permeability coefficient (Papp) was determined according to the formula:

\[ \text{Papp} = \frac{\text{dQ/dt}}{\text{ACo}} \times \frac{1}{\text{ACo}} \]

Where Papp (cm/s) is the apparent permeability coefficient, dQ/dt (mg/s) is the amount of drug transported across the membrane per unit of time, A (cm²) is the surface area available for permeation and C0 (mg/ml) represents the initial concentration of the drug outside the everted gut sacs.

The percentage of drug recovery (R %) was calculated according to the formula:

\[ R\% = \frac{C_r \times V_r + C_d \times V_d}{C_0 \times V_r} \times 100 \]
where Cr,end and Cd,end (mg/ml) are the drug concentrations measured at the end of the experiment inside and outside the sacs, respectively; Cd,0 (mg/ml) is the initial concentration of the drug outside the everted gut sacs; Vr and Vd (ml) are the volumes of the mucosal and the serosal media, respectively.

- The percentage of drug retained (Ad%) on the intestinal tissues was determined according to the formula:

\[
Ad\% = 100 - R\%
\]

**STATISTICAL ANALYSIS**

- The pharmacokinetic parameters of the in-vivo absorption studies were computed using KINETICA software and the model was non-compartmental extravascular.
- The apparent permeability average values (P_{app}) and the in-vivo absorption studies values were compared for each sample using one way analysis of variance (ANOVA) test.
- The difference was considered significant at p ≤ 0.05.

**RESULTS AND DISCUSSION**

**Determination of Absorption maxima**

**Phase Solubility Profile of Olmesartan and β-cyclodextrin**
GIBBS FREE ENERGY TRANSFER ($\Delta G^{\circ\text{tr}}$) FOR SOLUBLIZATION PROCESS OF OLMESARTAN MEDOXOMIL IN AQUEOUS SOLUTION OF $\beta$-CYCLODEXTRIN AT 37°C

<table>
<thead>
<tr>
<th>Percentage of $\beta$-CD in water</th>
<th>$\Delta G^{\circ\text{tr}}$(KJ/MOL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>-4.367</td>
</tr>
<tr>
<td>0.4</td>
<td>-9.345</td>
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<tr>
<td>0.6</td>
<td>-12.358</td>
</tr>
<tr>
<td>1</td>
<td>-20.569</td>
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<tr>
<td>1.5</td>
<td>-32.659</td>
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<tr>
<td>2</td>
<td>-35.485</td>
</tr>
<tr>
<td>3</td>
<td>-39.378</td>
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</tbody>
</table>

Saturation solubility of Olmesartan medoxomil inclusion complexes

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>% Drug content(ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM1</td>
<td>37.28±0.836</td>
</tr>
<tr>
<td>PM2</td>
<td>62.96±0.767</td>
</tr>
<tr>
<td>PM3</td>
<td>94.67±0.878</td>
</tr>
<tr>
<td>SD1</td>
<td>59.85±0.507</td>
</tr>
<tr>
<td>SD2</td>
<td>117±0.191</td>
</tr>
<tr>
<td>SD3</td>
<td>118±0.66</td>
</tr>
<tr>
<td>IC1</td>
<td>89.56±0.67</td>
</tr>
<tr>
<td>IC2</td>
<td>99.81±0.78</td>
</tr>
<tr>
<td>IC3</td>
<td>107.11±0.89</td>
</tr>
</tbody>
</table>

XRD Diffractogram
DISSOLUTION PROFILE OF DIFFERENT COMPLEXES OF APPARENT PERMEABILITY CO-EFFICIENT OF COMPLEXE OLMESARTAN AND β-CYCLODEXTRIN

ONE WAY ANOVA INTESTINAL PERMEABILITY STUDIES DATA

HPLC CHROMATOGRAPH

HPLC CHROMATOGRAM
PHARMACOKINETIC PROFILE OF OLMESARTAN PHARMACOKINETIC PARAMETERS

ONE WAY ANOVA

CONCLUSION

The present study showed the suitability of β-cyclodextrin as a carrier to prepare Olmesartan medoxomil inclusion complexes. As demonstrated by both XRD and FTIR the amorphization of Olmesartan medoxomil offered an explanation for better dissolution rate with the enhancement of oral absorption.

It revealed that naringin (P-glycoprotein inhibitor) can be used a pharmaceutical excipient for inclusion complex to increase in intestinal permeability and in-vivo pharmacokinetic performance of olmesartan.

However, further studies are required for comprehensive, systematic, multi-disciplinary evaluation of various claims to make effective use of these products.
REFERENCES


