IN-VIVO ABSORPTION STUDY OF RISEDRONATE SODIUM DOSAGE FORMS IN RATS
Thosar Milind M¹*, Pancholi Shyam Sunder²
¹Shree Swaminarayan Pharmacy College, Kevadiya Colony, Garudeshwar, Narmada-393151, India.
²College of Pharmacy, Jazan University, Jazan-82822, Kingdom of Saudi Arabia.
Email: amthosar@gmail.com

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Abstract
Risedronate sodium is generally used clinically to treat osteoporosis and other bone diseases. On the other hand, its oral efficacy is limited due to its low absorption due to poor permeability. This study was aimed to know the enhancement in permeability of Risedronate sodium after sublingual and oral administration and the extent of absorption was determined. Animals were divided in different groups and blood samples were collected at regular interval for 8 hours. Various pharmacokinetic parameters like Cmax, Tmax and AUC were found 1300 ± 80 ng/ml, 4 hrs and 4425 ± 200.64 for multiple emulsion formulation and 920 ± 45 ng/ml, 5 hrs and 3710 ± 18 ng/ml for sublingual formulations. The enhancement in permeability of Risedronate sodium was evident from pharmacokinetic data when compared with drug solution and marketed preparation. The study suggested sublingual route and multiple emulsion formulation may be an alternative way of administration of Risedronate sodium, providing enhanced bioavailability.

Key Words: Risedronate sodium, Sublingual formulation, Multiple emulsion, Poor permeability.

Introduction:
Risedronate sodium (RIS) belongs to BCS class-III, is a well known inhibitor of osteoclastic activity and is widely used in the clinical treatment of various system metabolic bone diseases, mainly osteopenia and osteoporosis. In both animals and humans, RIS inhibits bone resorption and thereby slows down the process of bone loss and also maintains bone mass, bone microstructure and strength in the relevant anatomical sites such as the femur and vertebra. RIS works by blocking the action of the enzyme farnesyl pyrophosphate synthase (FPPS).¹ This reduces osteoclastic bone resorption via accumulation of unprenylated small GTPases within the osteoclast. However, RIS and other bisphosphonates (BPs), which have been chosen for the treatment of osteoporosis, have a number of
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limitations in administration. RIS is soluble in water, but practically insoluble in almost all types of organic solutions. Moreover, RIS is a highly ionized and very acidic molecule. Therefore, it may have a low permeation flux in oral administration. RIS has a bioavailability of less than 0.5% and its absorption rate is reduced by the co-administration of other drugs or food and due to low lipophilicity with poor permeability due to high ionization at physiological pH values. RIS can also cause esophagitis, abdominal pain, acid reflux, acute phase reactions etc. \(^2,^3\) Thus, it is necessary to develop a dosage form with permeability enhancer and/or distinctive pharmaceutical means of drug delivery which may change environment around drug moiety and increase the lipophilicity to eliminate the problems caused by oral administration of RIS and enhances the permeability of the drug. An example of the later case is the concept of multiple emulsion formulations which generally involves the entrapping of a highly hydrophilic drug in a water-in-oil-in-water (w/o/w) or a solid-in oil-in-water (s/o/w) emulsion. The drug enclosed in the inner water phase is coated with oil and then dispersed in the outer water phase. If the drug is absorbed directly as an oil droplet from the intestinal tract due to increased lipophilicity, the permeability of the drug will increase remarkably. \(^4\) In addition, sublingual route of drug administration has been proven the alternate route for drugs which are poor permeable, having food interaction and unpredictable absorption. Further sublingual route offers ease of administration to patients, relatively rapid onset of action and large contact surface contributes to rapid and extensive drug absorption. \(^5,^6\) In the present study Risedronate sodium was incorporated in w/o/w multiple emulsion as well formulated in sublingual spray and their absorption was studied to obtain various pharmacokinetic data.

Materials and Methods:

Risedronate sodium (RIS) was received as gift sample. Sublingual spray pump devices were procured from local market. All other chemicals and reagent were of AR grade. Test samples for HPLC were investigated in Molecule lab, Ahmadabad. Animal tissue experiments were carried in accordance to proposal no. PhD/13-14/22 and approved by IAEC.

In Vivo Absorption Study:

In vivo absorption study was carried out for optimised formulations of RIS along with plain drug solution and marketed formulation. Study protocol is described as under.

Experimental Animals

The study protocol was approved by the IAEC proposal no. PhD/13-14/22. The study was carried out on healthy male Wistar rats weighing 200-250 g. Rats were housed in polypropylene cages, maintained under standard condition (12 h
light/dark cycle, 25°C, 35-55 % humidity) and allowed free access to diet. The animals were fasted at least 12 h prior to dose administrations and for 4 h after dosing with free access to water.

Experimental design

Animals were divided into four groups each consisting of 3 animals. All animals were given different formulation group wise as described underneath.

1. Group I: Control group (Plain RIS solution, 5mg/kg, p.o.)
2. Group II: Optimized Multiple emulsion Formulation equivalent to RIS to 5mg/kg, p.o.)
3. Group III: Optimized sublingual spray formulation equivalent to RIS to 5mg/kg, p.o.)
4. Group IV: Marketed tablet formulation made in suspension form to RIS to 5mg/kg, p.o.)

Serial blood samples (0.5ml) were withdrawn through capillary inserted in to retro orbital plexus under mild ether anesthesia at a time interval of predose 1, 2, 4, 8, 12 and 24 h post dose.

Sample extraction procedure

Blood samples were collected in micro centrifuge tubes containing anticoagulant (1.2% w/v EDTA disodium). The samples were centrifuged at 3000 RPM at room temperature for 10 min, then 150 µl of plasma were collected and stored at -18°C until analysis. Three rats were employed for each single administration.

Preparation of standards

RIS stock solutions was prepared at a concentration of 1mg/ml in deionized water. Working standards, at concentration of 0.1, 0.5, 1.0, 2.5 and 5.0 µg/ml of RIS, were obtained from stock solutions by serial dilutions with water. Drug-free plasma samples were spiked with Stock solutions before protein denaturation.

Bio analytical Method

Chromatographic technique was used as described in earlier work. HPLC system consisted of a Series I binary gradient system, a model 525 Dual-wavelength UV detector, Hypersil C18 reverse phase column (i.d. 5mm, 4.6mm×250 mm, ODS-2) and a Hypersil guard column (5 µ, 4.6mm×10 mm) at room temperature. The mobile phase for separation of RIS in samples consisted of buffer (5mMTBABion-pair reagent, 11mM sodium phosphate and 1.5M EDTA-2Na) – methanol (88:12,v/v), adjusted to pH 6.75with 0.2 M NaOH and was pumped at a flow rate of 1.0 ml min⁻¹. The injection volume was 10 µl and the detection wavelength was 262 nm. Peak areas were used for quantitative analyses.
Pharmacokinetic data analysis

PK solver 2.0 add-in program for Microsoft Excel was used for the estimation of Pharmacokinetic parameters. Various parameters like maximum plasma concentration ($C_{\text{max}}$), time for achieving maximum plasma concentration ($T_{\text{max}}$), Area under curve [AUC]$_{0-8}$ and relative bioavailability ($F$) were determined. Each experiment was carried out in triplicate and treated statistically.

Result

RIS being a BCS-III drug having high solubility and low permeability, which often results in a low oral bioavailability of drug. RIS was formulated in w/o/w multiple emulsion to increase the lipophilicity around the drug molecule which may enhance the permeability and increase absorption through intestinal track and lymphatic uptake. On the other hand RIS was also formulated in sublingual spray since the permeability of sublingual mucosa is very high allowing permeation of poorly permeable drug. Study results obtained were presented in table 1 and graphically in figure 1.

Table 1: Pharmacokinetic parameters of RIS administered in rats.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$C_{\text{max}}$</th>
<th>$T_{\text{max}}$</th>
<th>AUC$_{0-8}$</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ml</td>
<td>hr</td>
<td>µg*hr/ml</td>
<td></td>
</tr>
<tr>
<td>Plain drug solution</td>
<td>0.42±0.02</td>
<td>8</td>
<td>1.63±0.08</td>
<td>--</td>
</tr>
<tr>
<td>Conventional formulation</td>
<td>0.46±0.023</td>
<td>6</td>
<td>1.855±0.10</td>
<td>1.13</td>
</tr>
<tr>
<td>Drug loaded W/O/W Multiple emulsion</td>
<td>1.3±0.50*</td>
<td>4</td>
<td>4.425±0.20*</td>
<td>2.71</td>
</tr>
<tr>
<td>Sublingual spray formulation</td>
<td>0.92±0.045*</td>
<td>5</td>
<td>3.71±0.18*</td>
<td>2.27</td>
</tr>
</tbody>
</table>

Value are expressed as mean ±SD; n=3, $F$–Relative bioavailability, *$p<0.05$ compared with Plain RIS solution

![Plasma concentration versus time profile](image-url)

**Fig 1.** Plasma concentration versus time profile.
Discussion

The effect of the formulation on bioavailability of RIS was determined by the total amount of drug present in the plasma after oral administration of different formulations of the drug to rats. As summarized in Table 1 and Figure 1, compared with plain drug solution and the conventional formulation (untreated drug), the multiple emulsion formulation and sublingual formulation significantly \((P<0.05)\) improved drug absorption in rats. In particular, drug loaded multiple emulsion showed 3 fold increases in the total amount of drug present in the plasma in rats compared with the plain solution of drug and conventional formulation, whereas sublingually administered drug showed increase it by 1.9-fold. This result may be explained by several assumptions. Enclosing the hydrophilic charged drug RIS in the inner oil phase could have increased the lipophilicity there by increasing the permeation across gastric lumen in addition slow release of drug from the oil globules might have allowed prolong absorption of the drug. Increase in uptake of sublingually administered drug could be due to high permeability of that region and avoidance of the food interaction. Longer residence of the spray droplets as result of the addition of spreading agent could have increased the permeation of the drug.

Conclusion

In this study, two different formulations of Risedronate sodium were developed with promising drug delivery properties. The drug loaded multiple emulsion and sublingual formulations showed good in vivo absorption in rats compared to plain and conventional formulation. Therefore, both formulations appear to have the potential to improve the mucosal permeability of Risedronate sodium.

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References


Corresponding Author:
Thosar Milind M*,
Email: amthosar@gmail.com