PHARMACOKINETIC INTERACTION OF MOXIFLOXACIN AND MELOXICAM FOLLOWING INTRAMUSCULAR ADMINISTRATION IN RATS

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ABSTRACT
Pharmacokinetics of Moxifloxacin following intramuscular administration of Moxifloxacin (5 mg/kg) alone and in Meloxicam-treated (0.5 mg/kg, Intramuscular) wistar rats was evaluated. The plasma drug concentration of Moxifloxacin was assayed by HPLC. The mean peak plasma concentration ($C_{\text{max}}$) of Moxifloxacin following its administration as single drug and in combination with Meloxicam in male rats were $301.41 \pm 25.07$ and $375.72 \pm 44.97$ ng/ml respectively, which was observed at 1 h. while in female rats mean $C_{\text{max}}$ of Moxifloxacin were $251.43 \pm 46.36$ and $152.95 \pm 4.45$ ng/ml respectively, which was observed at 0.67 and 1 h respectively. Following intramuscular administration of Moxifloxacin (5.0 mg/kg) as single drug and in combination with Meloxicam (0.5 mg/kg) in male wistar rats the mean values of half-life ($t_{\frac{1}{2}}$), volume of distribution ($V_z$), clearance (Cl) and area under plasma drug concentration-time curve ($AUC_{(0-\infty)}$) of Moxifloxacin were $2.27 \pm 0.14$ and $1.98 \pm 0.31$ hr, $11051.18 \pm 530.11$ and $9728.50 \pm 1533.39$ ml, $3442.16 \pm 255.67$ and $3632.24 \pm 700.00$ ml/hr and $1496.67 \pm 120.04$ and $1575.18 \pm 222.45$ hr.ng/ml, respectively. Whereas in female rats mean values of Moxifloxacin were $1.95 \pm 0.22$ and $3.56 \pm 0.95$ hr, $19526.48 \pm 3171.76$ and $36868.52 \pm 6032.37$ ml, $6005.43 \pm 1134.19$ and $1575.18 \pm 222.45$ hr.ng/ml, respectively. The respective values were not significantly altered in Moxifloxacin alone and Meloxicam-treated female rats compared to male rats. Volume of distribution ($V_z$) of Moxifloxacin was significantly higher ($P < 0.05$) in female rats following concurrent intramuscular administration of Moxifloxacin and Meloxicam. It was concluded that concomitant administration of Meloxicam alter the disposition of Moxifloxacin in female rats.

Key words: Pharmacokinetics, Moxifloxacin, Meloxicam, wistar rats, interaction
INTRODUCTION

Moxifloxacin is a novel fourth generation fluoroquinolone with a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, anaerobes and atypical organisms such as Mycoplasma and Chlamydia spp. [1] It has the highest potency against Staphylococcus aureus and Staphylococcus epidermidis. The drug thus seems to be extremely useful in a variety of infections including those of urinary tract, respiratory tract, soft tissues, bones and joints. Meloxicam, a new anti-inflammatory drug is a member of the oxicam family of NSAIDs. [2] The advantage of Meloxicam over the traditional NSAIDs is that it has greater in vitro and in vivo inhibitory action against the inducible COX–2 isoform, which is implicated in the inflammatory response.

Pharmacokinetic interaction is result of alterations of drug absorption, distribution, metabolism and elimination in combination therapy. The study was planned to determine effect of co-administered Meloxicam on pharmacokinetic of Moxifloxacin following intramuscular administration in male and female rats.

MATERIAL AND METHODS

Animals

The present study was conducted on 24 adult healthy male and female wistar rats. The rats were procured from Animal Research Facility, Zydus Research Centre (ZRC) and housed in individually ventilated polysulphone cages (IVC) under standard laboratory conditions at experimental animal room at Animal Research Facility, ZRC, Ahmedabad. The full system was kept in environmentally controlled room with 22 ± 3 °C temperature and 30-70% humidity. Light/dark cycles of 12/12 hours were provided throughout the study period. Rats of 6-8 weeks age were selected after physical and behavioral examination. Nulliparous and non pregnant female rats were used in the present experiment. The live body weight range was within ± 20 % of the mean body weight for each sex at the time of randomization. Food and water were provided ad libitum. The experimental protocol for general procedures and use of animals was approved by the Institutional Animal Ethics Committee (IAEC). The rats were kept under constant observation for 7 days before commencement of experiment. All necessary managerial procedures were adopted to keep the rats free from stress.
Drugs and Chemicals

Moxifloxacin and Meloxicam pure base powder were obtained from Ms. Zydus Research Centre, Ahmedabad. Acetonitrile of HPLC grade was purchased from Merck India Ltd., Mumbai. Methanol was procured from SD Fine Chemical Ltd., Mumbai. Dimethyl sulphoxide (DMSO) and Twin-80 were procured from Qualigens fine chemicals, Mumbai.

Experimental Design and Drug administration

All animals of either sex were divided into two groups. Animals (6 male and 6 female) of group I and group II were treated with Moxifloxacin alone (5 mg/kg, intramuscular) and Moxifloxacin along with Meloxicam (0.5 mg/kg, intramuscular), respectively. Moxifloxacin and Meloxicam were formulated for dose administration in vehicle 5% twin-80, 5% DMSO (dimethyl sulphoxide) in milli-Q water (w/v). Doses were calculated according to body weight of animals and administrated as per concentration strength of formulation. The drugs were administered by deep intramuscular injection using sterile 1 ml syringe and needle (26 G, 0.45mm x 13mm) at gluteal muscle.

Blood samples (0.25 ml) were collected from retro-orbital plexus using ether anesthesia in heparinized centrifuge tube (0.5 ml capacity) at 0 minute (before drug administration), 10, 20, 40 minutes, 1, 2, 4, 6, 8, 12, 18, 24, 30 and 48 hours after intramuscular administration of drugs. The plasma was separated by centrifugation at 6000 rpm for 6 minutes at ambient temperature and stored in labeled cryovials at -70°C in deep freezer until assay of drug.

Moxifloxacin assay

One hundred microlitre of the plasma samples were transferred in 2 ml micro centrifuge tube. Ten microlitre of working solution of internal standard was added to each sample. The contents were mixed by vortexing for 30 sec. 1 ml of Acetonitrile was added to each prepared plasma sample. The contents were vortexed for 1 minute. Samples were centrifuged at 10000 rpm for 5 min. Supernatant was transferred in drying tubes and dried under steam of nitrogen at 40°C and 15 PSI pressure in evaporator for 20 min. Dried sample were reconstituted in 100 microlitre of diluents and transferred it in HPLC vials for HPLC analysis. 50 microlitre was injected using auto sampler in HPLC system (LC-2010C, Shimadzu Corporation, Japan). The temperature of auto sampler was kept at 10°C. The
methodology was validated by spiking rat plasma samples with known amounts of Moxifloxacin. The plasma drug concentration of Moxifloxacin was determined using the conditions described below.

RP- C\textsubscript{18} (250 x 4.6 mm, 5\textmu m, Phenomenex) column was used for separation of compounds at ambient temperature (25±5°C) using column oven. Moxifloxacin eluted at 7.76 min, Meloxicam at 12.6 min and Levofloxacin at 6.66 min, with a total run time of 18 min. The mobile phase consisted of 0.05 % Trifluoroacetic acid in water (solution A): 0.05 % Trifluoroacetic acid in ACN (solution B) (v/v). Above solutions were used to make mobile phase using gradient system with different ratio of solution A and B programmed at time 0.01 (85:15), 1:00 (85:15), 10:00 (30:70), 14:00 (30:70), 14.50 (85:15) and 18:00 (85:15) minutes respectively. The mobile phase was pumped at the flow rate of 1.1 ml/min. Wave length were used 296 nm for Moxifloxacin and Levofloxacin where as 355 nm for Meloxicam.

Accurately weighed 1 mg of Moxifloxacin pure API grade powder was dissolved in diluents (acetonitrile: methanol: water in the ratio of 40:40:20) to make concentration of 100 \( \mu \)g/ml (Stock solution A). Similarly, 1.0 mg of Levofloxacin was dissolved in the diluents to make concentration of 100 \( \mu \)g/ml (Stock solution B). Working solutions for calibration standards and quality control samples of Moxifloxacin were prepared from stock solution A in the range of 250 to 50,000 ng/ml. Working Solution of Internal Standard was prepared by taking 1 ml of stock solutions B and add 10 ml diluent to get concentration of 10 \( \mu \)g/ml. Calibration samples consisted of 8 different concentrations of Moxifloxacin over the range of 250 to 50,000 ng/ml in diluents and plasma. The low, medium and high quality control samples (750, 7500, 35000 ng/ml) were prepared independently in diluent and rat plasma. All QC samples in plasma were treated similar to method described above.

**Pharmacokinetic analysis:**

Various pharmacokinetic parameters like absorption, distribution, elimination half-life, apparent volume of distribution and total body clearance were determined using WinNonlin software (version 5.2, Pharsight Corporation, USA), using non compartmental analysis.

**RESULTS AND DISCUSSION**
The mean recovery of Moxifloxacin and Meloxicam from plasma was 85.14\% and 83.54\% at 25 ng/ml. The sensitivity of Moxifloxacin and Meloxicam assay was 25 ng/ml. The assay was sensitive, reproducible and linearity was observed from 25 to 5000 ng/ml. The mean correlation coefficient ($r^2$) of Moxifloxacin and Meloxicam was 0.9992 and 0.9995. The lower limit of quantitation (LLOQ) was 25 ng/ml. Intra-day and inter-day precision (±15\%) and accuracy (±10\%) were within standard limits. Validation parameters indicated that the method was reliable, reproducible and accurate.

Following intramuscular administration of Moxifloxacin alone and in Meloxicam-treated male rats, the mean observed peak plasma Moxifloxacin concentration ($C_{\text{max}}$) were $301.41 \pm 25.07$ and $375.72 \pm 44.97$ ng/ml, respectively observed at 1 h ($T_{\text{max}}$). Where as in female rats, respective mean values were $251.43 \pm 46.36$ and $152.95 \pm 4.45$ ng/ml respectively, which was observed at 0.67 and 1 h respectively ($T_{\text{max}}$). Following intramuscular administration of Moxifloxacin alone and in Meloxicam-treated male and female rats, peak plasma drug concentration was not significantly altered in female rats compared to male rats. Plasma concentration-time profile of Moxifloxacin in male and female rats of both treatment groups are illustrated in Figure 1 and Figure 2, respectively.

**Figure 1.**
Moxifloxacin concentration following intramuscular administration of Moxifloxacin alone (5 mg/kg) and in combination with Meloxicam (0.5 mg/kg) in male wistar rats (n=6). Each point represents mean ± SEM.
Following intramuscular administration of Moxifloxacin (5.0 mg/kg) as single drug and in combination with Meloxicam (0.5 mg/kg) in male and female wistar rats the mean values of pharmacokinetic parameters of Moxifloxacin rats are summarized in Table 1.

**TABLE 1 PHARMACOKINETIC PARAMETERS OF MOXIFLOXACIN IN MALE AND FEMALE RATS (MEAN ± SEM)**

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Unit</th>
<th>Moxifloxacin</th>
<th>Moxifloxacin + Meloxicam-</th>
<th>Moxifloxacin</th>
<th>Moxifloxacin + Meloxicam-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male (n=6)</td>
<td>Female (n=6)</td>
<td>Male (n=6)</td>
<td>Female (n=6)</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>hr</td>
<td>2.27 ± 0.14</td>
<td>1.95 ± 0.22</td>
<td>1.98 ± 0.31</td>
<td>3.56 ± 0.95</td>
</tr>
<tr>
<td>Kel</td>
<td>hr⁻¹</td>
<td>0.31 ± 0.02</td>
<td>0.35 ± 0.07</td>
<td>0.39 ± 0.05</td>
<td>0.20 ± 0.05</td>
</tr>
<tr>
<td>$AUC (0-∞)$</td>
<td>hr.ng/ml</td>
<td>1496.67 ± 120.04</td>
<td>1076.79 ± 282.55</td>
<td>1575.18 ± 222.45</td>
<td>1028.86 ± 218.83</td>
</tr>
<tr>
<td>$V_z$</td>
<td>ml</td>
<td>11051.18 ± 530.11</td>
<td>19526.48 ± 3171.76 *</td>
<td>9728.50 ± 1533.39</td>
<td>36868.52 ± 6032.37 *</td>
</tr>
<tr>
<td>CL</td>
<td>ml/hr</td>
<td>3442.16 ± 255.67</td>
<td>6005.43 ± 1134.19</td>
<td>3632.24 ± 700.00</td>
<td>5974.10 ± 1108.59</td>
</tr>
</tbody>
</table>

* Significant at $p < 0.05$
Following intramuscular administration of Moxifloxacin with Meloxicam-treated female rats, the mean volume of distribution (Vz) was significantly higher (p<0.05) compared to female rats given Moxifloxacin alone. Results of our study were supported by report of change in pharmacokinetic of tetracycline following concomitant administration with Naproxen and Diclofenac in rats. Total clearance of tetracycline was decreased and half life \(t_{1/2}\) and AUC were increased in the presence of Naproxen and Diclofenac.\(^3\) Moreover, co administration of Pepto-Bismol (50 mg/kg of bismuth Subsalicylate) and Maalox (50 mg/kg of aluminum hydroxide and 50 mg/kg of magnesium hydroxide) also altered pharmacokinetic of Moxifloxacin in rats.\(^4\) In contrast, non significant change in pharmacokinetic of Enrofloxacin following its co administration with Diclofenac in buffalo and cow calves was reported.\(^5,6\) Similarly no alteration in pharmacokinetic of Tolfnamic acid was found following its co administration with Marbofloxacin in goat and calves.\(^7\)

**CONCLUSION**

In conclusions, administration of Meloxicam caused alteration in pharmacokinetics of Moxifloxacin in rats. It would be prudent to raise the awareness about the potential drug-drug interaction between Moxifloxacin and Meloxicam.

**REFERENCES**

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