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Impact on pharmacokinetics of erlotinib by meloxicam and metformin administration in SCID mice

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Abstract
The study was carried out to evaluate the impact of meloxicam and metformin administration on pharmacokinetics of erlotinib in SCID mice. The plasma concentration of erlotinib was assayed by LC-MS/MS. Following oral administration of erlotinib as single drug in male SCID mice, the mean values of peak plasma drug concentration (Cmax), time of maximum concentration (Tmax), area under plasma drug concentration-time curve ($AUC_{0-\infty}$), volume of distribution (Vz), half-life (t½) and total body clearance (Cl) were 17272.59 ± 640.80 ng/ml, 0.29 ± 0.09 h, 84784.69 ± 3209.24 ng.h/ml, 3083.37 ± 247.42 ml, 6.08 ± 0.59 h and 356.48 ± 14.03 ml/hr, respectively. Significant alteration in area under curve and total body clearance of erlotinib were observed when administered along with metformin as well as meloxicam and metformin in SCID mice. Thus, concomitant administration of these drugs for prolong period requires close therapeutic monitoring for potential pharmacokinetic drug interaction. However, effect of long term administration of metformin and meloxicam on pharmacokinetics of erlotinib in mice needs to be evaluated.

Keywords: Pharmacokinetic, erlotinib, meloxicam, metformin, SCID mice

1. Introduction
Several malignancies in human and animals are associated with aberrant or over expressed epidermal growth factor receptor (EGFR) and HER-1/ErbB1 [1]. EGFR tyrosine kinase serves as a potential target for therapeutic intervention in tumors including ovarian, head and neck, breast, bladder, lung and other squamous cell carcinomas [2]. COX-2 is also an important enzyme in carcinogenesis and expression of COX-2 is up-regulated in cancer [3, 4]. COX-2 inhibitors exhibit anticancer effect by induction of apoptosis, inhibition of angiogenesis, and decreased invasiveness and metastatic potential. COX-2 inhibitors have been reported to have chemo-preventive role in cancers and also for treatment of non-small cell lung cancer (NSCLC) [5-8]. Therefore, a combination of EGFR (i.e. erlotinib) and COX-2 inhibitors (meloxicam) may be a reasonable combination for the chemoprevention.
A combination of anti-diabetic drug (i.e. metformin) along with chemotherapeutic drugs is routinely used to alleviate suffering in diabetic patients having cancer. Drug that activates the AMP activated protein kinase (AMPK) pathway or inhibit m TOR, a major sensor of the energetic status of the cell, which has been proposed as a promising therapeutic agent as it lead to improve in survival rate of cancer patients [9-11]. Diabetic patients with lung cancer who are previously exposed to metformin are less likely to present with metastatic disease and may survive longer [12-14].
Erlotinib is a quinazolinamine, potent selective inhibitor of the epidermal growth factor receptor tyrosine kinase used for the treatment of patients with metastatic non–small cell lung cancer [15]. Erlotinib prevents phosphorylation of the receptors and the subsequent cascade of signaling events [16]. Pharmacokinetic interaction of erlotinib with meloxicam and metformin has not been evaluated so far. The severe combined immunodeficient (SCID) mice is considered one of the worthy laboratory animal models used for the research in the field of oncology. Therefore, the present study was carried out to evaluate the impact of administration of meloxicam and metformin on pharmacokinetic profile of erlotinib in male SCID mice.

2. Materials and Methods
2.1 Experimental animals and conditions
The present study was conducted in adult healthy SCID male mice. Total of 96 SCID male mice of 5-8 weeks of age were obtained from in-house breeding stock as well as maintained at

Animal Research Facility, Zydus Research Centre, Cadila Healthcare Ltd, Moraiya, Ahmedabad, India. All mice were housed in groups of 3 mice in sterilized solid floor conventional polypropylene cages in aseptic environment with animal isolators throughout the study period. Autoclaved corncob was used as bedding material (changed twice a week), which was analyzed for pesticide and microbial. The temperature and humidity in the experimental room were 22 ± 3 ºC and 30-70%, respectively. The light: dark cycle of 12:12 hours throughout the study period was followed. The experimental animals were provided with gamma irradiated standard laboratory animal diet (Teklad 18, Rodent Pellet feed, Harlan laboratories, USA) and purified autoclaved drinking water *ad libitum*. All necessary standard husbandry procedures were adopted to keep the mice free from stress. All animals were acclimatized to experimental conditions for seven days before grouping and dosing. The experimental protocol for general procedures and use of animals for conducting this study was approved by the Institutional Animal Ethics Committee (IAEC).

2.2 Randomization and grouping of animals

Total 96 SCID male mice were randomly assigned to four treatment groups as shown in Table 1. Each group consisting of total 24 mice, which were further randomly divided into 4 sub-groups (A, B, C and D) of 6 animals each. Total twelve blood sampling time points were distributed among the 4 sub-groups of 6 animals each (each animal/sub group was not bled more than three time points). The same procedure of blood collection was repeated for other sub group of each treatment groups. The single time point concentration data set was made by clubbing and sequential arranging of all samples as per time points from different sub group.

2.3 Drugs and chemicals

Pure powders of erlotinib, meloxicam, metformin and Bisoprolol were obtained from M/s. Zydus Research Centre, Ahmedabad. Acetonitrile of HPLC grade was purchased from Merck India Ltd., Mumbai. Methanol, Ammonium For mate and Formic acid were procured from SD Fine Chemical Ltd., Mumbai. Twin-80 (polysorbate) was procured from Merck, Germany and hydroxypropyl methylcellulose (HPMC) was procured from Colorcon Asia Pvt. Ltd.

2.4 Administration of drugs and sampling

Erlotinib, meloxicam and metformin were formulated in Tween 80 and in 2% hydroxypropyl methylcellulose (HPMC). The concentration strength of drug was adjusted with 2% HPMC by keeping Tween 80 concentration at 0.25% in final formulation. Erlotinib (30 mg/kg) and metformin (100 mg/kg) were administered by oral route using oral gavage needle, whereas meloxicam (20 mg/kg) was administered by intra peritoneal route with sterile 1ml tuberculin syringe and needle of 26G (0.45mm x 13mm). Blood samples (0.25 ml) were collected from retro-orbital plexus under light anesthesia in 0.5 ml capacity centrifuge tube at 5, 10, 20, 40 minutes, 1, 2, 4, 6, 8, 12, 24 and 48 hours after administration of drug and/or combination of drugs. From each sub group, blood samples at 3 time points were collected. Blood samples were collected from all animals in heparinized tubes and centrifuged at 5000 rpm for 10 minutes to obtain plasma. Separated plasma was transferred to labeled cryovials and stored at - 70 °C until analysis of drug.

2.5 Erlotinib Assay

The plasma samples (150 µL) were transferred in 2 ml micro-centrifuge tube to which 5 µL of working solution (10µg/ml) of Bisoprolol as an internal standard was added. The contents were mixed by vortexing for 30 seconds. Each sample was added with 800 µL of acetonitrile for plasma protein precipitation and vortexed for 1 minute. Samples were centrifuged at 10,000 rpm for 5 minutes. The supernatant was transferred in HPLC vials and 5 µL was injected using auto sampler (SIL-HTc Auto sampler, Shimadzu, Japan) in LC system (Shimadzu Corporation, Koyoto, Japan) with MS-Ms (API 3200 LC-MS/Ms system, PE SCIEX, Canada). The temperature of auto sampler was 10°C. YMC CN (150 mm x 4.6 mm), 5µm column was used for the separation of erlotinib at ambient temperature (25±5 °C). Erlotinib eluted at 3.11 ± 0.5 minutes and Bisoprolol at 2.75 ± 0.5 minutes with a total run time of 6.2 minutes. Ions were generated using electro spray ionization and detected in the positive-ion mode. The mobile phase consisted 5mM Ammonium for mate + 0.13% Formic acid in water (Solution A) and Acetonitrile (Solution B). Both solutions were used to make the mobile phase using gradient system with different ratio of solution A and B at time 0.01 (45:55), 3 (20:80), 4 (20:80) and 4.5 (45:55) minutes. The mobile phase was pumped at the flow rate of 1.0 ml/min (LC-20-AD, Prominence, Pump, Shimadzu, Japan). Ion spray voltage and temperature in detector were 4500 V and 550 ˚C, respectively. Dwell time was 300 Seconds. Dec lustering, entrance, collision cell entrance and collision cell exit potentials were 70, 12, 20.77 and 1 V, respectively.

The methodology was validated by spiking the mice plasma samples with known amounts of erlotinib. Accurately weighed 10 mg of erlotinib pure API grade powder was dissolved in diluent (methanol: water in the ratio of 80:20) to make the concentration of 1,000 µg/ml (Stock solution A). Similarly, 1.0 mg of Bisoprolol was dissolved in the diluents to make the concentration of 10 µg/ml (Stock solution B). Working solutions for calibration standards and quality control (QC) samples of erlotinib were prepared from the stock solution A in the range of 1000 to 80,000 ng/ml. Working solution of internal standard was prepared by diluting 0.5 ml of stock solution B up to 10.0 ml with diluent in volumetric flask to get final concentration of 0.5 µg/ml. Calibration samples consisted of eight different concentrations of erlotinib over the range of 1000 to 80,000 ng/ml in the diluents and plasma. The low, medium and high quality control samples (1500, 8000, 80,000 ng/ml) were prepared independently in diluent and plasma. All QC samples in the plasma were treated similar to the method described above. The mean recovery of erlotinib from the plasma was ≥ 80% at 100 ng/ml (LLOQ). The linearity was observed from 1000 to 80,000 ng/ml with mean correlation coefficient (R^2) of 0.9998. 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.

2.6 Pharmacokinetic and statistical analysis

Various pharmacokinetic parameters were determined using Win Nonlin software (Version 5.2.1, Pharsight Corporation, USA) by non-compartmental analysis. The data obtained for pharmacokinetic parameters were presented as Mean ± SEM and analyzed statistically using unpaired two tail `t` test. Where $p \leq 0.05$ was considered as statistically significant and $p \leq 0.01$ was considered as statistically highly significant.

3. Results and Discussion

Co-administration of several drugs may result in unpredictable therapeutic outcome (diminished/synergistic therapeutic efficacy or increased/decreased toxicity of one or more of the administered drugs). Use of non-steroidal anti-inflammatory drugs (NSAIDs) are frequently recommended with antineoplastic agents for the treatment of various carcinogenic condition accompanied by pain and other inflammatory conditions. The carcinogenic conditions are mainly observed at old ages, where there are more chances of having metabolic disorders like diabetes. In such condition, there are always chances of combinational drug therapy like EGFR inhibitors, COX-2 inhibitors as well as m TOR inhibitors for different perspective of treatment. Based on this consideration, the study was planned to evaluate impact of meloxicam and metformin administration on pharmacokinetics of erlotinib in SCID mice, a laboratory animal model to study the anticancer activity of novel compound.

In the present study, the erlotinib in plasma was detected in samples collected up to 24 h in animals of all groups. Semi logarithmic plot of plasma erlotinib concentration following single dose administration of erlotinib alone, in combination with meloxicam, in combination with metformin and in combination with meloxicam and metformin is depicted in Figure 1. Various pharmacokinetic parameters calculated from plasma concentration–time profile after single dose administration of erlotinib alone and in combination with meloxicam and/or metformin in male SCID mice are presented in Table 2.

Following oral administration of erlotinib as single drug in male SCID mice, the mean values of observed peak plasma drug concentration (Cmax), time of maximum concentration (Tmax), area under plasma drug concentration-time curve ($AUC_{(0-\infty)}$, volume of distribution (Vz), half-life (t½) and total body clearance (Cl) were 17272.59 ± 640.80 ng/ml, 0.29 ± 0.09 h, 84784.69 ± 3209.24 ng.h/ml, 3083.37 ± 247.42 ml, 6.08 ± 0.59 h and 356.48 ± 14.03 ml/hr, respectively. Non-significant difference in mean peak plasma concentrations (C_{max}) of erlotinib following its oral administration alone and in combination with meloxicam and/ or metformin was observed in SCID male mice. The mean peak plasma value of erlotinib was observed considerably high in combination with meloxicam and lower in combination with metformin as well as both in comparison to alone. The plasma concentration of erlotinib with metformin and in combination with meloxicam and metformin both were significantly low ($P < 0.05$) than erlotinib alone treatment group at 0.33 h and 4 h post administration of drug. The lower plasma concentration of drug in the present study might be due to increase in elimination of the drug in presence of metformin which increases the micro-vascular blood flow and ultimately enhances the micro-circulation towards kidney, which may leads to fastened the clearance of the drug [17].

The value of $AUC_{(0 - \infty)}$ was significantly lower in animals treated with erlotinib and metformin ($p<0.05$) as well as erlotinib with both meloxicam and metformin ($p<0.01$) which indicates that less area of body is covered with erlotinib concentration when given in combination with metformin and all three drugs together. This significant change in $AUC_{0-\infty}$ of erlotinib can be correlate with other pharmacokinetic parameter like C_{max}, volume of distribution and clearance. Significant reduction in $AUC_{0-\infty}$ of erlotinib following co-administration of erlotinib with temozolomide (12.00 ± 4.00) compared to erlotinib alone (22.30 ± 11.00) has also been reported in human [18].

The total body clearance of erlotinib was also significantly higher in animals treated with erlotinib along with metformin ($p<0.01$) and all three drugs together ($p<0.01$) compared to erlotinib alone treated animals. Higher values of clearance of erlotinib were observed in animals treated with metformin and with both meloxicam and metformin in present study suggest that drug is rapidly eliminated from body. The alteration in pharmacokinetic parameters of erlotinib following administration along with meloxicam may be due to decrease in renal blood flow to kidney and also due to reduction in glomerular filtration rate (GFR) by meloxicam [19]. However, similar alterations have not been observed with animals treated with erlotinib along with meloxicam and metformin which may be due to improved micro-circulation to kidney by metformin (17). This hypothesis is additionally also supported activate AMPK by metformin and the subsequent up-regulation of small heterodimer partner (SHP) [20]. SHP suppresses the functions of several nuclear receptors involved in the regulation of hepatic metabolism, including pregnane X receptor (PXR), which is referred to as a ‘‘master regulator’’ of drug/xenobiotic metabolism.

Table 1: Experimental outline showing animal number and data set number for various treatment groups.

Group No.	Treatment Group	Cage No.	Animal No.	Data Set No.
I	Erlotinib	1-8	1-24	1-6
II	Erlotinib + Meloxicam	9-16	25-48	7-12
III	Erlotinib + Metformin	17-24	49-72	13-18
IV	Erlotinib + Meloxicam + Metformin	25-32	73-96	19-24

Final data set was prepared for six animals by pooling data of sub groups.

Table 2: Pharmacokinetic (PK) parameters of erlotinib following single dose administration of erlotinib (30 mg/kg, p.o) and in combination with meloxicam (20 mg/kg, i.p) and/or metformin (100 mg/kg, p.o) in male SCID mice

PK parameters	ERT	ERT+ MEX	ERT+ MET	ERT+ MEX+ MET
T_{max} (h)	0.29 ±0.09	0.21±0.04	0.27±0.09	0.21±0.04
C_{max} (ng/ml)	17272.59±640.80	19865.31± 2154.82	13840.11±797.51	15080.29±828.43
$T_{1/2}$ (h)	6.08±0.59	8.24±1.48	4.79±0.71	6.63±0.72
$AUC_{(0-\infty)}$ (ng.h/ml)	84784.69±3209.24	91056.92±5850.21	66493.10±3418.82*	62452.70±2400.15**
Vz (ml)	3083.37± 247.42	3865.44±584.29	3052.14±279.98	4559.44±446.48
Cl (ml/h)	356.48±14.03	335.50±18.90	456.74±21.69**	483.92±18.55**
MRT (h)	5.14±0.36	6.12±0.56	4.63±0.53	5.16±0.38

*significant at p<0.05, **significant at p<0.01 (ERT: Erlotinib; MEX: Meloxicam; MET: Metformin; T_{max}: Time of maximum drug concentration; C_{max}: Maximum drug concentration; $T_{1/2}$: Elimination half-life; $AUC_{(0-\infty)}$: Area under the curve from zero to infinity; Vz: Volume of distribution; Cl: Total body clearance; MRT: Mean residence time)

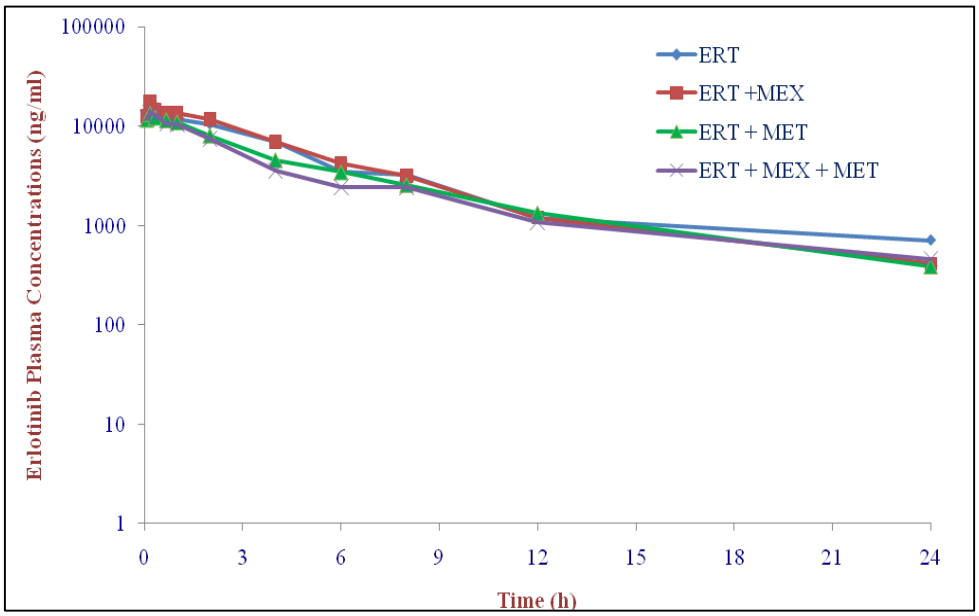

Fig 1: Semi-logarithmic plot of erlotinib plasma concentrations following single dose administration of erlotinib alone (30 mg/kg, p.o), in combination with meloxicam (20 mg/kg, i.p), in combination with metformin (100 mg/kg, p.o) and in combination with meloxicam (20 mg/kg, i.p) and metformin (100 mg/kg, p.o) in SCID male mice (n=6).

4. Conclusions

The present study revealed that administration of meloxicam and metformin in combination with erlotinib altered pharmacokinetic profile of erlotinib up to certain extent in mice. However, effect of long term administration of metformin and meloxicam on pharmacokinetics of erlotinib in mice needs to be evaluated.

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