ABSTRACT

Thesis aimed to investigate possible pharmacokinetic (PK) drug-drug interaction and PK studies of CDRI novel trioxane antimalarial with antiepileptic drugs (AEDs). Thesis has been divided into two parts. Part-I contains PK drug-drug interactions between CDRI novel trioxane (99/78 and 99/411) antimalarials and selected AEDs in male and female rats. Part-II contains PK studies of 97/63 in rats and in-vitro protein binding study of 97/78 and its metabolite 97/63 in human plasma. At first 97/63 was synthesized but due to lower bioavailability it has been derivatised as hemi-succinate derivative; coded 97/78 and used as prodrug. Molecule 97/78 was unstable in rats and gets instantaneously and completely converted into 97/63 which necessitated to carry-out the estimation of 97/63 instead of 97/78. Therefore the PK profile of 97/78 was generated in terms of its metabolite 97/63.

Today, drug-drug interactions are subject of great attention worldwide. Drug interaction at clinical level is indeed the only valid way of establishing drug interaction in human. However, ethical guidelines don't allow direct drug interaction on humans. So drug interactions in animal models are planned. Rats are the most convenient animal models for PK studies due to ease of handling, dosing and sampling.

97/78 and 99/411 have emerged as lead compound due to their excellent and potent antimalarial activity, in both in-vitro and in-vivo malarial models. Regulatory preclinical studies have been successfully performed and the compound is currently in phase I clinical trials. So, it is pre-requisite to carry drug interaction studies of these molecules as per drug discovery and development program and FDA guidelines. Antimalarial drugs shows interaction among themselves as well as with non-antimalarial drugs. So single dose PK drug interaction study in male and female SD rats has been performed after oral administration of antimalarial molecule alone and coadministered with AEDs, as part of regulatory preclinical studies. To determine the PK parameters and see interaction effects; sensitive, selective and precise assays were developed and validated for 97/78, 97/63 and 99/411 separately as well as for simultaneous estimation with AEDs. Separate baseline (control) PKs of 97/78 and 99/411 were performed in male and female rats. Then these antimalarials were separately coadministered with AEDs. PK parameters of 97/78 alone; 99/411 alone and coadministered AEDs were
statistically compared by “Student t-test”. If P value was >0.05, then no significant difference exists and Vise-Versa.

For PK drug interaction studies, the revalidated LC–MS/MS assay of 97/63 and 99/411 in male and female rat plasma was linear, sensitive, accurate and precise over the range 1.56-200 ng/mL. We also developed and validated LC-MS/MS assay for simultaneous quantification of 97/63 and gabapentin in male and female rat plasma which was linear, sensitive, accurate and precise over the range 1.56-200 ng/mL (97/63) and 62.5–4000 ng/mL (gabapentin).

After oral administration of 97/78 at 40 mg/kg in male and female rats, 97/63 exhibited irregular plasma concentration-time profile and multiple peaks were observed. PK profile of 97/63 demonstrates that metabolite mean T\\textsubscript{1/2} and MRT in male rats were 6.98 ± 0.63 h and 8.47 ± 0.93h while in females were 5.45 ± 0.76 h and 9.35 ± 0.93 h respectively after oral administration of 97/78 alone. In individual male rats, the C\\textsubscript{max} were 291 ng/mL, 818 ng/mL, 1720 ng/mL, 619 ng/mL at T\\textsubscript{max} of 4 h, 0.75 h, 1.5 h and 0.75h while in female rats were 808 ng/mL, 764 ng/mL, 481 ng/mL, 438 ng/mL at T\\textsubscript{max} of 8 h, 6 h, 8 h and 8 h respectively. PK parameters of 97/78 alone in male and female rats were compared statistically by “two-tailed Student’s t-test”. Intersex statistical analysis of PK parameters of 97/63 after oral administration of 97/78 alone showed no significant difference for all PK parameters (P >0.05) with exception to T\\textsubscript{max} (P <0.05)

Then 97/78 was coadministered with phenytoin (42 mg/kg), carbamazepine (42 mg/kg), gabapentin (42 mg/kg), topiramate (10 mg/kg), divalproex sodium (52 mg/kg), lamotrigine (42 mg/kg) respectively. Statistical analysis was performed for 97/63 after oral coadministration of 97/78 and AEDs as well as intersex comparisons were also considered.

Statistical analysis of PK profile of 97/63 after oral administration of 97/78 alone and coadministration of 97/78 and phenytoin; 97/78 and carbamazepine; and 97/78 and topiramate in male and female rats demonstrates that profiles are similar (P>0.05) except MRT (P<0.05) in female rats (97/78 and carbamazepine) and T\\textsubscript{max} (P<0.05) in female rats (97/78 and topiramate). Also, intersex statistical analysis of PK parameters of 97/63 after oral coadministration of 97/78 and phenytoin, 97/78 and carbamazepine and 97/78 and topiramate showed no significant difference for all PK parameters (P>0.05) except MRT (P<0.05) for coadministered 97/78 and carbamazepine. So coadministration of these drugs does not require any dose adjustment.
Statistical analysis of PK profile of 97/63 after oral administration of 97/78 alone and coadministration of 97/78 and gabapentin in male and female rats demonstrates that profiles are similar (P>0.05). Also, intersex statistical analysis of PK parameters of 97/63 after oral coadministration of 97/78 and gabapentin showed no significant difference (P>0.05) for all PK parameters except MRT (P<0.05). Intersex statistical analysis of PK parameters of gabapentin after oral administration of gabapentin alone showed no significant difference (P>0.05) for all PK parameters except MRT (P<0.05). Statistical analysis of PK profile of gabapentin after oral administration of gabapentin alone and coadministration of 97/78 and gabapentin in male and female rats demonstrates that profiles are similar (P>0.05) except T_{1/2} and MRT (P<0.05) in male rats; and V_d, Cl and MRT (P<0.05) in female rats. Intersex statistical analysis of PK parameters of gabapentin after oral coadministration of 97/78 and gabapentin demonstrates that profiles are similar (P>0.05) except V_d and Cl (P<0.05). Since C_{max} and T_{max} are not significantly different, their coadministration does not require any dose adjustment.

Statistical analysis of PK profile of 97/63 after oral administration of 97/78 alone and coadministration of 97/78 and divalproex sodium in male and female rats demonstrates that profiles are not similar (P<0.05) except T_{max}, C_{max} and MRT (P>0.05) in male rats; and except T_{1/2}, T_{max} and C_{max} (P>0.05) in female rats. Also, intersex statistical analysis of PK parameters of 97/63 after oral coadministration of 97/78 and divalproex sodium showed significant difference for all PK parameters (P>0.05) except T_{1/2} and MRT (P>0.05). Since C_{max} and T_{max} are not significantly different, they can be coadministered without any dose adjustment.

Statistical analysis of PK profile of 97/63 after oral administration of 97/78 alone and coadministration of 97/78 and lamotrigine in male and female rats demonstrates that profiles are not similar (P<0.05) except T_{max} and C_{max} (P>0.05) in male rats and except T_{1/2}, T_{max} and C_{max} (P>0.05) in female rats. Also, intersex statistical analysis of PK parameters of 97/63 after oral coadministration of 97/78 and lamotrigine showed no significant difference for all PK parameters (P>0.05). Since C_{max} and T_{max} are significantly different in female rats, hence their coadministration requires dose adjustment. Precaution should be taken in clinical phase drug interaction study for divalproex sodium and lamotrigine.

Similar to 97/78; after oral administration of 99/411 alone at 12 mg/kg in male and female rats exhibited irregular plasma concentration-time profile and multiple peaks were observed. In individual male rats, the C_{max} were 424 ng/mL, 429 ng/mL, 326 ng/mL and 338...
ng/mL at $T_{\text{max}}$ of 2 h, 2 h, 2 h and 2 h respectively while in female rats were 304 ng/mL, 615 ng/mL, 1010 ng/mL and 316 ng/mL at $T_{\text{max}}$ of 1.5 h, 6 h, 6 h and 6 h respectively. Statistically no significant difference ($P>0.05$) was observed for all PK parameters.

Then 99/411 was coadministered with phenytoin (42 mg/kg), carbamazepine (42 mg/kg), gabapentin (42 mg/kg). Statistical analysis of PK profile of 99/411 after oral administration of 99/411 alone and coadministered of 99/411 and phenytoin; 99/411 and carbamazepine; and 99/411 and gabapentin in male and female rats demonstrates that profiles are similar ($P>0.05$). Significant difference was observed for $T_{\text{max}}$ ($P<0.05$) in male rats and MRT ($P<0.05$) in female rats after coadministration of 99/411 and gabapentin, but still they can be coadministered without any dose adjustment. Also, intersex statistical analysis of PK parameters after oral coadministration of 99/411 and AEDs showed no significant difference ($P>0.05$) for all PK parameters. So their coadministration does not require dose adjustment.

For PK study of 97/63 in rats, developed and validated HPLC-UV assay was linear, sensitive, accurate and precise over the range 10-500 ng/mL in rat serum. Moreover, an LC-MS/MS assay was also developed and validated for simultaneous quantification of 97/78 and its metabolite 97/63 over the range 1.56-200 ng/mL in human plasma and was applied for their plasma protein binding study.

Following oral and intravenous dose administration in male rats, the levels of 97/63 could be monitored in serum from the first dose sampling point i.e., 5 min. After oral administration 97/63 was rapidly absorbed from the gastro-intestinal tract, attaining maximum serum level concentration ($C_{\text{max}}$) at 1 h post oral dose administration. A dominant $\beta$ phase characterized much of the profile and exhibited clear log-linear behavior from ~6 to ~18 h post dose. No metabolite peaks were observed in rat serum. PKs of 97/63 in rats showed low bioavailability (15.66%) and high variability which may be due to lower log P value (<3.5) which indicates the poor absorption of the compound in the aqueous medium and less lipophilic behavior.

*In-vitro* plasma protein binding was performed for 97/78 and 97/63 at 0.5 and 1 µg/ml concentration levels. Compound 97/78 and its metabolite 97/63 showed plasma protein binding 85.25±7.63 % and 54.22±9.43 % respectively at concentration of 0.5µg/ml and 73.90±3.66 % and 56.57±2.05 % respectively at 1µg/ml. Compound 97/78 showed higher plasma protein binding compared to 97/763 respectively.