Chapter 3

Pharmacokinetic studies of osteoprotective candidate K058

3.1. Experimental methods

3.1.1. Animals

Pharmacokinetic studies were performed in young and healthy female *S.D* rats weighing 250±20 g. Animals were housed in well-ventilated cages and kept at room temperature on a regular 12h light–dark cycle at Laboratory Animal Division of the institute. Animals were cared for in accordance with the guidelines laid by the Institutional Animal Ethics Committee (IAEC) for animal experimentation. All experimental animals were acclimatized for a minimum period of three days prior to the experiment. Standard pellet food and water were allowed freely. Animals in oral dose group were starved for 8-12h before dosing but allowed free access to water. Prior approval from the IAEC was sought and the study protocols were approved before the commencement of the studies.

3.1.2. Drug formulation, administration and sampling schedule

Oral pharmacokinetics study was carried out at the dose of 5mg/kg and the intravenous study at the dose of 1mg/kg. The oral formulation of K058 (20mg) was prepared in 4mL of 0.5% sodium methyl cellulose suspension at the concentration of 5mg/mL. The dosing volume of oral suspension was 0.25mL, which contained 1.25mg. The intravenous formulation of K058 (5mg) was prepared in dimethyl acetamide (40%), Tween 20 (10%) and saline (q.s. to 5mL) at the concentration of 1mg/mL. The intravenous formulation was sterilized by filtration through 0.45µm filter. The dosing volume was 0.25mL, which contained 0.25mg of K058.

Animals were divided into three groups with three animals in each group. Blood samples (0.5mL) were collected by cardiac puncture. Sparse sampling approach was followed so that not more than three samples were collected from each animal. The oral formulation was administered to rats by oral feeding needle after shaking well to ensure uniformity in content. Samples were collected by cardiac puncture in heparinised tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, 24 and 30h. Intravenous formulation was administered in tail vein after swapping the tail region with alcohol. Samples were collected at 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, 18, 24 and 30h.

3.1.3. Sample clean-up and analysis

Blood samples were processed by SPE method as described under the chapter 2 of part-II. 100µl of blood samples were processed similar to the processing of calibration standards. The dried residue obtained after evaporation of organic solvent was reconstituted in 200µl of reconstitution solvent. 20µl of the processed samples were analyzed by validated LC-MS/MS.

3.1.4. Data analysis

The plasma concentration of K058 was plotted against the sampling time to obtain the plasma concentration-time profile. The pharmacokinetic parameters were derived by fitting the plasma concentration-time data using WinNonlin software, Ver 5.1.

3.2. Results and Discussion

The plasma concentration profile of K058 after intravenous and oral dosing is shown in Fig-3.2. The pharmacokinetics parameters were obtained after non-compartmental analysis of its plasma concentration-time profile.

3.2.1. Intravenous pharmacokinetics

The half life of K058 following i.v. dosing was found to be 1.65h and its MRT was 1.62h (Table-3.1). The systemic clearance (Cl=0.38L/h/kg) was very low and K058 could be detected in plasma till 18h. The volume of distribution at steady state were also very low (V_{ss}=0.98L/kg), which indicates that K058 has low tissue distribution. This was in contrast to the behaviour of isoflavones, which generally show extensive tissue distribution. The systemic exposure of K058 was high after i.v. dosing and the value of $AUC_{0-\infty}$ was 2737.54ng.h/mL.

3.2.2. Oral pharmacokinetics

K058 reached the maximum concentration (C_{max}= 40.67ng/mL) at 1h (T_{max}). The half life and MRT were 5.07h and 5.77h respectively. These values were greater than the corresponding values obtained after i.v. dosing. The value of $AUC0_{-\infty}$ was

138.09ng.h/mL. The percentage bioavailability of K058 was 1.04%, which is very low when compared to that of isoflavones (Ch-3, Part-I).

Though the absorption of the glycoside K058 was moderate and its clearance was slow, the systemic exposure was not high but the circulating levels of K058 were detected beyond 24 hours. The studies reported on the bioavailability of O-glycosides have shown that absorption of O-glycosides is generally low because of their highly polarity [1-4]. Moreover, the O-glycosides have been reported to get hydrolysed by intestinal enzyme β-glucosidase to aglycone and the released aglycones enter the systemic circulation [5-7]. As a result, the presence of intact glucosides in plasma is low, resulting in poor bioavailability of glycosides [8-12]. In the case of C-glycosides, the report on bioavailabilty study is limited. One of the studies has reported on the poor absorption of C-glycosides [13]. In the present study, the low bioavailability of K058 might be due to very low absorption of intact glycosides. However, the prolonged systemic availability and low clearance correlate well with pharmacodynamic response observed with single dosing of K058, thus establishing the PK-PD correlation.

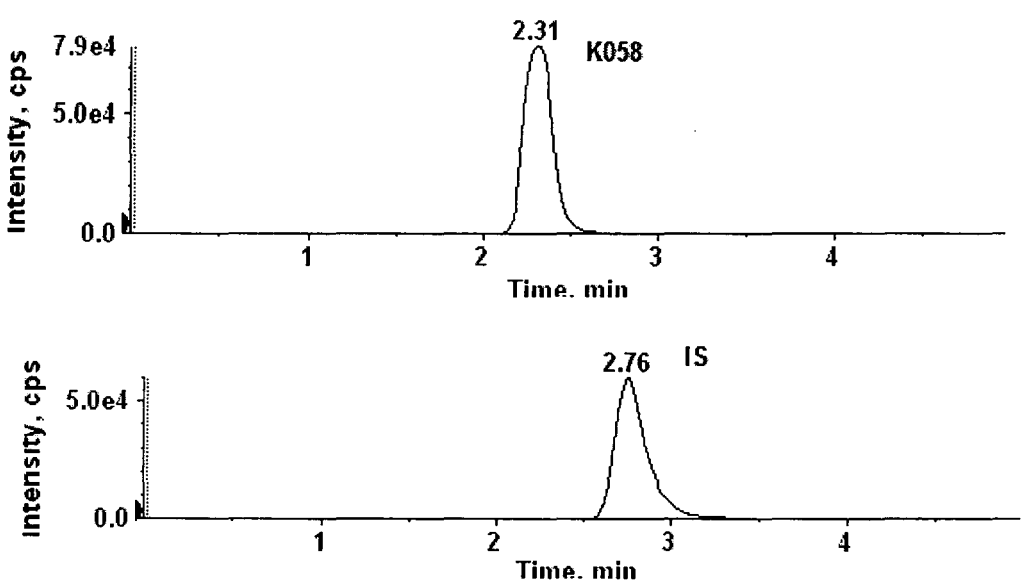


Fig-3.1.1: LC-MS/MS chromatogram of K058 after i.v. dosing in rats

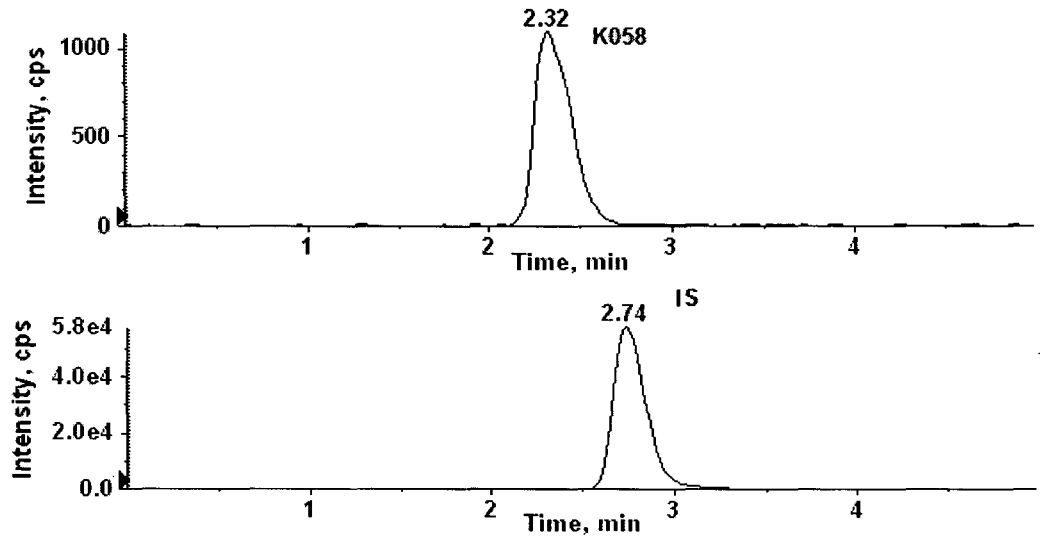
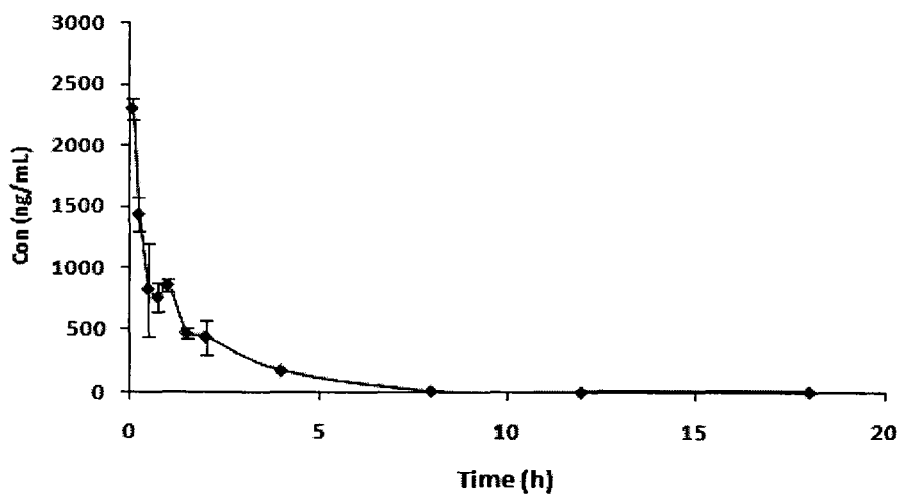


Fig-3.1.2: LC-MS/MS chromatogram of K058 after oral dosing in rats

(A)



(B)

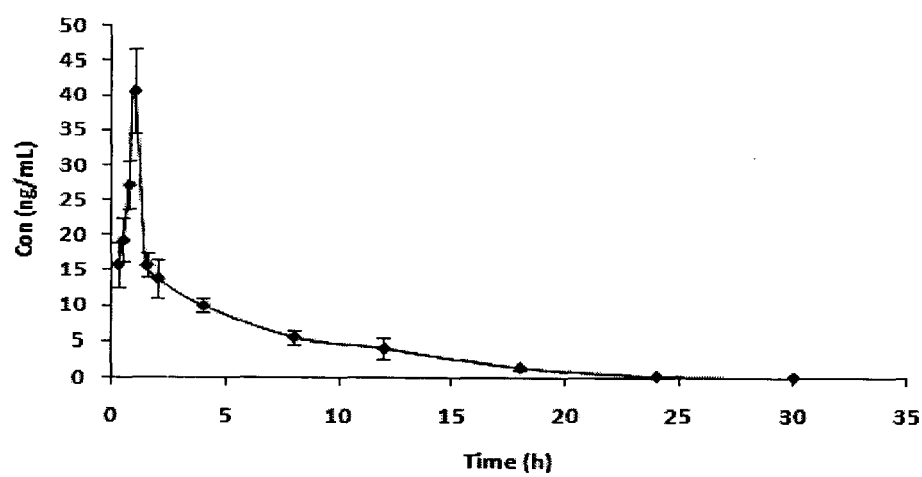


Fig-3.2: Plasma c-t profile of K058 after (A) i.v. and (B) oral dosing in rats

Table-3.1: Pharmacokinetic parameters of K058 after i.v. and oral dosing in female S.D. rats

Pharmacokinetic parameters	Intravenous (1mg/kg)	Oral (5mg/kg)
C_{max} (ng/mL)	--	40.67
T_{max} (h)	--	1.0
C_0 (ng/mL)	2873.92	--
$AUC_{0-\infty}$ (ng.h/mL)	2737.54	138.09
$T_{1/2}$ (h)	1.65	5.07
K_a (1/h)	0.42	0.14
MRT (h)	1.62	5.77
Cl (L/h/kg)	0.38	0.37
V_{ss} (L/kg)	0.98	1.13
% F	--	1.04

3.3. Conclusion

The intravenous and oral pharmacokinetic studies of K058 were performed in female S.D. rats at the dose of 1mg/kg and 5mg/kg respectively. The oral absorption of K058 was moderate with the T_{max} of 1.0h and the C_{max} was 40.66ng/mL. The volume of distribution at steady state was low (0.98 L/kg) indicating lower tissue distribution of K058. The clearance of K058 was found to be very low (0.38 L/h/kg) and could be detected in plasma till 30h in oral pharmacokinetics study. The half life of K058 was 1.65h and 5.07h after i.v. and oral dosing respectively. The bioavailability was determined to be 1.04%, which shows that the systemic exposure of K058 was very low. The low clearance of K058 is a desirable pharmacokinetic behaviour but its tissue distribution and bioavailability were low, which might affect its activity. However, the *in vivo* pharmacodynamic studies at the dose of 5mg/kg have shown that K058 exhibit potent osteoprotective activity despite low tissue distribution and bioavailability. This indicates that K058 may be effective even at very low concentration *in vivo*. Therefore, if suitable dosage form is formulated incorporating K058 to enhance its bioavailability, it may be more, or equally effective at still lower doses.

References

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