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Studies on the Pharmacokinetic Interaction between Meloxicam and Quercetin, A CYP2C9 and CYP3A4 Inhibiting Flavonoid, in Rabbits

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A B S T R A C T

The effect of quercetin pre-treatment (10 and 20 mg.Kg^{-1}), 30 minutes before the single oral administration of meloxicam at the dose rate of 1.5mg.Kg^{-1},was studied on the pharmacokinetics of meloxicam. Eighteen adult male rabbits were divided into 3 groups of 6 animals each. Group 1 received meloxicam alone @ 1.5 mg.Kg^{-1} b. wt. orally, group 2 received meloxicam @ 1.5 mg.Kg^{-1} b. wt. orally 30 min post-treatment with quercetin @ 10 mg.Kg^{-1} b. wt. orally, while group 3 received meloxicam @ 1.5 mg.Kg^{-1} b. wt. orally 30 min post-treatment with quercetin @ 20 mg.Kg^{-1} b. wt. orally. Plasma concentrations of meloxicam were determined at specified time intervals by using high performance liquid chromatography(HPLC). The plasma concentration versus time data of meloxicam was adequately described by a non compartment model. The area under plasma concentration – time curve(AUC_{0-t}) ($p<0.01$), area under first moment curve ($AUMC_{0-t}$) ($p<0.05$), volume of distribution at steady state(V_{dss}) ($p<0.05$) and peak plasma concentration(Cl_B) ($p<0.01$) in quercetin 20 mg.Kg^{-1}pre-treatment group were significantly different from the corresponding values of control group. Quercetin pre-treatment @ 10 mg. Kg^{-1} had no significant effect on the pharmacokinetic profile of meloxicm in rabbits.

Introduction

Cytochrome P450 enzymes (CYP) represent a large family of proteins involved in the metabolism of drugs and other xenobiotics, as well as some endogenous substrates (1). CYP 2C9 isoform is considered a key enzyme and is second only to CYP 3A4 in terms of total human liver microsomal P450 content. CYP 2C9 is responsible for phase I metabolism of approximately 15% of clinically used drugs (2). Pharmacokinetic interactions can frequently arise when drugs are co-administered due to modification of a specific

CYP enzyme activity (3). Non-steroidal anti-inflammatory drugs (NSAIDs) are routinely used to provide analgesia. Meloxicam, is a novel preferential cyclooxygenase-2 (COX-2) inhibiting NSAID that is used extensively as an analgesic agent in humans andin some companion animals. Unlike many other NSAIDs, meloxicam has high oral bioavailability and has a long half-life, making it an attractive analgesic.

In all species studied, meloxicam undergoes extensive hepatic metabolism into 4 inactive metabolites that are excreted in both urine and faeces (4).*In vitro* and *in vivo*, it is mainly metabolized to a 5'-hydroxymethyl metabolite that is further converted to a 5'-carboxy metabolite (5). The 5'-hydroxylation of meloxicam is predominantly catalyzed by CYP 2C9 and with a minor contribution by CYP 3A4 (6).

Flavonoids represent a group of phytochemicals that are produced by various plants in high quantities (7). Quercetin, a polyphenolic flavonoid,is ubiquitous in plants and is the major bioflavonoid in the human diet. It has been reported that quercetin inhibits the P-gp (8, 9) and CYP 3A4 *in vitro*(10, 11), and CYP 2C9 (12).

This study was taken up to assess the pharmacokinetic alterations of meloxicam if any, following interaction with quercetin pre treatment (10 $mg.kg^{-1}$and20 $mg.kg^{-1}$) in rabbits.

Materials and Methods

The study was conducted on 18 healthy adult male rabbits aged above 3months weighing between 2.0 to 2.5 kg which were procured from Department of Animal Genetics and Breeding, College of Veterinary Science, Hyderabad, India. The rabbits were randomly divided into 3 groups of six animals each and were maintained in well ventilated small animal house. Standard feed and clean water were provided ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee.

All the rabbits in three groups received meloxicam (Melonex® bolus, M/s Intas Pharmaceuticals Ltd, Ahmadabad, India) @ 1.5 $mg.Kg^{-1}$ b. wt. orally. In addition, rabbits in group 2 and 3 received quercetin @ 10 and 20 $mg.Kg^{-1}$ b. wt. orally 30 minutes prior to the administration of meloxicam, respectively.

Blood was collected from the ear vein of the animals by vein puncture in heparinised vials at 0, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24h after the oral administration of meloxicam. Plasma was separated by centrifugation at 3000 rpm for 10 min and the plasma samples were stored at -20ºC till analyzed for meloxicam.

Assay of meloxicam in plasma

Acetonitrile (0.5ml) was added to plasma (0.5ml) in the ratio of 1:1 after vortex mixing at high speed for 1 min. The tubes were subjected to centrifugation for 10 min at 10000rpm. 0.5ml of clear supernatant thus obtained was transferred to a tube and 0.5 ml of HPLC-grade water was added. The aliquot was filtered through a 0.22 µm nylon membrane syringe filter and then loaded into the HPLC sampling vial.

The plasma concentration of meloxicam was determined by using reverse phase-high performance liquid chromatography (HPLC) method described by Baert and De Backer (13) with certain modifications. Separation of meloxicam was achieved by using C_{18} reversed-phase column (Phenomenex, particle size 5µm, 4.6mm x 250mm) as the stationary phase. The mobile phase consisted of a mixture of acetonitrile and a buffer prepared, in the ratio 4:6 (v/v). The flow rate was

adjusted to 1ml.min^{-1} with the run time of 6 min. Chromatography was performed at 35ºC with detection at 355 nm using PDA detector. Meloxicam (Technical grade, generous gift from M/s PVS laboratories pvt Ltd., Vijayawada) was quantified from their respective peak heights / areas and the concentration in the plasma samples was determined by references to calibration curves based on the analysis of blank plasma samples spiked with meloxicam and analyzed as for the test samples. Standard calibration curve for meloxicam was linear from 0.156to 2.5µg.ml^{-1} with regression coefficient of 0.999. Limit of detection was 0.078µg.ml^{-1} with the achieved recovery.

Pharmacokinetic analysis

Non compartmental analysis

Plasma concentration versus time data of meloxicam obtained for three groups in the present study were utilized for calculating various pharmacokinetic parameters in rabbits with an interactive programme for personal computer software (PK Solver, version. 2.0, 2010 by Zhang Yang).

Statistical analysis

The data were expressed as mean ± SE. Differences in pharmacokinetic data between meloxicam alone and quercetin pre-treated groups were analyzed for statistical significance using unpaired student's 't' test with Welch's correction using 'Instat' software. The level of significance was $p < 0.05$.

Results and Discussion

The mean plasma concentrations of meloxicam versus time of single oral administration of meloxicam in control group and in groups pre-treated with quercetin at the dose rate of 10 and 20 mg. kg^{-1} are presented in Table I and Fig. 1. The mean plasma concentration of meloxicam in the group pre-treated with quercetin at the dose rate of 20 mg.kg^{-1} was1.03 ± 0.46, 1.15 ± 0.51, 0.94 ± 0.42 and 0.30 ± 0.13 µg.ml^{-1} at 3, 4, 8 and 24 h, respectively and these values were significantly ($p<0.05$) different form the corresponding values of the control group.

Pharmacokinetic parameters of meloxicam after single oral administration of meloxicam (1.5 mg.kg^{-1}) in control group and in quercetin (10 and 20 mg.kg^{-1}) pre-treated rabbits are given in Table II.

The value of C_{max} in quercetin (20 mg.kg^{-1})pre-treated group (1.22 ± 0.07 µg.ml^{-1}) was significantly ($p<0.01$) higher than the value in control group (0.84± 0.06 µg.ml^{-1}).

AUC_{0-t} (16.78 ± 1.28 µg.h.mL^{-1}) was significantly ($p<0.01$) higher than the corresponding control value (10.94 ± 0.75 µg.h.mL^{-1}) and the increase in AUC_{0-t} was by 53% as compared to the control group.

The $AUMC_{0-t}$ and V_{dss} values in quercetin (20 mg.kg^{-1})pre-treated group were 160.77 ± 12.76 µg.h^2.ml^{-1} and 1.14 ± 0.11 L.kg^{-1}, respectively that differed significantly ($p<0.05$) from those of the control group.

Meloxicam undergoes extensive metabolism, primarily by cytochrome P450 isozyme CYP2C9 and to a minor extent by CYP3A4 (6*).*

It was also reported that voricanazole, a known CYP2C9 and CYP3A4 inhibitor, increased the plasma meloxicam concentrations, while itraconazole, a CYP3A4 inhibitor, decreased the same in humans (14) and this decrease was attributed to some unknown mechanism by which itraconazole inhibited the gut absorption of meloxicam.

Flavonoid quercetin has strong inhibitory effect on CYP3A4 and CYP2C9 activity (15) and hence, drug interactions are bound to arise when quercetin is given along with drugs that are substrates for these CYP enzymes.

It was reported that quercetin has increased the oral bioavailability of various drugs that have been substrates for CYP3A4 like pioglitazone and diltiazem (16, 17).

Table.1 Mean plasma concentration of meloxicam (µg.ml^{-1}) in different groups of rabbits after single oral administration of meloxicam at 1.5 mg.kg^{-1}

(n=5)

Time (h)	Group 1	Group 2	Group 3
0.25	0.18 ± 0.02	0.19 ± 0.06	0.21 ± 0.09
0.5	0.27 ± 0.03	0.24 ± 0.08	0.35 ± 0.16
1	0.34 ± 0.04	0.37 ± 0.11	0.59 ± 0.26
2	0.47 ± 0.08	0.53 ± 0.09	0.81 ± 0.36
3	0.56 ± 0.09	0.64 ± 0.05	1.03 ± 0.46*
4	0.70 ± 0.13	0.71 ± 0.07	1.15 ± 0.51*
8	0.63 ± 0.07	0.69 ± 0.11	0.94 ± 0.42*
12	0.49 ± 0.08	0.51 ± 0.11	0.73 ± 0.33
24	0.22 ± 0.02	0.23 ± 0.01	0.30 ± 0.13*

Values are Mean ±SE

*Significantly different ($p<0.05$) from respective values of control group.

Table.2 Pharmacokinetic parameters of meloxicam in different groups of rabbits after single oral dose administration of meloxicam (1.5 mg.kg^{-1})

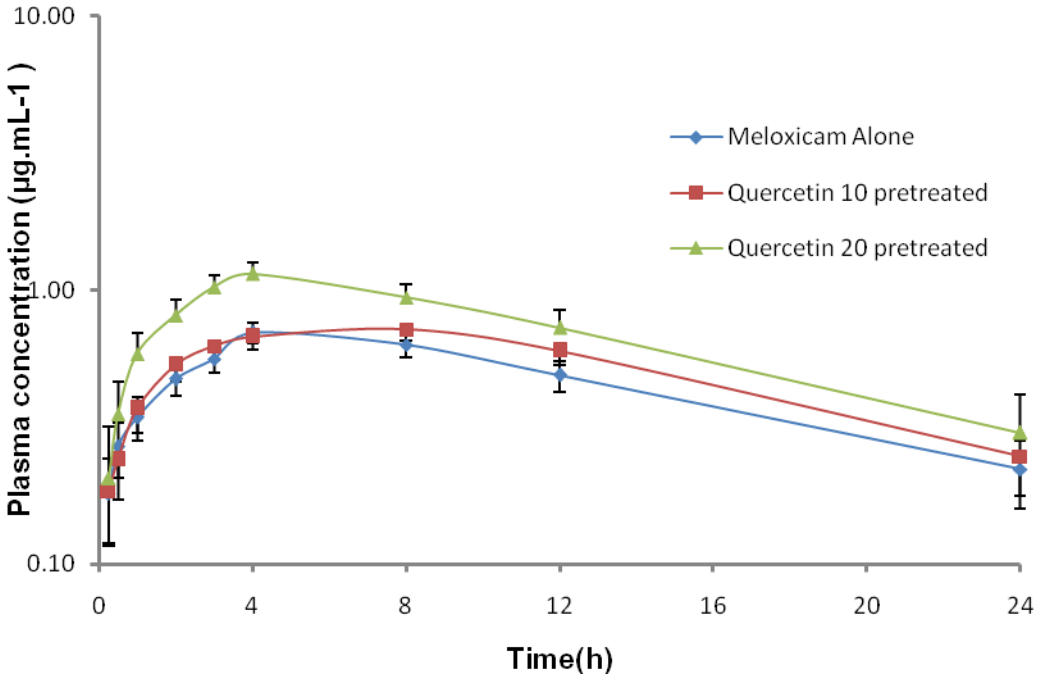
Parameter	Unit	Group 1	Group 2	Group 3
B	h^{-1}	0.07 ± 0.01	0.065 ± 0.008	0.07 ± 0.004
t½β	H	14.52 ± 4.40	11.46 ± 1.62	9.93 ± 0.59
AUC_{0-t}	µg.h.mL^{-1}	10.94 ± 0.75	11.57 ± 1.30	16.78 ± 1.28**
$AUC_{0-\infty}$	µg.h.mL^{-1}	16.02 ± 1.95	15.40 ± 1.18	21.08 ± 1.36
$AUC_{0-t}/AUC_{t-\infty}$	Per cent	28.0 ± 7.00	25.49 ±3.64	20.66 ± 1.62
$AUMC_{0-t}$	µg.h^2.mL^{-1}	108.84 ± 8.06	114.90 ± 14.43	160.77 ± 12.76*
$AUMC_{0-\infty}$	µg.h^2.mL^{-1}	394.86 ± 147.73	275.23 ± 26.83	326.21 ± 19.35
MRT	H	21.89 ± 5.32	18.10 ± 1.80	15.58 ± 0.64
V_{dss}	L.kg^{-1}	1.97 ± 0.24	1.83 ± 0.24	1.14 ± 0.11*
Cl_{β}	L.kg^{-1}.h^{-1}	0.10 ± 0.01	0.10 ± 0.01	0.07 ± 0.01
C_{max}	µg.mL^{-1}	0.84 ± 0.06	0.83 ± 0.06	1.22 ± 0.07**
t_{max}	H	6.40 ± 0.90	4.2 ± 0.93	4.4 ± 0.85

Values are Mean ±SE

*Significantly different ($p<0.05$) from respective values of control group

** Significantly different ($p<0.01$) from respective values of control group

Fig.1 Semi logarithmic plot of meloxicam concentrations in plasma versus time after single oral bolus administration of meloxicam (1.5 mg.kg-1) in control (Blue plot), quercetin (10 mg.kg-1) pretreated (Red plot) and quercetin (20 mg.kg-1) pretreated (green) in adult rabbits. Each point represents the mean ± SE of six rabbits



The plasma concentration of meloxicam in quercetin (20 mg.kg^{-1})pre-treated group(3) at 3, 4, 8 and 24 h was significantly ($p<0.05$) higher from corresponding values in control group, while the concentrations at other time intervals were non-significantly higher in group 3 as compared to the corresponding values in control group. The $AUC_{0-t,}$ $AUMC_{0-t,}$ V_{dss} and C_{max} in quercetin (20 mg.kg^{-1}) pre-treated group were significantly ($p<0.05$) different from the corresponding values of the control group. The values obtained for remaining parameters as shown in Table 2 were non-significantly different in quercetin (20 mg.kg^{-1}) pre-treated group as compared to that of control group.

There was no significant difference in the pharmacokinetic parameters of meloxicam in the group 2 that was pre-treated with quercetin @ 10 mg.kg^{-1} in comparison to the meloxicam control group.

The results suggest an interaction between meloxicam and quercetin, which was evident with higher test dose of quercetin (20 mg.Kg^{-1}), which may be due to inhibition of CYP2C9 and CYP3A4 that mediate metabolism of meloxicam, while 10 mg.Kg^{-1} dose of quercetin did not influence the pharmacokinetic profile of meloxicam.

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