BIOAVAILABILITY OF DICLOFENAC SODIUM AFTER PRETREATMENT WITH DIOSMIN IN HEALTHY VOLUNTEERS

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SUMMARY

Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID). It undergoes extensive Phase I and Phase II metabolism and *in vitro* it is a specific CYP2C9 substrate. The first part of the study consisted of oral administration of 100 mg of diclofenac sodium (Voveran®100) to 12 healthy male volunteers. Blood samples were collected from the antecubital vein at intervals of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours. The second part of the study was conducted after a washout period of 7 days. Treatment with 500 mg p.o. of diosmin (Venex®500) was given daily for 9 days. On day 10, 100 mg of diclofenac sodium (Voveran®100) was administered. Blood samples were obtained as mentioned earlier and pharmacokinetic parameters of diclofenac before and after pretreatment with diosmin analyzed by HPLC. Diosmin pretreatment significantly enhanced AUC, C_{max} and $t_{½}$ with a concomitant reduction in CL/f. Diosmin might have inhibited the microsomal CYP2C9 mediated oxidation of diclofenac sodium.

KEY WORDS

diclofenac sodium, CYP2C9, diosmin, bioavailability

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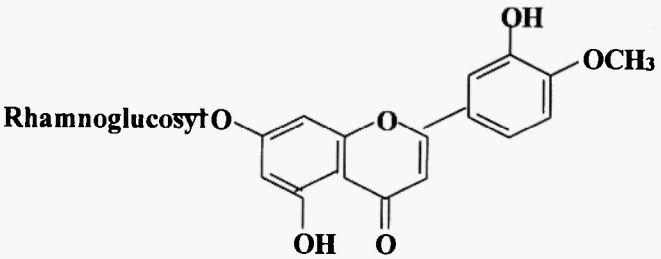
INTRODUCTION

Diosmin (3′,5,7-trihydroxy-4′-methoxyflavone-7-rutinoside) is a flavone. Diosmin and diosmetin (shown in Fig. 1) are natural dietary agonists of the aryl hydrocarbon receptor (AhR) /1/, reported to potentiate CYP1A1 transcription and its activity /1/. However, diosmetin is only capable of inhibiting CYP1A1 enzyme activity, thus inhibiting carcinogen activation. The pharmacokinetics of diosmin and diosmetin have been extensively studied. Both flavanoids are rapidly metabolized and the parent compounds are completely absent in urine. Diosmetin presents a long plasma elimination half-life ranging from 26 to 43 hours /2,3/. CYP2C9, a major enzyme in human liver, metabolizes a wide range of therapeutic agents. Allelic variants of CYP2C9 influence its catalytic activity towards various substrates.



(A)

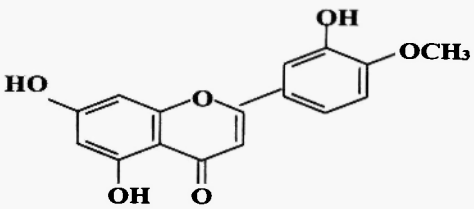


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Fig. 1: Structures of diosmin (A) and diosmetin (B).

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Diclofenac, sodium [(2,6-dichloro-phenyl)-amino]phenyl acetate, is a non-steroidal anti-inflammatory drug (NSAID). It is the first of a series of phenyl acetic acid derivatives that have been developed as anti-inflammatory agents. Diclofenac shows potent anti-inflammatory, analgesic and antipyretic activity in various experimental models. It undergoes extensive Phase I and Phase II metabolism /4/, and is an *in vitro* specific CYP2C9 substrate /5/. The major metabolite, 4′-hydroxydiclofenac, and the minor metabolites, 3′-hydroxydiclofenac and 3′-hydroxy-4′-methoxydiclofenac, are formed by hepatic cytochrome P450 2C9 (CYP2C9) /6/. However, the formation of 5-OH-diclofenac is catalyzed by several CYP enzymes in humans, including CYP3A4, CYP2C8, CYP2C18, CYP2C19 and CYP2B6 /7,8/. The hydroxy metabolites are further conjugated and excreted in urine and bile /9/. From earlier studies it is obvious that flavonoids inhibit a number of enzymes in the body, which include oxidative metabolic enzymes. We hypothesized that diosmin (a flavone) might interact with diclofenac sodium at the metabolism level. In the present study diclofenac sodium was used as a probe drug for CYP2C9 in human volunteers.

MATERIALS AND METHODS

Materials

Diclofenac sodium tablets (Voveran®100) were purchased from Novartis India Limited, Mumbai, India. Diosmin tablets (Venex®500) were procured from Elder Pharmaceuticals Limited, Mumbai, India. Chlorzoxazone and diclofenac sodium pure substance were a kind gift from Biological-E Limited, Hyderabad, India. Acetonitrile and methanol (HPLC grade) were purchased from E. Merck Limited, Mumbai, India.

HPLC instrumentation

A Shimadzu high performance liquid chromatography unit equipped with a LC-8A solvent delivery module, SPD-10AVP UV-visible spectrophotometer detector, class CR-10 data processor, rheodyne (with 20 µl capacity loop) injection port and Wakosil II C-18 column (stainless steel column, 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5µ diameter, 100 Å pore

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diameter) was used for analysis of samples. Mobile phase consisting of acetonitrile and 0.5% glacial acetic acid (70:30 v/v) at 1 ml/min flow rate, UV detection set at 280 nm, and sensitivity of 0.001 a.u.f.s was used for the analysis.

Study design

Healthy volunteers were briefed about the study and written informed consent was obtained. The local ethics committee approved the study protocol. Study drugs were taken in the morning with 100 ml of tap water just after voiding. Twelve healthy male volunteers with a mean age of 24.3 ± 3.3 years (range 20 to 30 years), a mean height of 172.4 ± 5.0 cm (range 165 to 180 cm) and a mean body weight of 61.8 ± 6.6 kg (range 54 to 70 kg) participated in the study after undergoing a thorough physical examination. The first part of the study consisted of oral administration of 100 mg of diclofenac sodium (Voveran®100) alone. Blood samples were obtained from the antecubital vein after 0, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hours of drug administration. At the end of a washout period of 7 days, 500 mg diosmin (Venex®500) was administered daily for 9 days. On day 10, a tablet of 100 mg of diclofenac sodium (Voveran®100) was administered Blood samples were collected as described above. Blood samples were centrifuged at 3,000 rpm for 15 min and serum samples were stored at -80°C until analysis.

Method of analysis

Diclofenac in the serum samples was estimated by reversed phase high pressure liquid chromatography /10/. To 250 µl of serum, 20 µl of internal standard chlorzoxazone (1 mg/ml) were added and vortexed for 2 min. An equal volume of methanol was added to serum samples for protein precipitation and vortexed for one minute and centrifuged at 13,000 rpm for 8 min using a Biofuge Fresco centrifuge (Heraeus, Germany). The retention times of diclofenac sodium internal standard were 7.8 and 4.2 minutes, respectively, shown in Figure 2 (I). Test and blank chromatograms of diclofenac sodium are shown in Figure 2 (II) and (III).

Calibration curve of diclofenac sodium in human serum

Different concentrations (0.5, 1, 2, 4, 8, 12, 16, 24 µg/ml) of diclofenac sodium in serum were prepared for the calibration curve.

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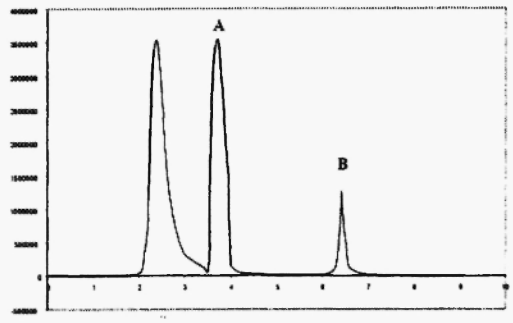
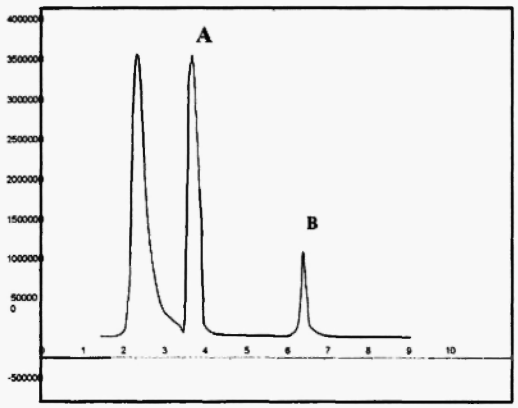


(I)



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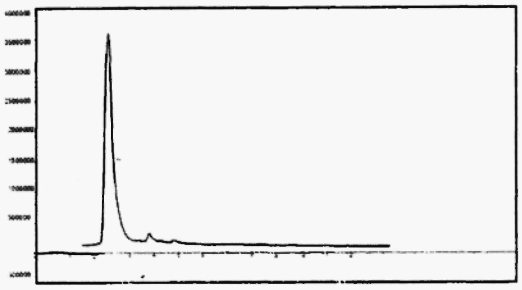

(III)

Fig. 2: I. Serum standard chromatogram (**A** = chlorazoxazone, **B** = diclofenac). II. Serum test chromatogram (**A** = chlorazoxazone, **B** = diclofenac). III. Serum blank chromatogram.

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The samples were extracted as described and peak areas of diclofenac and chlorzoxazone were obtained. The peak area ratios obtained at different concentrations of the drug were plotted against the concentration of drug. The slope of the plot determined by the method of least square regression analysis ((r^2 = 0.9981) was used to calculate the diclofenac sodium concentration in the unknown sample.

Statistical analysis

Pharmacokinetic parameters: area under the curve (AUC), elimination half life ($t_{½}$), volume of distribution (V/f) and total clearance (CL/f), were calculated for each subject using a non-compartmental pharmacokinetic program RAMKIN /11/.

RESULTS

The mean ± standard deviation (SD) serum concentrations of diclofenac at different time points before and after diosmin pretreatment are shown in Figure 3. The pharmacokinetic parameters of diclofenac before and after diosmin pretreatment are presented in Table 1. There was a significant ($p < 0.01$) increase in $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}\infty}$ of diclofenac by 56.34% and 59.19%, respectively. There was a concomitant decrease in clearance by 40.27% and increase in half-life by 20.0%. Upon statistical analysis of the data using ANOVA, considering diclofenac sodium administration as reference and co-administration with diosmin as test, indicated that there was a significant increase in bioavailability. ANOVA of log-transformed data resulted in confidence intervals (both Westlake's and Classical) lying beyond 80-125%, indicating that the bioavailability of diclofenac sodium was significantly altered in the presence of diosmin.

DISCUSSION

The apparently decreased metabolism of diclofenac sodium and increased serum levels of diclofenac after pretreatment with diosmin may be due to decreased expression of CYP2C9 in liver or intestine. It is evident that the metabolism of diclofenac by human liver microsomes is primarily mediated by CYP2C9 /12/. Accordingly, it has been suggested that the metabolism of diclofenac sodium may provide

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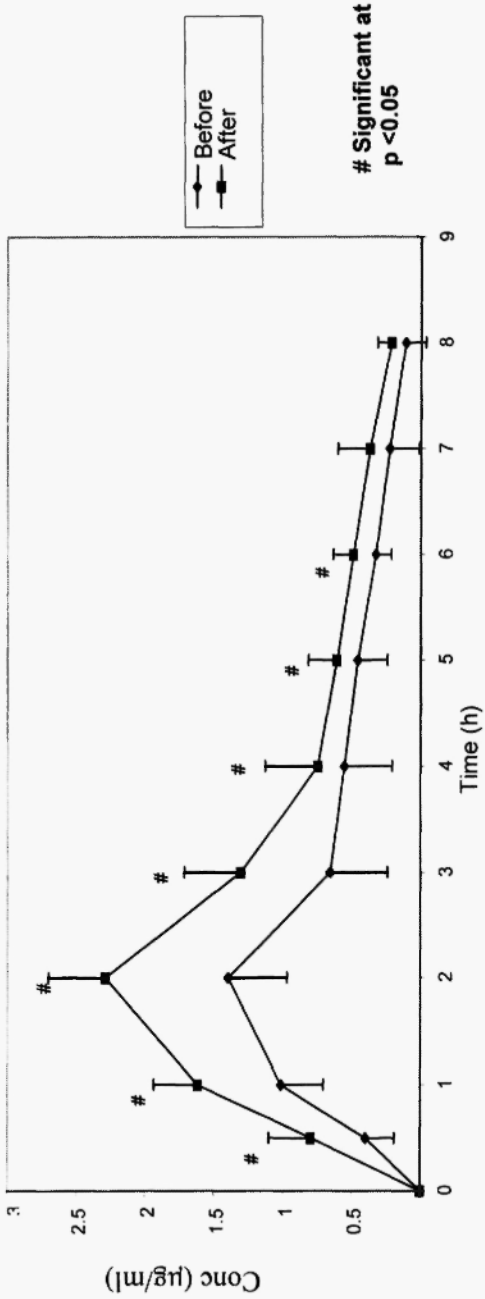

Fig. 3: Diclofenac levels before and after pretreatment with diosmin.

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TABLE 1

Pharmacokinetic parameters of diclofenac in human serum before and after pretreatment with diosmin

	Before	After	P value
C_{max} (μg/ml)	1.522 ± 0.94	2.368 ± 1.06	<0.00008
t_{max} (h)	1.833 ± 0.38	1.916 ± 0.288	NS
AUC_{0-8} (μg//ml/h)	4.634 ± 4.25	7.246 ± 5.78	<0.0003
$AUC_{0-\infty}$ (μg/ml/h)	5.080 ± 5.048	8.089 ± 7.37	<0.002
$t_{½}$ (h)	1.58 ± 0.70	2.067 ±0.641	<0.004
CL/f (ml/h/kg)	304.41 ± 186.87	167.39 ± 107.51	<0.0002
Vd/f (ml/kg)	851.36 ± 412.06	438 ± 229.97	NS

Values are means ± SD (n =12).
$AUC_{0-\infty}$ = area under the time-concentration curve from 0 to infinity; AUC_{0-8} = area under the time-concentration curve from 0 to 8 hours; CL/f = apparent systemic clearance; C_{max} = peak serum concentration; t_{max} = time to reach C_{max}; $t_{½}$ = elimination half-life; Vd/f = apparent volume of distribution; SD = standard deviation; NS = not significant.

an *in vivo* probe for the enzyme's activity in humans. It was reported that the citrus phyto-flavonoids, naringin, naringenin, quercetin and rutin, inhibited the metabolic activation of tobacco specific nitrosamine by inhibiting cytochrome P450 isoforms 1A1, 1A2, 2B1, 2D6 and 2E1, and may afford protection against nitrosamine induced carcinogenesis /13/.

Diosmin exhibits potent inhibition of ethoxyresorufin-*O*-deethylase (EROD) activity, by 11% at 0.25 mM concentration and by 61% at 0.5 mM. It inhibits methoxyresorufin-*O*-demethylase (MROD) by 47% and 54% at the two concentrations tested but did not significantly alter benzyloxyresorufin-*O*-dealkylase (BROD) activity. The alkoxyresorufin-*O*-dealkylase reactions are selective for different isoforms of cytochrome P450, and therefore diosmin might have varied effects on the metabolism of substrates for these isoforms /14/.

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CONCLUSION

Diosmin might have inhibited the microsomal CYP2C9 mediated oxidation of diclofenac sodium. The elimination half-life, AUC and C_{max} showed a significant increase due to decreased clearance.

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