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In - Vivo Assessment of Enhanced Bioavailability of Metronidazole with Piperine in Rabbits

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ABSTRACT

Piperine was established as being able to increase the bioavailability of a large number of drugs. So an attempt has been taken to study changes in bioavailability of metronidazole in presence of piperine in rabbits. Male New Zealand white rabbits (2.0-2.5 kg body weight) used for pharmacokinetic and bioavailability study. Three groups of rabbits were formed from which one group was considered as control and received only vehicle (distilled water) orally. Remaining two groups were treated with metronidazole and combination of metronidazole and piperine respectively. About one ml of blood sample was collected at the different time intervals and analyzed spectrophotometrically by HPLC. A C_{max} value of 3,805.89 ± 233.8 ng/ml was obtained with metronidazole alone (20 mg/ kg) and 6,007.07 ± 348.8 ng/ml was obtained with a combination of metronidazole (20 mg/kg) and piperine (10 mg/kg). This represents an increase of 57% in peak plasma levels of metronidazole. Reduction in total clearance from 0.06 ± 0.02 to 0.04 ± 0.02 ml/h, and a volume of distribution from 2.69 ± 0.23 to 1.48 ± 0.65 L resulted in a net increase of 88.53% in AUC (45,073.75 ± 713.7 to 84,980.98 ± 345.6 ng*h/ml). So we can conclude that bioavailability of metronidazole was significantly enhance in the presence of piperine.

Key words: Bioavailability; Metronidazole; Piperine

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INTRODUCTION

About 60% of world population and almost 80% of third world population still depend upon plant based medicines. The modern pharmacopoeias too contain almost 25% drugs of plant origin [1, 2]. In allopathic system of medicine the concept of bioenhancer appears to be comparatively of recent origin where as this concept had been use in Ayureveda since centuries and called it "*Yogvahi"* e.g. use of '*trikatu'* [3]. Black pepper is the supporting evidence where piperine was one of the ingredients as "*Yogvahi"* [4]. In Ayurvedic system of medicines, black pepper/ long pepper/ trikatu was prescribed routinely for a variety of diseases as part of multidrug formulations [5].

Piperine is a major alkaloid of Pepper fruits belonging to family Piperaceae which has a number of medicinal properties [6]. Pepper is used in different food, drink, dessert, perfume as a brandy flavors and preservative of pickles. It is known as "*King of spices*". Black pepper alone accounts for about 35% of the world's total spice [7]. Mode of action of any drug mainly depends upon its bioavailability which in turn depends upon the rate at which the unchanged drugs are made available to the body and the extent to which the dose is ultimately absorbed after administration. Piperine enhances the bioavailability of structurally and therapeutically different drugs, either by increasing the absorption or by delaying the metabolism of the drug or by a combination of both processes [8- 13]. It is evident that black pepper has been used as bio-enhancer for a number of drugs in allopathic system of medicine like oxyphenylbutazone [14], phenytoin [15], aflatoxin B_1 [16], beta-carotene [17] propanolol and theophylline [18]. Piperine has been used as bioenhancer for certain antibacterial -antibiotics with promising results e.g. rifampicin [19, 20], dapsone [21], curcumin [22], ciprofloxacin [23], cefotoxime sodium and cyclosporine A [24].

However hardly any such information seems to have been available about the bioenhancing property of pepper for antiprotozoal drugs like Metronidazole. Metronidazole used widely as antiprotozoal drug and primarily metabolized by oxidation of side chain. Based on the reported interaction of piperine with drug–metabolizing enzymes responsible for oxidation, hydroxylation and glucuronidation [25, 26], the present investigation was undertaken to study changes in bioavailability of Metronidazole in combination of piperine in rabbits.

MATERIALS AND METHODS

Materials

Fresh Fruits of *Piper nigrum* were collected from different locations around Cochin in February. The plant for this study was identified by Mr. M.H. Parabia of the Shri Bapalal Vaidya Botanical Research Center, Department of Bioscience, Veer Narmad South Gujarat University, Surat, India. Chemicals like acetone, chloroform, dichloromethane, ethanol, methanol, picric acid, silica gel, potassium hydroxide, hydrochloric acid, Phloroglucinol and glycerine were of analytical grade (AR) and obtained from M/S E. Merck and M/S Loba Chemie (India).

Isolation of piperine

Around Four kg of black pepper fruits were crused and powdered. Powdered material was soxhlated in 1000 ml of solvent mixture of 95% methanol and n-hexane (9:1) for 8 h. The solvent extract of siphon becomes colorless in appearance on complete soxhlation. The methanol and n-hexane mixture (solvent extract) was separated by centrifugal separator. The hexane part of the solvent was discarded and alcoholic part containing piperine was decolorized by charcoal filtration. The extract was finally concentrated in Rotary evaporator (Yamato, Japan). The concentrated extract was kept in the freeze for 24 h for crystallization. The crystals of piperine would stand out as greenish yellow needle like crystals. These crystals were dissolved in alcohol and filtered. The filtrate was again kept in the freeze for 24 h for re-crystallization and this process was repeated twice to have maximum yield of piperine. Crystals of pure piperine so obtained were air dried.

Animals used

Healthy male New Zealand white rabbits (n = 9) weighing 2.0 - 2.5 kg and age 4 to 6 month were selected for pharmacokinetic and bioavailability study. All 9 rabbits were kept for acclimatization for seven days prior to start pharmacokinetic study. Body weight variation among the animals did not exceed 20% of the mean body weight. The room was environmentally-controlled at a temperature of 22 ^{o}C with relative humidity of 60 %. The photoperiod was 12 h light and 12 h darkness. Prescribed feed and *ad libitum* drinking water were provided during experimental period. The experiment was carried out in accordance with the guidelines of Institutional Animal Ethics Committee.

Experimental design

Prior to initiate pharmacokinetic study, animals were fasted overnight and the fasting continued up to 4 h post dosing. Three groups of rabbits were formed each group having three rabbits. Group I received vehicle (water) only, and was used for obtaining control plasma; group II was administered Metronidazole at a dose of 20 mg/kg orally, and group III received a combination of Metronidazole (20 mg/kg) and piperine (10mg/kg) orally. About one ml of blood was collected from each rabbit at time interval of 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 16.0 and 24.0 h from marginal ear vein with the help of Scalp Vein Set and disposable syringe of 2 ml. Heparin was used as anticoagulant at the rate of 20 µl/ ml.

Estimation of metroidazole in blood

The blood samples so collected were stored in 1.5 ml plastic tube and centrifuged at 3000 rpm for 5 min. Separated blood plasma (0.5ml) was then mixed with methanol (5 ml) and vortexes for 10 min. These samples were left aside for 15 min which resulted into separation of plasma and solvent (methanol) layer. Solvent layer containing respective drug was allowed to evaporate on water bath to have the final volume of 1 ml.

Samples having Metronidazole was analyzed by HPLC. Instrument used was Gilson HPLC system model 302 with isocratic mode having UV-Visible detector model 118

interfaced with integration software Gilson Unipoint (v1.9). Kromasil (C_{18}) column was used for detection of Metronidazole at 300 nm. The composition of mobile phase was acetonitrile and ammonium acetate (0.1M) in the ratio of 30:70 & pH was adjusted at 4.3. The flow rate 1.0 ml/min, sensitivity 0.01 and retention time 3.4 min was maintained.

Pharmacokinetic analysis

Pharmacokinetic analysis was carried out using the TOPFIT (v.2) kinetic software. Peak serum concentration (C_{max}) and time to reach the peak serum concentration (T_{max}) were calculated from the actual plasma data. Elimination rate constant i.e. K_{el} was calculated by least square regression analysis, while elimination half life ($t_{1/2el}$) was obtained using the formula $t_{1/2el} = 0.693 / K_{el}$. Area under the serum drug concentration verses time curve (AUC) was calculated by trapezoidal rule.

RESULTS AND DISCUSSION

HPLC chromatogram of Metronidazole in blood plasma shows a sharp peak at 3.861. The peaks obtained from the metronidazole reference standard as well as metronidazole extracted from the blood plasma were matched. Typical plasma profile of metronidazole is shown in Figure 1.

Plasma levels of Metronidazole for the different regimens are presented in Figure 2. A C_{max} value of 3,805.89 ± 233.8 ng/ml at 2 h was achieved when Metronidazole was administered alone. While C_{max} after administration with piperine was 6,007.07 ± 348.8 ng/ml; representing an increase of 57%. Higher Metronidazole plasma concentration was found in the piperine experiment at every time point up to 24 h after administration. Other pharmacokinetic parameters comparing the bioavailability of the Metronidazole alone and combination with piperine are given in Table 1. A higher C_{max} value, reduced total clearance and reduced volume of distribution contributed to an apparent increase in AUC about 88.53 % were achieve when Metronidazole give in combination with piperine. However the elimination half-life remained unchanged.

The above findings show that piperine significantly improve the bioavailability of metronidazole.. This can be explained on the basis of its effect on gastric and intestinal mucosal cells. Transepithelial electrical resistance (TER) is responsible factor in controlling the permeability of intestinal epithelia. Piperine decreases the TER and thus increases the pore size between the cells and in turn the permeability of the intestinal milieu resulting in higher rate and extent of absorption of Metronidazole.

Metronidazole extensively metabolized by oxidation of side chain in phase I metabolism and by glucuronidation in phase II. Pharmacokinetic results showed that plasma $t_{1/2}$ of metronidazole increases from 11.48 to 12.24 h when it was administered with piperine. This shows that Piperine delaying the metabolism of metronidazole by inhibiting the enzymes responsible for oxidation and glucuronidation of metronidazole. Inhibition of drug- metabolizing enzymes ultimately leads to enhanced plasma level of metronidazole.

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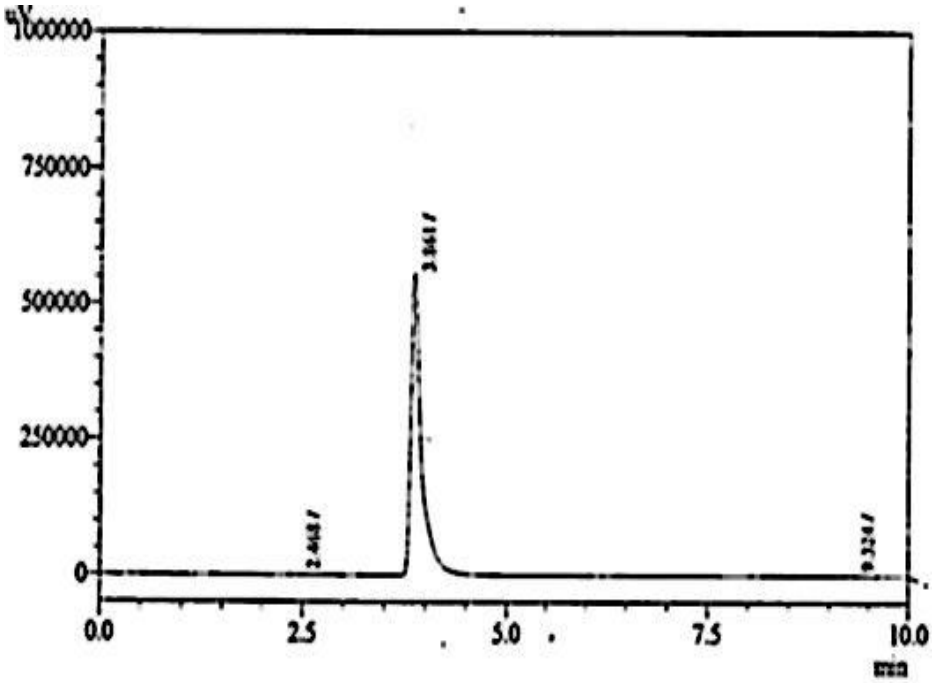

Figure 1: HPLC profile of metronidazole from blood plasma.

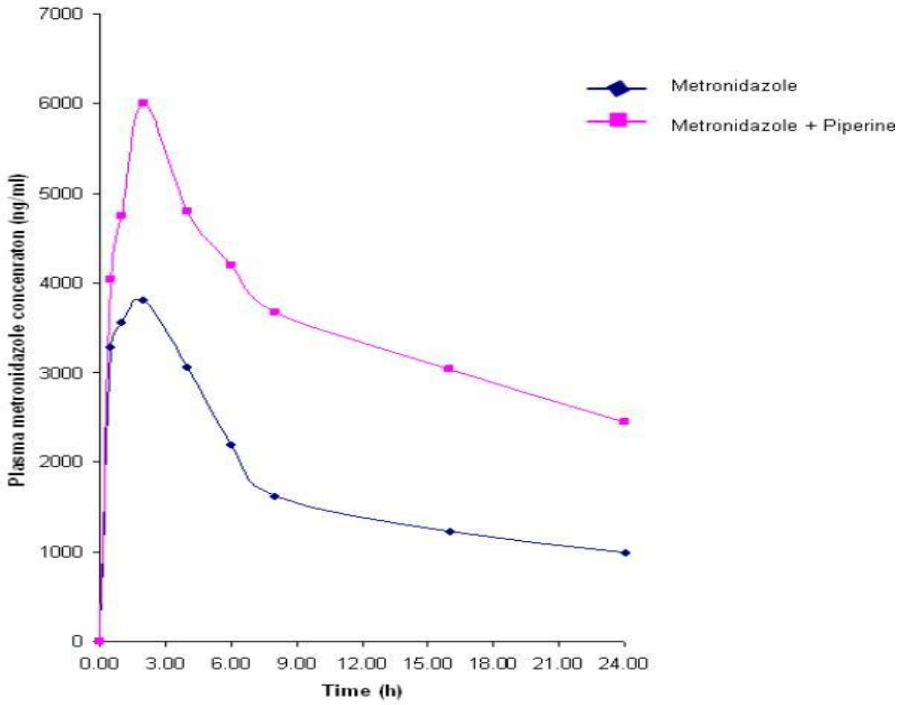

Figure 2: Comparison of mean plasma concentration of metronidazole alone and with piperine.

CONCLUSION

Results of the present study indicate that piperine significantly increase the absorption of metronidazole and retarding its metabolism. So we can conclude that the Metronidazole and piperine combination may result in a reduced strength dosage form and also reduced dose dependent side effects.

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Table 1: Comparative pharmacokinetic data of metronidazole alone and in presence of piperine.

Pharmacokinetic Parameters	Metronidazole[g]	Metronidazole[g] + Piperine
C_{max} (ng/ml) [a]	3,805.89 ± 233.8	6,007.07 ± 348.8
AUC 0-24 (ng*h/ml) [b]	45,073.75 ± 713.7	84,980.98 ± 345.6
T_{max} (h) [c]	1.66 ± 0.57	1.66 ± 0.57
Kel (ml/h) [d]	0.06 ± 0.02	0.04 ± 0.02
$t_{½}$ (h) [e]	1.48 ± 1.23	12.24 ± 1.04
Vd (L) [f]	2.69 ± 0.23	1.48 ± 0.65

[a]Maximum concentration, [b]Area under curve, [c]Time at maximum drug concentration, [d]Elimination rate, [e]Half life, [f]Volume of distribution, [g]mean ± sd (n = 3)

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