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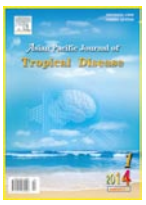

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Enhanced pharmacokinetics of omeprazole when formulated as gastroretentive microspheres along with piperine

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PEER REVIEW

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Comments

The nicely designed experiment demonstrates three concepts at once: leveraging the potential of piperine as permeability enhancer as well as inhibitor of metabolism, to enhance the bioavailability of omeprazole: demonstrating the usefulness of such combinations; showing the potential use of piperine and omeprazole to improved therapeutic efficacy.
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ABSTRACT

Objective: To investigate the formulation of gastroretentive microspheres of omeprazole along with piperine and estimate the pharmacokinetic parameters in comparison with omeprazole alone.
Methods: In our present investigation, gastroretentive microspheres of omeprazole were prepared with the inclusion of piperine. Pharmacokinetic parameters like C_{max}, T_{max} and area under curve were estimated by administering the prepared microspheres to rabbits and the results were compared with omeprazole alone.
Results: There was a significant increase in area under curve from 3.441±1.093 mg·h/mL to 14.422 ±0.708 mg·h/mL along with an increase in C_{max}.
Conclusions: This clearly shows the increased absorption and decreased metabolism of omeprazole when administered along with piperine as gastroretentive microspheres.

KEYWORDS
Bioavailability, Omeprazole, Piperine, Gastroretentive microspheres

1. Introduction

Bioavailability plays an important role in achieving the required plasma level of drug especially when administered orally. Poor bioavailability of this route may be due to several reasons, and absorption is considered as the major reason. Further efflux activity of P-glycoprotein (P-gp) is another potential reason for decreased absorption. The desired plasma levels after oral administration also depends on extent of hepatic first pass by cytochrome P450 monooxygenase enzymes[1]. Omeprazole is a proton pump inhibitor that inhibits H^+/K^+ ATPase activity in parietal cells. Its oral bioavailability was found to be around 40%, suggesting pronounced first pass metabolism by CYP1A2, CYP3A1 and efflux by P-gp[2,3]. An extensive research is going on in the area of novel drug delivery and targeting towards herbal active principles. Herbal active principles have enormous therapeutic benefits and they need to be explored. However, the research is still at the exploratory stage[4]. Piperine extracted from pepper is the

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most abundantly available active principle of Indian food. Piperine was found to be the inhibitor of both P-gp and CYP3A4 and thus can enhance the bioavailability of many drugs[5]. It was also reported that, piperine inhibits gastric mucosal damage by the inhibition of volume of gastric juice, gastric acidity and pepsin A activity and can be used against gastric ulcers[6]. The present investigation is focused on the formulation of gastroretentive microspheres of Omeprazole along with Piperine and estimating the pharmacokinetic parameters in comparison with Omeprazole alone.

2. Materials and methods

Omeprazole was a kind gift sample from Dr. Reddy, Bachupally, Hyderabad, piperine (98%) from Alfa Aesar, UK and all the other solvents and chemicals used were of analytical grade.

2.1. Compatibility studies

Individual and physical mixture of omeprazole-piperine (1:1) were processed for Fourier Transform Infra Red (FT-IR) studies between 400-4 000 cm^{-1}.

2.2. Preparation of omeprazole gastroretentive microspheres with piperine

Emulsification-solvent evaporation method was used for preparing the microspheres. The quantity of ethyl cellulose (250 mg), calcium carbonate (50 mg), hydroxyl propyl methyl cellulose (100 mg), piperine (50 mg) and omeprazole (50 mg) were added to the acetone (dispersed phase) and stirred well to get homogenous mixture. This mixture was then added to 50 mL of light liquid paraffin containing 3% of span 80 (dispersion medium) with continuous stirring at the pre optimized speed of 950 r/min. The emulsion was stirred for 4.5 h at room temperature to facilitate complete evaporation of acetone and the formation of microspheres. The prepared microspheres were filtered and washed with petroleum ether to remove the traces of light liquid paraffin[7].

2.3. Bioavailability studies in rabbits

A total of 2 groups of rabbits (3 per group) weighing 1.5 to 2 kg were housed at room temperature, light (10 h) and dark (14 h) cycle throughout the experimental period. All animals were maintained and experimented as per the protocol approved by Institutional Animal Ethical Committee, Nalanda College of Pharmacy, Nalgonda (Ref. No. NCOP/IAEC/Approval/41A/2011, dated 20/09/2011). Group I and II were administered with omeprazole (10 mg) alone and omeprazole (10 mg) along with piperine (10 mg) as gastroretentive microspheres respectively. Exactly 0.5 mL of blood samples were collected from the ear veins of rabbits at regular time intervals of 0.5, 1.5, 2, 2.5, 3, 6 and 12 h and immediately transferred to sterile tubes of 2 mL capacity with 10 μL anticoagulant solution (10% w/v sodium citrate). The samples were centrifuged at 3 000 r/min for 20 min to separate the plasma. Thus obtained plasma samples were stored in refrigerator till further use.

2.4. HPLC estimation of omeprazole

Plasma (500 μL) was extracted with 4 mL of dichloromethane and centrifuged. About 3 mL of the dichloromethane extract was evaporated to dryness and reconstituted with 250 μL of mobile phase. The samples were eluted using C18 column (4.6 mm×150 mm and 5 μm) at 30 °C under isocratic conditions with a mobile phase of 50 mmol/L sodium dihydrogen phosphate (pH 7.2): acetonitrile (75:25) at a flow rate of 1 mL per minute. Eluted omeprazole was detected by UV at 302 nm[8].

2.5. Estimation of pharmacokinetic parameters

Individual plasma concentrations and time curves were plotted. C_{max}, T_{max} were directly obtained from them and area under curve (AUC) was calculated by using trapezoidal rule. From the terminal log decay phase, the elimination rate constant was estimated using linear regression[9].

2.6. Effect of piperine on stomach surface

Two avian stomachs upto 3 cm of proximal part of intestine were hanged in 100 mL of phosphate buffer solution with a pH of 7.4 filled inside with 10 mg of piperine in 5 mL of 0.1 mol/L HCl in test and plain 5 mL of 0.1 mol/L HCl. After 12 h the stomach at fundus region was studied for histopathology[10].

2.7. Statistical analysis

The data was expressed as mean±SD. Data was analyzed by Dunnet's multiple analysis of variance (ANOVA) to compare all groups against control. Results were

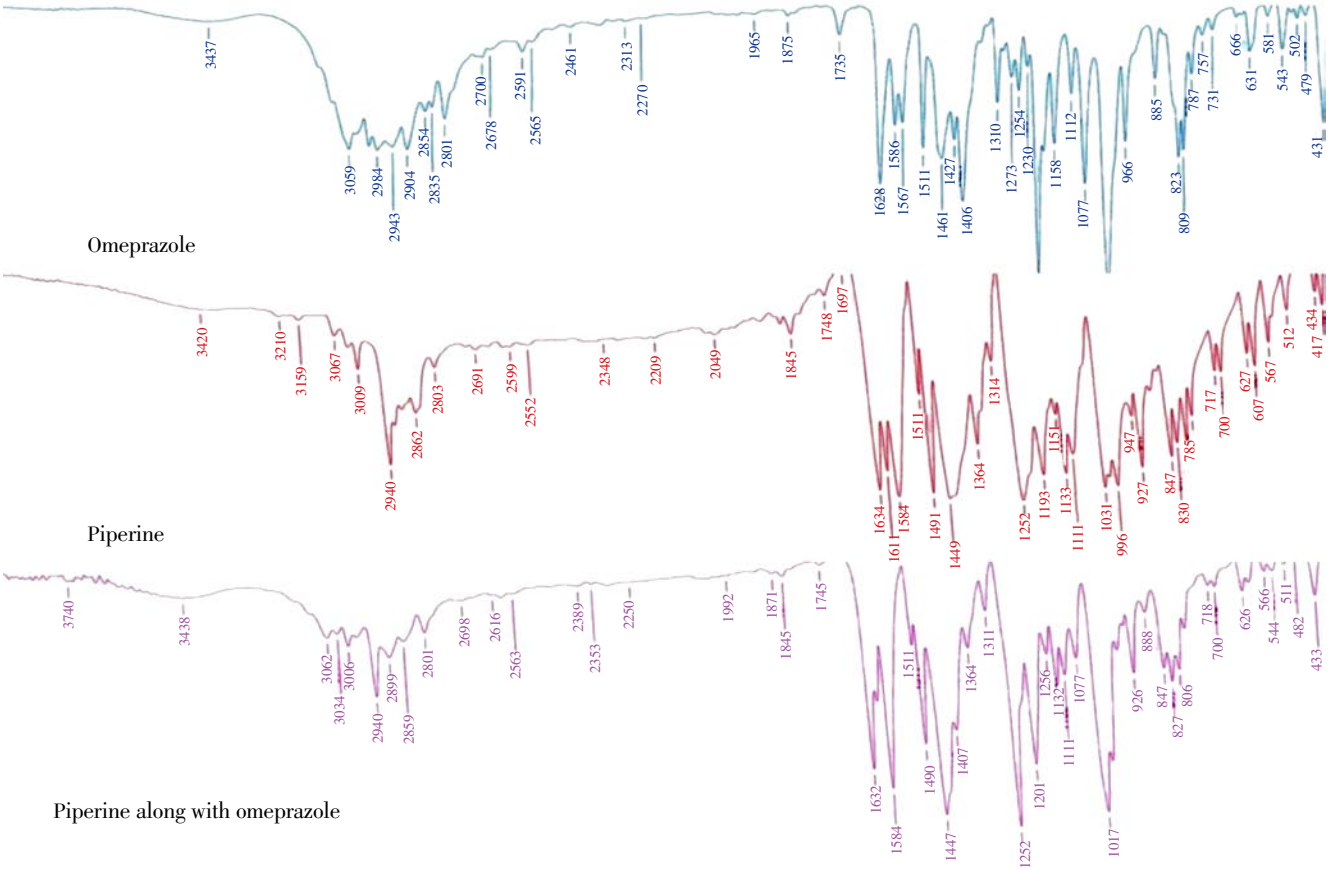

Figure 1. FTIR details of pure omeprazole and piperine with important IR bands.

Table 1
Pharmacokinetic parameters of omeprazole in plasma samples.

Treatment	AUC (mg·h/mL)	C_{max} (mg/mL)	T_{max} (h)
Omeprazole alone	3.441±1.093	0.473±0.186	3
Gastroretentive formulation along with piperine	14.422±0.708[a]	1.899±0.139[b]	6[b]

a: the values are significant at $P<0.05$; b: values are significant at $P<0.01$ by Dunnet's multiple comparison test.

considered statistically significant at $P<0.001$ and $P<0.005$.

3. Results

The FTIR spectrum was given in Figure 1. The characteristic peaks of omeprazole, 1 075 cm^{-1} (resonance band), 1 203.4 cm^{-1} (aromatic C-O-CH3 vibration), 1 626.7 cm^{-1} (C=C-N) and S-C=N stretching and for piperine at 930 cm^{-1} (C=O stretching), 1 030 cm^{-1}, 1 250 cm^{-1} (=C-O-C stretching), 1 635 cm^{-1}, 1 603 cm^{-1} (C=C diene stretching) 2 840 cm^{-1}, 2 925 cm^{-1}(C-H stretching) and 3 000 cm^{-1} (aromatic C-H stretching) were present in the combined and individual FTIR spectra of omeprazole and piperine. Pharmacokinetic parameters were estimated and given in Table 1 and Figure 2. From the data it was clearly understood that, there is an increase in the AUC by 4 times when omeprazole was administered along with piperine.

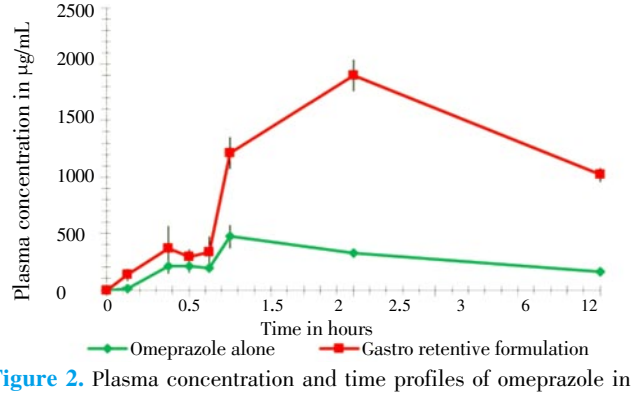

Figure 2. Plasma concentration and time profiles of omeprazole in plasma samples in rabbits.

Microscopic pictures of histopathology of avian stomach wall with and without piperine treatment was given in Figure 3. From the pictures, it is clearly evident that, there were intact cells without any fluidity in the untreated

stomach and lose of integrity, loose fibrous connective tissue and gaps between cells in piperine treated stomach.

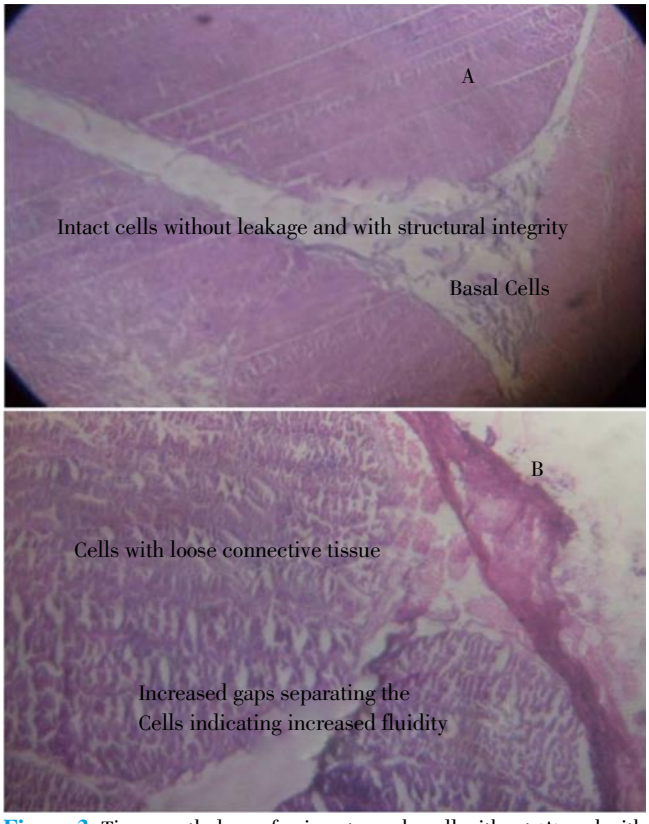

Figure 3. Tissue pathology of avian stomach wall without (A) and with (B) piperine treatment.

4. Discussion

FTIR analysis clearly shows that, there were no interactions between omeprazole and piperine. There were no displacement of charecteristics peaks and hence no compatibility problem between them. In bioavailability studies, there was almost 4 times increase in AUC in the animals administered with gastroretentive formulation of omeprazole along with piperine. C_{max} achieved was also 4 times greater than the administration of omeprazole alone. The T_{max} was delayed by 3 h and this may be because of the sustaining release property of the gastroretentive formulation. The increase in bioavailability may be because of ability of piperine to increase membrane fluidity and inhibition of efflux protein, so that more amount of drug reaches to systemic circulation. From this it can be considered that, there is an increase in absorption. Furthermore, in the study of piperine treated stomach histopathology, there is a significant increase in the fluidity of cells. This increased permeability may be due to the ability of piperine to induce alterations in membrane cytoskeleton and synthesis of proteins associated with increased absorption. Its apolar nature also supports increased fluidity because of the configuration that it forms with the lipid bilayer of the membrane[11]. There will be additive effect of inhibition of metabolism and hence drug reaching systemic circulation won't be metabolized. There will be another clinical significance for the adjuvant therapy of piperine with omeprazole. Omeprazole leads to mucosal inflammation by damaging parietal cells and further may result in gastritis. This effect may be because of oxidative damage and can be prevented by antioxidant therapy[12]. Piperine being good antioxidant[13] can prevent inflammation and gastritis. The prepared gastroretentive formulation contains hydroxyl propyl methyl cellulose as a polymer that swells in slightly alkaline pH (≈7)[14] and the formulation tends to stay in the proximal part of intestine. Piperine has maximum absorption (97%) in upper duodenal part than in lower segments[15]. Omeprazole rapidly degrades in acidic environment and it is beneficial to be formulated as retentive system at slightly alkaline pH[16].

The current drug discovery approach of finding new entities, if shifted to combining existing agents, may be helpful. Omeprazole along with piperine in gastroretentive formulation can be beneficial as there will be increased bioavailability of omeprazole because of the mechanisms of piperine to increase absorption and also to decrease the metabolism.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Interaction of piperine analogs with intestinal membrane and metabolic enzymes is a widely published and accepted fact. In the present study, it was elegantly demonstrated that piperine can be utilised to enhance the bioavailability

of compounds which were limited by metabolism, permeability or efflux transport.

Research frontiers

Since many failure in new product development is due to low oral bioavailability, any method to improve bioavailability is need of the hour. In that aspect, this research article presents a strong evidence of enhancing bioavailability through simply combining two well established known compounds.

Related reports

It is reported that omeprazole is a proton pump inhibitor which inhibits H^+/K^+ ATPase activity in parietal cells. And piperine inhibits gastric mucosal damage by the inhibition of volume of gastric juice, gastric acidity and pepsin A activity and can be used against gastric ulcers.

Innovations & breakthroughs

No such distinct innovation is observed, however, this could serve as an important piece of research showcasing the way of increasing bioavailability, leveraging biological factors.

Applications

The outcome of this research can be applied to the following areas: combination drugs; bioenhanced products; dose reduction methods.

Peer review

The nicely designed experiment demonstrates three concepts at once: leveraging the potential of piperine as permeability enhancer as well as inhibitor of metabolism, to enhance the bioavailability of omeprazole; demonstrating the usefulness of such combinations; showing the potential use of piperine and omeprazole to improved therapeutic efficacy.

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