Natural bioenhancers are drug facilitators, they are the molecules which by themselves do not show typical drug activity at given dose, but when used in combination enhances the permeability or bioavailability of drug molecules (1). Piperine (PI) is a bioenhancer found in plants Piper nigrum L. commonly known as black pepper (Fig.1), and Piper longum L. commonly known as long pepper (2). PI is used as spices and as ingredients in Indian traditional systems of medicine (3). PI is one of natural bioenhancer which has been patented and reported as bioavailability enhancer for some food nutrients and drugs. It acts as bioenhancer by various known and unknown causes such as it inhibits the functions of both P-glycoprotein and CYP3A4 and increase permeation of P- glycoprotein and CYP3A4 substrates. It stimulates metabolism and thermogenesis in conjunction with increased nutrient absorption. It may enhances bioavailability of these drugs by various mechanisms such as increase in splanchnic blood flow, decreased hydrochloric acid secretion, delay in gastric emptying and an alteration of membrane dynamics which aid in efficient permeability through membranes which may increase bioavailability (4-6).

Atenolol (AT) is very well used to treat mild to moderate hypertension and stable angina pectoris. It is a BCS class III drug which poorly absorbs in lower intestinal tract (50%). It is hydrophilic in nature and absorbs through paracellular pathway (7).

In present study, effect of PI on permeability and bioavailability of AT has been evaluated by performing in vitro Caco-2 cell line studies and in-vivo pharmacokinetics studies in wistar rats.

Methods

Caco-2 Cell Culture

Cell culture plates (8-wells, from European collection of cell cultures) were cultured at 37°C in Dubosso's modified Eagle medium supplemented with 10% fetal bovine serum, 1% nonessential amino acid, 1% glucose and 1% antibiotics-gentamycin in 5% CO2 and 95% relative humidity. Cells were seeded at 1 X 10⁶ cells/cm² on 6-well Transwell™ plates and cultured for 21-28 days prior to transport experiments. Integrity of IEC monolayers was checked by measuring TEER. Caco-2 monolayers with TEER values over 750 Ωcm² were used for transport experiments.

Drug Transport Studies in Caco-2 Cell Monolayers

Permeation of 10 AT across Caco-2 cells was measured in presence and absence of five different concentration of PI and EDTA. Samples were withdrawn from basolateral compartment at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. Sample from apical compartment was withdrawn at 0 and 180 min. Withdrawn samples were mixed with StarText acidification cocktail, and pHAT were quantified using a Beckman Coulter L50000T (Buckinghamshire, UK). TEER was measured after final sample withdrawal. After completion of the experiment, cell monolayer with insert membrane was removed with help of scapper to measure the amount of drug retained in the cell monolayer. Results of experiments performed (n = 9) are presented as mean ± SD. Apparent permeability coefficients (Papp) and transport enhancement ratios were calculated in presence and absence of PI.

In-vitro Pharmacokinetics Studies

In-vitro experiments were conducted as per CPCSEA (Committee for Prevention, Control and Supervision of Experimental Animals, Reg No. 40/04/CPCSEA) guidelines. Each group (four groups) of wistar rats (300-350gm) has six rats. Drug and bioenhancer (three concentrations) were administered to rat orally. Blood samples were collected from retro-orbital plexus of rats at 0, 0.5, 1, 2, 3, 6, 8, and 24 hour time points into heparinized collection tubes. Plasma samples were processed to determine pharmacokinetics parameters in AT of presence in PI.

Statistical Analysis

In-vitro studies: Significance between the mean values was calculated using ANOVA with SPSS v12.0 (SPSS Inc., USA). Further post-hoc analysis was done to localize significant differences (P<0.05) between permeability of AT with and without PI. In vivo studies: Primary pharmacokinetic parameters were determined using non compartmental analysis. Maximum plasma concentration Cmax and time to reach maximum concentration Tmax were estimated directly from plasma concentration time curve. All the data were expressed as mean ± SD. Plasma drug concentrations as well as pharmacokinetic parameters were compared by student’s t-test at p<0.05.

Results

Pmax, values and transport enhancement ratio of [H]AT is significantly higher (p<0.01) with 1.0 and 50μM concentrations of PI and with 10μM EDTA. Pmax, values and transport enhancement ratio of [H]AT was equal or less with higher concentration of PI. Reduced %TEER values of Caco-2 cell monolayers post transport studies in presence of PI suggests that PI may cause opening of tight junctions that lead to enhanced Pmax, values of [H]AT. PI (except 1μM concentration) could not cause enhancement of [H]AT across Caco-2 cells by itself. EDTA, Permeation reduction effect of higher concentrations of PI remains still unclear but it may be due to saturation of paracellular pathways.

Conclusions and Discussion

In-vitro studies postulates that PI can modulate paracellular openings which led to increases permeation of AT significantly in presence of PI. Decreased PI effect on tight junction remain to be elucidated but it is believed that subtle changes in cell membrane caused by cytoskeletal changes may result in TEER reduction and enhanced permeability of atenolol.

Pharmacokinetic results show more enhancing effect of PI on AT bioavailability. This may be due various mechanisms. PI can increase splanchnic blood flow, decreased hydrochloric acid secretion, delay in gastric emptying and an alteration of membrane dynamics which aid in efficient permeability through membranes (4-6). PI also increases the absorption of many drugs due to its easy partitioning ability, induction of synthesis of proteins associated with cytoskeletal functions resulting in an increase in the small intestinal surface, thus assisting efficient penetration through epithelial barriers. An enhanced absorption of AT can be due to brush broader mechanism of PI (6). It has been found that PI doesn’t affect the excretion of AT, but successively enhance permeation and absorption of AT.

This studies suggest that PI can be utilized as the novel excipient as bioenhancer for class III & IV drugs. PI is of herbal origin and also listed by FDA as Generally Recognized as Safe (GRAS), excludes need for sophisticated and costly drug delivery system.

References


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