

Role of Bioenhancers in Tuberculosis

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ABSTRACT

Bioenhancers are such agents, which by themselves are not therapeutic entities but when combined with an active drug lead to the potentiation of the pharmacologic effect of the drug. They act through several mechanisms which may affect mainly drug metabolism, absorption process, or action on drug-target. Bioenhancers can be herbal and animal origin. Herbal bioenhancers have been shown to enhance bio-availability and bio-efficiency of different class of drugs such as anti-tubercular drugs, antibiotics, antiviral, antifungal and anticancer drugs. Scientists at Indian Institute of Integrative Medicine, Jammu, discovered and scientifically validated piperine as the world's first bioavailability enhancer in 1979. They have been found to increase the bioavailability of a number of drugs including anti-tubercular drug, Rifampicin, even when reduced doses of drugs are present in such formulations. Bioenhancers will reduce the dose and toxicity of drugs; it may shorten the treatment period. Immunomodulatory and hepatoprotective properties of piperine may further benefits in treatment of tuberculosis. Extensive research on these bioenhancer is need of the hour so that they could be utilized in drug formulations in future.

Keywords: Bioenhancers, Piperine, Rifampicin.

INTRODUCTION

The bioenhancers are chemical entities which promote and augment the bioavailability of the drugs which are mixed with them and do not exhibit synergistic effect with the drug. [1,2]

The concept of bioenhancers of herbal origin can be tracked back from the ancient knowledge of Ayurveda system of medicine. In Ayurveda, this concept is known as 'yogvahi' and is used to increase the therapeutic effect of drugs by enhancing the oral bioavailability, tissue distribution especially for those drugs with poor oral bioavailability, decrease their dose and adverse effects, and to circumvent the parenteral routes of drug administration. Use of ayurvedic preparation "Trikatu" from the period between the 7th century

B.C. and the 6th century A.D., which is a Sanskrit, word meaning three acids. It refers to a combination of black pepper (*Piper nigrum*), long pepper (*Piper longum*), and ginger (*Zingiberofficinale*), which contains active component piperine, which enhances the bioavailability of drugs, nutrients, and vitamins. Concept of bioenhancers first time reported by Bose in 1929, who described the increase in the anti-asthmatic effects of vasaka (*Adhatodavasica*) leaves by the addition of long pepper to it. The term bioavailability enhancer was first coined by Indian Scientists at the Regional Research laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine). They discovered and scientifically validated piperine as the

world's first bioavailability enhancer in 1979. [3]

Ideal Bioenhancer

Ideal bioenhancers should have novel properties such as: They should be nontoxic to humans or animals, should be effective at a very low concentration in a combination, should be easy to formulate, Most importantly, they should enhance uptake/absorption and activity of the drug molecules.

Need for Bioenhancers

The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, pre-systemic metabolism and susceptibility to efflux mechanisms. Bioenhancers modulate one or more of these factors to enhance absorption and decrease the metabolism to reduce dose, toxicity, and

cost of drugs. Need for bioenhancers arises for drugs which are poorly available, administered for long periods, toxic and expensive.

Effect of bioenhancers on drugs

The dose of the drug is reduced and risk of drug resistance is minimized. There will be reduction in the dose-dependent toxicity and cost of the drug, especially of anti-tubercular drugs. Research showed that bioenhancers enhanced bioavailability and bio-efficacy of different classes of drugs, such as anti-tubercular, antibiotics, antiviral, anti-fungal, and anticancer drugs at low doses. [4,5]

Classification of Bioenhancers

Bioenhancers can be classified based on their natural origin (Table 1) as well as based on the mechanism of action (Table 2).

Table 1: Classification of Bioenhancers based on origin.

Plant Origin	Animal Origin
Piperine [<i>Piper longum</i> (long pepper) and <i>Piper nigrum</i> (black pepper)]	Cow urine distillate: (Kamdhenu ark)
Curcumin [<i>Curcuma longa</i> (Turmeric)]	
Allicin [<i>Allium sativum</i> (garlic)]	
Genistein [<i>Glycine max</i> (soybean) and <i>Puerarialobata</i> (kudzu)]	
Naringin [grapefruit, apples, onions and tea.]	
Quercetin [Citrus fruits]	
Caraway/cumin [<i>Carum carvi</i>]	
Black cumin [<i>Cuminumcuminum</i>]	
Niaziridin [Drumstick pods]	
Lysergol [Morning glory plant]	
Glycyrrhizin [<i>Glycyrrhizaglabra</i> (Liquorice)]	
Gingerol [<i>Zingiberofficinale</i> (Ginger)]	
Stevia [Honey leaf]	
Sinomenine [<i>Sinomeniumacutum</i>]	
Capsaicin [<i>Capsicum annum</i>]	
Capmul [edible fats and oils]	
Ammoniac multiflora [<i>Ammannia multiflora</i>]	
Peppermint oil	
Aloe vera	
Gallic acid	

Table 2: Classification of Bioenhancers based on mechanism of action [6]

Mechanism of action	Examples
1.Inhibitors of P-glycoprotein (P-gp) efflux pump and other pumps:	Cuminumcuminum (black cumin), Carum carvi (Caraway), Genistein, Sinomenine, Naringin, Quercetin.
2.Inhibitors of CYP-450 enzyme and its isoenzymes:	Quercetin, Naringin, Gallic acid and its esters.
3.Regulators of GIT function to facilitate better absorption:	Niaziridin (drumstick pods), Glycyrrhizin (liquorice), Aloe vera (Aloe), Zingiberofficinale (ginger).

Mechanism of Action of Bioenhancers

The following are the chief mechanisms via which the various bioenhancers exert their bioavailability enhancing properties on the drug molecules. [7] Bioavailability enhancement can be done

by the following by several ways such as enhancement of gastrointestinal absorption of the drugs, alteration in pharmacokinetics as well as pharmacodynamics properties of various drugs, immunomodulation for facilitating interaction between drugs,

pathogens as well as host, enhancing the penetration into the pathogens even where they become persistors within the macrophages such as for *Mycobacterium tuberculosis* leading to enhanced killing of these organisms is well secured within the places otherwise inaccessible to the active drug and also increasing penetration of drug into cerebrospinal fluid. They enhance the absorption of orally administered drugs from gastrointestinal tract by increasing in blood supply. They modulate the active transporters located in various locations e.g. P-glycoprotein (Pgp) is an efflux pump which pumps out drugs and prevent it from reaching the target site. Bioenhancers in such case act by inhibiting the P-glycoprotein. They also decrease the elimination process thereby extending the sojourn of drug in the body by inhibiting the drug metabolizing enzymes like CYP 3A4, CYP1A1, CYP1B2, CYP2E1, in the liver, gut, lungs, and various other locations. This will in addition help to overcome the first pass effect administered drugs. Bioenhancers inhibit the renal clearance by preventing glomerular filtration, active tubular secretion by inhibiting P-glycoprotein and facilitating passive tubular reabsorption. Sometimes biliary clearance is also affected by inhibiting the Uri dine diphosphate (UDP) glucuronyl transferase enzyme which conjugates and inactivates the drug.

In addition to the above mentioned mechanisms, few other postulated theories for herbal bioenhancers include: reduction in hydrochloric acid secretion and increase in gastrointestinal blood supply, inhibition of gastrointestinal transit, gastric emptying time and intestinal motility, modifications in GIT epithelial cell membrane permeability, cholagogous effect, bioenergetics and thermogenic properties, suppression of first pass metabolism and stimulation of gamma glutamyl transpeptidase (GGT) activity which enhances uptake of amino acids. Recently, pharmaceutical industries have introduced several concepts for enhancing bioavailability like salt preparation, prodrug

formation, chemical alteration, micronization, sustained drug delivery and liposomal microencapsulation.

Drugs Bioenhanced by Various Bioenhancers

Extensive research has been published regarding bioenhancing effect of herbal products on modern medicine drugs. [5] Studies done in vitro, animals or humans has proved bioenhancing properties of bioenhancers along with drugs such as Macrolides (Azithromycin, Erythromycin, Roxithromycin), Cephalosporins (Cefalexin, Cefadroxil), Penicillins (Amoxycillin, Cloxacillin), Aminoglycosides (Kanamycin) Fluoroquinolones (Ciprofloxacin, Pefloxacin), Antifungal (Fluconazole, Ketoconazole), Antiviral (Acyclovir, Zidovudine), CNS drugs (Alprazolam), Anticancer (Methotrexate, 5-Fluorouracil, Doxorubicin, Cisplatin), Cardiovascular (Amlodipine, Propranolol, Lisinopril), Anti-inflammatory / Antiarthritic (Diclofenac, Nimesulide, Piroxicam), Antituberculosis / Antileprosy (Rifampicin, Dapsone, Ethionamide, Cycloserine), Antihistamines, Salbutamol, Theophylline, Bromhexine, Corticosteroids (Dexamethasone, Betamethasone), Immunosuppressants (Cyclosporin A, Tacrolimus), Antiulcer (Ranitidine Cimetidine) Vitamins (Vitamin A, Vitamin E, Vitamin C, Folic acid, Antioxidants (β -Carotene, Silymarin).

Bioenhancers and Antitubercular Drugs Piperine

Piperine (1-piperoyl piperidine) is an amide alkaloid found in plants of Piperaceae family like *Piper longum* (long pepper), *Piper nigrum* (black pepper). Indian Scientists at the Regional Research laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine) discovered and scientifically validated piperine as the world's first bioavailability enhancer in 1979. [3] Zutshi RK et al, [8] in 1985, reported that piperine enhances bioavailability of rifampicin in patients of pulmonary tuberculosis. Piperine is the first and most potent bioenhancer to this date. Piperine increases bioavailability of

rifampicin by about 60%. Therefore adding bioenhancer 'Piperine' reduces the dose of rifampicin from 450 to 200 mg. [9] This reduces dosage, cost and toxicity of rifampicin. Rifampicin considered to be a most potent anti-tubercular drug, primarily metabolised in liver microsomal enzyme system. Piperine increases bio-availability of drugs [10] by inhibiting Cytochrome P-450 enzymes thus decreasing metabolism of many drugs, including that of rifampicin. Piperine improves intestinal permeability and inhibits P-glycoprotein and thus prevents efflux of absorbed drug from enterocytes. In addition to above; Piperine has potential immune-modulatory activity and has protective efficacy against Mycobacterium tuberculosis in a study done in mice. [11] Protective immunity against Mycobacterium tuberculosis requires the generation of cell-mediated immunity. Secretion of Th-1 cytokines such as gamma interferon (IFN- γ) by antigen-specific T cells plays important role in protective granuloma formation and stimulates the antimicrobial activity of infected macrophages. Piperine demonstrated augmentation of Th-1 response. Thus piperine can be synergistically combined with rifampicin to improve its therapeutic efficacy in immune-compromised TB patients. Sharma S et al [12] studied piperine against M. tuberculosis H37Rv and rifampicin resistant M. tuberculosis. A laboratory-generated rifampicin-resistant mutant of M. tuberculosis was tested for drug susceptibility and the expression level of the putative efflux protein by real-time PCR. Rifampicin in combination with piperine reduced the MIC and mutation prevention concentration (MPC) of rifampicin for M. tuberculosis H37Rv, Rifampicin-resistant M. tuberculosis and M. tuberculosis clinical isolates. Moreover, piperine effectively enhanced the bactericidal activity of rifampicin in time-kill studies and also significantly extended its post-antibiotic effect. Rifampicin in combination with piperine exhibited a significantly lower mutation frequency and

there was no mutant detected even at a concentration of 2 mg/L. The MPC of the combination was much lower than the C_{max} of rifampicin, indicating the clinical relevance of these combinations in restricting the selection of resistant mutants. Thus use of piperine can prevent drug resistance. Kapil RS et al [13,14] studied effect of piperine on pharmacokinetic parameters of rifampicin, isoniazid, and pyrazinamide combination in human volunteers. C_{max} ($\mu\text{g/mL}$) and AUC_{0- ∞} ($\mu\text{g}\cdot\text{h/mL}$) were significantly higher for formulation of these drugs with piperine than in those without piperine. The results from study indicate that piperine significantly increases the bioavailability of anti-tubercular drugs like rifampicin, isoniazid, and pyrazinamide. A Hepatoprotective effect of piperine on D-galactosamine induced hepatotoxicity in rats has been demonstrated. [15] Anti-tubercular drugs including rifampicin, isoniazid and pyrazinamide have known hepatotoxic effects. Thus use of piperine along with anti-tubercular drugs may prevent hepatotoxicity and increase compliance of patients with these drugs, however human studies are needed to provide enough evidence. Risorine is a formulation containing rifampicin (200 mg), isoniazid (300 mg), and piperine (10 mg) developed by Indian Institute of Integrative Medicine, Jammu, and marketed in India in November 2009. Risorine has been approved for marketing by Drug Controller General of India, after successful completion of all the phased clinical trials. [16]

Black cumin [*Cuminumcyminum*]

The Bioenhancer chemical constituent present in cumin is 3', 5-dihydroxyflavone-7-O- β -D galactouronide-4'- β -O-Dglucopyranoside. The effective dose of the bioenhancer extract is in the range of 0.5-25 mg/kg body weight. Percentage enhancement of bioavailability for rifampicin is 250%, for cycloserine is 89%, for ethionamide is 78%. [17,18] Sachinet al [19] studied the enhancement of rifampicin

levels in rat plasma by 3', 5-dihydroxyflavone-7-O-β-D-galactouronide-4'-β-O-D-glucopyranoside. The results obtained revealed that the C_{max} of rifampicin was enhanced by 35% and the AUC was enhanced by 53%.

Drumstick pods

It contains niaziridin, a nitrile glycoside which is a powerful bioenhancer. It regulates GIT functions to facilitate better absorption. It enhances the bioavailability of rifampicin by 38.8 folds at 1.0 μg/ml. [20]

Caraway (*Carum carvi*)

C. carvi is a prized culinary herb that has been used extensively since ages for many ailments worldwide. It has been used as a carminative, stomachic, aromatic, and diuretic. This herb has been extensively studied along with antibiotics, anti-fungals, anti-virals, anti-cancer, anti-inflammatory and anti-histaminics. It acts as bioenhancer and modifies the pharmacokinetics of anti-tubercular drugs favourably by enhancement of mucosal to serosal permeation (major mechanism), modification of permeation characteristics of the intestine and also by interacting on the P-glycoprotein. Choudhary et al. observed in 20 healthy human volunteers that an addition of *C. carvi* extract has led to increase in plasma levels of rifampicin, isoniazid, and pyrazinamide. [21] The bioavailability indices C_{max} of rifampicin increased from 4.57 ± 0.19 to 5.95 ± 0.19 (*P* = 0.000) and AUC increased from 40.11 ± 1.69 to 53.01 ± 1.88 (*P* = 0.000), C_{max} of isoniazid increased from 2.66 ± 0.16 to 3.62 ± 0.16 (*P* = 0.000) and AUC from 17.72 ± 0.78 to 22.87 ± 0.94 (*P* = 0.000) and C_{max} of pyrazinamide from 18.81 ± 0.79 to 25.06 ± 1.14 (*P* = 0.000) and AUC from 107.65 ± 4.42 to 137.71 ± 5.92 (*P* = 0.000), respectively.

Cow's Urine Distillate

Cow (*Bos indicus*) urine/*gomutra* has been elaborately explained in Ayurveda and described in "*Sushruta Samhita*", "*Ashtanga Sangraha*" and other Ayurvedic texts as an effective medicinal substance/secretion of animal origin with innumerable therapeutic properties. [22]

Cow's urine distillate is found to be more effective as bioenhancer than cow's urine. Cow urine has several medicinal properties, particularly as a bioenhancer and as an antibiotic, antifungal and anticancer agent. It enhances the transport of antibiotics like rifampicin across the gut wall by 2-7 folds. [23] Cow urine distillate increased the activity of rifampicin by about 5-7 times against *Escherichia coli* and 3-11 times against Gram-positive bacteria. It facilitates absorption of drugs across the cell membrane. Cow urine distillate along with Rifampicin in *Mycobacterium tuberculosis* is a potential subject of research.

Hurdles with use of bioenhancers

Although bioenhancers gained success in drug delivery there are still some challenges for new bioenhancers that are being developed, such as: to improve the properties of drug formulation like circulation in blood, increased functional surface area, protection of drug from degradation, crossing biological barriers and site specific targeting. The research and development of bioenhancers on large scale production is a problem. The next posed problem is of regulatory control. There need to have regulations for physiochemical and pharmacokinetic properties of newer bioenhancers. [4]

CONCLUSIONS

Bioenhancers are chemical entities which enhances bio-availability and bio-efficiency of a particular drug with which it is combined with typical pharmacological activity of its own at the dose used. Piperine increases bioavailability of rifampicin by about 60%. Therefore adding bioenhancer 'Piperine' reduces the dose of rifampicin from 450 to 200mg. Immunomodulatory and hepatoprotective properties of piperine can have further benefits in treatment of tuberculosis. This reduces dosage, cost and toxicity of rifampicin. Extensive researches are needed for finding out beneficial effects of different bioenhancers so that they could be utilised in drug formulation in

management of tuberculosis and other diseases in future.

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