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TARGETING THE ENZYME - THE NEW TOOL FOR DRUG DELIVERY

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ABSTRACT: Enzymes are the protein molecule that acts as a catalyst for various biological reactions. The enzymes have a major role in designing the dose of the drug. Enzymes have a vital role as metabolizer of the xenobiotic. The activity or the mode of action of enzymes determines the bioavailability of drugs. The presystemic metabolism of the drug monitored by the gut wall and hepatic enzymes can increase or decrease the bioavailability. The drastic presystemic metabolism of the drug may reduce the amount of the drug reaches into the systemic circulation. Means enzymes are one of the keys that control the therapeutic action. It can be assumed that an increase in drug concentration followed by an oral administration of the drug can be achieved by inhibiting the metabolizing enzymes. A study has been conducted on this which claims so many therapeutic applications of enzyme inhibition. The principle of enzyme inhibition can be used for treating various diseases like mammary cancer various infectious diseases and even for chemoprevention. The enzyme inhibition achieves a greater increase in the bioavailability of drugs. Thus, inhibiting the metabolizing enzyme can be an optional pathway for enhancement of bioavailability by reducing the dose dumping. The chance of developing the drug resistance can be reduced, and ADR can be properly monitored.

INTRODUCTION: Enzymes are the protein molecules that act as a catalyst in various biological reactions. The enzymes can complex with a specific set of a substrate to form an enzyme-substrate complex. The complex formed can be reversible or irreversible. These complexes can catalyze the reaction which leads to the production of a metabolite and a free enzyme [1]. The binding of a third molecule to the active site of the enzyme can change its activity which may lead to increase or decrease the enzyme activity depends on the nature of the molecule called as induction or inhibition [2].

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The enzymes are the key catalyst for the drug metabolism. The hepatic enzymes govern this process, and gut wall enzymes. Several types of drugs are extensively degraded in the gut wall before reaching into the systemic circulation due to extensive metabolism by the gut wall enzymes [3]. The enzymes can also act as activators of drug molecules if it is administered as a prodrug [4]. Thus, these enzymes have a unique role in therapeutic delivery of drug either by activating the drug molecule or by metabolizing it.

If the enzymes are induced, it may activate or degrade the drug to a long extent, whereas if it is inhibited the metabolic process may reduce resulting in accumulation of drug or in case of prodrug the drug remains inactive, thus identifying the enzyme for its specific catalytic reaction and altering its functionality may help to find out new approaches for drug delivery [5].

The enzymes also have other functionalities. An enzyme can metabolize the drug without changing its pharmacological activity. It is both the active drug and its metabolite exerts a pharmacological action. An example is a codeine metabolized by the enzyme CYP2D6 and CYP2D7 to form morphine. The codeine and the metabolite exert the same pharmacological activity [6]. Metabolism also results in toxicological activation. The metabolic intermediate formed may be the highly reactive lead-in tissue toxicity. Isoniazid metabolized by the enzyme CYP2E1 changes to tissue acylating intermediate. It may produce nerve and liver damage [7]. Dapsone antibacterial agent, due to slow acetylation process results in systemic lupus erythematosus [8,] and primaquine metabolite produces acute hemolytic anemia [9]. The metabolism also results in a change in pharmacological activity. The drug, as well as its metabolite, also exerts a pharmacological activity iproniazide an anti-tubercular drug metabolized to isoniazid an antidepressant drug [10].

Enzymes in Drug Metabolism:

Cytochrome P450: Cytochrome P450 (CYP) is a heme-containing an enzyme that catalyzes the oxidative biotransformation of drugs facilitating their removal from the cells. The CYPs were first identified by Martin Klingenberg [11] while studying the spectrophotometric properties of pigments in a microsomal fraction prepared from rat livers.

The diluted microsomal preparation when reduced by sodium dithionite and exposed to carbon monoxide gas, a unique spectral absorbance band with a maximum absorbance at 450 nm appeared. The ferric ion in the resulting heme, binds with CO following the reduction reaction, and the complex's maximum absorbance band were unique amongst the heme proteins, this serves as the signature of CYP450 enzymes.

CYPs are mostly located in the endoplasmic reticulum, and in mitochondrial fractions of hepatic and extra-hepatic tissues to some extent. Even though these enzymes are ubiquitous in the body, of the 18 families in mammals identified, 11 are expressed in a typical human liver (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1, and CYP3A4/5) [12]. Also, five of these enzymes (CYPs 1A2, 2C9, 2C19, 2D6 and 3A4) expressed at high levels in the liver demonstrate a broad substrate selectivity which accounts for about 95% of drug metabolism [13].

The metabolism of a drug can be altered by another drug or foreign chemical, and such interactions can often be clinically significant. As a result, the FDA (Food and Drug Administration) and other regulatory agencies such as the Department of Health and Human Services (DHHS), Centers for Disease Control and Prevention (CDS) and Hazard Analysis Critical Control Point (HACCP) among others expect information on the relationship between each new drug to CYP enzymes (substrate, inhibitor and or inducer) making these enzymes vital in the process of drug discovery.

Organ CYPs Detected: Cytochromes are present in almost all the tissue in the body. A major part is present at small intestine and lungs.

- ✓ Nasal mucosa 2A6, 2A13, 3A, 2B6, 2C, 2J2,
- ✓ Trachea 2A6, 2B6, 2A13, 2S1
- ✓ Lung 1A1, 1A2, 1B1, 2A6, 2A13, 2E1, 2B6, 2C8, 2D6, 2F1, 2J2, 2S1, 3A4, 3A5, 4B1
- ✓ Oesophagus 1A1, 1A2, 2E1, 2A, 2J2, 3A5
- ✓ Stomach 1A1, 1A2, 2C, 2S1, 2J2, 3A4
- ✓ Small Intestine 1A1, 1B1, 2C19, 2D6, 2E1, 2J2, 2S1, 3A4, 3A5, 2C9
- ✓ Colon 1A1, 1A2, 2J2, 3A5, 1B1, 3A4

Enzyme Inhibition and Bio Applications P450s as therapeutic targets: Some P450s are targets for rational drug design. Among this CYP19A1, CYP19 and CYP 5A1 is more prominent. CYP 19 catalyze androgens to estrogens by oxidation, the decreases expression of CYP19 is essential for estrogen-dependent tumors. Other P450s are less well developed as a target for drug delivery. One possibility is a study on CYP3A4, the main human P450 in the liver and small intestine. Research has been conducted to find safe and effective inhibitors of CYP3A4 to enhance the bioavailability of drugs such as HIV protease inhibitors. Another obstacle to greater availability of P450 inhibitors is the general reluctance of the FDA and other regulatory bodies to approve mixtures of P450-inhibiting drugs without extensive testing and confirming its safety. For example, the Australian Medicines Handbook notes that ketoconazole and diltiazem have been used as cyclosporine sparing agents.

Another set of theoretical targets for inhibition includes the P450s that produce biologically active oxidize eicosanoids (*e.g.*, 20-hydroxy arachidonic acid and various arachidonic acid epoxides). Most of these studies are just starting, and the application of P450-inhibiting drugs in humans is not yet a reality [14].

Enzyme Inhibition for Treatment of Mammary Cancer: The metabolites of enzyme reaction can be a prominent cause of cancer. A typical example of it is the development of mammary cancer by activation of 17-estradiol (E2) to 4-hydroxy E2.CYP450 1B1 present in the extrahepatic tissues places a major role in this activation. The study conducted for inhibition of CYP450 1B1 were very effective. The inhibition of recombinant human P450 1B1 by 2, 4, 3', 5'-tetramethoxy-stilbene (TMS) was investigated using either bacterial membranes from a human P450/NADPH-P450 reductase bicistronic expression system or using purified enzymes. 2, 4, 3', 5'-tetramethoxy-stilbene showed potent and selective inhibition of P450 1B1 with an IC_{50} value of 6 nM. TMS reported to exhibit 50-fold selectivity for P450 1B1 over P450 1A1 (IC_{50} = 300 nM) and 500-fold selectivity for P450 1B1 over P450 1A2 (IC_{50} = 3µM) [15].

Enzyme Inhibitors are Therapeutic Agents for Treating Infectious Disease: Glycosidase inhibitors are proven to be therapeutic agents in various infectious diseases. Glycosides are involved in the biosynthesis of the oligosaccharide chains and quality control mechanisms in the endoplasmic reticulum of the n-linked glycoproteins. Inhibition of these glycosides can have profound effects on quality control, transport, maturation and secretion of glycoproteins and can alter cell ± cell or cell ± virus recognition processes.

This principle is the basis for the potential use of glycosidase inhibitors in viral infection, genetic disorders, and cancer. Glycosidase inhibitors could have many kinds of beneficial effects as therapeutic agents, such as antibiotics, antifungal agents, insecticides, and antivirals, anti-obesity drugs, and therapeutic agents for some genetic disorders. The *in-vitro* and *in-vivo* studies have established the inhibitors are effective and safe antiviral drugs [16].

Enzyme Inhibitors as Bioavailability Enhancers: The metabolism of a drug can be altered by another drug or foreign chemical, and such interactions can often be clinically significant. The observed induction and inhibition of CYP Enzymes by various traditional remedies have led to the general acceptance that natural therapies can have adverse effects. Drug-herb interactions may involve competitive, non-competitive, or uncompetitive inhibition of drug-metabolizing enzymes or enzyme induction by the phytopharmaceutical [17].

Several epidemiological surveys, including ones conducted in a laboratory, have indicated higher usage of herbal medicines along with prescription medicines with low physician awareness [18]. With over 80% of the prescription medicine users also seeking some form of herbal remedy in Jamaica, the chances of a drug interactions rise and this motivated investigation into likely pharmaco-kinetics, metabolism-based interactions between the two types of medicines [19]. The inhibition of CYP3A4 by furanocoumarin's found in grapefruit juice leading to clinically observable toxicities with drugs and the induction of the same CYP3A4 enzyme by ingredients found in St. John's wort leading to sub therapeutic interferences with cyclosporine provide suitable examples for the involvement of CYP enzymes in drug-herb interactions [20].

Enzyme Inhibition in Chemoprevention: The CYP 1 family is linked activation of pro-carcinogenic. The inhibition of CYP1 enzymes inhibits induction of cell cycle arrest and induction of phase II. Various researches proved that the inhibition of CYP1 enzymes can protect healthy cells from carcinogens. The CYPs IA1 and 1B1 act on polycyclic hydrocarbon to form benzo-a-pyrene, which on metabolize to form phenols, polyphenols, quinines, epoxides, and dihydrodiols. The various dihydrodiols like (-)-benzo[a]pyrene-trans- 7, 8-dihydrodiol (BPD) and (+)-anti-benzo[a]pyrene-trans-7, 8-dihydrodiol-9, 10-epoxide (anti BPDE) are carcinogenic [21].

Some examples of drugs that act as chemo protectants *via* enzyme inhibition are Disulfiram, ginseng, flavonoid, resveratrol. Disulfiram inhibits the action of dimethylhydrazine through CYP1 inhibition [22]. Ginseng reduces the occurrence of

7,12 dimethyldenz (a) anthracene initiated cancer by inhibiting CYPs1A1, 1A2 and 1B1 [23]. The flavonoid, Galangin act as a chemoprotective agent by an increased level of CYP1A1 activity [24]. Resveratrol acts as chemopreventive by inhibiting CYPs1A1 and 1B1 [25].

Inhibition of Matrix Metalloproteinase (MMP): Matrix metalloproteinase (MMP) constitute a family of 23 zinc- and calcium-dependent Endopeptidases that play a major role in various physiological processes such as wound healing, embryogenesis, vasculogenesis or stem cell mobilization [26]. MMP exerts their degradative function extracellularly against matrix macro-molecules or at the pericellular microenvironment. MMP cleaves intracellular substrates belonging to any subcellular compartments [27].

Thus, MMP can be considered as proteases mainly controlling signaling events through processing cytokines, chemokines and degrading matrix, liberating matrikines in the extracellular space, or turn, cleaving enzymes involved in signal transduction inside the cells thus the need of inhibiting the MMP play a major role in maintaining the healthy tissues. It made the development of various drugs such as Marimastat® Batimastat®, Solimastat®, Galardin®, Prinomastat®, Trocade®, Tanomastat®, Rebimastat®, Metastat® for various indications like Cancer, Eye disease, COPD, Macular degeneration and Rheumatoid arthritis. But most of the drugs discontinued in clinical trials due to various other toxic reactions associated with it. Up to now, one selective MMP-9 inhibitor is a monoclonal antibody binding to N-terminal part of catalytic domain [28].

Inhibition of Nitric Oxide Synthesis: Nitric oxide is an uncharged free radical abundant in nanomolar quantities and detected by measuring nitric oxide synthases (NOS). Nitric oxide is a gas highly reactive, short-lived free radical generated enzymatically by NOS involved in diverse physiological (neurotransmission, immune system) and pathophysiological (tumor progression) mechanisms.

Nitric oxide is a biological mediator for its role as EDRF (endothelial-derived relaxing factor) responsible for the regulation of blood vessel relaxation and blood pressure maintenance [29]. Recent years, inhibition of NOS gene expression has become a scientific interest to measure NO in tissues, and synthetic NOS/NO inhibitors have revolutionized molecular imaging of tissues [30].

CONCLUSION: Cytochromes are the most abundant and highly metabolizing enzymes in humans. It has a variety of therapeutic applications. These enzymes can either activate the prodrug or it can either deactivate the drug. Activation of a prodrug increases the bioavailability of drug whereas the deactivation reduces the active drug content in the body. Enzymes are the key players in increasing or decreasing the drug content. Thus to increase the bioavailability of drugs the enzymes can be tuned. The inhibition of enzyme activity can increase the bioavailability. With this, the patients are most benefited by reducing the dose dumping. The inhibition should be modulated so that the long term inactivation should not produce an adverse effect.

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CONFLICT OF INTEREST: Nil

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