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Enzymes as targets of Drug Action: an Overview

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ABSTRACT

Enzymes are known to catalyse thousands of biochemical reaction types. They are used for in various industries when extremely specific catalysts are required. They play a very important role as drug targets to manufacture drugs in the medical field. The review was hence aimed at compiling the various mechanisms and strategies through which drugs are targeted against enzymes in the field of medicine.

Drugs acting on enzymes can either inhibit them or activate them with the former being more common. Drugs inhibiting enzymes prevent the formation of the product by inhibiting the enzyme-substrate interaction competitively, non-competitively or uncompetitively. The type of inhibition by the drug is hence classified accordingly. Enzyme activity can be accelerated through biochemical modification of the enzyme by activators such as heparin which activates Anti-Thrombin III, etc.

Microsomal enzymes are also used as drug targets in certain clinical scenarios such as neonatal jaundice & cushing's disease.. Transmembrane receptors linked to enzymes are currently the most explored drug targets. They include Receptor Tyrosine Kinases, JAK-STAT receptors, receptor Serine-Threonine Kinases, Toll-like receptors & TNF-α Receptors. They are widely used in the field of oncology & immunology.

Keywords: Enzyme inhibitors, Pharmacodynamics, Tyrosine Kinase, JAK-STAT, TNF-α

INTRODUCTION

Enzymes are biologic polymers that catalyse chemical reactions facilitating the conversion of substrates to products which plays a very integral role in our existence.[1] For example, acetylcholinesterase causes breakdown of acetylcholine to acetate & choline which prevents acetylcholine from causing a prolonged depolarisation at the neuromuscular junction.[2] It is known that enzymes currently catalyse thousands of biochemical reaction types.[1]

Enzymes enhance rates of reactions by lowering the activation energy required by reactions & stabilising the reaction molecule at their activated complex states for example the enzyme thymidylate synthase (which is a target of anti-cancer drug 5-fluorouracil) catalyses a reaction in which deoxyuridine monophosphate (dUMP) is converted to deoxythymidine monophosphate (dTMP) in milliseconds which would have otherwise taken millions of years to occur.[3] Enzymes play an essential role in biological life processes & pathophysiology e.g. cyclo-oxygenase enzyme (COX) causes breakdown of arachidonic acid into prostaglandins, prostacyclins & thromboxanes. It contains two well-known iso-types. COX1 is a

constitutive enzyme and it plays an important role in normal physiology. COX2 is an inducible enzyme which is produced under certain inflammatory conditions by macrophages, leukocytes & fibroblasts.[4]

Role of Enzymes in various industries

Enzymes are used by various industries when extremely specific catalytic actions are required.

- Food industry: the enzymes lactase & protease are used by the milk & meat industry respectively.
- Textile industry: enzymes like cellulase are used very commonly.[5]
- Diagnostic: Various enzymes are commonly used for diagnostic purposes for e.g. Alkaline Phosphatase is used in bony metastasis, Creatine Kinase- MB is used in myocardial infarction.
- Drug targets: Pharmaceutical industries use enzymes as drug targets to manufacture drugs e.g Dihydrofolate Reductase inhibitors such as methotrexate, Phosphodiesterase inhibitors such as theophylline, etc.
- Therapeutics: Role of enzymes is also extended to therapeutics for example serratiopeptidases as antii-inflammatory, β-lactamases for penicillin allergy, tissue plasminogen activators as fibrinolytics.[6]

Despite enzymes playing such a crucial role there are hardly any articles or chapters in textbooks which summarise the pharmacodynamic role of enzymes in detail. Hence this article aims to collect data from various articles & textbooks in order to provide a concise summary on enzymes as targets of drug action.

Enzymes as drug targets, why?

Medicine in the 21st century has become a science in which drugs are being targeted against macromolecules such as receptors (e.g opioids), ion channels (e.g. calcium channel blockers), transporters (e.g. diuretics) & enzymes (e.g. ACE inhibitors).[7] Enzymes hold a prominent position among such macromolecules because altering the activity of enzymes has immediate and defined effects which make them good therapeutic agents/targets & they remain prime targets for drug design, for example inhibition of cyclooxygenase by NSAIDS can give an immediate analgesic, anti-inflammatory, antipyretic & anti platelet effect.[4] The structure of active sites of enzymes & ligand binding pockets are highly amenable for high affinity interaction with small drug like molecules.[8] The binding of molecules to allosteric site produces a change in the conformation or dynamics of the enzyme for example statins bind to the allosteric site of HMG CoA reductase enzyme which causes a change in the dynamics of the enzyme which is transduced to the active site because of which its substrate HMG CoA is unable to bind to it which hence reduces the reaction rate & in this way Statins inhibit HMG CoA reductase enzyme.[9]

Mechanism of action of enzymes

Drugs acting on enzymes can either inhibit them or activate them. Inhibition of enzymes is a strategy which is more commonly used rather than activation of enzymes. 47% of all current drugs inhibit their enzyme targets.[10]

Enzyme Inhibition

Drugs inhibiting enzymes bind to the enzyme and decreases its activity. It hence slows down or blocks enzyme catalysis.[10] The strategy of enzyme inhibition is used to produce various effects in different clinical scenarios.

- Levels of physiological cellular molecules can be altered by enzyme inhibitors in pathologies. The enzyme phosphodiesterase causes degradation of the active second messenger cyclic AMP in cardiac cells. Cyclic AMP plays a role in controlling cardiac contractility and sinus rhythm. In congestive heart failure, a therapeutic augmentation of contractility can be obtained by blocking the enzymatic degradation of cyclic AMP. Hence phosphodiesterase-III inhibitors such as milrinone, levosimendan are useful in congestive heart failure.[11]
- Chemical deficiencies can be corrected by using enzyme inhibitors for enzymes which use that chemical as its substrate. Deficiency of GABA mediated Cl^- channel opening plays a role in the pathophysiology of epilepsy. Hence prevention of degradation of GABA by the enzyme GABA Transaminase by the drugs vigabatrin & valproate enhance the levels of GABA in the brain which is

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followed by enhancement of GABA mediated Cl^- channel opening.[12]

- Activity of enzymes that play a role in pathophysiology can be blocked by enzyme inhibitors for example the renin-angiotensin system is responsible for blood pressure & fluid balance & hence Angiotensin Converting Enzyme Inhibitors (ACE Inhibitors) can be used to manage hypertension.[13]

- Chemical excess can be corrected by inhibiting the enzyme that produces the molecule itself for example excess uric acid which is produced from hypoxanthine by the enzyme xanthine oxidase plays a role in the pathophysiology of Gout & hence xanthine oxidase inhibitors such as allopurinol & febuxostat are used to treat Gout.[14]

- Biochemical pathways unique to a pathogen which can be inhibited to reduce the growth or kill a pathogen (bacteria, virus or a parasite) in infectious diseases. Human cells are freely permeable to exogenously given Folic Acid but bacterial cells are not hence bacteria needs to synthesise their own folic acid from PABA. Sulfonamide drugs structural analogues of PABA inhibit Dihydropteroate synthetase which is required for the folic acid synthesis in bacteria.[15]

Mechanism of inhibition of enzymes by drugs

The substrate molecule reacts with the enzyme to form an enzyme-substrate (ES) complex which at the end results in the formation of the final products. Drugs inhibiting enzymes prevent the formation of the product by inhibiting the enzyme-substrate interaction competitively, non-competitively or uncompetitively. The type of inhibition by the drug is hence classified accordingly.[10]

Competitive Enzyme Inhibition by Drugs

Competitive inhibition is caused by compounds which resemble the chemical structure & molecular geometry of substrate molecule.[Figure I] In this type of inhibition the inhibitor binds only to the enzyme & not to the enzyme-substrate complex. The inhibitor gets strongly stuck on the enzyme which prevents any substrate molecules from further reacting with the enzyme.[16] However competitive inhibition is usually known to be surmountable if sufficient substrate molecules are available to ultimately displace the inhibitor molecule from complex. If the drug binds very strongly to the active site, it can result in irreversible competitive inhibition.[16]

If the concentration of the substrate is increased, it will decrease the chance of inhibitor binding to the enzyme.[16] Hence, if the substrate concentration is high enough the enzyme will reach the same Vmax as without the presence of the inhibitor.[16] However a higher concentration of the substrate will be required to achieve this and so the Km of the enzyme will also be higher than usual. The Lineweaver-Burk double reciprocal plot for this set of data when platted shows inhibitor line crossing the y (1/v) axis at the same point - i.e. Vmax is unchanged, but with a decreasing value of 1/Km (and hence a higher Km) in the presence of the inhibitor.[Figure I] Enzyme inhibitor depends on: Inhibitor concentration, substrate concentration & relative affinities of inhibitor & substrate for active site.[16]

Methanol poisoning causes retinal damage & lactic acidosis due to its metabolites; formaldehyde and formic acid respectively. It is treated by competitive inhibition of alcohol dehydrogenase by ethanol & fomepizole. Ethanol metabolism is 7 times faster than methanol hence ethanol is the preferred substrate. Fomepizole is less likely to cause cerebral depression.[17] Methotrexate is known to have 50,000 times higher affinity for dihydrofolate reductase than the normal substrate DHFA. Hence it competitively inhibits DHFR & deprive cells of folate enzymes & essential components such as purines & pyrimidines leading to cell death. Administration of leucovorin (folinic acid) rescues normal cells from toxicity & allows them to form purines & pyrimidines even in the presence of methotrexate.[18] Levodopa if administered alone, only 1-3 % of it enters the brain unaltered as most of it is metabolised by peripheral decarboxylase enzyme. Drugs such as carbidopa & benserazide have been specially developed to inhibit this enzyme peripherally and increase the availability of levodopa at the site of action. The benefit of this combination in Parkinson's disease is due to reduced metabolism of levodopa to dopamine in the peripheral blood but not in the brain as carbidopa does not cross the blood-brain barrier.[19] Diisopropyl-fluorophosphate (DIFP) organophosphates react with the esteretic site of the enzyme cholinesterase & irreversibly inhibit the enzyme by undergoing the process of ageing.[20]

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Some common examples of competitive inhibitor drugs include cholinesterase inhibitors such as physostigmine and neostigmine, MAO A inhibitors such as moclobemide, dihydropteroate synthase inhibitors such as sulfadiazine, xanthine oxidase inhibitors such as allopurinol, angiotensin converting enzyme (ACE) inhibitors such as captopril, 5α-Reductase inhibitors such as finasteride, aromatase inhibitors such as letrozole and anastrozole, etc.[21]

Non-Competitive Inhibition by drugs:

Non Competitive inhibition is caused by a substance that reacts with the enzyme but at the allosteric site.[Figure II] It binds to the Enzyme as well as the ES complex with equal affinity. Drug need not have a similar structure as that of the substrate of the enzyme.This type of inhibition is unsurmountable. The shape of the active site changes so that the substrate can no longer react with the enzyme to give the response. Non-competitive inhibition is usually irreversible but there are few cases where it is reversible e.g. carbonic anhydrase inhibition by acetazolamide.

If the concentration of substrate is increased, it will not reverse the inhibition, since the inhibitor reacts with the enzyme-substrate complex. Hence the enzyme will not reach Vmax. The Lineweaver-Burk double reciprocal plot when plotted for this set of data shows inhibitor line converging on the same point on the X (1/S) axis - i.e. Km is unchanged, but Vmax is reduced.[22][Figure II]

Disulfiram non competitively inhibits the enzyme Aldehyde Dehydrogenase, it forms an active metabolite diethylthiomethyl carbamate (DETMC). Conversion of acetaldehyde to acetic acid is stopped. Acetaldehyde accumulation causes nausea, vomiting, flushing, etc. which is used as a favouring factor in process of alcohol de-addiction. Disulfiram like reaction is also seen with metronidazole,chlorpropamide, glibenclamide, tolbutamide, griseofluvin, cefotetan, cefoperazone, etacrynic acid & urinary antiseptics such as nitrofurantoin because of inhibition of Aldehyde Dehydrogenase by them.[23] Echinocandins are 1,3-β glucan synthase inhibitors which can cause cell wall lysis by inhibiting synthesis of 1,3-β glucan an essential component of the cell wall of susceptible fungi. Mammalian cell wall does not require 1,3-β glucan.[24] Hence it is not toxic to the host. Aspirin irreversibly inhibits COX I present in the platelets for their total span of 7 days.[25]

Some common examples of non-competitive enzyme inhibitor drugs include carbonic anhydrase inhibitors such as acetazolamide, H+ K+ ATPase inhibitors such as omeprazole, Na+ K+ ATPase inhibitors such as digoxin, phosphodiesterase-III inhibitors such as theophylline, inhibitors of peroxidase enzyme in thyroid gland such as propylthiouracil, HMG-CoA reductase inhibitors such as lovastatin, phosphodiesterase-V inhibitors such as sildenafil, etc.[21]

Uncompetitive Inhibition by drugs:

Uncompetitive inhibition occurs when a drug binds reversibly to the enzyme when the substrate is already bound to the active site. The inhibitor binds to the Enzyme-substrate complex. Increasing the concentration of substrate will not overcome the inhibition in this case. The level of inhibition depends on the sufficient substrate being present at the active site to make E-S complex.[22]

Initially the reaction rate is speeded up to the formation of the enzyme substrate complex till a point after which the rate slows down & never reaches Vmax. Hence the enzyme will not reach Vmax. The Lineweaver-Burk double reciprocal plot for this set of data doesn't show inhibitor line converging on the same point on the X (1/S) axis or the Y (1/V) axis - i,.e. Km is reduced & Vmax is also reduced.[22]

This type of inhibition is very rare hence there are very few examples. Lithium uncompetitively inhibits hydrolysis of inositol monophosphate by inositol monophosphatease. As a result, the supply of free inositol for regeneration of membrane phosphoinositides which are sources of IP_3 & DAG is reduced. The hyperactive neutrons involved in maniac state may be preferentially affected.[26]

Suicide Inhibitor drugs:

Suicide inhibition, also termed as suicide inactivation or mechanism-based inhibition by many authors.[27] It causes an irreversible form of enzyme inhibition wherein an enzyme binds an inhibitor & forms an irreversible complex with the enzyme. The complex produces an irreversible reaction to form a stable inhibitor-enzyme complex & hence causes a stronger

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inhibition of the enzyme. β-Lactamase inhibitors are given with β-Lactams to inhibit β-Lactamase enzyme and hence prevent hydrolysis of β-Lactam drugs like penicillins. Clavulanic acid covalently bonds to a serine reside present in the active site of the β-lactamase, which results in restructuring of the clavulanic acid molecule. This results in creation of a much more reactive species which attacks another amino acid in the active site, permanently inactivating it, and thus producing inactivation of the enzyme β-lactamase.[27]

Some examples of suicide inhibitor drugs include aldehyde dehydrogenase inhibitor drug disulfiram, aromatase inhibitor drug exemestane, ornithine decarboxylase inhibitor drug eflornithine, viral DNA polymerase inhibitor drug acyclovir, CYP3A4 inhibitor drug erythromycin.[28]

Discovery and design of inhibitors:

In the past the only method which was available to discover new inhibitors was by trial and error which was carried out by by screening of huge libraries of compounds against a targeted enzyme and hoping for emergence of useful leads. This tried & tested approach is still successful in todays world and has even been extended by certain combinatorial chemistry approaches which can quickly produce large numbers of novel compounds and high-throughput screening technology which can rapidly screen these huge chemical libraries to discover useful drug leads as targeted enzyme inhibitors.[29]

More recently, an alternative approach has been applied called in-silico rational drug design or a computer aided drug designing (CADD) which uses the three-dimensional structure of an enzyme's active site to predict which molecules might be possible inhibitors. These predictions are then tested in-silico to check if any of these tested compounds may be a novel inhibitor at the active site. This new inhibitor is then used to try to obtain a 3D structure of the enzyme in an inhibitor/enzyme complex to show how the molecule is binding to the active site of the enzyme. The latter is studied to determine further changes to be made to the inhibitor to try to optimise binding. This test and improve cycle is then repeated continuously until a sufficiently potent inhibitor is produced which can produce the desired efficacy & potency. Computer-based methods can also predict the affinity of an inhibitor for an enzyme with the help of molecular docking and molecular mechanics.[29]

Enzyme Activation by drugs:

Enzyme activity can not only be inhibited, it can also be accelerated through biochemical modification of the enzyme (i.e., phosphorylation). Just as in the case of agonists of receptors, it is theoretically possible to bind molecules to enzymes to increase catalysis (enzyme activators). These molecules must bind to a site other than the substrate binding site, otherwise substrate binding cannot occur. There are conditions where enzyme activators could be of benefit therapeutically.[30]

Anti-Thrombin III plays a crucial role in natural endogenous anticoagulant mechanisms in the body by blocking the activity of the activated clotting factors XII, XI, X. IX & II. Under physiological conditions these interactions are slow & on a demand supply basis. Heparin accelerates the activity of Anti-Thrombin III by 1000 folds especially against IIa & Xa with IIa being more sensitive. Factors XII, XI & IX are also affected.[30] Pralidoxime (2-PAM) reactivates the enzyme AchE by attaching with the anionic site which lies vacant in the phosphorylated enzyme. Its oxime group which is now in close proximity with the phosphorylated esteric site attracts the phosphate group. The oxime-phosphate complex diffuses out leaving the regenerated AchE enzyme in the active form.[31]

Microsomal Enzymes as drug targets:

Microsomal enzymes are enzymes which are typically found in the endoplasmic reticulum of hepatocytes. They play a very important role in the metabolism of drugs & some endogenous substrates. These enzymes can be induced or inhibited by certain drugs in order to affect the metabolism of certain endogenous substrates or drugs.[32] Phenobarbitone is used in the prevention and treatment of unconjugated hyperbilirubinemia in preterm neonates because of its property of inducing the enzyme glucuronyl transferase which causes increased conversion of bilirubin to glucuronic acid & reduces the excess bilirubin.[33] Enzyme inducers enhance the hydroxylation of steroids in man. Phenobarbital, diphenylhydantoin, and phenylbutazone are examples of drugs that stimulate cortisol hydroxylase activity

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in liver microsomes and enhance the urinary excretion of 6 β-hydroxycortisol in man.[34]

Transmembrane Receptors Linked to Intracellular Enzymes:

Transmembrane receptors act in cell signaling by binding to extracellular molecules. Signal transduction is a process through membrane receptors which involves some external reactions, in which the ligands binds to a membrane receptor, and the corresponding internal reactions, in which intracellular response is triggered. Majority of the enzyme-linked receptors are protein kinases or are associated with it.[35] There are various types of Transmembrane Receptors Linked to Intracellular Enzymes:

I. Receptor Tyrosine Kinases.

II. JAK-STAT Receptor (Jannus Kinase-Signal Transducer & activator of transcription proteins)

III. Receptor Serine-Threonine Kinases

IV. Toll-like Receptors (TLR)

V. TNF-α Receptors (Tumor necrosis factor alpha receptors)

I. Receptor Tyrosine Kinase:

This consists of a signaling molecule, signal binding site, alpha helix containing region embedded inside the cell membrane & a signal relay part which is made up of tyrosine kinase molecules.[36]

Tyrosine Kinase receptors include receptors for neurotrophin, growth factors (epidermal growth factor [EGF], platelet-derived growth factor [PDGF]), as well as insulin and many other trophic hormones. These receptors shift from an inactive monomeric state to an active dimeric state upon agonist binding (dimerization).[Figure III] This is followed by autophosphorylation of the intracellular domain of each receptor and binding of SH2-domain proteins that are themselves phosphorylated. Depending on the receptor subtype, SH2-domain proteins allow the phosphorylated receptor to activate other functional proteins, which eventually results in either stimulation of gene transcription, or enzymes such as phospholipases, leading to the formation of second messengers.[36]

One important pathway involved in the transduction mechanisms of tyrosine kinase receptors include the (Ras/Raf/MAP) kinase pathway which is important in cell division, growth, and differentiation.[36]

Functioning of kinase-linked receptors. The main steps are dimerization of the receptor, autophosphorylation, and phosphorylation of targeted proteins. The growth factor pathway is shown with the kinase cascade involving the successive phosphorylation of many enzymes (Raf, Mek, Map kinase), eventually leading to gene transcription. GDP, guanosine diphosphate; GTP, guanosine triphosphate.[36]

Examples of drugs acting on tyrosine kinase pathway include Monoclonal antibodyEGFR kinase inhibitors such as cituximab indicated for metastatic colorectal cancer with wild type KRAS, small molecule EGFR Kinase Inhibitors such as erlotinib indicated for advanced pancreatic cancer, small molecule HER2 Kinase inhibitors such as lapatinib indicated for HER2 positive breast cancer, monoclonal antibody HER2 kinase inhibitors such as trastuzumab indicated for HER2 positive breast cancer with gastric cancer, platelet derived growth factor inhibitors such as olaratumab indicated for soft tissue sarcoma, mutant B-RAF kinase Inhibitors such as vemurafenib indicated for BRAFV600E/K mutant melanoma, MAP kinase Inhibitors such as cobimetinib indicated for BRAF600E/K Mutant melanoma, Cyclin dependent kinase 4/6 inhibitors such as palbociclib indicated for advanced ER positive, HER2 negative breast cancer, VEGF inhibitors such as bevacizumab indicated for metastatic colorectal cancer, bruton tyrosine kinase inhibitors such as zanubrutinib indicated for mantle cell lymphoma and CLL, etc.[28]

Insulin:

The effects of insulin are initiated by its binding to a receptor present in the cell membrane. The receptor molecule contains an α- and β subunits. Two molecules are joined to form what is known as a homodimer. Insulin binds to the α-subunits of the homodimer, which faces the extracellular side of the cells. The β subunits have tyrosine kinase enzyme activity which is triggered by the insulin binding. This activity provokes the autophosphorylation of the β subunits and subsequently the phosphorylation of proteins inside the cell known as insulin receptor

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substrates (IRS). The phosphorylation of the IRS activates a signal transduction cascade that leads to the activation of other kinases as well as transcription factors that mediate the intracellular effects of insulin.[37]

II. JAK-STAT Receptor Pathway:

Cells express a family of receptors for cytokines such as γ-interferon and hormones such as growth hormone and prolactin. They signal to the nucleus by a more direct manner than the receptor tyrosine kinases.[Figure IV] These receptors have no intrinsic enzymatic activity; rather, the intracellular domain binds a separate, intracellular tyrosine kinase termed a Jak. On dimerization induced by ligand binding, Jaks phosphorylate other proteins termed STATs, which translocate to the nucleus and regulate transcription. The entire pathway is termed the Jak-STAT pathway.[38]

JAK-STAT: There are four Jaks and six STATs in mammals that, depending on the cell type and signal, combine differentially to activate gene transcription.[38]

Examples of drugs acting on JAK-STAT pathway include JAK1/JAK2 inhibitors such as ruxolitinib (indicated for polycythemia vera and myelofibrosis) and baricitinib (indicated for rheumatoid arthritis), JAK1 inhibitors such as oclacitinib (indicated for pruritus associated with allergic dermatitis) and newly approved drug upadacitinib (highly selective JAK1 inhibitor indicated for rheumatoid arthritis) JAK2 inhibitors such as newly approved drug fedratinib (indicated for primary myelofibrosis or secondary myelofibrosis), JAK3 inhibitors such as tofacitinib (indicated for psoriasis and rheumatoid arthritis), etc.[28][38]

III. Receptor Serine-Threonine Kinases:

They are analogous to the receptor tyrosine kinases except that they have a serine-threonine kinase domain in the cytoplasmic region of the protein. They are already present as homodimers. 2 homodimers dimerise with each other to form tetramers. Activin/TGFβ/ BMP binds to type II receptors which recruits type I receptors & phosphorylates it. It then activates Smad 2/3 0r Smad 1/5 depending upon the ligand, after which Smad 4 is activated resulting in activation of transcription.[39]

Examples of drugs acting on serine threonine kinase pathway are currently under clinical trials, some of them include drugs such as sorafenib (Phase III trial: indicated for Renal cell carcinoma, unresectable hepatocellular carcinoma, and advanced thyroid carcinoma), enzastaurin (Phase II trial: Indicated for newly diagnosed GBM, vemurafenib (Phase I trial: Indicated for BRAFV600E/K mutant melanoma, etc.[39]

IV. Toll-like Receptors:

The MyD88-dependent response occurs on dimerization of the TLR receptor. Its primary effect is activation of NFκB and Mitogen-activated protein kinase. Lipids, peptidoglycans, viruses, etc. act as ligands for this receptor which causes dimerisation of the receptor. Ligand binding and conformational change that occurs in the receptor recruits the adaptor protein MyD88, a member of the TIR family. MyD88 then recruits IRAK4, IRAK1 and IRAK2. IRAK kinases then phosphorylate and activate the protein TRAF6. It interacts with the protein TAK1, as well as adapter proteins TAB1 & TAB 2 in order to facilitate binding to IKK-β. On binding, TAK1 phosphorylates IKK-β, which then phosphorylates IκB causing its degradation. This allows NFκB to diffuse into the cell nucleus and activate transcription and consequent induction of inflammatory cytokines.[40}

Examples of drugs acting on Toll-like receptor pathway are currently under clinical trials, they include drugs such as pembrolizumab (Phase II, TLR 3 agonist, indicated for metastatic colon cancer), romidepsan (Phase I, TLR 3 agonist, indicated for cutaneous T cell lymphoma), eritoran (Phase II, TLR 4 antagonist, indicated for insulin sensitivity), imiquimod (Phase II, TLR 7 agonist, indicated for Human papillomavirus (HPV) infection, ibudilast (Phase II,TLR 4 antagonist, indicated for glioblastoma, entolimod (Phase I, TLR 5 agonist, indicated for unspecified adult solid tumour, hydroxychloroquine (Phase III, TLR 9 inhibitor, indicated for Sjogren's syndrome.[40]

V. TNF-α Receptors:

TNF can bind two receptors, TNFR1 and TNFR2. TNFR1 is expressed in most tissues, and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF. Whereas TNFR2 is found typically in cells of the immune system, and respond

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to the membrane-bound form of the TNF homotrimer. As most information regarding TNF signaling is derived from TNFR1, the role of TNFR2 is likely underestimated.[41]

Upon contact with their ligand, TNF receptors also form trimers, their tips fitting into the grooves formed between TNF monomers. This binding causes a conformational change to occur in the receptor, leading to the dissociation of the inhibitory protein SODD (Silencer of Death Domain) from the intracellular death domain. This dissociation enables the adaptor protein TRADD (TNFR associated death domain) to bind to the death domain, serving as a platform for subsequent protein binding. Following TRADD binding, three pathways can be initiated.[41]

1) Activation of NF-κB[41] :

TRADD recruits TRAF2 and RIP. TRAF2 in turn recruits the multicomponent protein kinase IKK, enabling the serine-threonine kinase RIP to activate it. An inhibitory protein, IκBα, that normally binds to NF-κB and inhibits its translocation, is phosphorylated by IKK and subsequently degraded, releasing NF-κB. NF-κB is a heterodimeric transcription factor that translocates to the nucleus and mediates the transcription of a vast array of proteins involved in cell survival and proliferation, inflammatory response, and anti-apoptotic factors.

2) Activation of the MAPK pathways[41] :

Of the three major MAPK cascades, TNF induces a strong activation of the stress-related JNK group. JNK translocates to the nucleus and activates transcription factors such as c-Jun and ATF2. The JNK pathway is involved in cell differentiation, proliferation, and is generally pro-apoptotic.

3) Induction of death signaling[41] :

Like all death-domain-containing members of the TNFR superfamily, TNFR1 is involved in death signaling. However, TNF-induced cell death plays only a minor role compared to its overwhelming functions in the inflammatory process. Its death-inducing capability is weak compared to other family members (such as Fas), and often masked by the anti-apoptotic effects of NF-κB. Nevertheless, TRADD binds FADD, which then recruits the cysteine protease caspase-8. A high concentration of caspase-8 induces its autoproteolytic activation and subsequent cleaving of effector caspases, leading to cell apoptosis.

Examples of drugs acting on TNF-α Receptor pathway include etanercept (indicated for rheumatoid arthritis, psoriatic arthritis,☐ ankylosing spondylitis, plaque psoriasis), infliximab (indicated for rheumatoid arthritis,☐ psoriatic arthritis, ankylosing spondylitis, plaque psoriasis

Crohn's disease,☐ pediatric RA & pediatric Crohn's), adalimumab (indicated for rheumatoid arthritis, psoriatic arthritis, plaque psoriasis, active ankylosing spondylitis, crohn's disease,☐ juvenile idiopathic arthritis, golimumab (indicated for rheumatoid arthritis, psoriatic arthritis, plaque psoriasis, ulcerative colitis), certolizumab Pegol (indicated for rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease), etc.[41]

Conclusion:

There have been many advancements since enzymes were first discovered in 1833. They are used in various industries & especially for drug design because altering enzyme activity has immediate & defined targets. Enzymes can be inhibited competitively, non-competitively & uncompetitively or be activated by drugs. Microsomal enzymes can also be used as drug targets.Trans-membrane receptors linked to enzymes are currently the most explored drug targets since they play an important role in the fields of oncology & immunology.

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Figures:

Figure I

Figure I: Kinetics of competitive enzyme inhibition by drugs along with Lineweaver-Burk Plot Km: Michaelis-Menten constant, Vmax: maximal rate of the reaction.

Figure I: Competitive enzyme inhibition

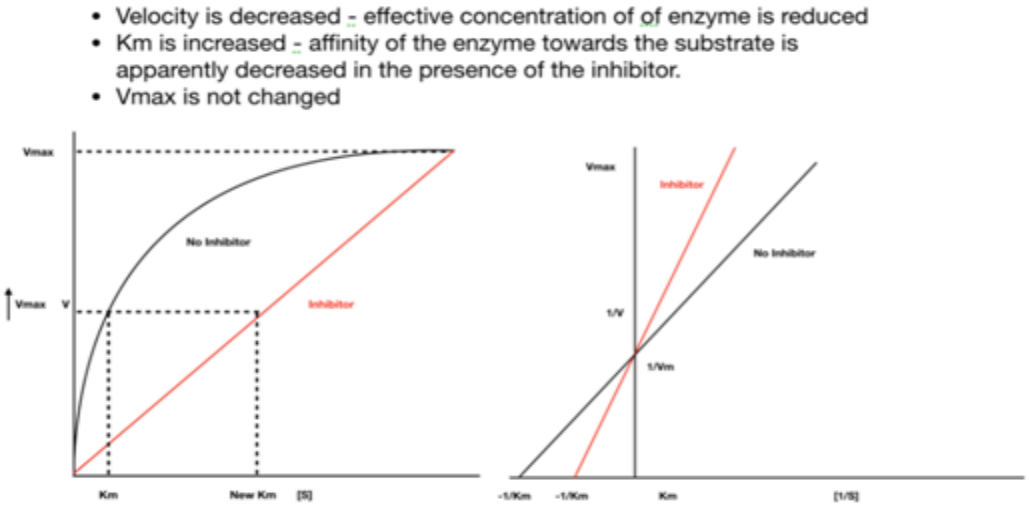

Figure II

Figure II: Non-competitive enzyme inhibition

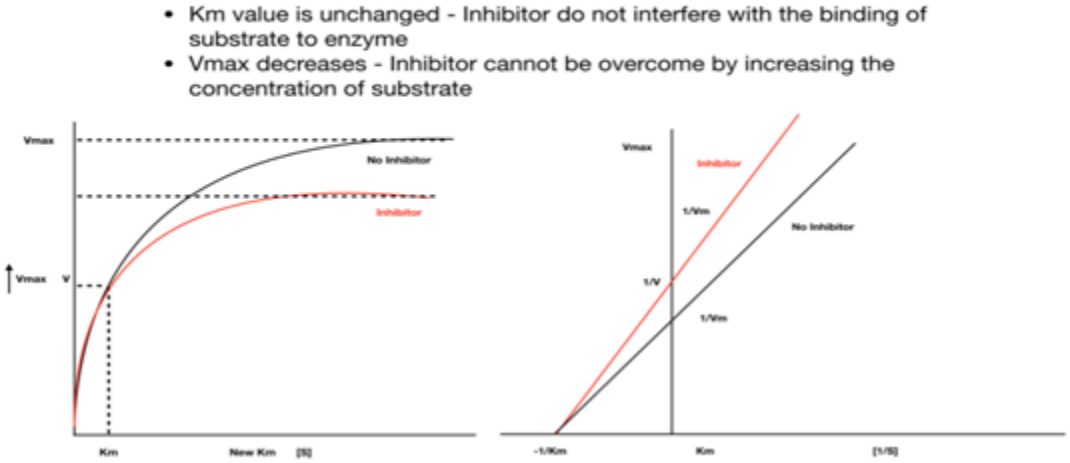

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Figure II: Kinetics of non-competitive enzyme inhibition by drugs along with Lineweaver-Burk Plot

Km: Michaelis-Menten constant, Vmax: maximal rate of the reaction.

Figure III

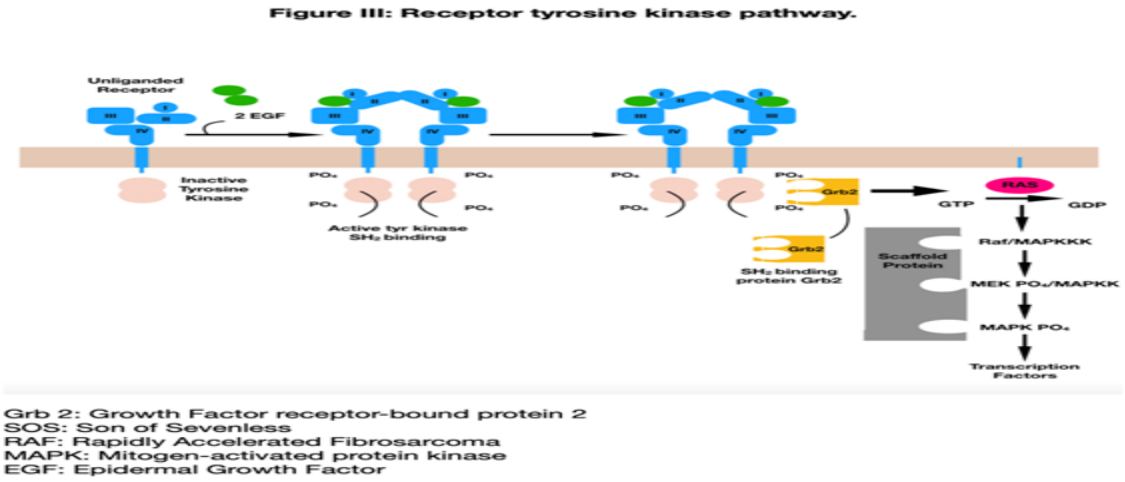

Figure III: Receptor tyrosine kinase pathway

Figure IV

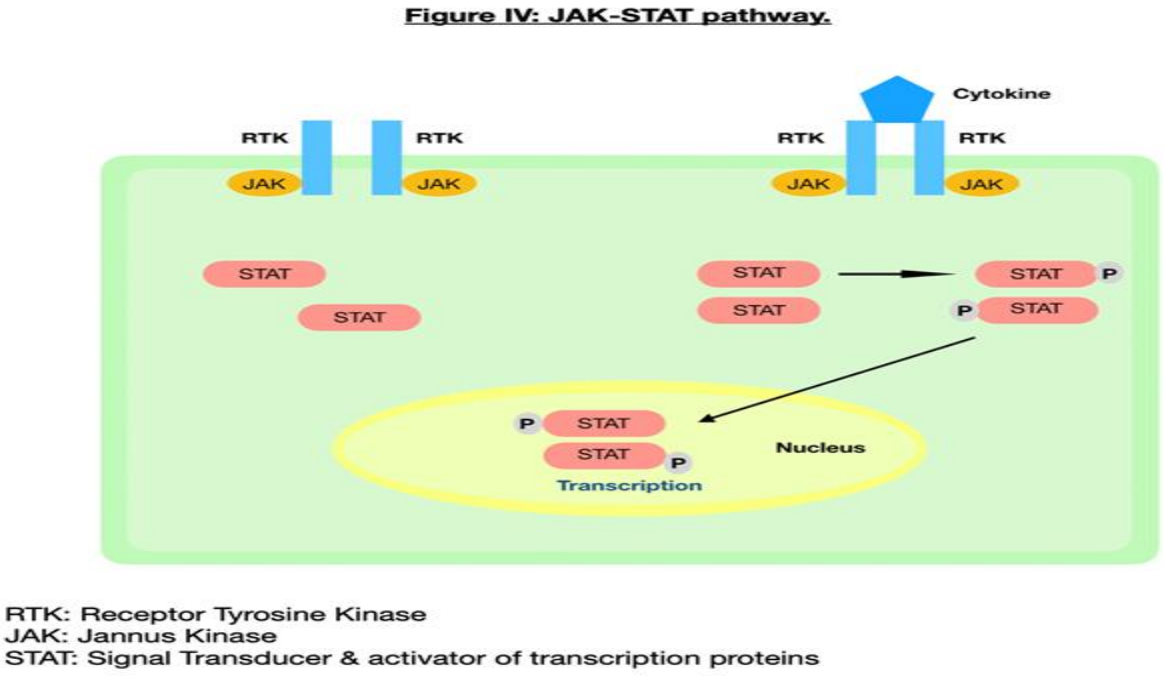

Figure IV: JAK-STAT pathway.

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