BIOTRANSFORMATION

Lecture

by

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- **Loss of parent drug from body**

- **Due to biotransf… of parent drug to metabolites/ or excretion of parent drug from body**

Metabolites

- They are products of enzyme catalyzed chemical changes in drug or toxicant.
- These products may vary in toxicity or therapeutic effects from parent drug/toxicant
- Biotransformation results in less toxic product . This is **detoxification.**
- Some reactions result in more toxic products than parent drug. These are called **Intoxication or bioactivation** reactions.

Detoxification

- Parent drug/toxicant is reversibly bound to a protein.
- metabolite released later into blood
- It may be parent compound itself
- Blood borne drugs/toxicants are capable of crossing cell membranes and binding to protein targets if lipophylic, small and neutral
- **Detoxification** is most efficient when parent compound is altered to a metabolite i.e. hydrophylic, large and carries a charge.

Changes occur in two stages

- **Phase 1 reactions**: Parent compound is hydrolyzed/reduced.
- Results in formation of metabolite that is conjugated to larger, much more hydrophyllic ,charged molecule .
- **Phase II reactions**: 2 conjugation step

- This stepwise biotransformation is necessary, as many compounds lack functional groups to which large molecule is attached.

- Phase I adds functional group
- Phase II attach the larger molecule.
- e.g carbohydrates involve in conjugation reactions/soluble in water/show little tendency to partition into lipids or membranes.
- e.g from molecular genetics enzymes involved in xenobiotic biotransformation are being studied by protein less sequencing.

*metabolomics

- Gene of interest is cloned and sequenced then protein sequence is infered by translation of codons present in sequence.
- Easy and fast method than enzyme purification.
- Imp for Diagnostic test for genetic deficiency.
- Patients with low detoxification ability can be identified and traced this to specific sequence of gene. urine analysis is done with nmr and urine profile is used for genetic studies.*

Primary biotransformation

- **Phase I reactions**
- Hydrolsis/hydrolases e.g amidase ,peptidase, lipase(digestion)cholinestrase(hydrolsis of choline esters. In liver hydrolases for detoxification of xenobiotic carboxylester.
- Drugs aspirin /pesticides,pyrethrins contain ester linkage and can be hydrolyzed.
- Insecticide once used **mirex** (carcinogen) has cage of only carbon and chlorine so impervious to biotransformation /no ester so no hydrolysis . Found to accumulate in adipose tissue due to lipophilicity.

Serine hydrolases

- These enzymes have catalytic site a serine residue that reacts with substrate to form alkylated enzyme.
- Shape is also important in the active sites of hydrolases. 3 dimensional structure of enzyme forces substrate into active site and formation into transition state.
- **Peptidases** e.g chymotrypsin,trypsin
- **Carboxylester hydrolases:** This group has more than 20 genes in mouse ,multiple genes in rat and humans.In mouse 2 clusters of carboxyl ester hydrolases genes. **Multigene family**

- **Cholinester hydrolases**: active against choline esters.Acetylcholinesterase is target of poisoning by organophosphate and carbamates. One gene for acylcholinesterase in humans.
- **Paraoxonase:** common in mammals in liver and serum. Catalyse organoph.. Hydrolysis. rare in birds and insects. Polymorphic activity in humans in serum. Caucasians have low activity allele etc.
- **Epoxide hydrolase:** acts on epoxides convert into diols.

Oxidation

- It is 2nd mechanism of primary metabolism by which xenobiotics(DRUGS,DYES, antibiotics, pesticides,petroleum) are detoxified or bioactivated to more toxic product.
- C**ytochrome p450**/ also known as **monooxygenases** found in microsomes(SER) of liver.contain heme (iron containing group O is bound to iron)absorbance at 450nm.co is bound to reduced enzyme called ferrous-Co 450nm soret band. 36 known families of p450 enzymes.

- **Oxidation reactions** catalyzed by p450 enzymes are: **Epoxidation**
- **Aromatic Hydroxylation: insertion of O** to form aromatic ring
- **Dealkylation:** of some insecticides lead to formation of RO intermediates which may be carcinogenic. **Desulfuration reaction**, malathion transform to more toxic.

- **Metabolic capabilities** of this enzyme family can vary from individual. Poor metabolizers may suffer from deficiency in drug detoxification. Poor metabolizers in nigerian smokers were less susceptible to cancer due to reduced bioactivation of smoke.

- **Flavin containing monoxigenases**: Known for N oxidation of tertiary amines. Lack heme group
- **ADH** carries out oxidation in liver, kidney and lungs. Converts alcohol to aldehyde.
- 4 classes of isozymes.
- Class I isozyme: in ethanol metabolism in liver.
- Class IV isozyme: gastrointestinal tract(ethanol metabolism) ,increase risk of gastric cancer due to production of acetaldehyde (carcinogen) in GI tract.
- **ALDH** converts aldehyde to COOH
- **Monamine oxidase**: enzyme in brain for deamination of neurotransmitters.play role in parkinsons disease.

Reduction in phase 1

- Reductions more likely to occur in environment due to low O.
- Azo reduct.. N-N
- Nitro reduct.. NO2 in GI tract
- Dehalogenation from CCl4
- Catalyzed by cytochrome p450

Secondary metabolism(phase II)

- Products of phase I enter secondary phase of biotransformation
- Now they are polar by conjugation to carbohydrates,aa or small peptides.
- These products excreted out of body more efficiently than parent molecule.
- **Glucoronidase**: conjugation of phase I product with UDGPA uridine diphospho glucoronic acid.cofactor, Catalyzed by UDPG transferase(found in RER,SER in liver etc
- Imp in conjugation of bilirubin(heme released from Hb of dead erythrocytes oxidized in spleen.
- Testosterone conjugates with UDGPA to form t glucuronide

Glutathione transferases

- Glutathione conjugation: has unusual glutamyl linkage with cysteine.
- Glutathione can react with phase I products catalyzed by G transferases(dimers)
- 7 classes: differ in isoelectric points,size 23000 Da per subunit.

- Activity in liver, kidney, intestine.
- Linoleic acid is a good substrate for several G transferases.
- G transferases has role in biotransformation of drug acetominophen to N acetyl benzoquinonimine a hepatotoxin and it is rapidly conjugated to glutathione.

- Depletion of glutathione by acetaminophen overdose can lead to toxicity and death.
- G transferase imp role in cancer research.AfflatoxinB1 is a mutagen biotransformed to less toxic.
- EDB fumigant is biotransformed to highly carcinogenic conjugate to alkylate DNA.

Acetylation

- For transformation of aryl amines and hydrazines catalyzed by acetyl co A dependant-Nacetyltransferase.
- Common recessive allele for slow acetylation in humans.

Factors influencing metabolism

- in Both phase I & II reactions
- **Species**: Qualitative differences in (differences in enzymes expressed) and quantitative differences in(differences in level of expression) exist between species.
- And within single species(polymorphic alleles for enzymes)
- **Age :** Capacity of drug metabolism is low during development, in humans it develops well after birth.
- **Gender**:differences also exist in metabolism in many species, but not in humans.
- **Diet & environmental factors** have impact in some cases.