The Royal College of Pathologists
Pathology: the science behind the cure

FRCPath Examination

Toxicology Speciality

Subspecialty
Biochemical and Molecular Toxicology
Part I, Paper II

Curriculum

General Principles of cellular biology
Specific Topics
Cellular biology • Protein and DNA structure and synthesis, and the basis for interaction of drugs/chemicals with proteins, DNA/RNA and laboratory methodologies • Receptors and their interaction with chemicals/drugs to induce toxicity • Types of binding of chemicals to macromolecules: covalent, ionic, hydrogen, van der Waals interactions, bond strengths

Mechanisms of Toxicity
Specific Topics
Mechanisms of Toxicity • Major determinants and mechanisms of toxicity including the MOA of classical toxicants, factors determining target organ toxicity and strategies for elucidating mechanisms of toxicity. Factors modulating toxicity and antidotes • Biochemical and molecular mechanisms by which xenobiotics dysregulate the control of the cell/tissue in structure or function. Environmental and genetic factors influencing individual or populations susceptible to toxicity. • Adaptive processes and their effect on xenobiotic exposure • The utility and limitations of species differences or methodological differences in determining or detecting mechanisms of toxicity. The evaluation of experimental data and its implication for human exposure

ADME: Drug absorption
Specific Topics
Absorption • Mechanisms, routes, factors affecting absorption, methods for studying absorption in vitro and in vivo, toxicological aspects

ADME: Drug distribution/disposition
Specific Topics
Toxicity is dependent upon disposition of drugs and varies according to: • Protein and macromolecule binding in tissues • Blood cell uptake • Blood brain barrier • Placental transfer • Absorption at the site of exposure • Tissue distribution (non-polar substance deposit in fat, skin) • Metabolism • Excretion Methods to assess distribution and toxicological aspects

ADME: Drug Metabolism
Specific Topics
Metabolism • Pathways of metabolism: Phase I, Phase II and Phase III processes • Tissue sites of metabolism, pharmacogenetics, polymorphisms, microflora metabolism and deconjugation methods for separation and identification of metabolites, species differences • In vitro methods, enzyme induction and inhibition, genetic-environmental interaction, interspecies comparison, prediction of drug-drug interactions, prediction of PK parameters in in vitro data • Phase 1 drug metabolising enzymes and their mode of action in inducing or reducing the toxicity of drugs/chemicals: CYPs and the types of metabolic reactions that they undertake: N-dealkylation, hydroxylation, oxidation and non-P450 mediated oxidations (e.g. monoamine oxidases and alcohol, and aldehyde, dehydrogenases) and their importance in toxicology • Phase 2 drug metabolic reactions and their modes of action: glucuronidation, sulphation, glutathione and its various enzymes, acetylation, amino acid conjugation, methylation • Metabolism of specific chemicals and chemical classes • Effects of "rates" of metabolism on determining tissue specific toxicity • Comparative animal metabolism and how specific chemicals may be handled differently dependent upon the animal species • Polymorphic distribution of metabolic enzymes and their influence on toxicity: slow and fast acetylation (isoniazid, debrisoquine) • Effect of stress and unbalanced, or nutritionally poor, diet on expression of enzymes involved in metabolism (Paracetamol increased; carbon tetrachloride reduced toxicity) • Effect of age on metabolism: infant and aged • Effect of pre-existing disease on metabolic enzyme expression (malabsorption syndrome; cirrhosis of liver) • Induction and inhibition of enzyme activity by xenobiotics • Suicide enzyme inhibition and its relevance to toxicity with chemical examples • Biochemical development of Tolerance: exposure at low doses leads to a tolerance whereby subsequent exposure to toxic dose levels prove to be less toxic than normal. • Extra-hepatic metabolism and biochemistry specific to blood, kidney, lung, cardiac and striated muscle, adipose tissue and their drug/chemical specific toxicities (e.g. statins, ACAT inhibitors)

ADME: excretion/elimination
Specific Topics
• Mechanisms, major and minor routes, factors affecting excretion, enterohepatic recirculation and implications, methods of study and toxicological aspects

Dose response
Specific Topics
• Dose response relationships and the dependence upon interaction between the receptor/specific target and the local concentration of the drug

- Toxicity and shape of dose-response curves (steep versus shallow dose-response curves)
- Factors affecting the dose-response: metabolism, absorption, excretion, reservoir effects and species-specific effects

Pharmacokinetics of Drug Metabolism-uptake and excretion

Specific Topics

Parameters: clearance, volumes of distribution, half-life

- Parameter changes in special populations
- Data modelling and interpretation
- Species scaling and prediction

Role of TK in the design of toxicity tests and their interpretation in Safety Assessment

- Pharmacological, toxicological impact of PK and metabolism.
- Strategies for investigating species differences in metabolism and PK
- Implication of species differences for the design and interpretation of safety evaluation studies.

Non-linear kinetics caused by:

- Saturation of a storage site (e.g. plasma protein)
- Accumulation (concentration) of a compound in a particular tissue
- The causes of threshold responses where doses below a toxic level do not induce toxicity and those of maximum responses (sigmoidal dose response curve)

Understanding of the following:

- ED50: effective dose. Generally used for drugs - the dose required to have a therapeutic or other effect in 50% of sample population.
- TD50: toxic dose. The dose required to elicit a defined toxic effect (e.g. fatty liver, fever, etc.) in 50% of the sample population
- LD50: lethal dose. The dose required for lethality in 50% of the sample population
- Factors influencing toxicity such as receptor/drug residency times and local concentration of toxin
- The concept of therapeutic index and its use in drug development and the use of LD50 & ED50 in deriving the therapeutic index
- Competitive versus non-competitive interactions of toxins with targets and the kinetics of interaction
- Drug kinetics and the factors influencing changes in rates of absorption, metabolism and elimination
- The use of “scaling” in the calculation of safe doses between preclinical and clinical studies

Mechanisms of absorption and distribution:

<u>Membrane structure</u>:

- Lipid bilayer and the role in transport of polar molecules and non-polar molecules.
- Protein channels and specific binding/transport provide transport of polar molecules across the membrane, both non-specific and specific.

<u>Mechanisms of transport</u>: Fick's Law governing diffusion

- Filtration
- Passive diffusion.
- Facilitated diffusion: catalysed diffusion dependent on specific binding to carrier molecules, commonly a protein e.g. the glucose carrier
- Active transport: particles are "pumped" against a concentration gradient with the expenditure of energy (e.g. ATP hydrolysis).

- Pinocytosis

Properties of drugs/chemicals that affect their disposition:

- size/shape of the drug
- lipid solubility/hydrophobicity
- structural similarity to endogenous molecules
- charge/polarity

PK calculations

- The significance, calculation and use of pH, pKa, LogD, in estimating kinetics of absorption and exposure of a drug in tissues (Henderson-Hasselbalch Equation)
- Volume of Distribution calculations and variables influencing change
- Effect of specific tissues concentrating drugs and acting as reservoir for continued prolonged exposure (TCDD) or for rapidly removing activity – adipose tissue accumulation of thiopental
- PB/PK mathematical modelling of exposure
- Zero and first order kinetics for drug exposure and their calculation and typical time course
- Single and two compartment models for absorption and distribution of drugs and chemicals

Drug-Drug Interactions in the Clinic
Specific Topics
Drug-drug interactions and how they can be predicted by preclinical studies (with specific examples) • Additivity: the overall effect of two toxins is the direct addition of the individual toxicities of the doses given. • Synergism: the toxicity of the doses of two toxins is greater than the sum of the toxicities of these two doses. Synergy gives a greater toxic effect than additive toxicities. • Potentiation: similar to synergism, but the toxins have different effects. Specifically, one substance leads to a greater effect by a second toxic substance. An example is "antabuse" in treating alcoholism - by itself at the dose given there is no effect, but when alcohol is ingested there is a toxic response to the alcohol at small doses. • Antagonism: one substance reduces the toxicity of another.

Organ-specific effects
Specific Topics

Kidney
• Excretion
• Blood flow through the kidney represents 25% of cardiac output
• Passive glomerular filtration
• Resorption of lipid soluble compounds.
• Concentrations of compounds in the filtrate approximate [unbound] in plasma.
• Fates of compounds in filtrate determined by lipid solubility, therefore urine pH is important factor in excretion.
  • bases secreted better if urine acidic
  • acids secreted better if urine is basic

- Passive diffusion into the tubule
- Organic acids and bases exchange by diffusion processes in the proximal convoluted tubule.
- Active tubular secretion - important for charged compounds

Liver and the biliary system

- Excretion
- Larger, polar compounds are often preferentially excreted via the biliary system into the duodenum.
- Usually need to have a MW > 300 for this system to be effective.
- Generally active processes are specific for acids, bases or neutral compounds.
- Charged compounds will not be resorbed from the intestinal lumen and so will be lost with faeces.
- Can get intestinal resorption however if the excreted conjugated compound is hydrolysed or metabolized to give a non-polar entity by intestinal flora.
- Additional excretion systems such as the lungs (volatile substances), sweat, and milk (particularly for lipid soluble substances).

Bioanalytical methodology
Specific Topics
• Understanding of the common molecular biology methodologies for separation and analysis of ribonucleic acids, proteins, lipids and carbohydrates and how they can be used to address complex problem solving in toxicology • Bioanalytical methodology: use of radioisotopes and techniques such as GC, HPLC, immunoassay (IA), CE, GC-MS, LC-MS/MS, method validation and quality control of the data. New techniques, method validation, QC • Appropriate use of statistics in analysing data and in giving confidence to dose extrapolation in prospective human studies