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BIOLOGISK INSTITUT
SYDDANSK UNIVERSITET

Eksamen i Farmaceutisk toksikologi A (BB523), 4. kvartal

Onsdag den 06. juni 2012

Eksamen er digital, på engelsk og uden hjælpemidler med undtagelse af ordbøger.

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1. Toxicology:

a) Which of the below is needed for LD50: dose

concentration
duration of exposure
species
volume
way of application
dose

organ failure
gender
mortality
tumor development
all of the above
dose

b) drug application: in which order will organs get affected?
Note, organs could be affected simultaneously

Oral (as a pill): Kidney
Liver
Heart
Lungs
Intestine
Intestine—lever—heart—kidney--lungs

Intravenous Kidney
Liver
Heart
Lungs
Intestine
instestine—lever-- heart—kidney--lungs

2. Biotransformation

a) name the 3 phases and the purpose of biotransformation, provide reason, why all 3 are necessary

Phase 1: Activation, the drug is activated by CYP450

Phase 2: conjugation, the activated chemical is conjugated
Phase 3: excretion, the more soluble product is know ready for excretion.

All 3 phases are necessary due to detoxification or else the substance will be taken by other cells.
The first to phases makes the molecule more water-soluble so they can be excreted in phase 3.

b) Provide one example of an environmental pollutant for phase 1 and 2 biotransformation, use chemical formula, name the enzymes facilitating the steps

See extra note

c) put together the correct enzyme, co-substrate and substrate
(note, there could be some that don't belong here at all)

UDP-GT, Glutathione, NADPH+H+, Isoniazid, PAPS, Acetyltransferase,
P-Glycoprotein, Aniline, GST, Sulfotransferase, Glucuronate, LDH, Aflatoxin, Acetyl-coA,
Phenol, Isoniazid, ALT, bilirubin, paracetamol

Phase II Enzymes	co-Substrates	substrate
Sulfotransferase	PAPS	Phenol
Acetyltransferase	Acetyl-CoA	Isoniacid
GST	P-Glycoprotein	Paracetamol
See extra note		

3. Oxidative Stress

a) list reactive oxygen species and name the damage they may cause

H_2O_2, $O_2^{\cdot-}$, NO, NO_2, $OH^{\cdot-}$

Oxidative stress can damage DNA, proteins and lipids. Leading to DNA adduct, Lipid peroxide.

b) name 3 antioxidant enzymes and their reaction mechanism

SOD: superoxide
Catalase
Peroxidase
Vitamin-E(alfa)

A natural pathway which may generate oxidative stress is redox cycling.
It is when an oxygen is reduced from NADH or NADPH. The drug takes the electrons from NADH or NADPH. If this reduced xenobiotic reduces another oxygen species, is it again ready to be reduced another time, this is called the redox cycling. the reduced xenobiotics can reduce the oxygen species to superoxide anion

c) describe how lipid peroxides in membranes will get repaired

4. Cytotoxicity

a) Membrane disruption:
Provide 3 examples of membrane disruption including a brief explanation and eventual an example of a very susceptible site inside the cell or a cell type.

Unstable membrane causes cell death. By loosing membrane cohesion, loosing semipermiabiltiy and concentration gradient thereby loosing homeostatis which causes cell death. Loosing communication. All this makes the membrane unstable because of lipophilic attack on the membrane.

Endocrine disturbers act on hormones. Hormone is responsible for development, maintenance of homeostatis, reproduction.

Susceptible parts of a nerve cell:

Ion channels (communication/nerveimpuls)
Myelin sheath.

When a substance solves in the membrane, a signal goes to the nerve and the speed in the myelin sheath increases through the myelin fiber. They don't need to go through it, but can spring over the myelin sheath. And then the synapse, releases neurotransmitters which

then binds to postsynaptic membrane. Acetyl cholin is released and binds to a ACH-receptor and it triggers. To stop it, there is a Acetylcholin esterase, which cleaves them, and prevent acetyl cholin to bind to ACH-receptor.

b) sort to apoptosis or necrosis or out
phagocytosis, heat shock proteins, energy, passive, swelling, biotransformation, caspase cascade, inflammation, vesicles, neurotransmitter, single cells, leaking of lysosomal enzyme, shrinking, base excision repair, fragmentation, redox potential, disruption, groups of cells,

see extra note

5. Genotoxicity

a) name possibilities of genotoxic changes/damages on the DNA-molecule

Mutagenic: altering the base sekvens. DNA changes
Clastrogenic: breakages of chromosomes.
UV light, radioation, xray, UV-b, UV-A. Oxidative stress in DNA. ROS which binds to DNA, and changes the nukleinsyre. Deamination of bases, methylation of bases, nitration of bases. DNA adduct.

Oxidative stress: Fenton reaction, occurs when hydroxyperoxid cannot be removed by catalase, and glutathione peroxidase. This hydroxyl radical will now damage DNA, bind to the bases of DNA.

b) briefly describe 2 DNA repair possibilities including the enzymes involved

Base- excision repair:
Replacement of A complex glykolase checks after, and when the damage is seen, it takes of the wrong one, and puts the right one in. DNA polymerase, DNA ligase enzymes is used.
Direct reversal: for pyrimidin dimmers. Often occurs in UV lightning. The light binds to thymine or cytosine. Photolyase repairs this in help with electrons from FADH and UV light.
Demethylation: for methylation of bases. Is removed via a protein: methyl guanine methyl transferase. Which takes the methyl group from the methylated base and in this way it repairs.

c) genotoxicity testing: describe one test procedure, explain what is it testing for in particular, and explain why in some of the test procedures a liver extract (=S9) is added to a subsample.

Test for DNA repair: Unscheduled DNA repair, in DNA there is thymine, and this thymine is tested with thymine. In S phase it is not tested. Is tested with and without S9.
Test for backward/forward mutation:

Ames test is for backward mutation. Uses histidin. And a bacteria named salmonella in a growth medium.
Mouse lymphoma L5178Y: is tested with TK (thymidine kinase) for forward mutation. TK+- is going to be TK--. With and without S9

Test for strand breakages/chromosomal aberrations:

Comet assay: tested in gel elektroforese

Micronucleus test: test for meiose/mitosis phase. Spindle damage.

6. Cancerogenicity

a) describe the steps of cancer development

Steps of cancer development:
Initiation: many mutations are required to start cancer initiation. DNA damage occurs in initiation. The gene in normal cells get affected. The cell initiates
Promotion: it uses a promoter. The initiated cell is growing in help with a promoter. Promotes the proliferation of the cell. It increases/grows. Altering gene expression. Giving rise to a large number of daughter cells.
Progression: the mutated cell becomes a oncogen. Protooncogen → mutation → oncogen. It affects the tumor suppressor gene, which is now switched off and can not help repairing it. Gene controlling growth is reduced: tumor suppressor gene, P53 gene.

b) name and describe characteristics of a cancer cell

Characteristic of a cancer cell:

Unchecked growth: mutation causes damage, initiates cancer

Immorality: limited growth

Specific growth features: invade neighboring cell, tumor, metastase

Genomic instability: loss of capacity to repair genetic errors

7. Teratology:

a) Name the 4 levels of teratogenic effects

Manifestation:

nature of drug will decide upon its effect. Type of drug and if it can entry the embryo.

Effects depend upon dose and duration of exposure.
Effects vary with developmental stage at time of exposure.

Manifestation varies with: growth retardation, functional defect, malformation, death of the embryo.

Toxicokinetics:

The Drug concentration in mothers blood is crucial.

c) list causes for teratogenic effects

Malnutrition, nutritive defect, genetics factor in mother and child, toxic chemicals like smoking, irradiation, metabolic disease of mother and child.

d) describe mechanisms of retinoids teratogenic effects

Retinoid(Vitamin-A) teratogenicity: is used for acne, and contains Vitamin A, which effects the embryo. Effect on the embryo development due to retinoids can cause. Risk of miscarriages or birth defect. Effect on reproduction, differentiation and proliferation of cells. It will mimick the normal signals that influence gene expression. It will bind to some receptors, retinoic acid receptor and these receptors act as transcription factors to activate specific genes. They will activate sonic hedgehog: responsible for the brain development. The brain will get way too big. Hox genes: formation of the body, makes thebody right.

8. Liver:

a) name and shortly describe the 4 main liver functions

Breakdown: hormone production, insulin. Protein (ammonia to urea), toxic substances breakdown. Storage: glycogen storage and used as glucose when needed. Minerals: iron. Synthesis: carbohydrate synthesis: gluconeogenese. glycolysis, glycogenesis, glycogenolysis., protein metabolism,lipid synthesis (lipogenesis, cholesterol synthesis). Bile synthesis. Hemoglobin synthesis to bilirubin.

b) By which mechanism(s) becomes paracetamol livertoxic? Describe toxicity, detoxication mechanism and possible antidote. Use formula.

Paracetamol. If taken in high dose. It cannot be fixed by GSH, because GSH gets depleted. Bioactivated by CYP450 and by a NAPQI and cannot be conjugated by GSH, but can now bind to cystein-thiol groups on proteins and DNA bases. That's why it is toxic, it can change the DNA sequence.

See extra note

9. Kidney:

a) name the 4 main kidney functions
kidney toxicity
kidney has a nephron which is located inside the kidney. It is made of browmanns capsule, which has 2 layers, inner and outer layer. inside it there is a glomerulus, which is made of capillaries and arterier. The browmanns capsule also has a tube, which goes down, up, down again, this tube is called the loop of henle. When it goes down, it is named the descending limb, when it goes up = ascending limb. And when it goes down the third time, it gets thicker and is names the collecting duct. There is a layer in browmanns capsule named: basal lamina. It holds big proteins and negatively charged proteins away. It is called filtration, where the wasted is filtrated. Proximale tubule: recovers glucose/aminoacid/lactate/vitamins/proteins.

reabsorption will occur, where the chemicals will be recovered by the proximal tubule.

Loop of henle: we have this structure to balance water. Ions/water will be recovered to keep it balanced in the body.
Filtration, the waste is filtrated
reorbsorbtion

b) How is primary urine produced and which ingredients need to be recovered?

Primary urine is produced by filtration. Blood will enter the kidneys to undergo filtration. Nephron forms urine by filtration, reabsorption. Filtration happens in glomerulus, the blood (containing useful chemicals) courses through it and dissolves waste materials such as glucose, amino acids. Then it is filtrated and flows into the browmanns capsule. When it is courses inside glomerulus we have capillary fenestrations,which allows the blood to come through with its chemicals. Under filtration: basal lamina, holds the big proteins and negative ones away. The result is then primary urine.

c) describe toxic effect in kidneys of Cisplatin, use formula, make a sketch drawing

10. respiratory system:

a) By which pathways of drug application is the lung directly concerned?

b) Describe cells with their function in the alveoli

c) Describe toxicity of paraquat, draw formula, what disease might be the outcome?

11. Chromatography:

a) tick the relevant characteristics for each chromatography:

Gel filtration	ligand
	ions
	C18
	V pores
	eluent
	pH

Affinity chromatography	ligand
	V ions
	C18
	pores
	eluent
	pH

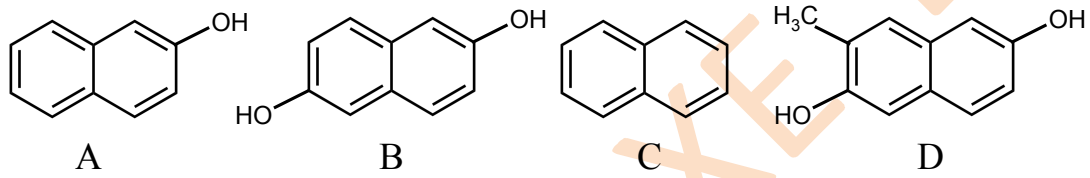
b) What is it important for the response factor?

V Peak height
Peak size
Retention time
Concentration of the substance

Injection Volume
All of the above

Provide a brief explanation
Hydrofil comes out first because of colum is hydrofob

c) sort according to increasing retention time on a RP C18 column:



A B C D

B DAC

12. SDS Polyacrylamid Electrophoresis (SDS PAGE)

a) briefly describe the function of SDS and DTT

DTT CLEAVES disulfidbonds and SDA charges the molecule

b) How is the protein size determined in SDS PAGE? Draw a sketch.