université PARIS-SACLAY

FACULTÉ DE PHARMACIE

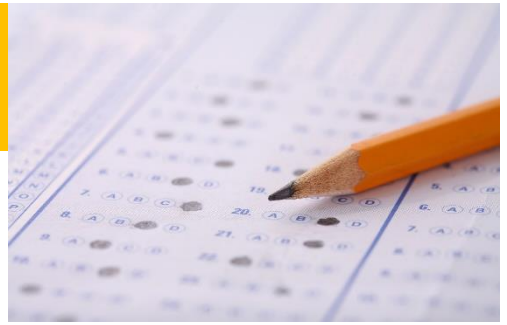
ED1 Metabolism

- **Master 1 D^2HP**

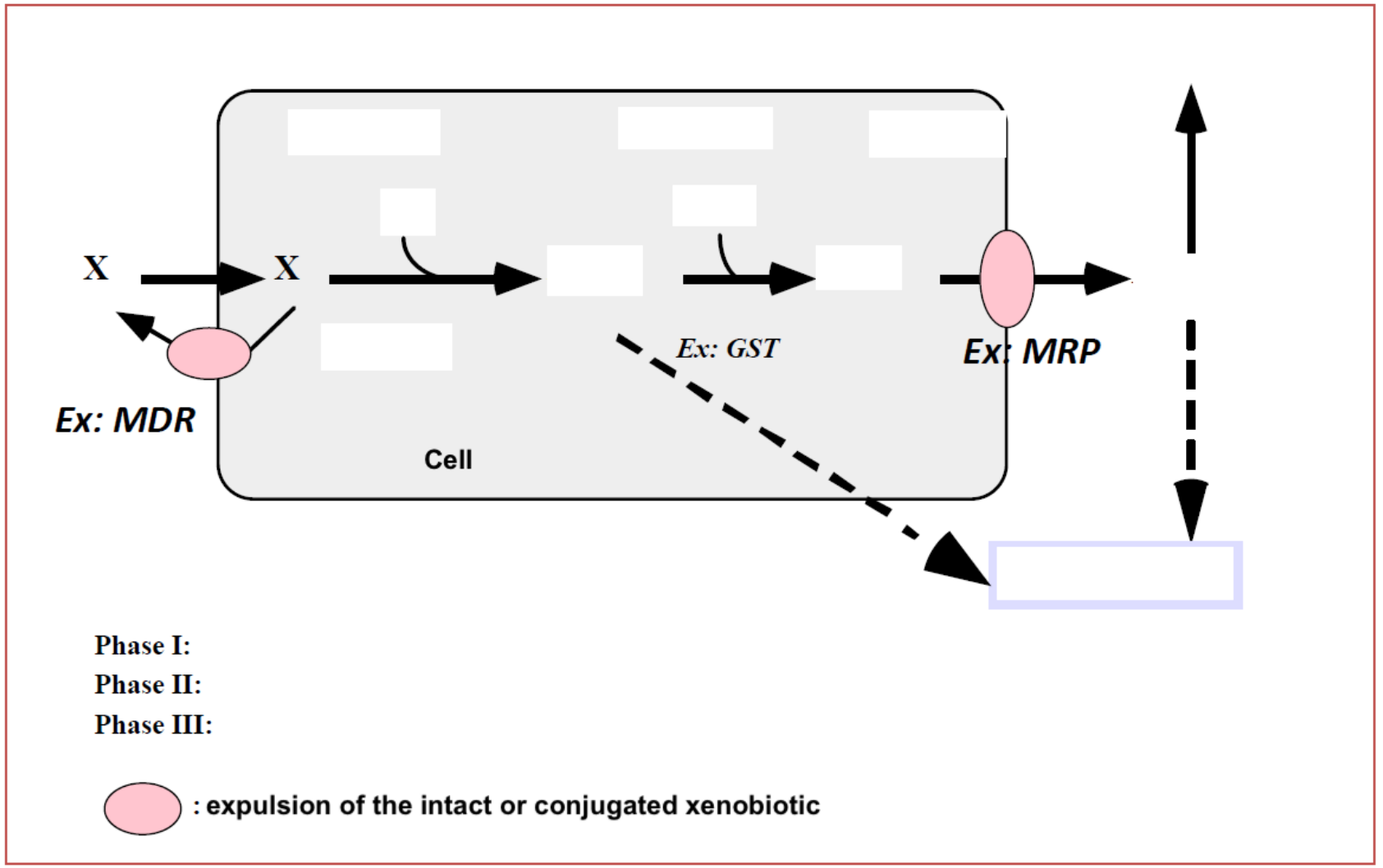
- **TU11**
- **Pharmacology/Toxicology**

Ghidaa BADRAN
30 January 2025

Quiz



Fill in the blanks.

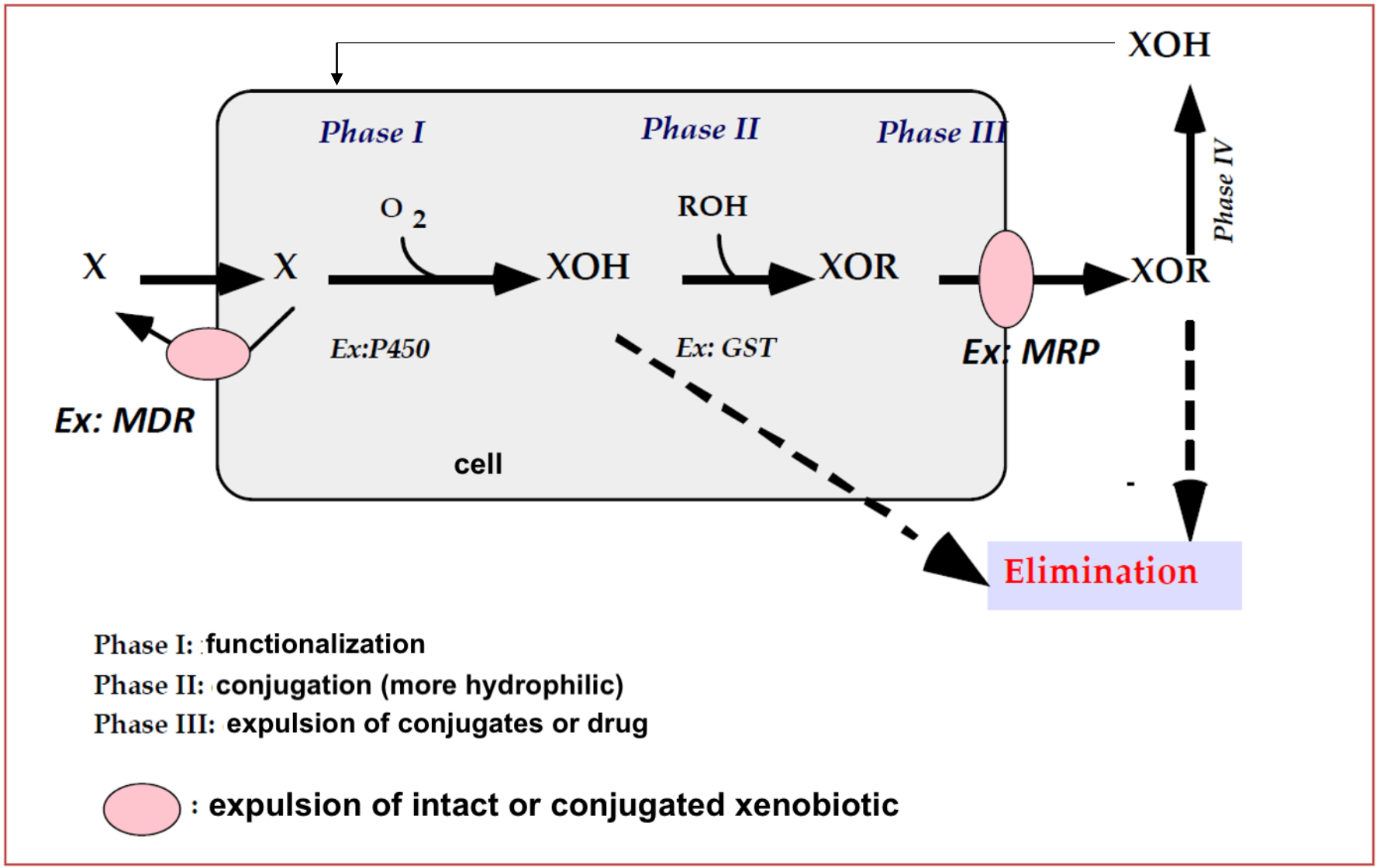


Phase I:

Phase II:

Phase III:

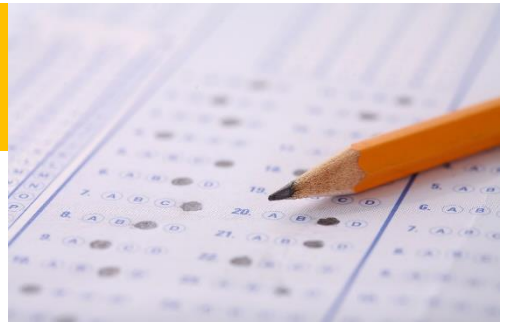
: expulsion of the intact or conjugated xenobiotic



Aim: formation of water-soluble derivatives with little or no activity and their elimination in urine and bile

$$RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$$

Quiz



Choose the correct answer(s):

A. The liver is a primary organ in the elimination of xenobiotics.

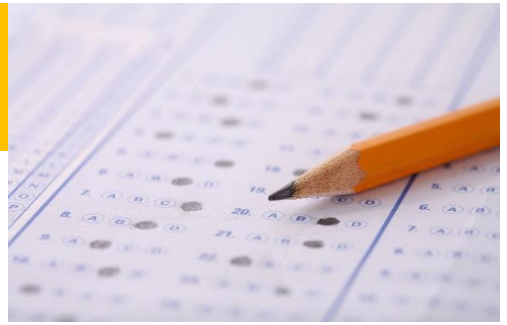
B. In hepatocytes, detoxification relies on a very diverse enzymatic equipment, whose functioning is inducible.

C. Phase I biotransformation consist in conjugation reactions.

D. Functionalization reactions consist of the addition of a functional group to the molecule to be eliminated.

E. During phase II reactions, the addition of endogenous molecules increases the hydrophobicity of the active ingredient, necessary for its elimination.

Quiz



Choose the correct answer(s):

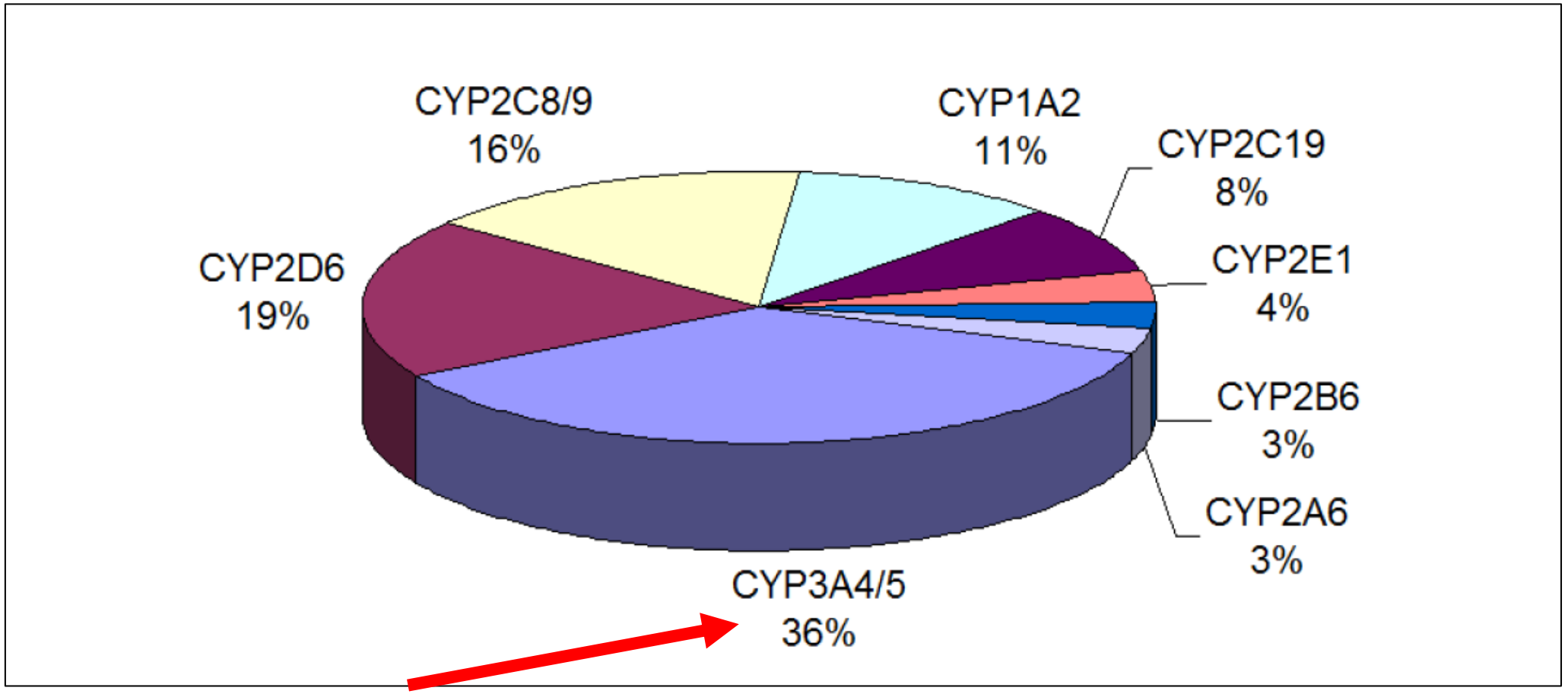
A. By transforming liposoluble apolar molecules into water-soluble polar metabolites, the metabolism facilitates the elimination of the drug.

B. A drug can be metabolized by several distinct CYPs, but it can also inhibit or induce the functioning of CYPs.

C. A drug that induces one cytochrome increases the plasma concentrations of another drug that is a substrate of that cytochrome.

D. Three isoenzymes of CYP450 metabolize 70% of drugs: CYP 2D6 is in the first position.

Percentage of drugs metabolized by different CYP450 families



Exercise n°1

Exercise 1

Mrs. B, 35 years old, has been treated for 2 months with isoniazid, rifampicin, pyrazinamide and ethambutol for *Mycobacterium tuberculosis* infection. She also lives with her partner and uses an estro-progestative pill as a contraceptive.

What advice can you give this patient regarding her method of contraception?

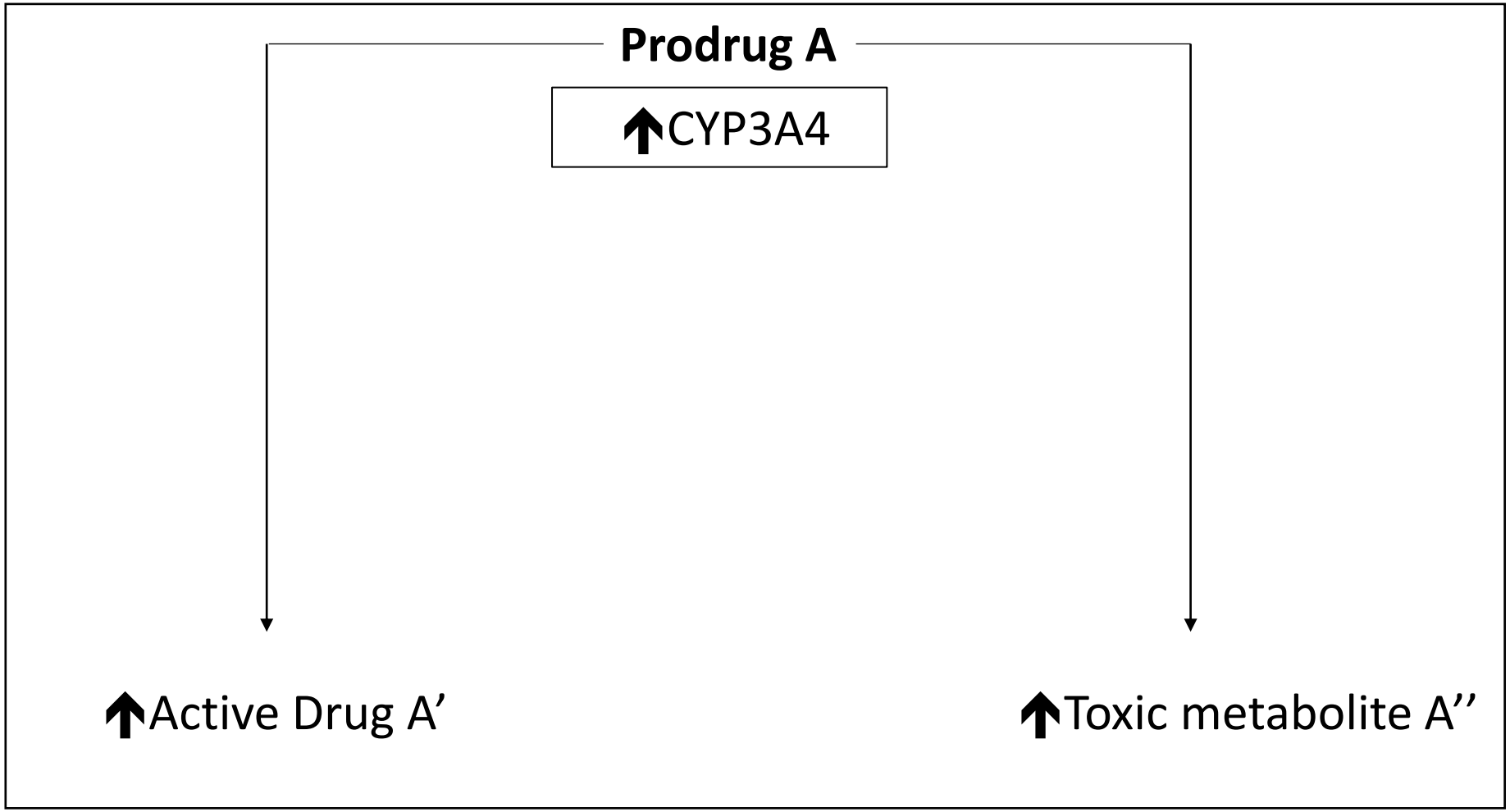
Enzymatic induction

- **Process** by which a substance (often a drug or other chemical) increases the activity of an enzyme in the body.
- Cytochrome P450 are inducible enzymes
- Possible drug interactions.

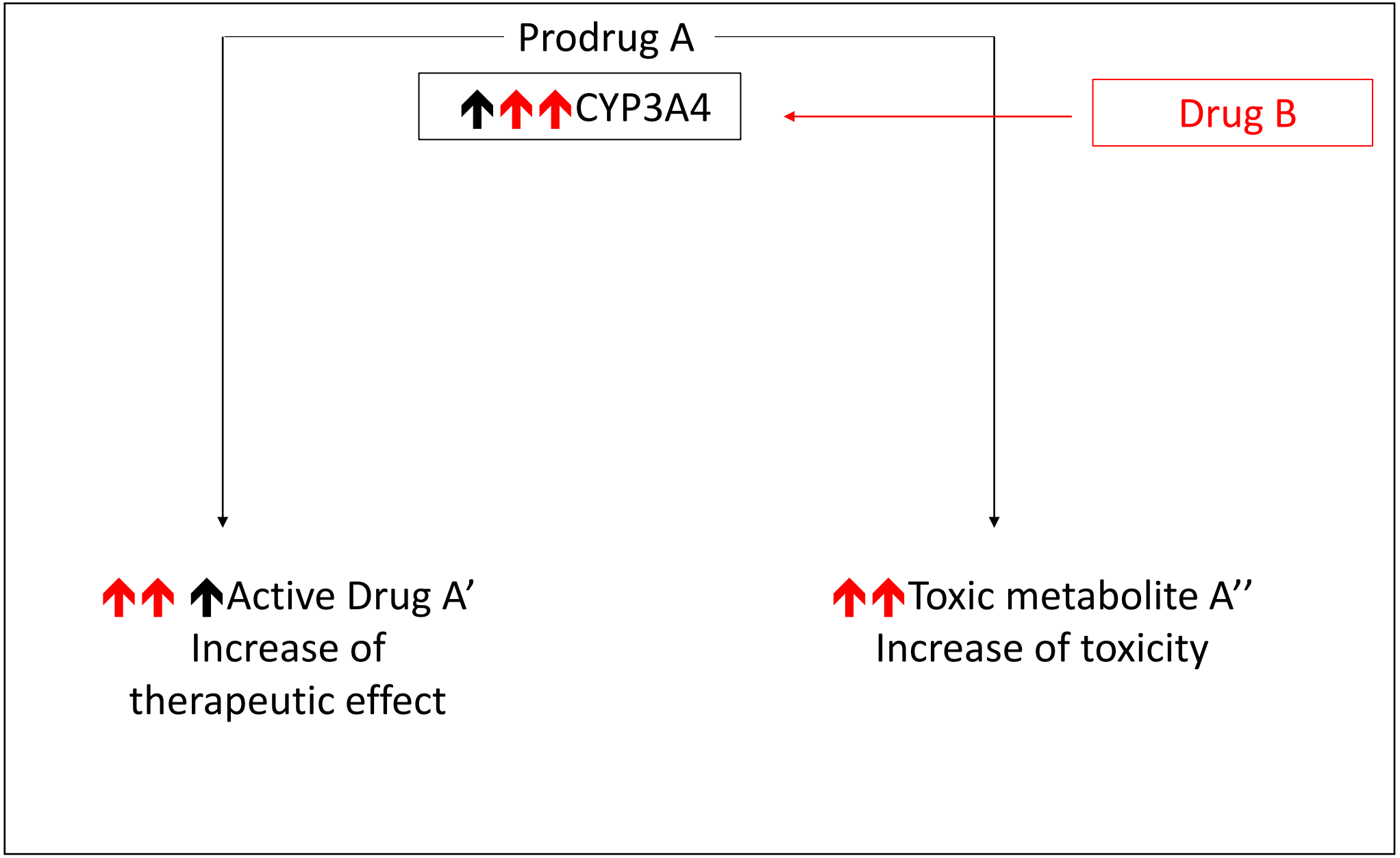
Consequences:

- Decrease in therapeutic effect for active drugs
- Increase of therapeutic effect and/or toxicity if the metabolites are more active than the drug.

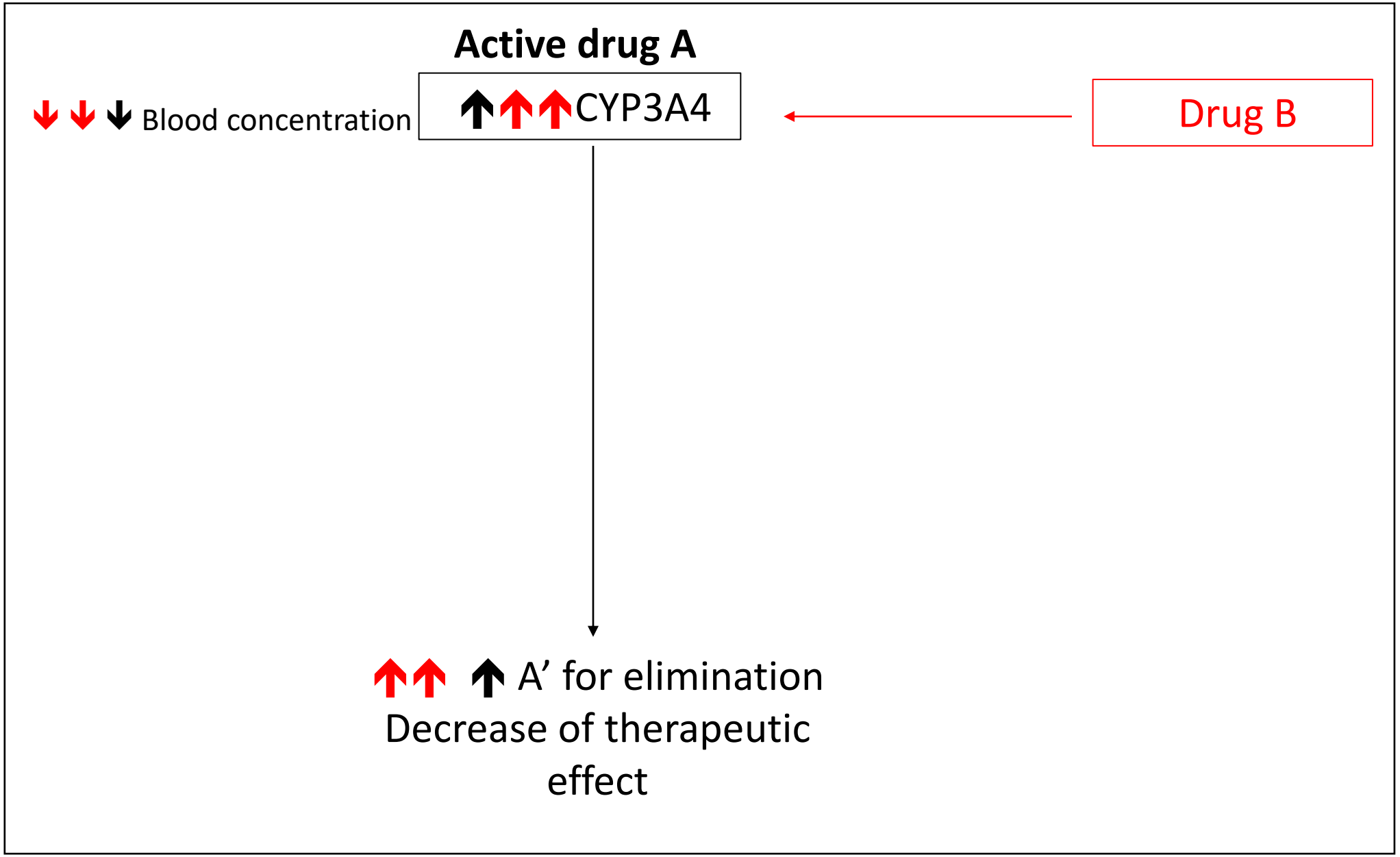
Enzymatic induction



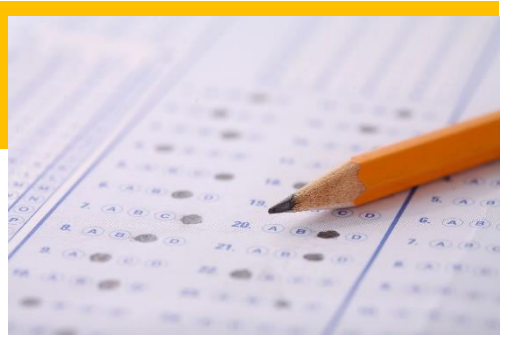
Enzymatic induction



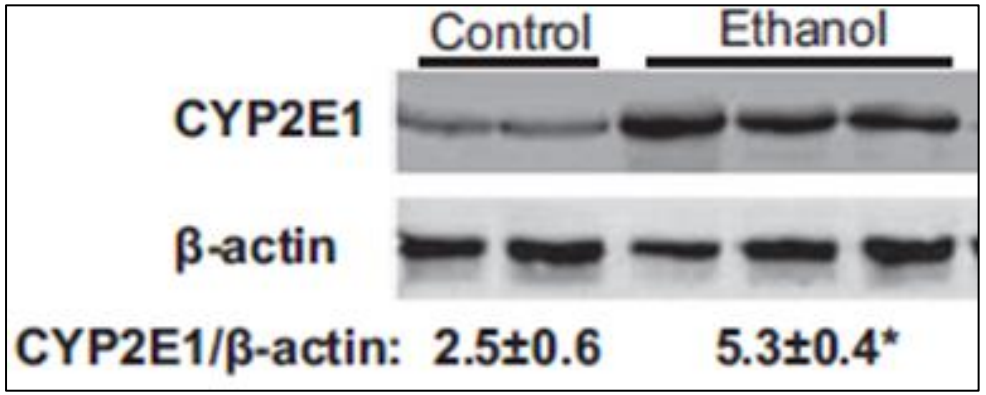
Enzymatic induction

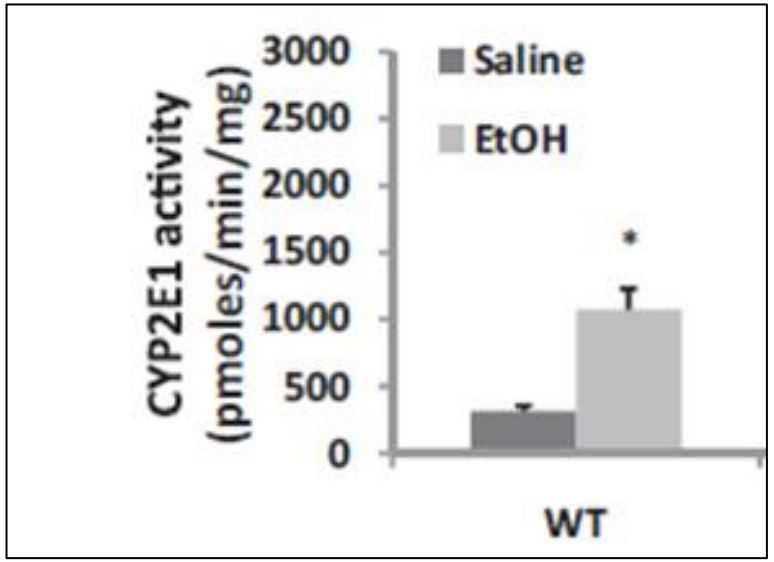


Quiz

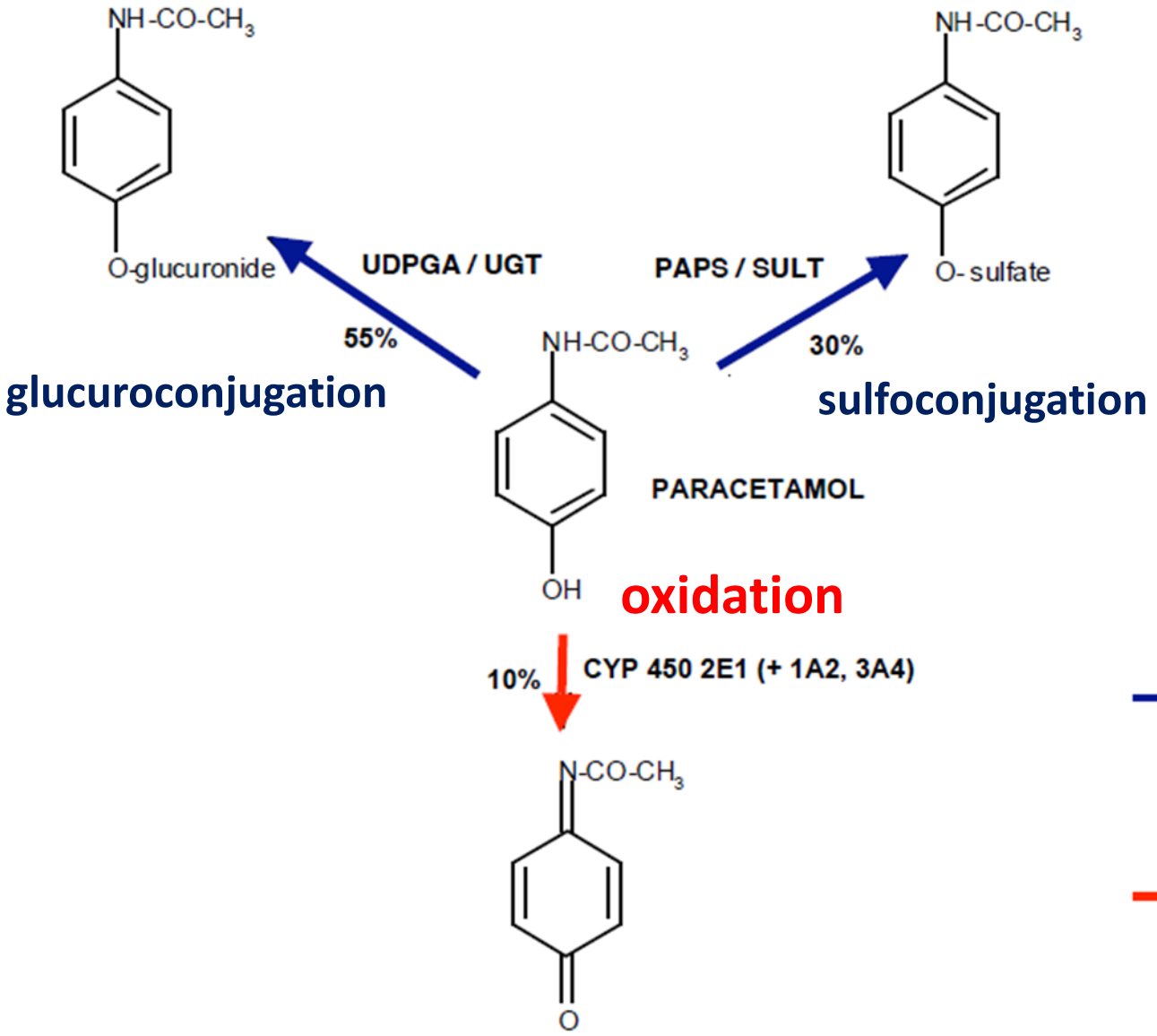


Paracetamol is metabolized by CYP2E1 into a toxic metabolite, NAPQI. The effects of ethanol on CYP2E1 protein expression (measured by western blot) in rats are shown in the following figure.





What can you conclude about the consequences of the concomitant consumption of paracetamol and ethanol on the toxicity of paracetamol?

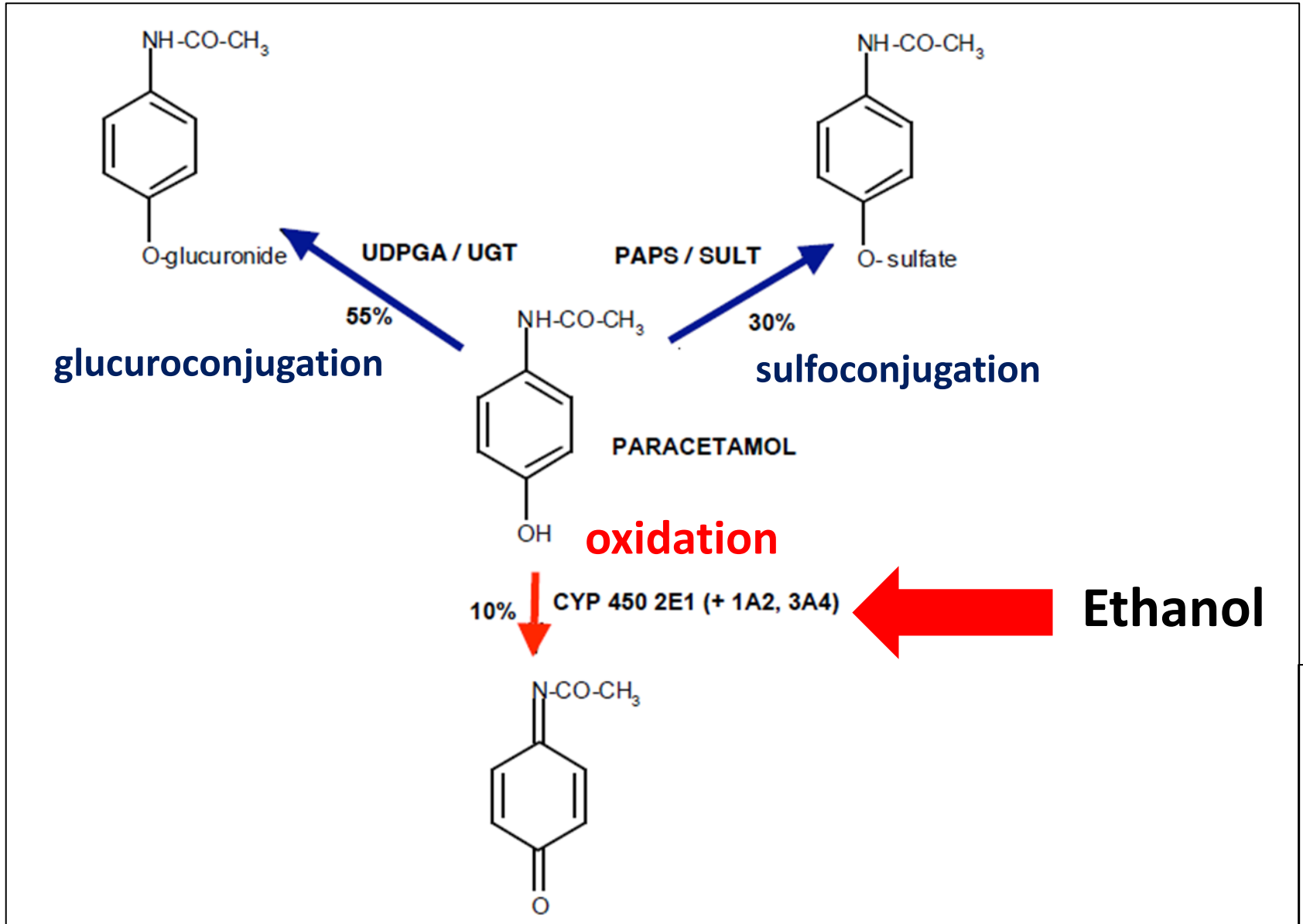


Formation of toxic metabolite

Target organ: liver

- 2 pathways of detoxification: conjugation ⇨ elimination
- 1 pathway of toxification: CYP450 2E1 (+1A2, 3A4)

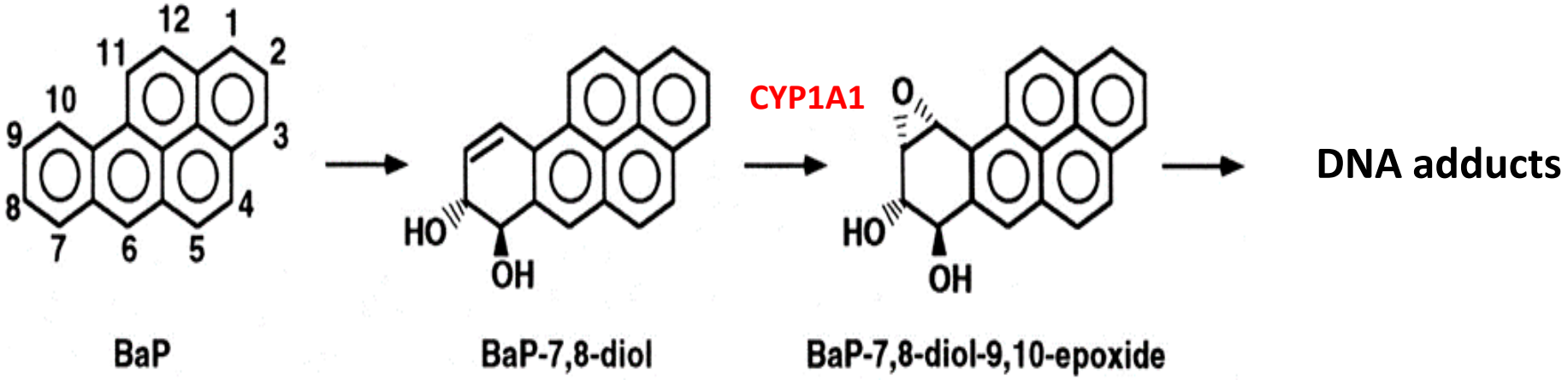
N-acetyl-p-benzoquinone imine (NAPQI)



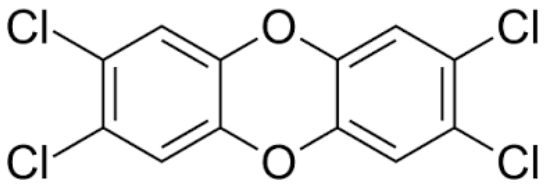
Increased formation of NAPQI

Increased hepatotoxicity

Benzo[a]pyrene metabolism



Examples of CYP450 inducers



Drugs

- Carbamazepine (1A2, 3A, 2C9, 2D6, 3A4)
- Omeprazole (1A2)
- Phenobarbital (2B, 2C9, 3A)
- Rifampicin (2C8, 2C19, 3A)
- Glucocorticoids (3A)
- Isoniazid (2E1)

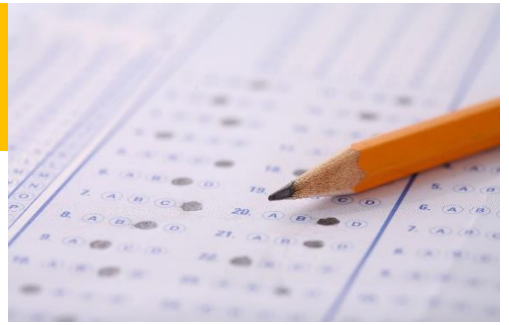
Food & additives

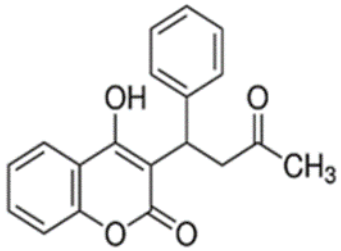
- Ethanol (2E1)
- Flavonoids, barbecued meats (polyaromatic hydrocarbons, 1A2)

Pollutants

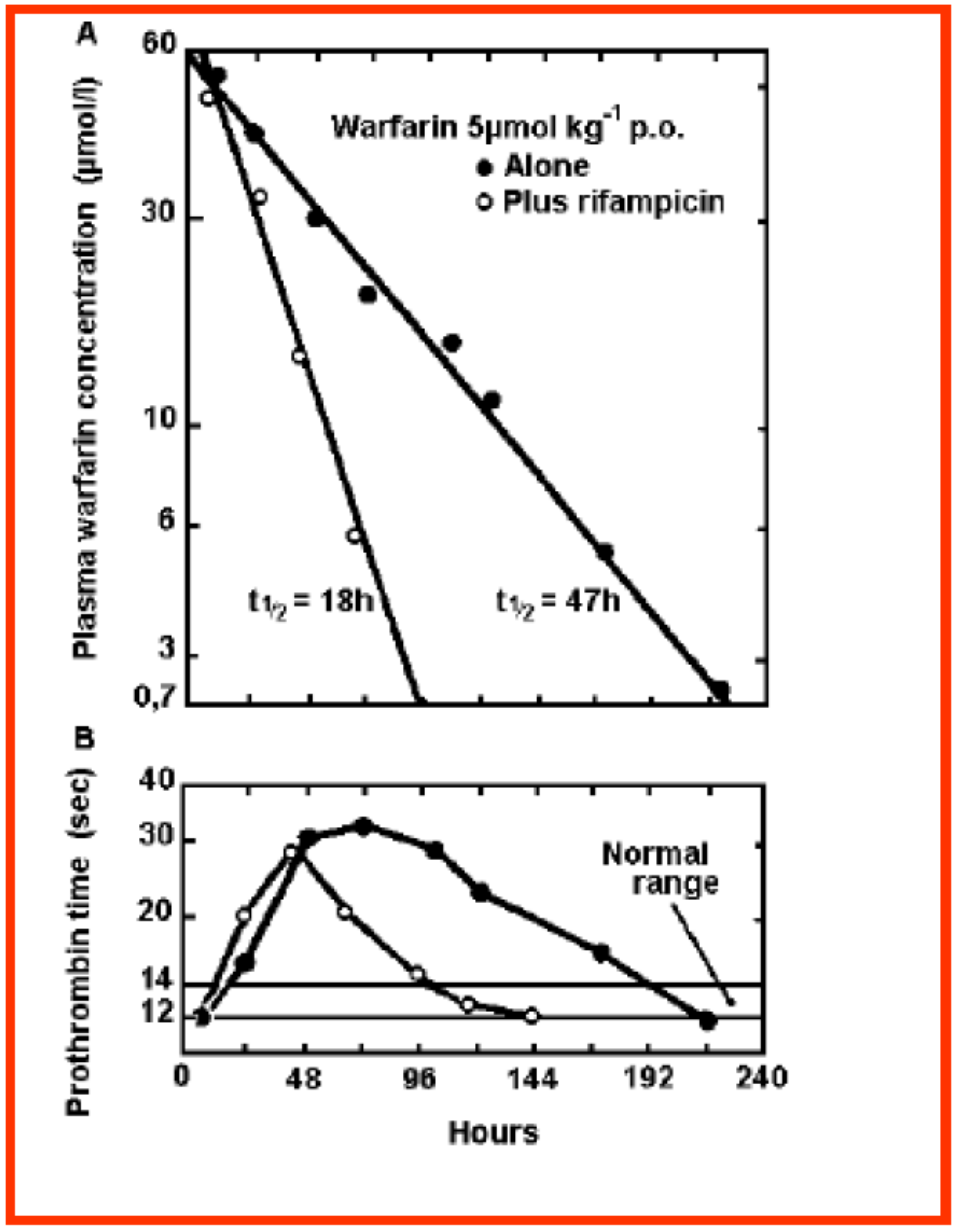
- Polyhalogenated hydrocarbons (dioxin, 1A)
- Polyaromatic hydrocarbons (B[a]P, 1A)

Quiz



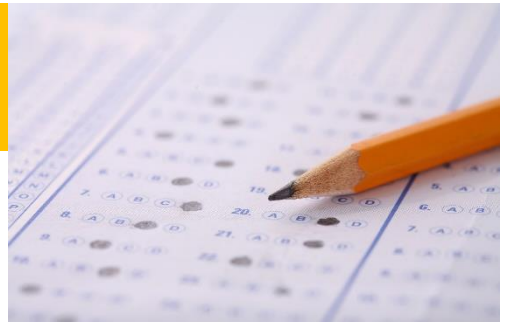


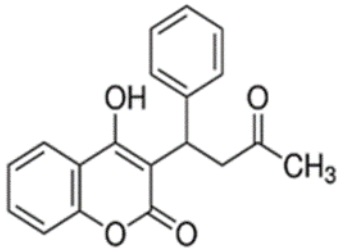
Warfarine



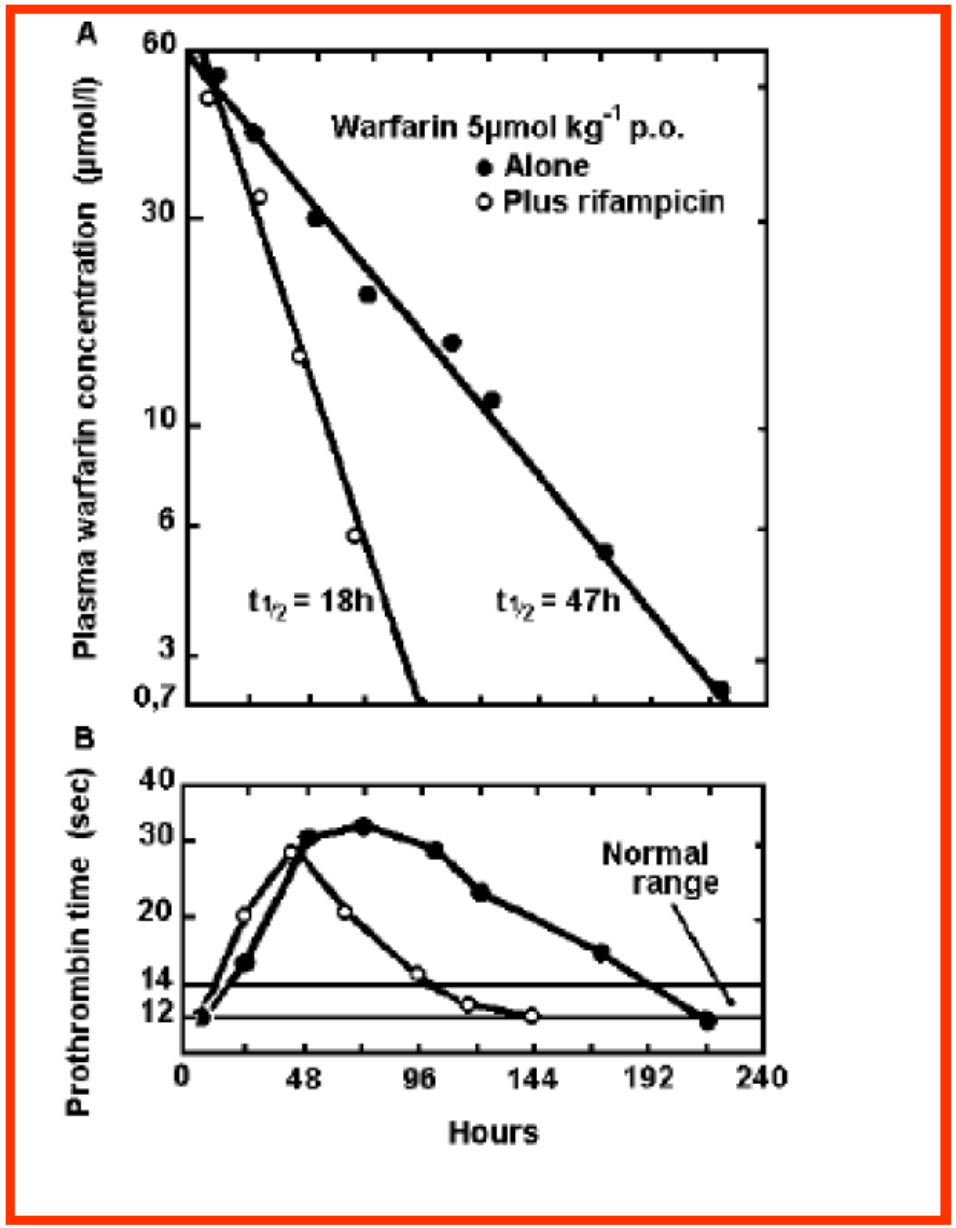
What type of interaction is detected?

Quiz





Warfarine



What type of interaction is detected?

- Drug interaction by enzymatic induction

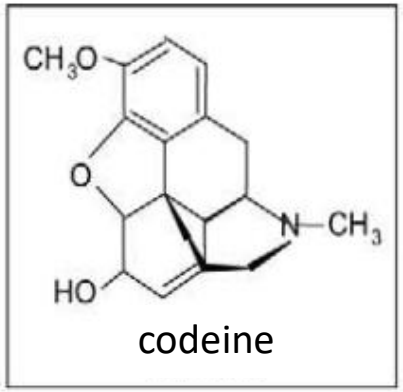
Exercise n°2

Exercise 2

M. X is a 75-years old patient presenting with a fracture and vertebral compression due to osteoporosis for which an analgesic treatment of codeine in combination with paracetamol has been started. Due to the ineffectiveness of the treatment, the doses were gradually increased to a maximum codeine dose of 180 mg/day with no benefit. The change to morphine therapy is necessary and is effective.

1. What are the possible hypotheses of the absence of effect of the codeine/paracetamol combination and the efficacy of morphine?

Codeine metabolism



codeine

CODEINE

GLUCURONIDATION
principal route (10–20%)

O-DEMETHYLATION
(5–15%)
CYP 2D6

N-DEMETHYLATION
(10–20%)
CYP 3A4

CODEINE-6-GLUCURONIDE

MORPHINE

NORCODEINE

(5–15%)

GLUCURONIDATION
+
N-DEMETHYLATION

UNCHANGED IN URINE

GLUCURONIDATION
+
O-DEMETHYLATION

morphine

MORPHINE 6 GLUCURONIDE
MORPHINE 3 GLUCURONIDE
NORMORPHINE

NORMORPHINE
NORCODEINE 6 GLUCURONIDE

Williams, Hatch and Howard, 2001

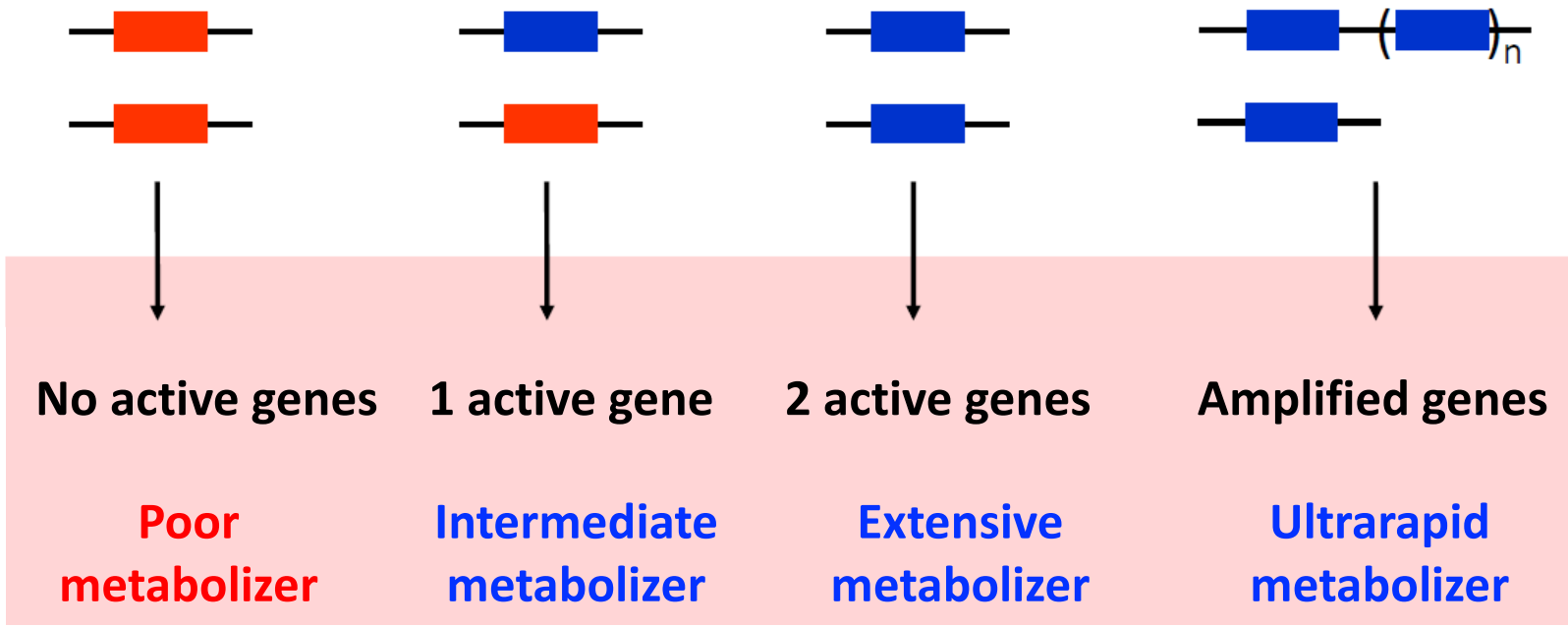
CYP 2D6 polymorphism

Table 1. Human polymorphic cytochrome P450 enzymes and the global distribution of their major variant alleles

Enzyme	Major variant alleles	Mutation	Consequences for enzyme function	Allele frequencies (%)			
				Caucasians	Asians	Black Africans	Ethiopians and Saudi Arabians
CYP2D6	*CYP2D6*2xN*	Gene duplication or multiduplication	Increased enzyme activity	1–5	0–2	2	10–16
	*CYP2D6*4*	Defective splicing	Inactive enzyme	12–21	1	2	1–4
	*CYP2D6*5*	Gene deletion	No enzyme	2–7	6	4	1–3
	*CYP2D6*10*	Pro34Ser, Ser486Thr	Unstable enzyme	1–2	51	6	3–9
	*CYP2D6*17*	Thr107Ile, Arg296Cys, Ser486Thr	Reduced affinity for substrates	0	ND	34	3–9

Ingelman-Sundberg, Oscarson and McLellan, 1999

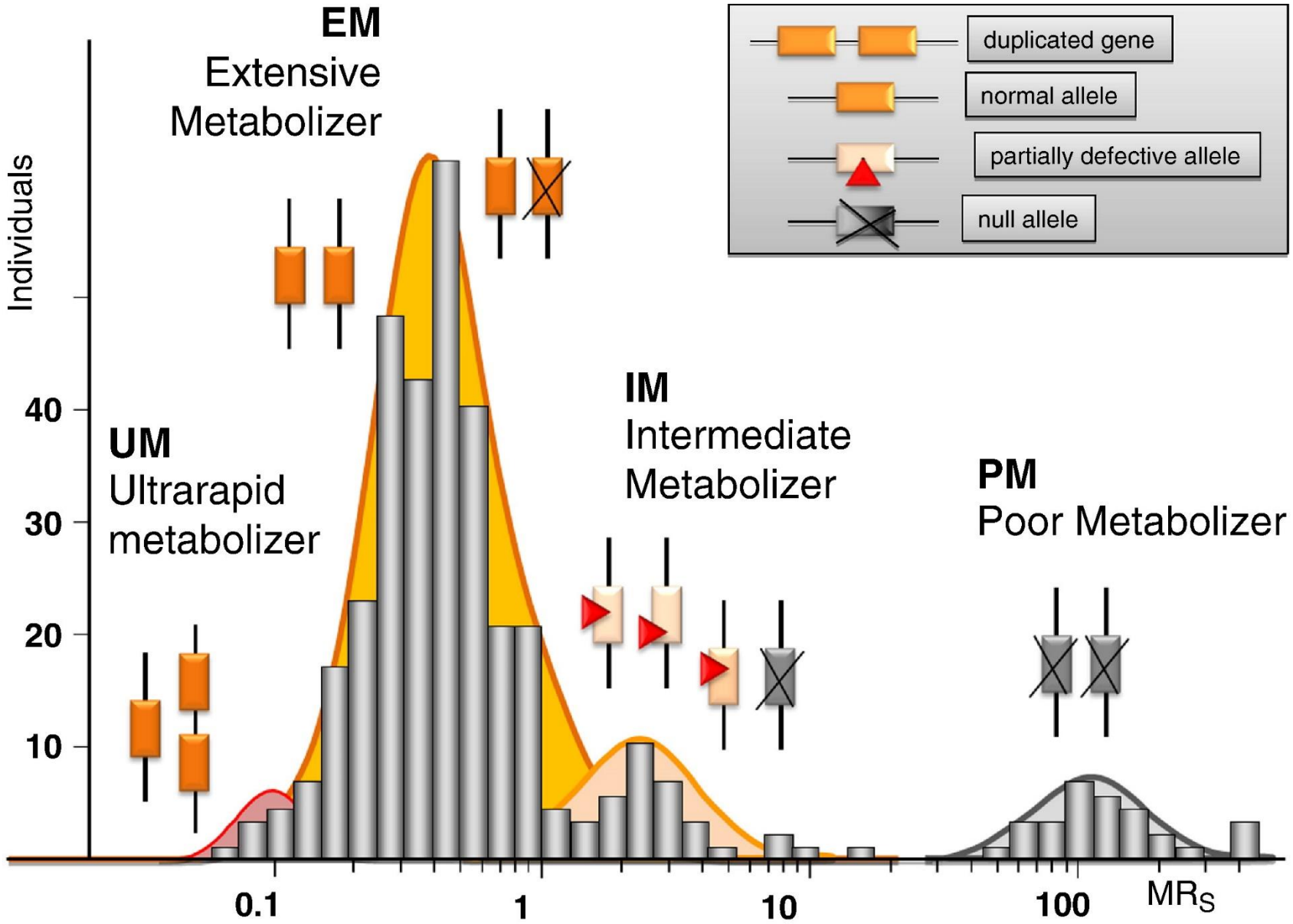
Genetic polymorphisms: molecular basis



Defective gene (deletion, inactivating mutation)

Active/normal gene

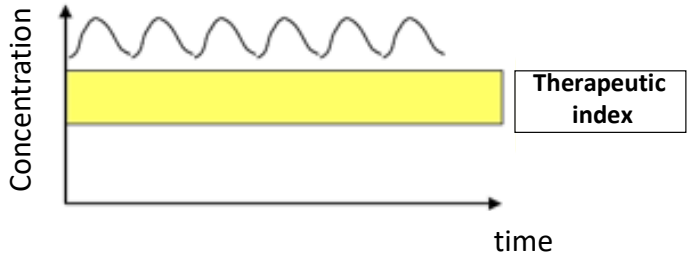
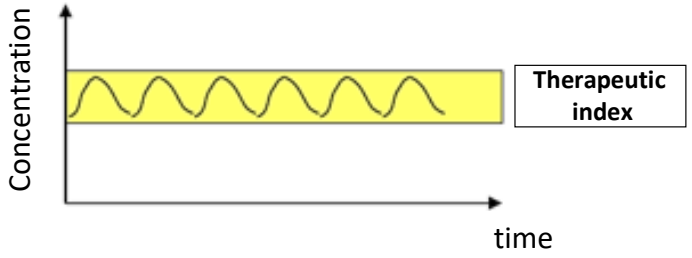
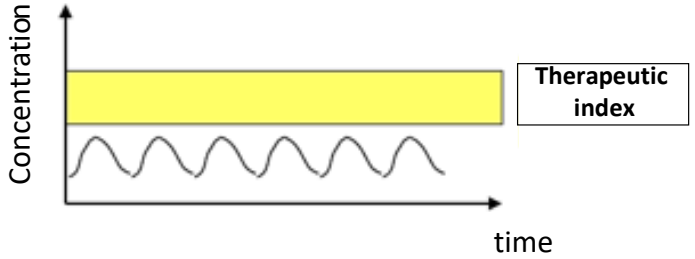
$(\quad)_n$ Amplification of active/normal gene

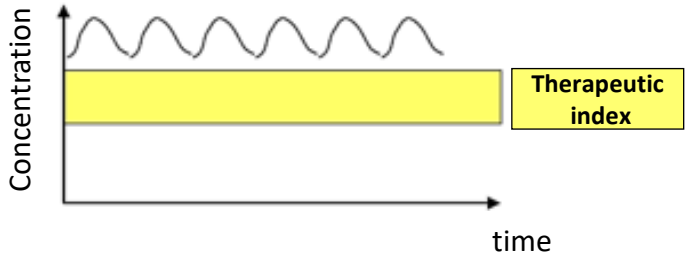
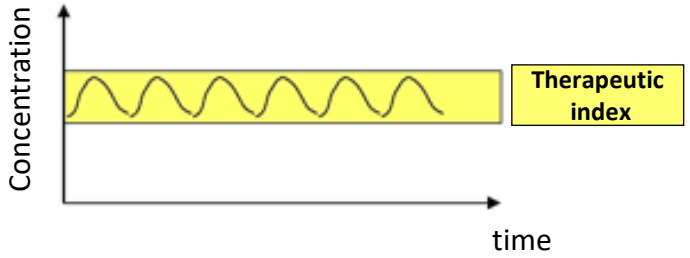
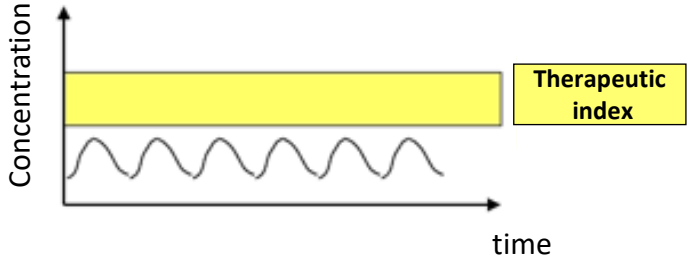


Zanger and Schwab, 2013

Quiz

Fill in the blanks with the therapeutic consequences:

Phenotype	Effects on [drugs]	Clinical consequences: Active drugs	Clinical consequences: Prodrugs
Poor metabolizers		1	2
Extensive metabolizers		3	
Ultrarapid metabolizers		4	5

Phenotype	Effects on [drugs]	Clinical consequences: Active drugs	Clinical consequences: Prodrugs
Poor metabolizers		Toxicity	No therapeutic effects
Extensive metabolizers		Therapeutic efficacy	
Ultrarapid metabolizers		No therapeutic effects	Toxicity

Exercise 2

M. X is a 75-year old patient presenting with a fracture and vertebral compression due to osteoporosis for which an analgesic treatment of codeine in combination with paracetamol has been started. Due to the ineffectiveness of the treatment, the doses were gradually increased to a maximum codeine dose of 180 mg/day with no benefit. The change to morphine therapy is necessary and is effective.

1. What are the possible hypotheses of the absence of effect of the codeine/paracetamol combination and the efficacy of morphine?

2. Several months later, M. X is treated with propafenone for ventricular rhythm disorders. M. X quickly complains of side effects such as blurred vision, dizziness and paraesthesia. Do you think the lack of effect of codeine may be related to these observations? If so, why?

CYP 2D6 Polymorphism

Drug	Metabolite	Active metabolite ?	Clinical consequences in poor metabolizers
Debrisoquine Antiarrythmic	OH benzylic	No	Hypotension
Perhexiline Calcium antagonist	OH aliphatic	No	Neurotoxicity Hepatotoxicity
Propafenone Antiarrythmic	OH aromatic	No	Neurotoxicity
Dextromethorphan Anticough	O-demethylation	No	Neurotoxicity
Encainide Na+ channels antagonist	O-demethylation	Yes	Decrease of pharmacological effect
Codeine Anticough, analgesic	O-demethylation	Yes (morphine)	Decrease of analgesic effect Respiratory depression

Exercise 2

M. X is a 75-year old patient presenting with a fracture and vertebral compression due to osteoporosis for which an analgesic treatment of codeine in combination with paracetamol has been started. Due to the ineffectiveness of the treatment, the doses were gradually increased to a maximum codeine dose of 180 mg/day with no benefit. The change to morphine therapy is necessary and is effective.

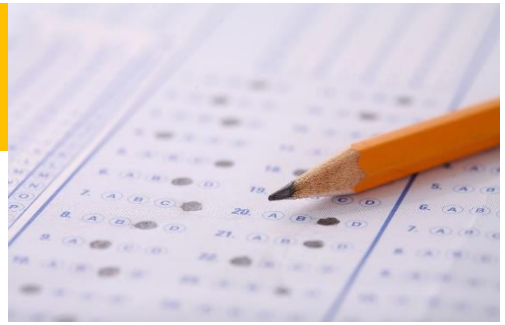
1. What are the possible hypotheses of the absence of effect of the codeine/paracetamol combination and the efficacy of morphine?

2. Several months later, M. X is treated with propafenone for ventricular rhythm disorders. M. X quickly complains of side effects such as blurred vision, dizziness and paraesthesia. Do you think the lack of effect of codeine may be related to these observations? If so, why?

3. What tests should be performed on this patient to explain this phenomenon?

- ✓ Phenotyping is a measure of the specific enzymatic activity following the ingestion of a test substrate (probe drug).
- ✓ Example of CYP2D6 such as dextromethorphan: you proceed by measuring its metabolic ratio (MR) in the urine.
- ✓ CYP2D6 substrates and inhibitors (quinidine, flecainide, paroxetine, moclobemide, fluoxetine, fluvoxamine ...) which have a high affinity for the enzyme, can modify the MR.
- ✓ Thus, a high-speed metabolizer may become sluggish under the action of a metabolic inhibitor (phenocopying).
- ✓ Phenotyping can therefore make it possible to detect drug interactions in patients whose phenotype is known beforehand, or by performing genotyping in parallel.

Quiz



Choose the correct answer(s):

A. Metabolites are always less active than the parent molecule.

B. Cytochromes, that are metabolism phase I enzymes, are hemoproteins.

C. Paracetamol can give a toxic metabolite.

D. The cytochrome P450 superfamily includes numerous isoenzymes, encoded by 481 genes.

E. Three cytochrome P450 isoenzymes metabolize 70% of drugs: CYP 3A4/5, CYP 2D6 and CYP 2C8/9.

F. Conjugation decreases the water solubility of the compound.

G. Alcohol is an enzyme inducer. It increases the activity of the enzymes of the metabolism.

Exercise n°3

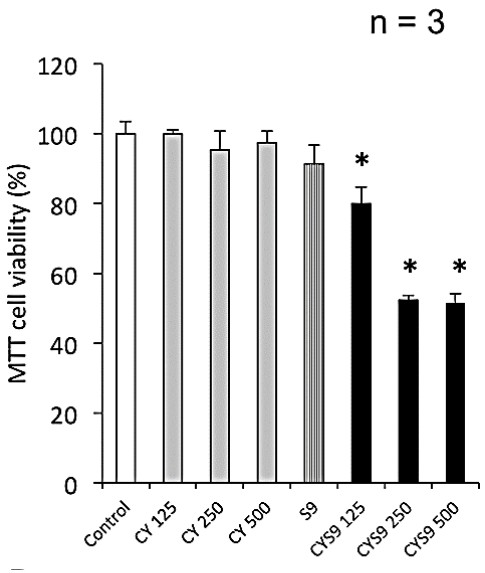
Context of the study

Cyclophosphamide (CY) is a medication used as chemotherapy but also as immunosuppressant. As chemotherapy it is used to treat lymphoma, multiple myeloma, leukemia, ovarian cancer, breast cancer, small cell lung cancer, neuroblastoma, and sarcoma. As an immune suppressor it is used in nephrotic syndrome, granulomatosis with polyangiitis, and following organ transplant, among other conditions.

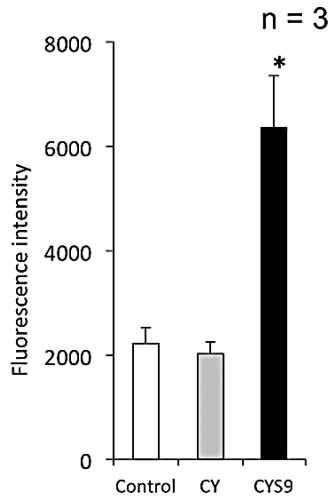
Observed only after administration of high doses, cardiotoxicity is the dose-limiting effect of cyclophosphamide (CY). We investigated the poorly understood cardiotoxic mechanisms of high-dose CY. A rat cardiac myocardial cell line, H9c2, was exposed to CY metabolized by S9 fraction of rat liver homogenate mixed with co-factors (CYS9).

Figure analysis

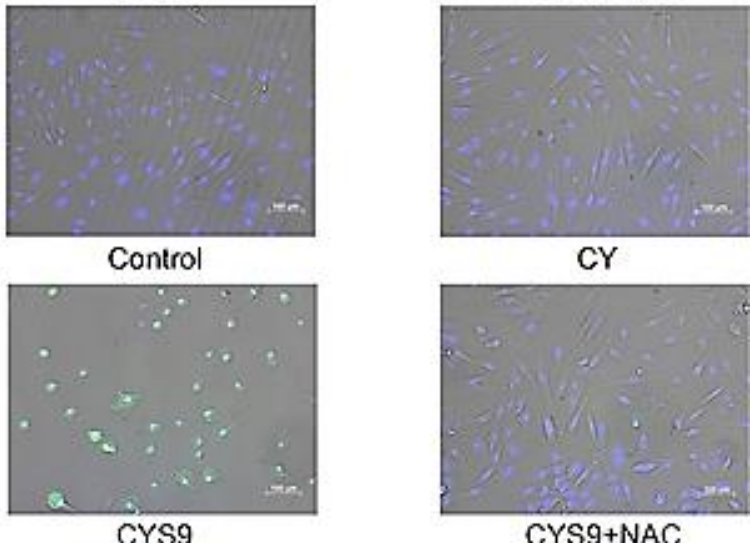
A



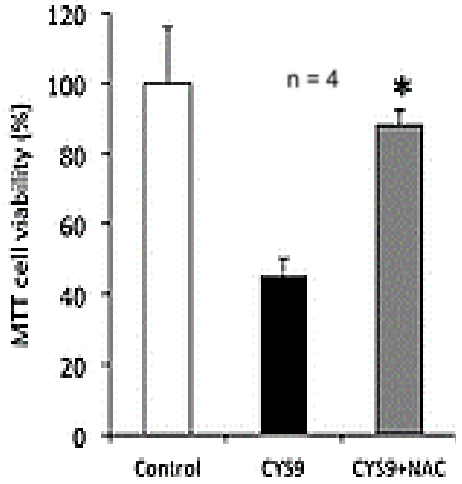
B



C



D



(A) H9c2 cell viability after 48-hour exposure to CY alone and CY metabolized by S9 fraction of rat liver homogenate mixed with co-factors (CYS9) was assessed by MTT assay (mean + SD from 3 independent experiments).

(B) Fluorescence intensities, corresponding to levels of H_2O_2, in control samples or cells exposed to 250 µM CY, CYS9 for 1 hour (mean + SD from 3 independent experiments). Fluorescence intensity is shown in arbitrary units.

(C) Apoptotic cells stained by FITC-conjugated probes are green. Control (unexposed H9c2 cells), H9c2 cells were exposed for 2 hours to 250 µM CY, CYS9 or CYS9 + 1 mM of NAC. Green dots indicate apoptotic cells. Magnification, 100×.

(D) The effect of N-acetylcysteine (NAC) on cytotoxicity of CYS9 in H9c2 cells was monitored after 24-hour exposure by MTT assy (mean + SD from 4 independent experiments).

Scheme

After analysing the figures of the previous slide,

suggest a diagram summarizing the results.