Metabolism of xenobiotics

FM 3.5-10
CHE 5-6
Metabolism of xenobiotics

Also called: Biotransformation, or detoxification

Primary metabolism – secondary metabolism

Goal: To make use of a compound or to facilitate its excretion

Increases the water solubility of a compound

Divided in two phases, phase I and phase II
Phase I and phase II, overview

Lipophilic → Phase I → Lipophilic with hydrophilic handle → Phase II → Hydrophilic conjugate

Water solubility increases
Phase I and phase II, overview

Toluen $\xrightarrow{\text{Ox.}}$ Bensylalkohol $\xrightarrow{\text{Ox.}}$ Bensaldehyd $\xrightarrow{\text{Ox.}}$ Bensoesyrat

Fas I $\xrightarrow{\text{}}$ + glycin $\xrightarrow{\text{}}$ Hippursyrat
Phase I and phase II, overview

Benzene $\rightarrow$ Phenol (major phase I metabolite) $\rightarrow$ Phenol-O-glucuronide (major phase II metabolite)

Many minor metabolites
Enzymes involved

Many different, in all parts of the body

Most important is cytochrome P-450
  - present as a number of isoenzymes
  - not unique to humans
  - highest levels in liver cells
  - can be induced
  - can catalyse oxidations as well as reductions
Cytochrome P-450

\[
\text{NADPH} + \text{H}^+ \quad \text{NADP}^+ \\
\text{R-H} + \text{O}_2 \quad \rightarrow \quad \text{R-OH} + \text{H}_2\text{O}
\]

A general oxidation performed by cytochrome P-450

The substrate R-H (as well as the cofactor NADPH) is oxidised, while oxygen is reduced to water.
Cytochrome P-450

An example of the natural function of cytochrome P-450

Dehydrocholesterol $\xrightarrow{\text{Sunlight}}$ Cholesterol $\xrightarrow{\text{H}}$ 7-Dihydrocholesterol $\xrightarrow{\text{P-450 (liver)}}$ Vitamin D$_3$ $\xrightarrow{\text{P-450 (kidney)}}$ 25-Hydroxyvitamin D$_3$ $\xrightarrow{\text{P-450}}$ 1,25-Dihydroxyvitamin D$_3$
Cytochrome P-450

1. The substrate comes in

[Chemical reaction diagram showing the process of substrate metabolism by cytochrome P-450.]
Cytochrome P-450

1. The substrate comes in

2. An electron is added
Cytochrome P-450

1. The substrate comes in

2. An electron is added

3. Fe(III) is reduced to Fe(II)

NADPH - Cytokrom P-450 reduktas
Cytochrome P-450

1. The substrate comes in
2. An electron is added
3. Fe(III) is reduced to Fe(II)
4. $O_2$ binds to the Fe(II)
1. The substrate comes in
2. An electron is added
3. Fe(III) is reduced to Fe(II)
4. $O_2$ binds to the Fe(II)
5. Fe(II) is oxidised to Fe(V)
Cytochrome P-450

1. The substrate comes in

2. An electron is added

3. Fe(III) is reduced to Fe(II)

4. O₂ binds to the Fe(II)

5. Fe(II) is oxidised to Fe(V)

6. The second O is reduced
Cytochrome P-450

1. The substrate comes in
2. An electron is added
3. Fe(III) is reduced to Fe(II)
4. O₂ binds to the Fe(II)
5. Fe(II) is oxidised to Fe(V)
6. The second O is reduced
7. Fe(V)=O oxidises R-H

NADPH - Cytokrom P-450 reduktas
Cytochrome P-450

1. The substrate comes in
2. An electron is added
3. Fe(III) is reduced to Fe(II)
4. \( \text{O}_2 \) binds to the Fe(II)
5. Fe(II) is oxidised to Fe(V)
6. The second \( \text{O} \) is reduced
7. Fe(V)=O oxideses R-H
8. The cycle is completed
Generation of ROS

ROS = Reactive Oxygen Species

At the bottom of the P-450 oxidation cycle…
Generation of ROS

- \( \cdot \bar{O} - \bar{O} \cdot \) Disyre (en diradikal)
- \( \cdot \bar{O} - \bar{O}^- \) Superoxidanjon (en anjonradikal)

\[ H - \bar{O} - \bar{O} - H \] Väteperoxid

För varje steg nedåt adderas en elektron

H \( - \bar{O} - H \) + \( \cdot \bar{O} - H \) Vatten samt hydroxylradikal

H \( - \bar{O} - H \) + H \( - \bar{O} - H \) Vatten
Generation of ROS

Superoxide dismutase (SOD): \[ 2 \cdot O_2^- + 2 \cdot H^+ \rightarrow H_2O_2 + O_2 \]

Catalase: \[ 2 \cdot H_2O_2 \rightarrow 2 \cdot H_2O + O_2 \]

Glutathione peroxidase: \[ H_2O_2 + G-SH \rightarrow 2 \cdot H_2O + G-S-S-G \]

Glutathione disulphide reductase

Spontaneous and enzyme-catalysed reactions
(Vitamins (C and E) also protect against radicals)
Generation of ROS

\[
\begin{align*}
\dot{\text{O}}_2^- + \text{H}_2\text{O}_2 + \text{H}^+ & \rightarrow \dot{\text{OH}} + \text{H}_2\text{O} + \text{O}_2 \\
\text{The Haber Weiss reaction} \\
\text{H}_2\text{O}_2 + \text{Fe}^{2+} + \text{H}^+ & \rightarrow \dot{\text{OH}} + \text{H}_2\text{O} + \text{Fe}^{3+} \\
\text{The Fenton reaction}
\end{align*}
\]

But superoxide and hydrogen peroxide may generate hydroxyl radicals via spontaneous reactions.....
Generation of ROS and radicals

1. Oxidative metabolism
2. Biotransformation of xenobiotics
3. Ionising radiation

CCl₄ → ·CCl₃ + Cl⁻
Carbon tetrachloride
Trichloromethyl radical

Benzene, phenol

OH
OH
Hydroquinone
Semiquinone

O₂
Ionizing radiation

H₂O → ·OH + H₃O⁺
Protection by vitamins

Semidehydro-ascorbic acid
Ascorbic acid

\[ R^* \]

\[ + \text{RH} \]

Ascorbic acid (vitamin C)
Semidehydroascorbic acid (a radical)
Dehydroascorbic acid

GSSG GSH
Ascorbic acid + Dehydroascorbic acid

Dismutation
Natural antioxidants

Astaxanthin
(Green algae, yeast, salmon, etc.)

Resveratrol
(Red grapes)

Quercetin
(Onions, black tea, etc.)

Ferulic acid
(Corn, tomatoes, etc.)
Effect of ROS: Lipid peroxidation

Takes place with unsaturated fatty acids
Autoxidations may be violent and cause fires
The same process makes food go rancid
Lipid peroxidation

Radikal

CAS
Centre for Analysis and Synthesis
Lipid peroxidation

- Membranes are frequently the target for ROS and other radicals
- The unsaturated fatty acids are oxidised by radical processes
- Membranes and membrane components are damaged
- Enzymes associated with the membrane are damaged
- Electrophilic products of the lipid peroxidation are formed
Lipid peroxidation

\[ \text{Oxidativ klyvning av alkoxy-radikalen, på andra sidan} \]

\[ \text{Resultatet blir goda Michael-acceptorer, omättade aldehyder} \]

\[ \text{Oxidation} \]

\[ \text{Illaluktande karboxylsyror} \]

Glycidaldehyd

Malonaldehyd
Phase I and phase II, details

Phase I:
- Oxidations
- Reductions
- Hydrolyses

Phase II: Conjugations
Phase I oxidations

Only 4 types to remember!!!

1. Epoxidation of alkenes and arynes
2. Carbon hydroxylation
3. Heteroatom oxidation
4. Oxidation of alcohols and aldehydes
A similar mechanism can be suggested for the case with two-electron transfer.

Epoxides are electrophilic, this is a metabolic activation!
Epoxidations of arynes

Bensenoxid → 2,4-Cyklohexadienon → Fenol
Carbon hydroxylation

Next to unsaturations
Carbon hydroxylations

X = heteroatom som N, O, S, Cl, Br

Väte på kol α
till heteroatom

Resonansstabiliserad radikal

α-hydroxylerad

Next to heteroatoms, α-hydroxylations
Carbon hydroxylations

The products of $\alpha$-hydroxylation may be unstable.
Carbon hydroxylations

An example from the primary metabolism:

\[
\begin{align*}
\text{Fettsyra} & \quad \overset{\text{ox.}}{\longrightarrow} & \quad \text{Enzymatisk nedbrytning av fettsyror} \\
\text{Ättiksyra} & \quad + & \quad \text{Kortare fettsyra} \\
\end{align*}
\]
Heteroatom oxidations

Amin → Hydroxylamin → Nitroso → Nitro

Oxidations of N and S most important
Alcohol and aldehyde oxidations

Primär alkohol

Sekundär alkohol

Aceton

Pyrodruvsysra
Phase I reductions

1. Reduction of ketones and aldehydes
2. Reduction of oxidised heteroatoms
Reduction of oxidised heteroatoms

Smörgult

Anilin
Phase I hydrolyses

- Hydrolysis of epoxides
- Hydrolysis of esters
- Hydrolysis of amides
Hydrolysis of epoxides

An important example of a metabolic detoxification
Hydrolysis of esters

Etylacetat $\xrightarrow{\text{Esteras}}$ Ättiksyra + Etanol

The products are much more soluble in water
Hydrolysis of amides

Dimethylacetamid $\xrightarrow{\text{Amidas}}$ $\text{Ättiksyra} + \text{Dimetylamin}$
Phase II reactions

Only 4 types to remember!!!

- Conjugation with glutathione
- Conjugation with sulphate
- Conjugation with glucuronic acid
- Conjugation with amino acids
Conjugation with glutathione

Glutation

= G-SH
Conjugation with glutathione

G\(-\text{SH}\) + H\(_3\text{C}-\text{Br}\) → G\(-\text{SCH}_3\) + H\text{Br}

G\(-\text{SH}\) + \(\text{CH}_2=\text{CH}-\text{CH}=\text{O}\) → G\(-\text{SCH}_2\text{CH}=\text{CH}-\text{CH}=\text{O}\)

G\(-\text{SH}\) + \(\text{O}\) \(\text{C}\) \(\text{C}\) \(\text{C}\) \(\text{C}\) \(\text{O}\) \(\text{O}\) \(\text{O}\) \(\text{O}\) \(\text{O}\) \(\text{O}\) \(\text{O}\) \(\text{O}\) \(\text{O}\) → G\(-\text{SCH}_2\text{CH}=\text{CH}-\text{CH}=\text{O}\)
Conjugation with glutathione

Merkaptursyraderivat

Acetylering

Tioeter
Conjugation with sulphate

Fenol → PAPS → Jonisk vid pH 7,4

Safrol → P-450 → OH → PAPS → Svagt reaktiv och cancerframkallande
Conjugation with glucuronic acid

Glukuronsyra

Glykosid mellan glukuronsyra och fenol, en glukuronid

Energirik bindning

UDP-GA
Conjugation with glucuronic acid

\[ \text{Conjugation with glucuronic acid} \]

\[ \text{Omsättning i tarmen} \]

\[ \text{Återupptag i tarmen} \]

\[ \text{Kemisk hydrolys i sur miljö} \]

\[ \text{Aktivering av hydroxylaminen} \]

\[ \text{Den verkliga carcinogenen} \]
Conjugation with amino acids

Bensoesyra

Glycin

Hippursyra
Phase I and II reactions

Only 4 types of phase I oxidations
Only 2 types of phase I reductions
Only 3 types of phase I hydrolyses
Only 4 types of phase II conjugations