Liver Cirrhosis and Drug metabolism

Reem H. Elbekai
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relbekai@pharmacy.ualberta.ca

Introduction: Cirrhosis

- End stage liver disease
- Top ten causes of death in N. America
- Commonly associated with alcohol abuse, chronic viral hepatitis, metabolic diseases, diseases of the bile duct
- Reduction of cell mass, collagen deposition

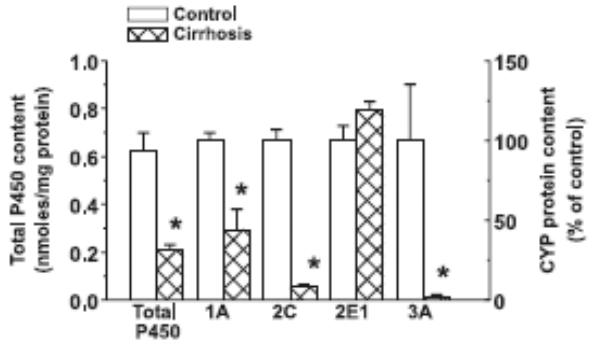
Introduction: Cirrhosis

- Child-Pugh classification
  - Child class A-C
- Inactive cirrhosis: no inflammation
- Severe disease: significant inflammation

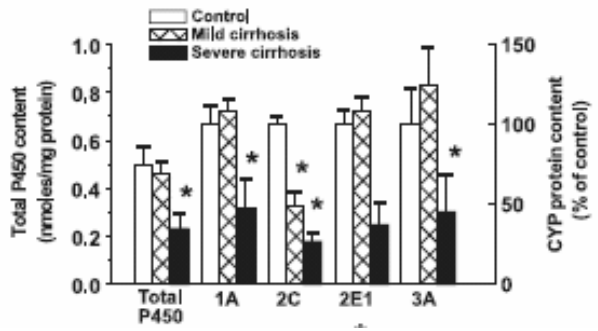
Cirrhosis: altered drug metabolism

- Multiple mechanisms:
  1. Reduction in absolute cell mass
  2. Impaired extraction of drug
  3. Change in enzyme expression
  4. Alteration in enzyme activity

Phase I: CYP450



Phase I: CYP450



Phase I: CYP450

1. CYP1A
   - Decrease in mRNA, protein and activity (in vivo/in vitro)
   - Similar results in animal models
2. CYP2A6
   - In vivo: reduced activity in patients
3. CYP2B
   - Not much information
   - Likely a decrease in activity with increasing severity

Phase I: CYP450

4. CYP2C
   - CYP2C19 only affected isoform
5. CYP2E
   - Etiology of cirrhosis determines the level of expression
   - Alcoholic liver disease: EtOH inducer of CYP2E1 thus levels may be increased or unaltered
   - Non-alcoholic liver disease: decrease in 2E1
6. CYP3A
   - Decrease in protein and activity

Phase I CYP450: mechanisms

1. Protein synthesis

- Poor nutritional status and ?defects in protein synthesis?
- Decreased synthesis of liver visceral proteins?

a. Total microsomal protein synthesis:
   cirrhotic=control livers

b. Total hepatic microsomal protein
   cirrhotic=control livers

c. Increase in total CYP450 by inducers
   cirrhotic=control livers

d. Individual CYP450 isozymes
   not affected to same degree in disease

Phase I CYP450: mechanisms

1. Protein synthesis?

Conclusions:

a. no abnormalities in protein synthesis

b. process of enzyme induction is intact

c. only basal levels of CYP450 are altered

d. CYP450 isoenzymes likely altered by various mechanisms

Phase I CYP450: mechanisms

2. mRNA turnover

- Decrease in transcription and/or decrease in mRNA transcript stability
- CYP1A2, 2C9, 3A4, 2E1 mRNA reduced in cirrhotic livers: correlated with protein and activity levels

3. Heme Oxygenase (HO)

- Rate limiting enzyme in metabolism of heme (protoporphyrin IX degredation)
- HO-1 isoform expression increased in animal models of cirrhosis

Phase I CYP450: mechanisms

5. Free Radicals

- Hydroxyl free radicals/lipid peroxidation markers complex with CYP450 proteins
- Epitopes formed lead to production of IgG antibodies

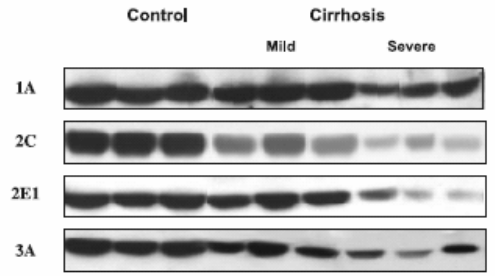
6. Accumulation of endogenous/exogenous agents

- Endogenous substrates of CYP450 may accumulate and modulate DMEs
- Estrogen accumulates and reduces CYP2C11 in rats

Phase I CYP450: mechanisms

7. **Inflammatory mediators**
   - Involved in modulation at transcriptional and post-transcriptional levels
   - IL-6 inhibits CYP3A4 transcription by inducing a repressor
   - Interferon decreases transcription of CYP1A1 and reduces mRNA stability
   - Inflammation may alter CYP2E1 mRNA stability
   - NO elevated in cirrhosis inhibits CYP450 by binding and ligating heme; peroxynitrite oxidizes proteins

Phase I and disease severity



Phase I: Other DMEs

Alcohol and aldehyde dehydrogenase (ADH/ALDH)

EtOH ⟶ acetaldehyde ⟶ acetate

ADH, catalase, CYP2E1 *ALDH*

- ↓ ADH alcoholic cirrhosis; ↓/↔ non-alcoholic cirrhosis
- ↓ ADH alcoholic cirrhosis vs. non-alcoholic cirrhosis
- ↓ ALDH in alcoholic and non-alcoholic cirrhosis, activity proportional to disease severity; systemic factors do not play a role in ALDH
- Alcoholic and non-alcoholic cirrhotics at increased risk of acetaldehyde toxicity

Phase II: Glucuronidation

Early studies:

- Mainly preserved in mild-moderate disease:
  - UGT mRNA, protein and activity unaltered
- Why?
  - Induction of UGT in remaining viable cells
  - Induction of extrahepatic UGT
    - Increase in extrahepatic morphine metabolism in cirrhosis
    - Induction of renal glucuronidation in cirrhosis

Phase II: Glucuronidation

Newer evidence:

Many factors determine impairment of glucuronidation :

- Disease severity
- Impairment of ester, not ether glucuronidation
  - Oxazepam, lorazepam (ether): preserved
  - Zidovudine (ester): significant decrease
- Differential effects on the various UGT isoforms
  - UGT isoforms differentially regulated

Phase II: Sulphation

- ↓ SULT activity in cirrhosis
  - ↓ acetaminophen sulphation
- Why?
  1. Reduced plasma sulphate level
     - ↓ cysteine dioxygenase activity (cysteine→sulphate)
  2. Impaired SULT activity
     - ↓ SULT protein
  3. Competition from endogenous susbtrates

Phase II: glutathione S-transferase

- GST activity decreased in liver
  - Consequence of ↓ GSH levels
  - N-acetylcysteine ↑ GSH and replenishes GST activity

Clinical implications

- **Liver cirrhosis:**

1. Decreased DME activity
2. Changes in PK:
   a. Absorption
   b. Protein binding
   c. Renal excretion

Clinical implications

Effect of cirrhosis on drug disposition dependent on

- Severity of disease
- DME family/isoform involved
- Extraction ratio (clearance dependent on blood flow or intrinsic clearance?)
- The presence of exogenous factors controlling enzyme induction

Examples

Drug	Metabolism	PK changes	Dose changes
Lamotrigine	UGT1A3, 1A4	↑ t1/2, ↑AUC, ↓CL	↓ 50-75%
Atomoxetine	CYP2D6	↑ t1/2, ↑AUC, ↓CL	↓ 25-50%
Budesonide	CYP3A	↑ t1/2, ↑AUC, ↑adverse effects	Not recommended

CYP450 and the pathogenesis of liver disease

- CYP-dependent formation of reactive metabolites may induce liver disease:

1. Drug-induced hepatitis
   - Reactive metabolites lead to toxic hepatitis
   - Toxicity increased by CYP inducers and decreased by inhibitors
   - Acetaminophen: oxidation by CYP2E1 to N-acetyl-p-quinone imine (NAPQ1)

CYP isozymes and hepatotoxic metabolite formation

Isoenzyme	**Drug**
CYP1A2	Dihydralazine Tacrine
CYP2E9	Tienilic acid Diclofenac
CYP2E1	Acetaminophen
CYP3A	Valproic acid Carbamazepine cocaine

CYP450 and the pathogenesis of liver disease

2. Autoimmune hepatitis
   - Presence of circulating auto-antibodies
   - Type II autoimmune hepatitis: anti-liver/kidney microsomal antibodies (anti-LKM)
   - Anti-LKM directed against CYP2D6 and may result in cell lysis by complement

3. Alcoholic liver disease
   - Chronic EtOH consumption induces CYP2E1 causing acetaldehyde and hydroxyl free radical formation
   - CYP2E1 increases formation of ROS and lipid peroxidation
   - ROS also lead to caspase activation and cell apoptosis

CYP450 and the pathogenesis of liver disease

4. Non-alcoholic Steato-Hepatitis (NASH)
   - Associated with obesity, diabetes and hypertriglyceridemia
   - Net retention of lipids in hepatocytes
   - Maybe simple but may be progressive and lead to fibrosis
   - CYP2E1 content and activity increased in NASH
   - CYP2E1 increases ROS leading to fibrosis and caspase activation

CYP450 in cardiovascular health and disease

Reem H. Elbekai, PhD candidate
4129 Dent/Pharm
relbekai@pharmacy.ualberta.ca

Introduction

- CVD is the leading cause of death in men and women worldwide
- Cardiovascular incidents account for 40% of all cause mortality in the US
- Increasing evidence emerging for the role of CYP450 in the onset, progression, and prognosis of CVD.

CYP450 and CVD?

- CYP450 isoforms have been detected in cardiovascular tissue
- CYP450 reaction products involved in the maintenance of cardiovascular health
- Dysregulation of CYP450 involved in cardiovascular disease?

CYP450 in cardiovascular tissue: CYP1 family

- CYP1A1 **inducible in blood vessels of heart, liver and kidneys**
  - **In the heart, it is inducible in the endothelial cells of arteries, veins, capillaries, and coronary vessels**
  - **Also found in coronary artery smooth muscle cells**
  - **In the kidney, found in afferent and efferent arterioles and glomelular and tubular capillaries**

CYP450 in cardiovascular tissue: CYP1 family

- **CYP1A1** also inducible in heart tissue
  - Ventricular tissue, specifically the left ventricle
- **CYP1A2** not detected in vascular tissue
- **CYP1B1** is constitutive and inducible in CV system
  - Left ventricular tissue
  - SMCs of coronary artery and aorta

CYP450 in cardiovascular tissue: CYP2 family

CYP2A

- CYP2A1/2/6/7 detected in left ventricles

- **CYP2B**
  - CYP2B in vascular endothelial cells; CYP2B6/7 also in right and left ventricle

CYP450 in cardiovascular tissue: CYP2 family

- **CYP2C**
  - Major subfamily
  - Found in vascular endothelial cells
  - CYP2C8 in left ventricle
  - CYP2C8/9 are responsible for most CYP activity in arteries under basal conditions.
  - CYP2C8/19 in right ventricle and aorta
  - CYP2C11 plays a major role in CYP activity in arteries under inducible conditions: detected in cerebral, renal, and skeletal muscle arterioles of the rat

CYP450 in cardiovascular tissue: CYP2 family

- **CYP2D**
  - CYP2D6 in right ventricle and aorta
- **CYP2E**
  - CYP2E1 in right and left atria, right and left ventricle, and ventricular septum: located most likely in endocardium. Also in aorta and coronary vessels

CYP450 in cardiovascular tissue: CYP2 family

- **CYP2J**
  - CYP2J2 in humans
  - CYP2J1 and CYP2J3 are the rabbit and rat analogues, respectively
  - CYP2J2 is highly and constitutively expressed in the heart
  - CYP2J3 present in low levels in rat heart: found in atrial and ventricular myocytes and in endothelial cells lining the endocardium

CYP450 in cardiovascular tissue: CYP4 family

- Highly expressed
- CYP4A1/2/3/8 are expressed in SMCs of renal, cerebral, pulmonary, and skeletal muscle arterioles
- CYP4A1 expression induced by fasting or diabetes
- CYP4A2 is constitutively expressed
- CYP4A1 and CYP4A2 activity in dog heart tissue
- CYP4F12 in human heart

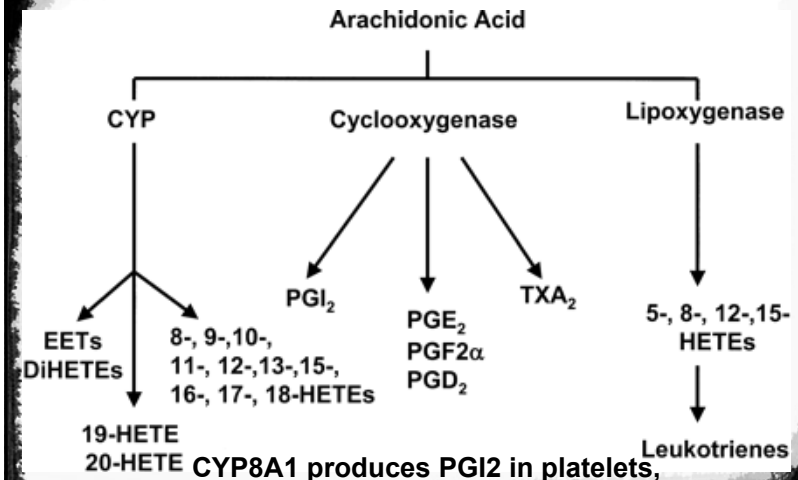
CYP450 in cardiovascular tissue: Other CYP family members

- CYP3A4 found in endothelial cells of endocardium and coronary vessels
- See table 1 of review

CYP450 metabolites in cardiovascular health

1. Arachidonic acid metabolites
2. Cholesterol and cholesterol derivatives

1. Arachidonic acid metabolites



CYP8A1 produces PGI2 in platelets, vascular endothelial cells, and XXXX

1. Arachidonic acid metabolites

A. Prostacyclin (PGI_2)
B. Thromboxane (TXA_2)
C. EETs
D. HETEs

A. Prostacyclin (PGI_2)

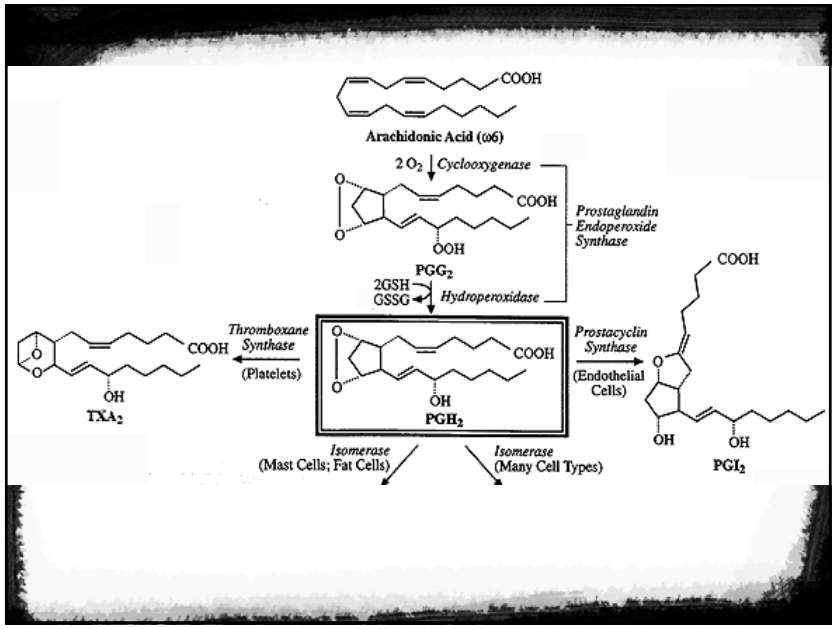
- Produced by CYP8A1 (prostacyclin synthase; PGIS)
- Multiple roles: vasodilator, inhibitor of platelet aggregation, VSMC proliferation, and fibrinolysis
- Cytoprotective: protects against reduction in blood flow during periods of ischemia and enhances post-ischemic neuronal recovery

A. Prostacyclin (PGI_2)

- Prostacyclin analogues (ie, beraprost):
  - Reduce bp, increase HR, prevent stroke in hypertensive rats
- Reduced PGI2 activity/deletion of its receptor:
  - Linked to hyertension, the formation of atherosclerosis, increase in thrombotic events, and myocardial infarction

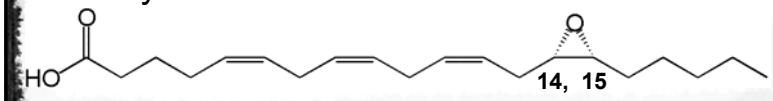
B. Thromboxane A_2 (TXA_2)

- Produced from PGH_2 by CYP5
- A vasoconstrictor and potent activator of platelets
- Cardiovascular homeostasis dependent on balance between PGI_2 and TXA_2



C. EETs

- CYP epoxygenases metabolize AA to epoxyeicosatrienoic acids (EETs) in the vascular endothelium
- The vascular endothelium can then metabolize EETs to respective regioisomers of DiHETEs by epoxide hydrolase

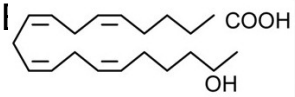


C. EETs

- Major EETs in rat heart are 14,15-EET and 8,9-EET, followed by 11,12-EET
- CYP2J2 is the major enzyme responsible for the formation EETs in the heart
- In the human liver, CYP2C9 or CYP2C8 are the major isoforms, followed by CYP1A and CYP3A
- CYP2B/2D/2E/4A are also involved in EET production

D. HETEs

- CYP hydroxylases metabolize AA to hydroxyeicosatetraenoic acids (HETEs) in the VSMCs
- 5-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 15-, 16-, 17-, 18-, 19-, and 20-HETE are products of CYP reactions
- CYP4A6/7/10/12 and CYP4F2 metabolize AA to 20-HI
- See table 2 in review



19-HETE

I. AA metabolites and cardiovascular health

i. Effect of AA metabolites on blood vessels
ii. Effect of AA metabolites on the heart
iii. Effect of AA metabolites on the kidney
iv. Effect of AA metabolites on cerebral blood flow
v. Others

i. Effect of AA metabolites on blood vessels

- 20-HETE involved in angiogenesis
  - Also a potent vasoconstrictor of small arteries with little effect on large arteries: increases intracellular calcium concentration and thus depolarization
  - Vasodilators such as NO inhibit 20-HETE formation
- EETs and DiHETEs are vasodilators

ii. Effect of AA metabolites on the heart

- EETs enhance cardiac calcium current and thus cardiac contractility
  - EETs also inhibit cardiac sodium channels
  - Regulators of cardiac electrical excitability: activators of ATP-sensitive potassium channels
- PGI_2 prolongs ventricular refractory period and increases QT interval
- TXA_2 increases calcium mobilization into ventricular myocytes

iii. Effect of AA metabolites on kidney function

- Involved in maintenance of renal blood flow
- EETs and HETEs vital regulators of renal hemodynamics: involved in autoregulation of glomerular capillary pressure
- 19- and 20-HETE decrease glomerular filtration rate by causing vasoconstriction; EETs antagonize this effect
- EETs and HETEs also play a role in regulation of ion transport in proximal tubule, Loop of Henle, and collecting duct

iii. Effect of AA metabolites on cerebral blood flow

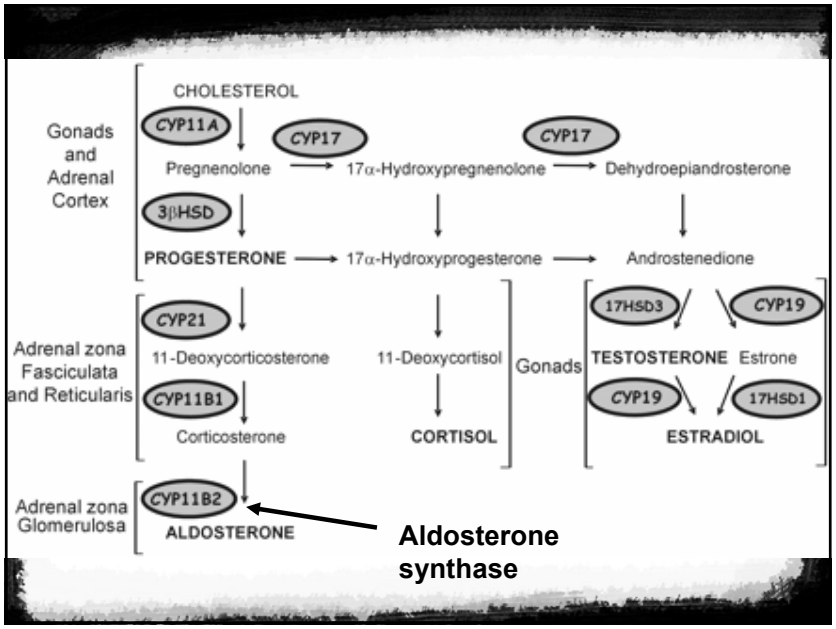
- 20-HETE and EETs play a role in autoregulation of cerebral blood flow
- EETs are stimulated by excitatory neurotransmitters (ie glutamate) and result in dilation of cerebral blood vessels
- EETs also involved in baseline cerebral blood flow
- 20-HETE involved in pressure-induced vasoconstriction

2. Cholesterol and cholesterol derivatives

- Cholesterol is the precursor for many biologically active compounds
- CYP involved in the synthesis and metabolism of cholesterol
- CYP51 is a key enzyme in the production of cholesterol
- CYP7A/B, CYP8B, and CYP27A are involved in bile acid synthesis from cholesterol

2. Cholesterol and cholesterol derivatives

- Cholesterol is also the precursor for steroid hormones, including the sex steroids and mineralocorticoids
- 1st step in cholesterol metabolism is mediated by CYP11A1



I. Effect of cholesterol metabolites on blood vessels

- Aldosterone blunts vascular response to vasodilators, inhibits fibrinolysis, and causes VSMC hypertrophy
- Testosterone stimulates proliferation of VSMCs
- At physiological levels in men, testosterone decreases vascular reactivity; causes vasodilation at supraphysiological levels
- Estradiol inhibits VSMC proliferation; causes vasodilation; decreases plasma levels of fibrinogen

ii. Effect of cholesterol metabolites on the heart

- Aldosterone involved in vascular inflammation in the heart which leads to fibrosis and necrosis
- Estrogen is protective on the heart: induces NO synthesis and decreases norepinephrine-induced vasoconstriction

iii. Effect of cholesterol metabolites on the kidney

- Aldosterone regulates extracellular fluid volume through its effects on the kidney
- Estrogen decreases Angiotensin converting enzyme activity

CYP450 and cardiovascular disease

1. Hypertension
2. CAD/MI
3. Heart failure
4. Stroke

1. Hypertension

- **Polymorphisms:**
  - CYP2J2*7 allele less frequent in hypertensive caucasian males
  - CYP3A5*1/*3 alleles more frequent in hypertensives
  - CYP3A5*1/*1 associated with blood pressure control in African-Americans
  - Mutations in CYP8A associated with hypertension and cerebral infarction

1. Hypertension

- **Polymorphisms:**
  - Glucocorticoid remediable aldosteronism (GRA): CYP11B1 and CYP11B2 cross over during meiosis resulting in chimeric gene. Associated with increased aldosterone, early onset hypertension and hemorrhagic stroke
  - Other polymorphisms in the CYP11B2 gene have been identified: those associated with increased transcriptional activity are associated with increased risk of hypertension

1. Hypertension

- **Altered expression**
  - Renal CYP2J, CYP3A and CYP4A increased in hypertensive rats
  - CYP1A1, 1B1, 2A1/2, 2B1/2, and CYP2J3 are increased in left ventricle of hypertensive rats

2. Coronary artery disease/ Myocardial infarction

- Aldosterone blockade reduces morbidity and mortality in patients with post-MI heart failure
- Testosterone exhibits pro-atherosclerotic properties; however, androgens decrease symptoms of CAD, especially exercise induced myocardial ischemia
- Estrogen exhibits anti-atherosclerotic properties; prevents the formation of atherosclerotic plaques

2. Coronary artery disease/ Myocardial infarction

- Reperfusion injury:
  - Restoration of blood flow to tissue after prolonged ischemia precipitates further tissue damage
  - 2 mechanisms: 1. CYP increases ROS production during reperfusion; 2. increase in intracellular calcium activates phospholipase A2 and causes the release of arachidonic acid, AA the metabolized by CYP450

2. Coronary artery disease/ Myocardial infarction

- Reperfusion injury:
  - Involves 20-HETE: blood 20-HETE levels are increased in late stages of ischemia and early stages of reperfusion; inhibition of CYP2C9 reduces infarct size
  - EETs are cardioprotective: they reduce contractile force and oxygen utilization
  - EETs also increased in reperfusion but at lower concentrations than 20-HETE
  - 11,12-EET pretreatment improves cardiac recovery after ischemia/reperfusion

2. Coronary artery disease/ Myocardial infarction

- Reperfusion injury:
  - Estradiol also protective: infusion of estradiol 1 h before experimental occlusion reduces infarct size
  - Enhanced production of PGI2 reduces infarct size and improves myocardial wall function

2. Coronary artery disease/ Myocardial infarction

- Polymorphism
  - Genetic variants of CYP2C8 and CYP2C9, conferring lower activity, associated with an increase in MI risk in females
  - CYP2C9*2 and CYP2C9*3 mutant alleles decrease MI risk in males
  - Mutations in CYP8A1 (PGIS) increases MI risk

3. Heart failure

- Increase in CYP2J2, 1B1, 2E1, 4A10, 2F2; decrease in CYP2C19 and 1A2 in failing heart
- Failing hearts express CYP11B1 and CYP11B2 which are not found in healthy human hearts; correspondingly, cardiac aldosterone production and plasma aldosterone levels are increased in heart failure. Aldosterone increases left ventricular volume and decreases ejection fraction

4. Stroke

- Following a hemorrhagic stroke, cerebral vasospasm and a rise in cerebral spinal fluid (CSF) pressure are responsible for high mortality. Delayed vasospasm up to 1 month after hemorrhage also responsible for high mortality rates
- 20-HETE increased in CSF following hemorrhage
- Inhibition of 20-HETE synthesis prevents fall in cerebral blood flow

4. Stroke

- 20-HETE may also be involved in ischemic stroke
- New 20-HETE synthesis inhibitor (TS-011) reduces infarct size in ischemic stroke and decreases degree of motor deficit following stroke in experimental animals
- Androgens and estradiol are neuroprotective against stroke
- Aldosterone increases stroke risk in hypertensive animals

4. Stroke

- Genetic polymorphism:
  - Polymorphism in CYP1A1 modulates stroke risk in hypertensive patients
  - Polymorphism in CYP8A1 leading to decrease in transcriptional activity also influences risk for cerebral infarct