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PHM142 - UNIT topics for 2017

- UNIT 5  Introduction and review of leukocytes (October 12, 2017)
- UNIT 6  Blood platelets and endothelial cells– properties and function (Oct. 19)
- UNIT 7A  Introduction to biochemistry of mitochondria (October 26)
- UNIT 7B  Mitochondria and hepatic detoxification (October 26)
- UNIT 7C  Discussion of reading assignments – part 1 (a) aspirin + cancer, (b) platelets and nanoparticle cloaking
- UNIT 8  Mitochondrial disorders – neurodevelopmental (November 2)
- UNIT 9  Mitochondrial disorders – neurodegenerative (November 9) Discussion of reading assignments – part 2
- UNIT 9  Mitochondrial Disorders – neurodegenerative (continued) (November 16)
- UNIT 10  Discussion of reading assignments – part 2 (November 16)

UNIT 6
Platelets use Oxygen to form Lipid Regulators while
Endothelial cells form NO

A) Platelets and their functions
   i. Blood clotting and stroke (anti-coagulant drugs)
   ii. Prostaglandin and thromboxane formation from polyunsaturated fatty acids
   iii. Nutritional approach to prevent heart disease
   iv. COX inhibitors (NSAIDs) (anti-platelet drugs)

B) Endothelial cell functions: Prostacyclin and plasminogen activator formation
   Role of endothelial cells in regulating blood pressure:
      - angiotensin and EDRF (NO)
   Nitric oxide (NO) formation, signaling and toxicity:
      - use of NO generators: (vasodilator/anti-hypertensive drugs)
Platelets

$\tau_{1/2} = 4$ days

Fragments of megakaryocytes of bone marrow

Contain: glycogen granules

mitochondria

lysosomes

No nucleus, DNA, protein synthesis

1) dense granules – ADP, ATP, serotonin (5HT)

2) $\alpha$ granules contain clotting factors and PDGF

(platelet derived growth factor)

80% ATP from glycolysis and 20% from mitochondria

Source and differentiation of blood cells
Thrombocyte (platelet)

Thrombocytopenia – platelet count < 50,000 per µl

- Life threatening platelet depletion caused by a drug or by heparin-induced antibodies (which bind to platelet factor 4 complex). This is not associated with immune memory.
- Hemorrhage, bruising, brain injury, nose bleeds, stools
- Drug-induced thrombocytopenia: methotrexate, chemotherapeutic agents, heparin, quinine, antimicrobials, NSAIDs, penicillin, ranitidine, furosemide

Platelet Function is to plug blood leaks (blood clotting)
Activated by collagen (damaged vascular surface) or thrombin which binds to receptors. Platelet disc shape changes to sphere i.e. swell, form pseudopods, become sticky and attach to collagen

Resting platelets

Activated Platelets
The BIG picture: Platelets Repair Broken Blood Vessels

a. Platelets secrete platelet derived growth factor (PDGF): a growth factor → migration and division of vascular endothelial cells, smooth muscle cells, fibroblasts → REPAIR OF DAMAGED VASCULAR WALLS

b. MEMBRANE ACTIN AND MYOSIN CONTRACT → platelet attached to fibrin are retracted → CLOT RETRACTS → edges of broken blood vessel are pulled together

c. Platelets are collected and stored for use in surgeries, transplants and cancer therapy to stop bleeding.

Structure of PDGF

Platelet-derived growth factors (PDGFs) and their receptors (PDGFRs) are prototypic growth factors and receptor tyrosine kinases, respectively, which have critical functions in development. Platelet-derived growth factors (PDGF-A, -B, -C, and -D) are major mitogens for connective tissue cells such as fibroblasts and smooth muscle cells, and are essential for regulating embryonic development.

PDGFs, and their relatives VEGFs, function as disulfide-linked dimers. They have an evolutionarily conserved cystine-knot fold growth factor domain of ~100 amino acids (aa), denoted the PDGF/VEGF homology domain, involved in receptor-binding and dimerization. PDGFs contain N-terminal prodomains of various length (70, 65, 212, 239aa for PDGF-A, -B, -C, and -D respectively, which are proteolytically cleaved for activation when secreted.
PDGF processing by proteases

- Intracellularly, the N terminal prodomains of PDGF-A and PDGF-B are removed by furin or related pro-protein convertases which is necessary for PDGF-A and PDGF-B to obtain receptor-binding ability.
- Prodomain in PDGF-C is removed proteolytically by plasmin and tissue plasminogen activator (tPA)
- PDGF-D activation can be done by plasmin cleavage, but not tPA cleavage.


Pro-opiomelanocortin (POMC) is a precursor polypeptide with 241 amino acid residues. POMC is synthesized in the pituitary from the 285-amino-acid-long polypeptide precursor pre-pro-opiomelanocortin, by the removal of a 44-amino-acid-long signal peptide sequence during translation. The cleavage products of POMC are all used in specific pathways.

cf. (compare) proteolytic processing of PDGF on previous slide where N-terminal fragment is removed but not used in PDGF receptor activation. Thus, POMC represents another facet of proteolytic processing of proteins and peptides.
Mechanism of platelet aggregation to form a clot

ADP released from platelet granule → receptor

\[ \text{Ca}^{2+} \text{ influx in other platelets} \]

Phospholipase A_2 activated

Aspirin acetylates cyclooxygenase

\[ \text{PGH}_2 \]

Thromboxane A2 (TxA_2)

Ca^{2+} influx

PLATELET AGGREGATION

Diacylglycerol (DAG) + inositol triphosphate (IP_3)

Phosphatidylinositol

Membrane

Collagen receptor

Phospholipase C

COLLAGEN

Fibrin clot formed by zymogen activation cascade

INTRINSIC PATHWAY

EXTRINSIC PATHWAY

FINAL COMMON PATHWAY

Modified from Fig. 10.37, Stryer (5th ed).
A fibrin clot (blood clot) is formed by the interplay of the intrinsic, extrinsic, and final common pathways.

Intrinsic pathway:
- initiated when factor XII is activated by contact with abnormal surfaces due to injury.

Extrinsic pathway:
- triggered by trauma, which activates factor VII which releases tissue factor.
Anticoagulant drugs (stroke treatment prevents new clots)\(^\text{13}\)

- Vitamin K is essential for prothrombin synthesis (and other clotting factors).

Abnormal prothrombin is formed in the absence of Vit. K (does not bind Ca\(^{2+}\)), or in the presence of Vit. K antagonists (see below).

- Warfarin for thrombosis but high CYP2C9 polymorphism
- Drug name: Coumadin (rat poison)
- 2-3 days before it works ::
- DON'T admin. again for 3d

Cyclooxygenases

Cyclooxygenase is an enzyme that is responsible for the formation of prostanoids.

The three main groups of prostanoids -- prostaglandins, prostacyclins, and thromboxanes -- are each involved in the inflammatory response.

- Two forms of the enzyme: cox-1 and cox-2

Assignment: find out the details of how these two isozymes differ
**Prostaglandins and thromboxanes**

roles in platelets and other cells

Prostaglandins and thromboxanes – structural components:

<> membrane phospholipid containing a glycerol backbone and

a fatty acid at the first position,

b arachidonic acid at the second position

c polar phospholipid moiety at the third position.

![Prostaglandin and Thromboxane Diagram](image-url)

X – Choline, Serine, Inositol, Ethanolamine

*Figure 1* (Head and Neck July 1988 p34-49)

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**Conversion of Arachidonic Acid to PGD\(_2\), PGF\(_{2\alpha}\), PGE\(_2\)**

![Conversion Diagram](image-url)

Prostaglandin synthase consists of cyclooxygenase (COX) and peroxidase

* NSAID now used as prophylaxis to prevent carcinogenesis
Multiple active metabolites of AA

Physiological functions – in many cells/tissues
- PG’s cytoprotective (stomach, kidney)
- TXA platelet (clotting function)

Inhibitors
- aspirin, NSAIDS, anti-thrombosis drugs but cause gastric/renal lesions ☹

Anti-platelet drugs:
Cyclooxygenase isozymes and inhibitors (NSAIDs)

**COX-1 (ALL CELLS)**
- Physiological functions – in many cells/tissues
  - PG’s cytoprotective (stomach, kidney)
  - TXA platelet (clotting function)

**COX-2 (INFLAMMATORY CELLS)**
- Inflammatory cells (also brain)
  - Mediate inflammation and pain
  - Inducible by cytokines, mitogens, endotoxins (contributes to rheumatoid arthritis)
  - Promote colon cancer by preventing apoptosis
  - Causes premature labour

Inhibitors
- Selective NSAIDS e.g. nimesulide
- Chemoprevention/colon cancer
- Anti-pyretic [knockout (KO) mice – infertile females]
NSAIDS
COX-1 inhibitors bind cyclooxygenase enzyme and prevents PGE formation)

GI toxicity because PGE normally protects intestinal mucosa

1) Aspirin - irreversible
   - acetylates Ser 530, prevents arachidonic acid binding
2) Mefenamate, Ibuprofen - reversible, competitive with fatty acid binding
3) Flurbiprofen - slow binding (salt bridge) competitive inhibition by binding in the hydrophobic channel
4) Indomethacin - non-selective
   - binds deepest in hydrophobic channel
   (incr. risk of hypertension, congestive heart failure unlike Celecoxib)

COX-2 inhibitors on the market – much less GI toxicity
4) Celecoxib (celebrex). Vioxx withdrawn
Side effects of cyclooxygenase-1 inhibitors

• e.g. aspirin may cause stomach bleeding and risk GI ulcer formation as a result of stomach cell mitochondrial uncoupling and acidosis.
• inhibits cox-1 activity thereby increasing tissue unsat. fatty acid levels and causing acidosis.
• decreases PGE2 levels that protect stomach membrane
• unsat. fatty acid + PGS (prostaglandin synthase) attacks protective mucous layer
• inhibits thromboxane formation and platelet aggregation

Role of endothelial cells in regulating blood pressure (e.g. angiotensin) and Nitric oxide formation, signaling, and toxicity
**Endothelial cells** (line vessel walls)

*Cell surface extracellular activity*

1) Angiotensin

2) Inactivate prostaglandins E and F and leukotrienes C4 and D4

*Intracellular endothelial cell activity*

3) Thromboplastin synthesis and secretion (activated state) initiate blood clotting (extrinsic pathway)

4) Synthesis and secrete plasminogen activators (resting state) initiates clot fibrinolysis

5) Nitric oxide system

**Function of Endothelial cells** (line vessel walls)

*Angiotensin converting enzyme formation and action*

Angiotensinogen (411 aa) synthesised in the liver → Plasma

Renin (synthesised in kidney, a protease) → Angiotensin I in plasma (10aa)

Angiotensin converting enzyme (endothelial cells) → Angiotensin II in plasma (8 aa)

ACE inhibitors

Angiotensin II in plasma (8 aa) → Vasoconstrictor → blood pressure
DRUG Molecular TARGET:
Inhibitors of angiotensin converting enzyme (ACE) used to lower blood pressure.

Captopril  25-50mg (but agranulocytosis risk, cough),

Enalapril  1-20 mg.

Ramipril  2-20 mg (10mg also used for preventing cardiovascular problems and stroke in diabetics.

Synthesis and Secretion of Plasminogen Activators
(Resting State) - initiate clot formation and reversed by clot busters e.g. plasmin & plasminogen activator)

<table>
<thead>
<tr>
<th>Stroke Therapy (Dissolve clots)</th>
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<tbody>
<tr>
<td>Plasminogen incorporated into clot fibrin</td>
</tr>
<tr>
<td>Endothelial cell</td>
</tr>
<tr>
<td>Dissolve fibrin Therefore activator</td>
</tr>
<tr>
<td>Fibrin</td>
</tr>
<tr>
<td>Fibrin-bound plasmin</td>
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<tr>
<td>Fibrinolysis</td>
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</tbody>
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Nitric Oxide (NO) = Endothelial-Derived Relaxing Factor

Endothelial cell regulation of blood pressure
i.e. effect on smooth muscle cells

NO
The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow.

Nitric oxide is a chemical radical thus highly reactive and has a lifetime of a few seconds - yet diffuses freely across membranes.

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Nitric Oxide Synthases: nNOS, iNOS, eNOS)

Analogous to "P450 + reductase"

L-Arginine → N-OH Arginine → L-Citrulline

Arginine (100 uM in blood, 2 mM in endothelial cells) - NO produced along with citrulline
**Drugs that act by Generating Nitric Oxide (NO•)**

1) **NITROGLYCERIN** – anti-anginal, anti-hypertensive, vasodilator

![Diagram of nitric oxide generation and metabolism]

**Adverse effect:** oxidative stress, HEADACHE unless tolerant

*Tolerance* because vascular ALDH in mitochondria reduces NG but is inactivated by peroxynitrite formed when NO reacts with O₂• (formed when NO inhibits respiratory chain) (J.Clin.Invest.113,482-9(2004);J.Am.Coll.Cardiol.57,93-8(2011).

*Tolerance reversed* antioxidants atorvastatin, carvediol, thiols, hydralazine, HMG-CoA red.inhib.

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**“AMYL” NITRITE** (i.e. isopentyl nitrite) - potent vasodilators which are inhaled

![Diagram of amyl nitrite metabolism]

**+ GSH transferase**

(GST 4-4)