The Non-steriodal Anti-inflammatory Drugs (NSAIDs): are a group of - chemically **dissimilar** agents that differ in their **antipyretic, analgesic, and anti-inflammatory activities**. They act primarily by inhibiting the cyclooxygenase enzymes that catalyze the first step in prostanoid biosynthesis. This leads to decreased prostaglandin synthesis with both **beneficial and unwanted effects**.

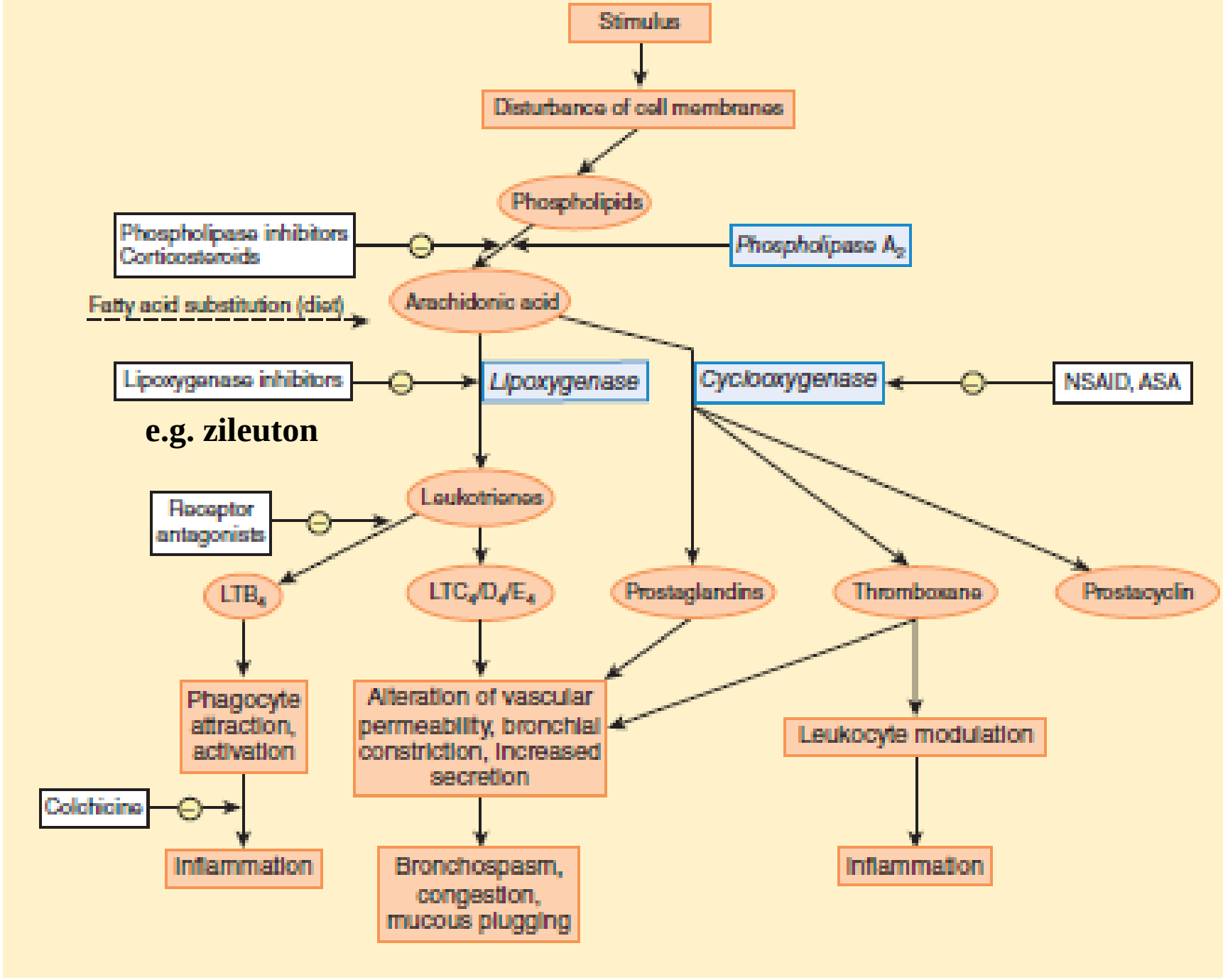
- Detection of **serious cardiovascular events** associated with **COX-2** - inhibitors have led to withdrawal of ***rofecoxib and valdecoxib*** *from the market (celecoxib is still available for use in patients with RA). Additionally, the* U.S. Food and Drug Administration (FDA) has required that the labeling of the traditional NSAIDs and *celecoxib be* updated to include the following:

1) a warning of the potential risks of serious cardiovascular thrombotic events, - myocardial infarction, and stroke, which can be fatal; additionally, a warning that the risk may increase with duration of use and that patients with cardiovascular disease or risk factors may be at greater risk;

2) a warning that use is **contraindicated for the treatment of perioperative** - **pain** in the setting of coronary artery bypass graft surgery; and

3) a notice that there is increased risk of serious gastrointestinal (GI) adverse events, including **bleeding, ulceration, and perforation of the stomach or intestines**, which can be fatal. These events can occur **at any time during use and without warning symptoms**.

- **Elderly patients are at greater risk for serious GI events**. ***Aspirin***, *however, has proven to be beneficial in patients for the primary and secondary prevention of* cardiovascular events and is most commonly used for this purpose rather than for pain control.


Stimulus
Disturbance of cell membranes
Phospholipids
Phospholipase inhibitors
Corticosteroids
Phospholipase A2
Fatty acid substitution (diet)
Arachidonic acid
Lipoxygenase inhibitors
Lipoxygenase
Cyclooxygenase
NSAID, ASA
e.g. zileuton
Leukotrienes
Receptor antagonists
LTB4
LTC4/D4/E4
Prostaglandins
Thromboxane
Prostacyclin
Phagocyte attraction, activation
Alteration of vascular permeability, bronchial constriction, increased secretion
Leukocyte modulation
Colchicine
Inflammation
Bronchospasm, congestion, mucous plugging
Inflammation

- Actions of NSAIDs:

1- Anti-inflammatory actions: **Because *aspirin inhibits cyclooxygenase activity, it diminishes the formation of*** prostaglandins and, thus, modulates those aspects of inflammation in which prostaglandins act as mediators. *Aspirin inhibits inflammation in arthritis, but it neither arrests the progress of the disease nor induces* remission.

2- Analgesic action: Prostaglandin E2 (PGE2) is thought to sensitize nerve endings to the action of bradykinin, histamine, and other chemical mediators released locally by the inflammatory process. Thus, by decreasing PGE2 synthesis, *aspirin and other NSAIDs repress the sensation of pain.*

3- Antipyretic action: Fever occurs when the set-point of the anterior hypothalamic thermoregulatory center is elevated. This can be caused by PGE2 synthesis, which is stimulated when an endogenous fever-producing agent **(pyrogen), such as a cytokine, is released from white cells that are activated by infection**, **hypersensitivity, malignancy, or inflammation**. The salicylates lower body temperature in patients with fever by impeding PGE2 synthesis and release. *Aspirin resets the thermostate toward normal, and it rapidly* lowers the body temperature of febrile patients by increasing heat dissipation as a result of peripheral vasodilatation and sweating.

4 - Respiratory actions: At therapeutic doses, *aspirin increases alveolar ventilation. [Note: Salicylates* uncouple oxidative phosphorylation, which leads to elevated CO2 and increased respiration.] Higher doses work directly on the respiratory center in the medulla, resulting in hyperventilation and respiratory alkalosis that usually is adequately compensated for by the kidney. At toxic levels, central respiratory paralysis occurs, and respiratory acidosis ensues due to continued production of CO2.

5- Gastrointestinal effects: Normally, prostacyclin (PGI2) inhibits gastric acid secretion, whereas PGE2 and PGF2Îα stimulate synthesis of protective mucus in both the stomach and small intestine. In the presence of *aspirin, these prostanoids are not formed, resulting in increased gastric acid secretion and diminished mucus* protection. This may cause epigastric distress, ulceration, hemorrhage, and iron-deficiency anemia. *Aspirin* doses of 1 to 4.5 g/day can produce loss of 2 to 8 mL of blood in the feces per day. Buffered and enteric coated preparations are only marginally helpful in dealing with this problem.

6- **Effect on platelets: TXA2 enhances platelet aggregation, whereas PGI2 decreases it. Low doses (60-81 mg** daily) of *aspirin can irreversibly inhibit thromboxane production in platelets via acetylation of* cyclooxygenase. Because platelets lack nuclei, they cannot synthesize new enzyme, and the lack of thromboxane persists for the lifetime of the platelet (3-7 days). As a result of the decrease in TXA2, platelet aggregation (the first step in thrombus formation) is reduced, producing an anticoagulant effect with a prolonged bleeding time. Finally, *aspirin also inhibits cyclooxygenase in endothelial cells, resulting in* reduced PGI2 formation; however, endothelial cells possess nuclei able to re-synthesize new cyclooxygenase. Therefore, PGI2 is available for antiplatelet action.

7 - Actions on the kidney: Cyclooxygenase inhibitors prevent the synthesis of PGE2 and PGI2 prostaglandins that are responsible for maintaining renal blood flow, particularly in the presence of circulating vasoconstrictors . Decreased synthesis of prostaglandins can result in retention of sodium and water and may cause edema and hyperkalemia in some patients. Interstitial nephritis can also occur with all NSAIDs except *aspirin.*

Classification of COX Inhibitors:

- **COX-1 Specific Inhibitors:** Inhibit COX-1 activity without any measurable effect on COX-2 activity. Aspirin at low dose (81 mg/day) is the only drug in this category.
- **COX-Non-Specific Inhibitors:** Reversibly and competitive inhibit both isoforms of COX. Example on these agents are ibuprofen & naproxen.
- **COX-2 Preferential Inhibitors:** At low doses, prefer inhibition of COX-2 while at higher doses they inhibit COX-1 as well. Meloxicam is an example.
- **COX-2 Specific Inhibitors:** These agents inhibit the COX-2 isoform but have no effect on the COX-1 isoform over the entire range of therapeutic serum concentrations. Example on this group of agents is rofecoxib, celecoxib & valdicoxib.

Methods for Assessment of Peripheral Analgesic Activity:

- The principle method intended for **assessment of peripheral analgesic activity** in this Lab. is the **writhing method** in mice, and **Paw-pressure (Randal-Selitto)** test in rats.

Writing Method

- The principle of this method depends on the induction of pain by injecting of irritants(e.g. phenylquinone and acetic acid solution) into peritoneal cavity of mice. The animals create a characteristics stretching behavior, which called **writhing**.
- A 1% acetic acid solution (10mL/kg), as an irritant agent, is injected intraperitoneally (I.P.) to mice & the stretching reaction is evaluated. The amount of writhing (**constriction of abdomen, turning of trunk (twist) & extension of hind legs**) due to acetic acid is expressed as a pain response. The amount of writhing per animal is counted during a 20 minutes period, beginning 3 minutes after injection of acetic acid, therefore it's a chemical method.

Paw-Pressure (Randall-Selitto) Test:

- This method for **measuring analgesic activity** based on the principle that "**inflammation increases the sensitivity to pain & that this sensitivity is susceptible to modification by analgesics**". Inflammation decreases the pain threshold that readily elevated by NSAIDs. Pain is induced by injection of freshly prepared egg-white (0.05ml) into the planter surface of the rat's hind paw and measured by Paw Pressure test described by Randal and Selitto. An anlagesia (analgesy) meter (Ugo-Basile, Italy) which a con-shaped paw pressure with a rounded tip is used to apply a linearly increasing force to the rat's right hind paw. The weight in grams required to elicit nociceptive response such as paw flexion or struggle is determine as nociceptive threshold. **A cut-off value of 300 gm** is used to prevent damage to the paw. This process is done at zero time and 1 hour and 2 hours.

Testing analgesic action using hot plate method on mice

- The hot-plate test is performed to **measure response latencies** according to the hot plate method. A hot-plate was maintained at 55.0 ± 0.2° C and the animals were placed into the Perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, prior to and after administration of the plan treatment. A latency period of 20 sec was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

Doses

Drug	Concentration	Dose
Diclofenac	75 mg/ ml	10 mg/ kg
Piroxicam	20 mg / 2 ml	2 mg/kg
Aspegic ®	500 mg as powder	25 mg/kg
Acetic acid	1%	10 ml/ kg

1- For Diclofenac : 10mg / kg

10 mg 1000 mg

X mg given wt of mice

X1= ? mg

Dilution : 75 mg 10 ml

? mg X2 ml

X2= ? ml

2- For Piroxiam : 2mg / kg

10 mg 1000 mg

X mg given wt of mice

X1= ? mg

Dilution : 20 mg 10 ml

? mg X2 ml

X2= ? ml

3- For Aspegic ® : 25mg / kg

10 mg 1000 mg

X mg given wt of mice

X1= ? mg

Reconstitution : 500 mg 10 ml

? mg X2 ml

X2= ? ml

1- For Acetic acid (1%) : 10ml / kg

10 ml **1000 mg**

X ml **given wt of mice**

X1= ? ml

Procedure and Handling of Mice

- Collect 12 mice and divide equally into 4 groups (3 mice for each) :

Gp1 : Control group (acetic acid 1% group)

Gp2: Diclofenac group

Gp3: Piroxicam group

Gp4: Aspegic® group

Gp1: **Acetic acid (1%) IP** 3 minutes → count the no. of writhing for 20 minutes.

Gp2: **Diclofenac IP** 10 minutes → acetic acid (1%) IP 3 minutes → count the no. of writhing for 20 minutes.

Gp3: **Piroxicam IP** 10 minutes → acetic acid (1%) IP 3 minutes → count the no. of writhing for 20 minutes.

Gp4**: Aspegic ® IP** 10 minutes → acetic acid (1%) IP 3 minutes → count the no. of writhing for 20 minutes.


THANKS FOR LISTENING