5.1: Pathology

Responding to tissue damage is usually accompanied by a physiological and psychological response via inflammation and pain. Although these are associated with negative responses, acute pain and inflammation are important in helping remove the negative stimuli, fighting off foreign invaders, and healing injuries. However, prolonged inflammation and pain can lead to additional damage and discomfort. The regimen of drugs developed for pain and inflammation are designed to deal with physiological and/or psychological responses.

In the simplest example, damage to a cell can occur through a puncture or rupture of the membrane which can lead to a cascade of different responses. For example, one initial aspect can involve the influx of Ca^{2+} into the cell. Ca^{2+} is a potent biochemical messenger and multiple proteins and transcription factors are sensitive to Ca^{2+} binding which can trigger different responses depending on the type of cell. One of the key responses is the activation of a protein called phospholipase A2 (PLA2). PLA2 interacts with phospholipids of the cell bilayer and will cleave fatty acids from the phospholipid.

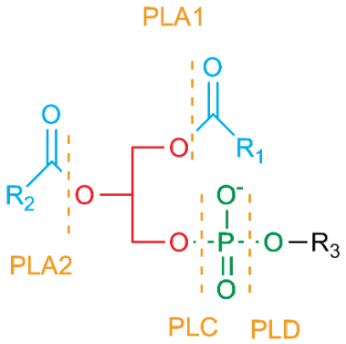

Figure 5.1 Sites of cleavage by the phospholipase family depicted on the phospholipid backbone. Image Source: Phospholipases2) by Roadnottaken is used under a CC BY-SA 3.0 license.

Recall that the structure of a phospholipid has a glycerol backbone with a phospholipid head group esterified to one oxygen of glycerol (called stereospecific numbering 1 or SN1) and fatty acid chains esterified to the other two oxygen atoms (called SN2 and SN3). (Figure 5.1) Phospholipase enzymes cleave at specific SN positions (PLA1 cleaves at the SN1 position, PLA-2 cleaves at SN2, PLAB cleaves at either SN1 or SN2, and PLAC and PLCD cleave on specific sides of the phosphate at the SN3 position). During the inflammatory responses, one of the most important phospholipases is PLA2 which cleaves the fatty acid in the middle (SN2) position. The fatty acid released is often an arachidonic acid. (Figure 5.2)

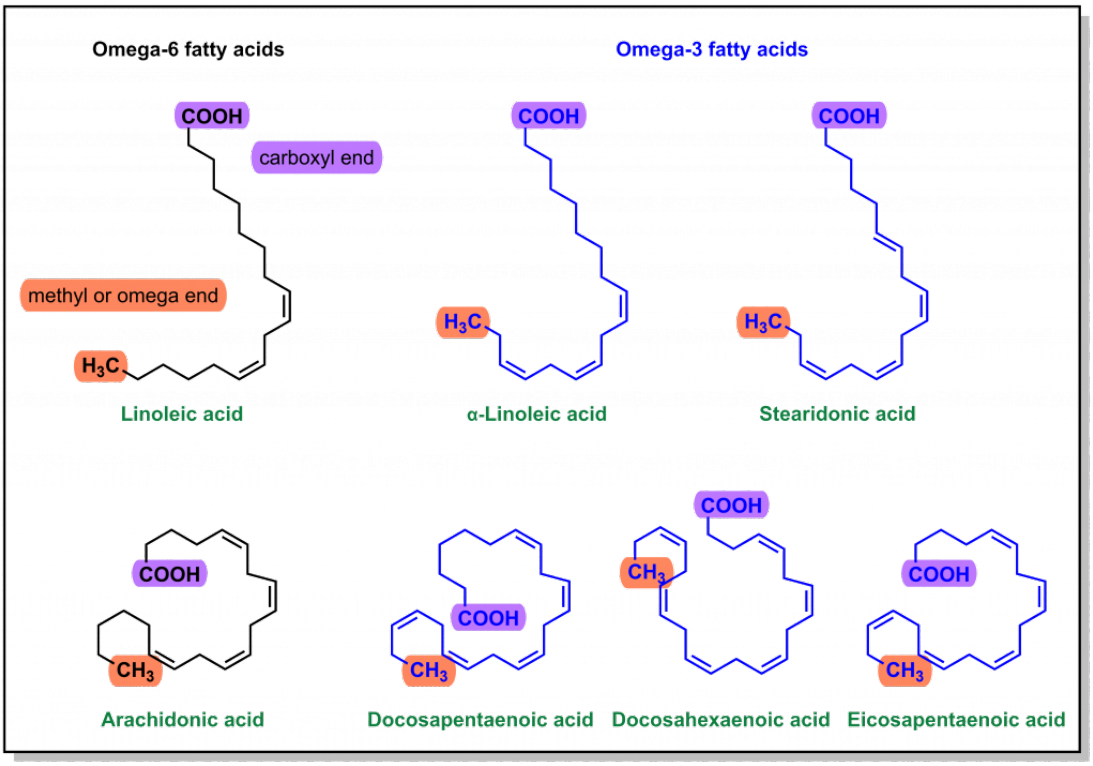

Figure 5.2 Structures of fatty acids.

Arachidonic acid and inflammation

There are multiple types of fatty acids that are incorporated into phospholipids. Since physiological fatty acids can have variable lengths and degrees of saturation, historically it has been more convenient to name fatty acids from the omega end (final carbon) rather than the alpha end. Omega-3 fatty acids have a double bond at the 3-C from the omega position and omega-6 fatty acids have a double bond at the 6-C from the omega position. (Figure 5.2) Arachidonic acid is an omega-6 fatty acid and it contains 4 double bonds, which are in the '*cis*' configuration, leading to a more closed geometry that contributes to cell membrane fluidity. The double bonds are also highly susceptible to oxidation, which makes arachidonic acid a good target for downstream signalling pathways. (Figure 5.3)

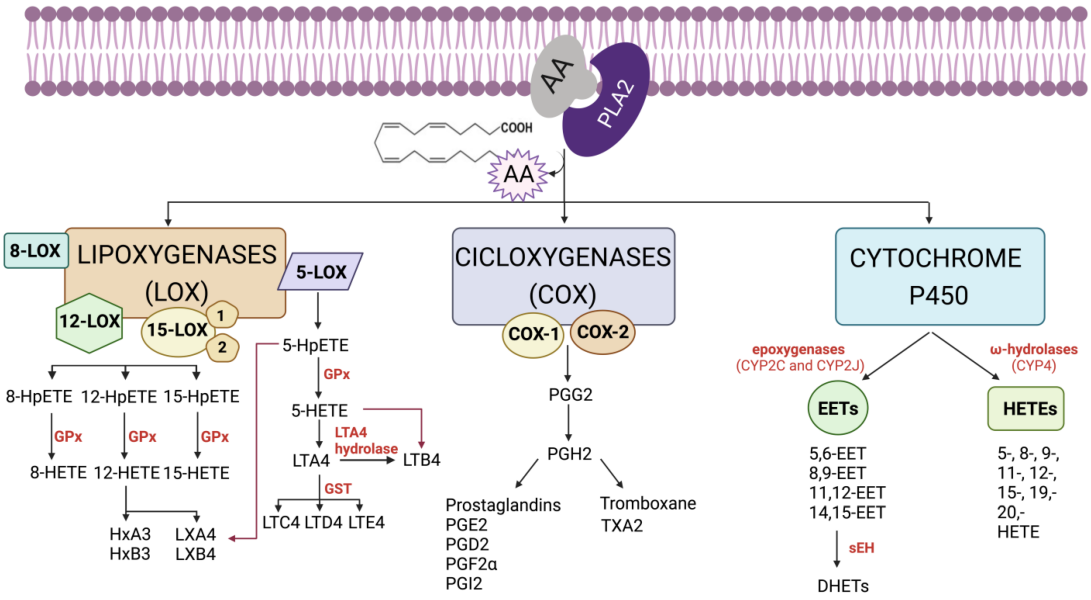

Figure 5.3 Arachidonic acid metabolism in response to cell damage. Image Source: (Fig 1) by Cándido Ortiz-Placín, Alba Castillejo-Rufo, Matías Estarás and Antonio González is used under a CC-BY 4.0 license.

Arachidonic acid signalling pathways begin following the influx of Ca^{2+} and activation of PLA2 which cleaves specific phospholipids and releases arachidonic acid from the SN2 position. Free arachidonic acid acts a substrate for many different proteins, which are mainly stratified into three classes, cyclooxygenases (**COX enzymes**), cytochrome P450 (CYP) enzymes, and lipoxygenases (LO enzymes). Arachidonic acid is oxidized into different molecules that are called eicosanoids and includes molecules such as prostaglandins and thromboxanes. Each of these molecules can have different effects, such as increased inflammation or platelet aggregation.

Inhibition of this pathway can occur at different points. At the top of the pathway is PLA2, and this enzyme is potently inhibited by steroids, which shuts down the inflammatory response. However, steroids are hormones, and they can have multiple effects throughout the body. Therefore, targeting enzymes lower in the biochemical response pathway such as arachidonic acid metabolizers represents a more selective strategy. The most common approach is inhibition of the COX enzymes which generate the prostacyclins, prostaglandins, and/or thromboxanes.

There are two key isoforms of the COX enzymes, referred to as COX1 and COX2. COX1 is shown to be constitutively active, albeit at low levels in multiple tissues, and helps maintain homeostasis. COX1 has important roles in maintaining the stomach lining, normal functioning of the kidneys, and platelet aggregation. COX2 is upregulated in response to inflammatory signals. Upon activation, both COX1 and COX2 are found at the cell membrane surface and are positioned in such a way that a long channel within the enzyme is oriented towards the membrane to accommodate any free arachidonic acid molecules. (Figure 5.4)

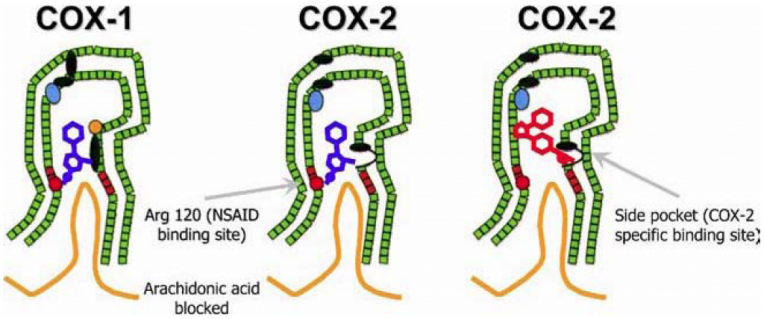

Figure 5.4 Schematics of COX1 and COX2 binding sites. Image Source: (Fig 1) by Inger L. Meek, Mart A.F.J. Van de Laar and Harald E. Vonkeman is used under a CC-BY 3.0 license.

When arachidonic acid is bound inside the COX enzymes, it is oxidized to form prostaglandin G2, which is converted into prostaglandin H2 and serves as a substrate for other enzymes. (Figure 5.5)

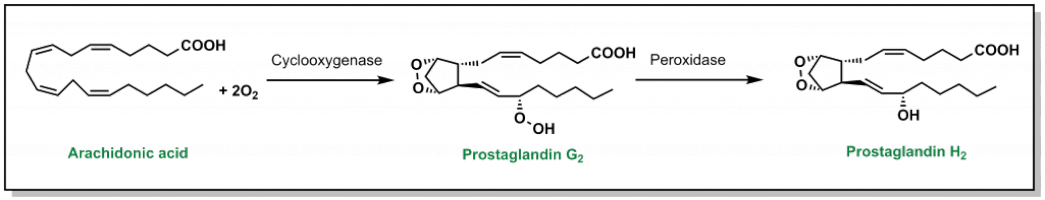

Figure 5.5 Oxidation of arachidonic acid by COX enzymes. The COX enzymes have allosteric side pockets that are sterically blocked by isoleucine residues in COX1, but available for binding in COX2.