Chapter 11

Clinical pharmacology of non-steroidal anti-inflammatory drugs: novel acquisitions

A. Sala, R. Ballerio, S. Viappiani, S. Zarini

Arachidonic acid metabolism and non-steroidal anti-inflammatory drugs; overview and novel perspectives

Prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), and hydroxyeicosatetraenoic acids (HETEs) are collectively referred to as eicosanoids because they are derived from 20-carbon essential fatty acids that contain three, four or five double bonds (i.e. 8, 11, 14-eicosatrienoic acid, 5, 8, 11, 14-eicosatetraenoic acid or arachidonic acid and 5, 8, 11, 14, 17-eicosapentaenoic acid). In humans, arachidonic acid represents the most abundant precursor.

Since the concentration of unesterified arachidonate in the cell is very low, the biosynthesis of eicosanoids depends primarily upon its availability to the eicosanoid-synthesizing enzymes. The first step is the liberation of arachidonic acid from membrane phospholipids, tightly regulated by acylhydrolases as a consequence of the interaction of hormones, autacoids or other substances with their receptors (or physical stimuli) which induce an elevation in cytosolic Ca$^{2+}$ [1].

Synthesis of PGs is accomplished in a stepwise manner by a ubiquitous complex of microsomal enzymes. The first enzyme, PGH synthase, possesses two distinct catalytic activities: it oxygenates and cyclizes unesterified arachidonic acid to form the cyclic endoperoxide PGG$_2$ (cyclo-oxygenase or COX activity) and it possesses peroxidase activity, reducing the –OOH group at position 15 of PGG$_2$ to OH in PGH$_2$ [2]. The endoperoxides are chemically unstable (t$_{1/2}$=5 min) and are transformed enzymatically into various products, including PGD$_2$, PGE$_2$, PGF$_{2\alpha}$, PGI$_2$, TXA$_2$. Whereas TX synthase and PGI synthase have been characterized, purified and cloned [3-6], the nature of the isomerases generating PGE$_2$, D$_2$ and F$_{2\alpha}$ is more elusive. The latter are relatively stable molecules, whereas TXA$_2$ (t$_{1/2}$=30 s) and PGI$_2$ (t$_{1/2}$=3 min) are chemically unstable and undergo non-enzymatic hydrolysis to TXB$_2$ and 6-keto-PGF$_{1\alpha}$, respectively. Although practically all tissues are capable of synthesizing the PGG$_2$/H$_2$ intermediates, their further metabolism varies in each tissue, depending on the specific enzymes that are present. For example, platelets contain TX synthase as the principal metabolizing enzyme of PGH$_2$ whereas endothelial cells contain primarily PGI synthase.
PGH Synthase-2 (PGHS-2, COX-2)

The oxygenation of arachidonic acid to PGH$_2$ is catalysed by two closely related enzymes, PGHS-1 and PGHS-2, also referred to as COX-1 and COX-2 [2]. Apart from red blood cells which do not make PGs or platelets that only contain COX-1, most nucleated cells have the gene for the inducible COX-2 which can be expressed in response to cell activation.

Human COX-2 has subsequently been cloned [7] and expressed. The primary sequence of the two isozymes presents approximately 60% homology between the deduced amino acid sequences within a given species. Mature COX-1 contains 576 residues compared to 587 for COX-2. COX-2 contains a C-terminal sequence of 18 amino acids, absent in COX-1; antibodies prepared against this peptide specifically identify COX-2 by western blotting.

The catalytic activities of the two COX isozymes are identical and all amino acids identified as important for catalysis in COX-1 are conserved in COX-2. The kinetic properties of the two isozymes are similar. However, the substrate binding sites exhibit subtle differences as COX-2 seems more permissive than COX-1 in its ability to transform 18-carbon polyunsaturated fatty acids [8] due to a somewhat larger, more accommodating, cyclo-oxigenase active site. Such differences may contribute to the development of highly selective inhibitors of the two isoforms [9].

PGH synthases are integral membrane proteins and they represent the first example of monotopic proteins (i.e. inserted only onto the inner membrane leaflet). The crystal structure of COX-1 suggests that three short amphipatic a-helices present in the amino-terminus of the enzyme interact with the membrane. Similar features have been found concerning the crystal structure of COX-2 [10]. COX-1 forms a dimer and these a-helices form two lipophilic poles which allow the protein to stand in the membrane. COX-1 appears to be located equally on the luminal side of the endoplasmatic reticulum and on the nuclear envelope, whereas COX-2 is slightly more abundant in the nuclear envelope.

Interactions of non-steroidal anti-inflammatory drugs with COX-1 and COX-2

Vane first demonstrated that aspirin inhibits the biosynthesis of prostaglandins [11]. A few months later, Smith and Lands reported that aspirin and indomethacin inhibited the oxygenation of arachidonic acid in a time-dependent manner [12]. Finally, in 1974, Roth and Majerus showed that $^{14}$C-acetylsalicylic acid selectively acetylated a platelet protein with a molecular weight of 70kDa [13]. This protein is now referred to as COX-1.