Metabolic pathways for diclofenac

Diclofenac (2-(2, 6-dichlo ranilino)phenyl acetic acid)

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) widely used to reduce inflammation and pain in conditions which include, but not limited to ankylosing spondylitis, arthritis, osteoarthritis and acute pain. Diclofenac, a phenyl acetic derivative, is a lipophilic and weakly acidic compound made up of two twisted aromatic rings.

Molecular structure:

Diclofenac exerts its pharmacological activity by non-selectively inhibiting cyclooxygenase (COX), an enzyme responsible for the conversion of the polyunsaturated fatty acid, arachidonic acid, into prostaglandins, thromboxanes and prostacyclins (Schweitzer et al., 2009). Prostaglandins are involved in maintenance of organ systems such as protection of the stomach wall or for the kidney function. They are also mediators of pain and inflammation.

The most commonly observed side effects of Diclofenac are gastrointestinal disturbances and these may include abdominal pain, constipation, flatulence, diarrhoea, dyspepsia, gross bleeding/perforation, heartburn, nausea, GI ulcers (gastric/duodenal) and vomiting. Other side effects that may occur include impaired renal function, anemia, dizziness, oedema, headaches, increased bleeding time and allergic reactions (pruritus, rashes and tinnitus).

Metabolism of Diclofenac

The metabolism of Diclofenac mainly takes place in the liver and involves two major pathways, oxidative metabolism and conjugation to glucuronic acid.

Metabolic pathways for Diclofenac (Vickers, 2008)

The oxidative metabolism (ring hydroxylation) of Diclofenac is catalysed by two enzymes of the cytochrome P450 family namely CYP2C9 and CYP3A4 (Boelsterli et al., 2007). This results in the formation of 4-hydroxydiclofenac and 5-hydroxydiclofenac as the major metabolites. A number of other mono- or dihydroxylated or methoxylated metabolites may also result. The 4-OH and the 5-OH metabolites of diclofenac can be further oxidized to a highly recative *p* -benzoquinone imine with great potential for redox cycling and hence oxidative stress. These metabolites are excreted from the body through the renal route.

Diclofenac is also conjugated to activated glucuronic acid (Uridine diphosphate glucuronic acid-UDPGA) in a reaction catalysed by UGT2B7 in humans and UGT2B1 in rats. This results in the formation of an acyl glucuronide which is a potentially reactive metabolite. The electrophilic carboxy carbon on the acyl glucuronide reacts with the sulfhydril group on proteins, forming covalent adducts. The acyl glucuronide can be further metabolized to 4_-OH-diclofenac acyl-glucuronide by CYP2C8, and subsequently to a benzoquinone imine which can pose oxidative stress to cells by redox cycling.

The conjugation of Diclofenac to acyl glucuronide (Boelsterli et al., 2007)

The acyl glucuronides are largely excreted from the body through the billiary system. The Diclofenac acyl glucuronide anions are transported from the hepatocytes into the biliary canaliculus by the canalicular anion transporter, multidrug resistace-associated protein (Mrp2).

The enterohepatic circulation of Diclofenac metabolites (Boelsterli et al., 2007)

During the transportation process, some of the most reactive metabolites will react to form covalent bonds with canalicular proteins and others in distal locations in the biliary tree. In the small intestines, a bacterial enzyme ß-glucuronidase, cleaves the acyl glucuronides to an aglycone which is readily reabsorbed. This phenomenon is referred to as enterohepatic cycling. ß-glucuronidase resistant iso-glucuronides are excreted. The overall exposure to Diclofenac and its metabolites is therefore increased.

Diclofenac-induced toxicities

The use of Diclofenac is often associated with certain toxicities, although some of them are not very common and therefore are not quite predictable. The major ones include gastrointestinal disturbances, hepatotoxicity and nephrotoxicity.

Gastrointestinal disturbances (dyspepsia and ulceration)

Diclofenac related gastrointestinal disturbances are thought to be related to its mechanism of action (Takeuchi et al., 2003). There are two important isoforms of the enzyme cyclooxygenase, COX-1 and COX-2. The inducible COX-2 is stimulated by tissue or cell injury to break down arachidonic acid to form prostaglandins involved in the regulation of pain and inflammation. The constitutive COX-1 is involved in the formation of prostaglandins and thromboxanes that take part in the normal tissue homeostasis and this include protection of the gastric mucosal lining from gastric acid. Upon inhibition of COX-1 by Diclofenac, the mucosal lining is left unprotected and disturbances may ensue. This may be related to the dose. Drugs with more selectivity towards the COX-2 isoform are safer in this regard.

Hepatotoxicity

The liver is highly susceptible to xenobiotic-induced toxic injuries because it is functionally interposed between the site of absorption and the systemic circulation, hence all drugs pass through the liver before reaching the systemic circulation. More so it is a major site of metabolism and elimination of foreign substances. Diclofenac is a generally safe drug within its therapeutic ranges, however its use can, in rare cases, result in severe hepatic injury (Boelsterli et al., 2003) Significant hepatotoxicity was also noted with the other pioneer NSAIDs and they were subsequently withdrawn from the market (Kaplowitz), for example benoxaprofen, piniprofen and fenclofenac to name but a few. Hepatoxicity is mainly characterised by jaundice, fatigue, anorexia nausea and vomiting. Liver toxicity is typical example of idiosyncratic drug toxicity because of liver injury is not a reproducible effect and lacks a simple dose-response relationship (Boelsterli et al. 2003).

Mechanism of Diclofenac induced liver injury

As with many xenoniotics, there is a casual link between the metabolism and binding of Diclofenac with its adverse effects and toxicities. The lipophilic nature of Diclofenac and its ability to form reactive metabolites (Diclofenac acyl glucuronides and the hydroxyl metabolites) are the features postulated to be associated with hepatotoxicity. Diclofenac is thought to induce liver damage through various mechanisms which may include acylation of hepatobiliary proteins, mitochondrial dysfunction, oxidative stress and immune response.

Acylation of hepatobiliary proteins (Boelsterli et al., 2007)

Diclofenac acyl glucuronides have electrophilic centers that can covalently bind with some proteins in the biliary tree. Most of the target proteins have sulfhydril groups.

The canalicular ectoenzyme, dipeptidyl peptidase IV (DPP IV) is an example of an important target protein for the acyl glucuronides. DPP IV is a multifunctional transmembrane glycoprotein and exopeptidase. DPP IV is more susceptible as a target protein for the acyl glucuronides because:

- It contains many sulfhydryl goups, making it a good target for the acyl glucuronides.
- DPP IV is located very close to the Mrp2, the pump responsible for the vectorial transport of the conjugates.
- The acyl glucoronides are highly concentrated in the canaliculus by the Mrp2 protein
- The slightly alkaline pH in bile favours hydrolysis of the acyl glucuronides, hence their possible reactions with target proteins.

A possible link between covalent binding and toxicity was delineated in rats, where inhibition of Diclofenac metabolism using the general CYP inhibitors greatly reduced hepatotoxicity (Vickers, 2008).

The mechanism of DPP IV covalent binding with Diclofenac acyl glucuronide(Boelsterli et al., 2007)

Mitochondrial Dysfunction (Boelsterli et al., 2003)

Mitochondrial dysfunction has long been implicated as a primary indicator of hepatotoxicity (Vickers, 2008). Diclofenac can act as a protonophoretic compound thereby uncoupling the electron transport chain. This dissipates the proton gradient required for ATP production. Diclofenac and its

metabolites may also produce oxidative stress which may affect mitochondrial membrane permeabilization (increased permeability of mitochondria outer membrane and opening of the mPT) (Woen Ping Siu et al, 2008). The formation of diclofenac cation radicals and quinone imine associated redox cycling produces a lot of oxidative stress to cells leading to Diclofenac toxicity. When rats and human liver cells were treated with Diclofenac, an increase in the expression of hemeoxygenase 1 (*Hmox1*) was noted in line with changes in the redox state and induction of oxidative stress (Vickers, 2008). These events lead to the release of pro-apoptotic proteins and bursting of the outer membrane.

Immune response

Some clinical features noted in some patients provided the evidence that the immune-allergic reactions are involved in Diclofenac-induced liver injury (Boesterli et al 2003). These include allergy symptoms, presence of IgM antibody, and hypersensitivity reaction in an inadvertent rechallenge to Diclofenac. All these are pointing towards immune response as a possible mechanism for Diclofenac-induced hepatotoxicity, however the real mechanism is still elusive.

Nephrotoxicity

Diclofenac is increasingly being associated with renal toxicity. Diclofenac-induced renal toxicity in humans has been cited in literature (Lin et al. 2008). In veterinary medicine, marked decreases in population of certain vulture species were attributed to ingestion of carcases contaminated with Diclofenac residues (Swan et al., 2006, Naidoo et al. 2007, Lin et al. 2008, Naidoo et al., 2009). In all the cases, the vultures died due to renal failure.

Most studies attributed this to high plasma uric acid levels and the production of ROS (Swan et al., 2006, Naidoo et al., 2007, Naidoo et al., 2009a, 2009b,). Diclofenac is said to inhibit the the p-amino-hippuric acid (PAH) channel and subsequently the transport of uric acid (Naidoo et al. 2009a). The resulting accumulation of uric acid in blood causes deleterious effects such as gout and increased intracellular ROS upon prolonged Diclofenac use (uric acid is an intracellular antioxidant).

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