



ISSN: 2231-3354
 Received on: 28-12-2011
 Revised on: 05-01-2012
 Accepted on: 10-01-2012

Toxicopathological overview of analgesic and anti-inflammatory drugs in animals

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ABSTRACT

Non-Steroidal Anti Inflammatory Drugs (NSAIDs) have been commonly used in both human and veterinary medicine to reduce pain and inflammation in different arthritic and postoperative conditions due to their three major activities, viz., anti-inflammatory, antipyretic, and analgesic. Phenylbutazone, diclofenac, meloxicam and some other NSAIDs are being used as therapeutic measures for pain, inflammation and fever in clinical veterinary medicine. Anti-inflammatory effect is mainly due to their ability to inhibit the activities of cyclooxygenases, enzymes those mediate the production of prostaglandins from arachidonic acid, a dietary fatty acid. Cyclooxygenase- 2 (COX-2) is the inducible form of the enzyme and is involved in inflammation. The widespread use of NSAIDs has meant that the adverse effects of these drugs have become increasingly prevalent. The two main adverse drug reactions (ADRs) associated with NSAIDs relate to gastrointestinal (GI) effects and renal effects of the agents. The most common is a propensity to induce gastric or intestinal ulceration that can sometimes be accompanied by anemia from the resultant blood loss. Large variety of compounds with similar actions but varying kinetics in different species thus varying risk of toxicity and variety of toxic effects. Review research relating to these adverse drug reactions, focusing on histopathological findings, which may contribute to the comprehension and possible avoidance of drug-induced disease.

Keywords: Analgesic, Anti-inflammatory drugs, Non-steroidal anti-inflammatory drugs, Toxicopathology.

INTRODUCTION

Analgesic and anti-inflammatory relieves mild to moderate pain, and reduces inflammation and fever. These agents are effective for somatic pain (e.g. musculoskeletal pain in joints, muscle and head ache). They are not effective in reducing discomfort from visceral organ (Heart, liver and lung). All these analgesic and anti inflammatory agent produce their therapeutic effects by inhibiting various prostaglandins substances involved in development of pain and inflammation as well as regulation of body temperature. Due to extensive use of analgesic and anti inflammatory agents, the toxicity and untowards effects do occurs many times especially when therapy of pain, inflammation and fevers involves use of higher dose for longer period. The common organs involved are liver, kidney and GIT. It has been estimated that 1 in 5 chronic users (lasting over a long period of time) of NSAIDs will develop gastric damage which can be silent (Rang *et al.*, 1999). The various approach used to assess the safety of such drug in patient are under practice. These includes biochemical analysis, haematological analysis, urine analysis and histopathological evaluation of various organs involved in toxicities. Biochemical, heamatological and urine analysis are primary tools used for evaluation of toxicity of analgesic and anti-inflammatory drugs.

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They indicate functional damage while toxicopathology helps to evaluate structural damage and tendency of pathological lesions caused due to toxicity of drug. Toxicopathology evaluation is many time essential for regulatory compliance. Due to this the significance of toxicopathological evaluation of analgesic and anti inflammatory agents is of paramount importance. Apart from regulatory compliance toxicopathology analysis helps to understand cellular and sub cellular mechanism of action of analgesic and anti inflammatory drugs. It also helps to understand relationship between quantitative and qualitative alteration caused due to excessive dose of drugs. The present review is focused on toxic pathological evaluation of analgesic and anti inflammatory drug.

CLASSIFICATION OF NSAIDs

Depending on their chemical structures, NSAIDs are broadly divided into two major classes like, non selective COX inhibitors and selective COX-2 inhibitors (Vane *et al.*, 1998).

Classification based on chemical nature

(I) Non-Selective COX Inhibitors

- Salicylic acid derivatives.
e.g. Aspirin, Sodium salicylate.
- Para-amino phenol derivatives.
e.g. Acetaminophen.
- Indol and Indane acetic acids.
e.g. Indomethacin, Sulindac.
- Heteroaryl acetic acids.
e.g. Tolmetin, Diclofenac, Keterolac.
- Aryl propionic acids.
e.g. Ibuprofen, Naproxen, Flurbiprofen, Ketoprofen, Fenoprofen, Oxaprofen.
- Anthranilic acids.
e.g. Mefenamic acid, Meclofenamic acid.
- Enolic acids (Oxicams)
e.g. Piroxicam, Meloxicam, Tenoxicam, Isoxicam.
- Alkanones.
e.g. Nabumetone.

(II) Selective COX-2 Inhibitors

- Indole acetic acids
e.g. Etodolac.
- Sulfonanilides
e.g. Nimesulide.
- Diaryl substituted furanones
e.g. Rofecoxib.
- Diaryl substituted pyrazoles
e.g. Celecoxib.

Classification based on mode of inhibition of COX

Class I: Simple, competitive reversible inhibition that competes with arachidonic acid for binding to the COX site.
e.g. Ibuprofen, Piroxicam, Sulindac, Meclofenamic acid

and Naproxen.

Class II: Competitive, time dependent reversible inhibitors that bind to the COX active site in the first phase to form reversible enzyme inhibitor complex.
e.g. Flurbiprofen, Diclofenac

Class III: Competitive, time dependent, irreversible inhibitors that form an enzyme inhibitor complex.
e.g. Aspirin

Classification on the basis of selective inhibition of COX

(I) Selective COX inhibitors

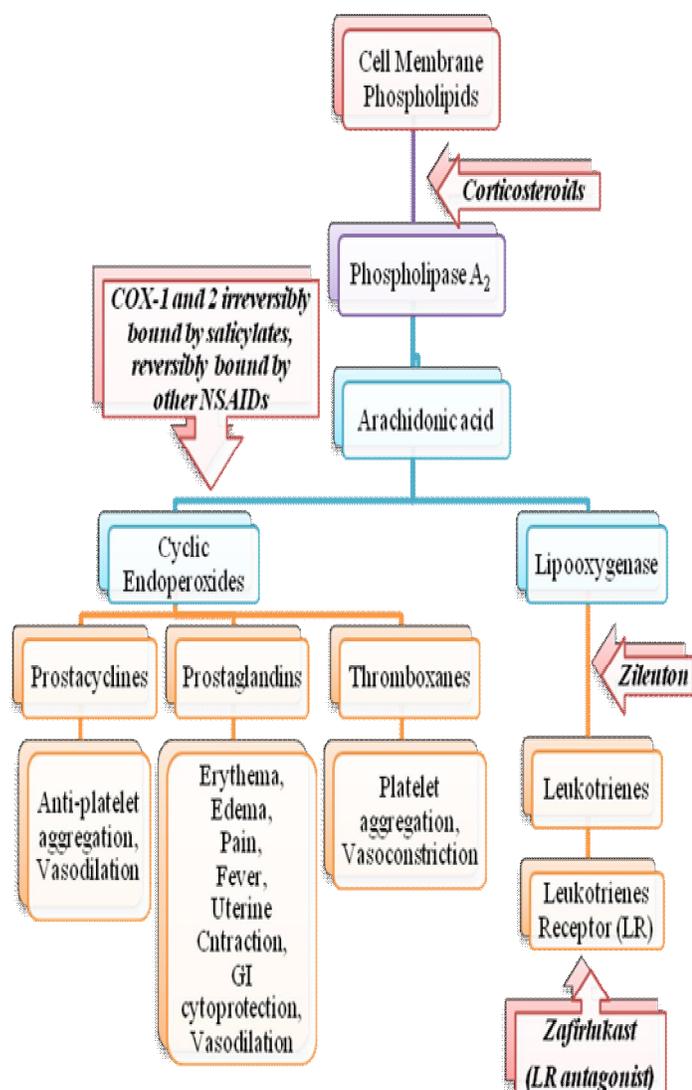
- Salicylates.
Acetylated: Aspirin.
Non acetylated: Salsalate, Trisalicylate.
- Nonsalicylates.
- Non selective COX-1/COX-2 or Traditional NSAIDs: Ibuprofen, Indomethacin, Naproxen, Silindac, Ketoprofen, Ketorolac, Fenoprofen, Diclofenac, Piroxicam, Oxaprazin
- Semi selective NSAIDs: Meloxicam, Etodolac, Nabumatone.
- COX-2 selective Inhibitor: Celecoxib, Rofecoxib, Enteroxib.

MECHANISM OF ACTION OF NSAIDs

All of the non-steroidal anti-inflammatory drugs (NSAIDs) appear to share at least one common mechanism, namely inhibition of cyclo-oxygenase (COX) enzyme(s) which leads to a decrease in the synthesis of various prostaglandins and thromboxanes. The main mechanism of action of NSAIDs was clarified by Sir John Vane in 1971.

He noted the inhibition by aspirin, of prostaglandin synthesis. Around 1972, the mechanism of action of NSAIDs was proposed that it works by inhibiting the cyclooxygenase enzymes (COX) or prostaglandin G/H synthase and subsequent prostaglandin formation (Vane, 1997). It was revealed that both their therapeutic benefits and toxicity are related to their ability to inhibit the COX.

It was noted that cells synthesized prostaglandins in response to tissue injury. Inhibition of these prostaglandins inhibited inflammation. Whilst the prostaglandin hypothesis is generally accepted as being the most important, we now know that the mechanisms are more complex (Abramson and Weissmann, 1989) and involve additional non-prostaglandin dependent pathways. In the prostaglandin mediated process, cyclooxygenase metabolises arachidonic acid to form prostaglandin endoperoxides, (Figure 2). These include the prostaglandins, prostacycline and thromboxane. Blocking of the cyclooxygenase enzyme results in a reduction in the formation of prostaglandins. The prostaglandins in fact are both physiologically important and potentially pathologically harmful. PGE₂ is the principal eicosanoid formed from arachidonic acid. It is a crucial mediator of inflammatory changes (Portanova *et al.*, 1996).



Prostaglandins are powerful signaling agents in the human and animal body. Prostaglandins are substantially involved in bringing about and maintaining inflammatory processes by increasing vascular permeability and amplifying the effects of other inflammatory mediators such as kinins, serotonin and histamine (Maundrell *et al.*, 1997). Hawkey (1999) stated that COX has a long hydrophobic channel that provides the site of binding for NSAIDs. Celotti and Laufer (2001) stated that COX-2 is known to be the dominant isoenzyme in the inflamed tissues where it is induced by a number of cytokines, including interleukin-1, tumor necrosis factor alpha (TNF- α) and bacterial toxins.

ROLE OF COX ENZYMES IN PATHOPHYSIOLOGY OF NSAIDS

Broadly speaking, anti-inflammatory and analgesic drugs are Non Steroidal Anti inflammatory drugs (NSAIDs). More than 20 drugs fall under the category of NSAIDs.

All NSAIDs produces anti-inflammatory effects by inhibiting cyclooxygenase enzymes which catalyses formation of prostaglandins and thromboxane from arachdonic acid. Exception to this is salicylic acid and acetaminophen which inhibits COX enzymes irreversibly for entire cell life and centrally respectively.

Two isoforms of cyclooxygenase have been identified. COX-1 has been proposed to generate prostaglandins that maintain organ function, protect the integrity of the gastric mucosa, and generate platelet-derived thromboxane responsible for platelet aggregation and vasoconstriction. COX-1 is expressed in all tissues, whereas COX-2 is induced during the inflammatory response and produces prostaglandins that mediate pain and inflammation.

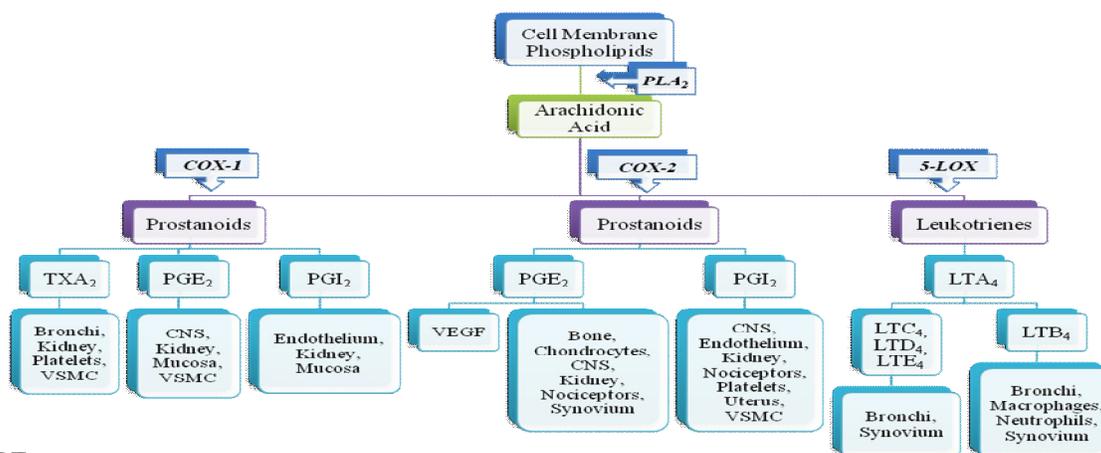
COX-2 is also expressed in kidneys and vascular endothelium. Classic, older NSAIDs (e.g, Ibuprofen) inhibit COX-1 more than COX-2, whereas the newer class of NSAIDs (e.g, Celecoxib, Valdecoxib, Rofecoxib) inhibit COX-2 predominately, decreasing gastrointestinal adverse effects. Selectivity of inhibition may be lost during overdose. The NSAID-induced COX inhibition causes decreased production of prostaglandins, which leads to decreased pain and inflammation. CNS, hemodynamic, pulmonary and hepatic dysfunction may occur with certain agents, but the relationship to prostaglandin production remains uncertain. Prostaglandins are involved in maintaining GI mucosal integrity as well as regulating renal blood flow and both acute and chronic toxicity often involves the GI and renal systems.

TOXICOPATHOGENESIS OF NSAIDs TOXICITY

NSAIDs inhibit enzymes activity of cyclooxygenase I & II leading to reduced synthesis of various inflammatory mediators. Based on selectivity of NSAIDs for cyclooxygenase enzymes, they are classified into (i) Conventional COX inhibitors which inhibit both enzyme COX1 and COX II. (ii) Novel COX inhibitors or Novel COX inhibitors which selectively inhibit COXII only (Albertsey, 2002). Both the COX-1 and COX-2 enzymes serve important homeostatic roles in many body systems. The inhibition of these enzymes produces the therapeutic benefits as well as the toxicities associated with NSAIDs. COX-1 is a constitutive enzyme that plays an important role in maintaining renal and gastrointestinal blood flow as well as ensuring platelet integrity. COX-2 has traditionally been thought to be an inducible enzyme that is upregulated in inflammatory disease, however, constitutive COX-2 expression has recensantly been shown in the renal and central nervous systems (Bergh and Budsberg, 2005).

Figure: Metabolic pathway of arachidonic acid and examples of some target tissues of the prostanoids and leukotrienes.

PLA₂ = Phospholipase A₂, COX = cyclooxygenase, 5-LOX = 5-Lipoxygenase, TXA₂ = Thromboxane A₂, PGE₂ = Prostaglandin E₂, PGI₂ = Prostaglandin I₂, LT= Leukotriene (A₄, B₄, C₄, D₄, E₄), VSMC = Vascular Smooth Muscle Cells, CNS = Central Nervous System, VEGF = Vascular Endothelial Growth Factor



NIMESULIDE

Nimesulide is a newer non-steroidal anti-inflammatory agent (NSAID) with additional anti-pyretic and analgesic activity. It is a NSAID of the sulfonilide class and is chemically 4-nitro-2-phenoxy-methanesulfonilide. The therapeutic efficacy of these drugs is usually due to reduction in prostaglandin levels. However, this effect is also responsible for the inhibition of gastroprotective prostaglandins leading to gastrointestinal intolerance. Potential hepatotoxicity of nimesulide is not uncommon phenomenon in human and veterinary clinical setup (Rainsford, 2006). These liver associated side effects have led much debate on safety of the drug and finally the withdrawal of nimesulide from the market in some countries. Multiple ulcers of varying sizes and shapes with haemorrhages and histopathologically, necrosis of mucosa involving superficial layers with intact glandular portion, tunica muscularis and serosal layers were evident in stomach of dogs treated with nimesulide. Other NSAIDs like aspirin have resulted in gastric ulceration in the dog (Lipowitz *et al.*, 1986).

But, the induction of gastric ulcers by nimesulide was unusual in that nimesulide rarely induces gastric ulcers in man at similar dose and duration of administration. A significant increase in BUN values in the nimesulide group with non significant histopathological findings were common findings in toxicity study. However, clinical dose of flunixin was found to be associated with acute renal failure in dogs. Affected kidneys showed the presence of both COX I and COX II. Inhibition of prostaglandins causes renal ischemia and leads to nephropathy (Vane and Botting, 1994). Nimesulide at the dose of 2 mg/kg *b.i.d.*, nimesulide was found to be toxic in dogs producing gastric ulcers and mild nephrotoxicity on a four day course of administration. A fifty seven year-old patient with jaundice and increased serum aminotransferases levels, when treated with nimesulide for 10 days. Liver biopsy of jaundice patient treated with nimesulide revealed lesions comparable with drug-induced hepatotoxicity (Cholongitas *et al.*, 2003). It has been recently linked with rare but serious and unpredictable adverse reactions in the liver, such as fulminant hepatic failure. It is thought that the hepatotoxicity induced by nimesulide is of the idiosyncratic type. In addition, the weakly acidic sulfonanilide (nimesulide) drug undergoes bioreductive metabolism of the nitroarene group to form reactive intermediates that have been implicated in oxidative stress,

covalent binding and mitochondrial injury (Boelsterli, 2002). Tian *et al.* (2002) investigated the effect of nimesulide on the proliferation and apoptosis of SMMC-7721 human hepatoma cells. Under the electron microscope, these cells exhibited characteristics of apoptosis, including plasma membrane blebbing, cytoplasmic condensation, pyknotic nuclei, condensed chromatin and apoptotic bodies. Compared with control groups, groups treated with 300 $\mu\text{mol/L}$ and 400 $\mu\text{mol/L}$ nimesulide had many more cells with apoptotic characteristics. This observation is of interest because of the putative antitumor effect of NSAIDs.

IBUPROFEN

Ibuprofen is (2*RS*)-1[4-(2-methyl propyl) phenyl] propionic acid (BP, 2004). It was the first member of propionic acid derivatives to be introduced in 1969 as a better alternative to Aspirin. It is the most commonly used and most frequently prescribed NSAID (Abraham and KI KD, 2005; Bradbury, 2004). It is a non-selective inhibitor of COX-1 and COX-2 (Chavez *et al.*, 2003). Although its anti-inflammatory properties may be weaker than those of some other NSAIDs, it has a prominent analgesic and antipyretic role. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation and subsequent heat dissipation. Gastric discomfort, nausea and vomiting, though less than aspirin or indomethacin, are still the most common side effects (Tripathi, 2003). Ibuprofen exhibits few adverse effects (Bateman, 1994). The major adverse reactions include the effects on the gastrointestinal tract (GIT), the kidney and the coagulation system (Rocca *et al.*, 2005). Based on clinical trial data, serious GIT reactions prompting withdrawal of treatment because of hematemesis, peptic ulcer (Tsokos and Schmoltdt, 2001) and severe gastric pain or vomiting showed an incidence of 1.5% with ibuprofen compared to 1% with placebo and 12.5 % with aspirin (Dollery, 1999). Ibuprofen was a potential cause of GI bleeding (Wolfe *et al.*, 1888; Oermann *et al.*, 1999). Increasing the risk of gastric ulcers and damage, renal failure, epistaxis (Gambero *et al.*, 2005; Kovesi *et al.*, 1998), apoptosis (Durkin *et al.*, 2006), heart failure, hyperkalaemia (Vale and Meredith, 1986), confusion and bronchospasm (Rossi, 2004) have been reported side effects of the drug. Less frequently reported side effects includes thrombocytopenia, rashes, headache, dizziness,

blurred vision and in few cases toxic amblyopia, fluid retention and edema. Patients who develop ocular disturbances should discontinue the use of ibuprofen (Burke *et al.*, 2006). Nephrotoxic side effects of ibuprofen are very rare but do occur and include acute renal failure, interstitial nephritis, and nephritic syndrome (Wagner *et al.*, 2004). Patients with hepatitis C may show increased serum transaminase concentrations when treated with ibuprofen. Ibuprofen is one of several drugs responsible for vanishing bile duct syndrome reported in children and also in adult with chronic rejection after transplantation (Mayoral *et al.*, 1999). More severe reactions, such as subfulminant hepatitis requiring liver transplantation following ibuprofen overdose, although very rare, have also been described (Laurent *et al.*, 2000).

KETOPROFEN

Ketoprofen, 2-(benzoyl-3-phenyl) propionic acid belongs to the class of arylpropionic acids of non-steroidal anti-inflammatory drugs. Like most NSAIDs, ketoprofen is advantageous because it lacks addictive potential and does not result in sedation or respiratory depression. Ketoprofen also exerts analgesic and antipyretic pharmacological properties (Green, 2001). It is having distinct advantage of being non addictive and non respiratory suppressant.

Ketoprofen is a chiral compound having two enantiomers namely S(+) and R(-). The toxicity profile and potency varies for both the types of isomers. Cabre *et al.* (1998) examined the intestinal ulcerogenic effects of single oral doses of S(+)-ketoprofen compared with racemic ketoprofen in the small intestine and caecum of rats. S(+)-ketoprofen had no significant ulcerogenic effect at 10 or 20 mg/kg. However, racemic ketoprofen was clearly ulcerogenic in the small intestine and caecum at the 40 mg dose. R(-)-ketoprofen at 20 mg/kg does not show any effect in the caecum and only limited ulcerogenesis in the small intestine.

Evaluation of intestinal damage and activities of oxidative stress related enzymes such as myeloperoxidase (MPO), xanthine oxidase (XO) and superoxide dismutase (SOD) in an experimental rats model revealed dose dependent occurrence longitudinal ulcers on the mesenteric side of the middle and lower intestine lumen. The intestinal toxicity caused by S(+)-ketoprofen was significantly lower when compared to racemate and R(-) enantiomer treatments Lastra *et al.* (2000). Shinichi *et al.*, (2002) studied the gastric ulcerogenic response to indomethacin, rofecoxib and celecoxib. Oral administration of indomethacin (3, 10, and 30 mg/kg) caused hemorrhagic lesions in the gastric mucosa of normal rats in a dose-dependent manner.

The gastric ulcerogenic response to indomethacin was markedly aggravated in arthritic rats, and severe lesions were observed even at the dose of 3 mg/kg. On the other hand, neither rofecoxib (3, 10, 30, and 100 mg/kg) nor celecoxib (100 mg/kg) induced any damage in the stomach of normal rats even at the highest dose (100 mg/kg). In arthritic rats, however, these drugs also provoked gastric lesions at the dose of 10 mg/kg or greater, similar to indomethacin, and for rofecoxib the severity of the lesions increased dose dependently.

MELOXICAM

Meloxicam is widely recognized as being one of the first commercially available selective COX-2 NSAIDs (Ogino *et al.*, 1996), has greater *in vitro* and *in vivo* inhibitory action against the inducible form of COX-2, which is implicated in inflammatory response, than against constitutive form of this enzyme COX-1 (Barners *et al.*, 1998). Meloxicam has anti-inflammatory effect better than other NSAIDs in animal models and a greater therapeutic ratio. Meloxicam has analgesic and antipyretic properties and is successfully used in cattle, buffaloes, goats, sheep, pigs, horses and dogs. Hakan, *et al.* (2008) investigated the effect of meloxicam on a rat femoral artery vasospasm model. Rats were randomly separated into subarachnoid haemorrhage (SAH), SAH + meloxicam and control groups. Rats in the SAH + meloxicam group were given meloxicam at 2 mg/kg daily for 7 days. Meloxicam treatment at the dose rate of 2 mg/kg for 7 days reduced ultrastructural and morphometric vasospastic changes.

DICLOFENAC

Diclofenac, a 2-arylacetic acid, nonsteroidal anti-inflammatory drug is widely used in human and veterinary medicine. It is known hepatotoxic drug in certain individuals. Diclofenac also causes deposition of urates crystals in kidneys, liver, heart and spleen. Acute hepatotoxic effects may be due to reduced / impaired ATP synthesis. But detailed toxicity study reveals that it doesn't induce significant oxidative stress or increase in intracellular calcium concentration at early stage. Additionally it causes cytotoxicity more extensively in drug metabolizing cells than to non metabolizing cells in in-vitro study using rat and human primary culture hepatocyte. Apart from these facts, the exact pathophysiology of cytotoxicity remains unexplained. Due to high gastric or hepatic and renal toxicity, the use of diclofenac declined (Green *et al.*, 2004; Oaks *et al.*, 2004 and Shultz *et al.*, 2004). Recently the environmental issues of declining vulture population due to use of diclofenac in veterinary medicine requires to be mentioned here. The wide spread use of diclofenac in treating fever and inflammation in live stock in South Asian and African countries exposed the many species of vulture to high level of diclofenac in carcasses. The exposed birds showed the nephrotoxicity and visceral gout (Oaks *et al.*, 2004; Shultz *et al.*, 2004). Major species affected are *G.bengalensis* and *G. indicus*. Diclofenac has been identified as a risk for three species of vultures in the Indian sub-continent, but diclofenac, as well as other NSAIDs, may pose a danger to five other *Gyps* vultures found in Asia, Europe and Africa (IUCN, 2004). Diclofenac induced hepatotoxicity and nephrotoxicity showed distinct patterns of histopathological lesions. Histopathologic changes in the liver sections showed cloudy swelling and hydropic degeneration of the liver cells, focal sinusoidal and vena centralis dilatation, proliferation of the bile duct in portal areas, enlargement of the periportal area with mononuclear cell infiltration, hyperemia and dose-dependent fibrous tissues proliferation and focal necrosis. Cloudy swelling and hydropic degeneration were seen in the tubular epithelial cells of the kidney tissue. Necrosis, peritubular

lymphocyte infiltration, stromal fibrous tissue proliferation and hyperemia were observed in rats. In the liver and kidney tissue of the high dose group, which was given a high dose of diclofenac sodium, necrosis, cloudy swelling and hydropic degeneration and inflammation were rather widespread and intensive, as compared to the group given a low dose. The increase in fibrous tissue in the kidney and liver that caused irregularities in the periportal areas was only seen in the diclofenac given at a high dose. High dose of diclofenac sodium causes meaningful changes in liver and kidney tissue (G.Isen aydin *et al.*, 2003). The scarce side effects of diclofenac are significant because of its worldwide use by many patients. Therapeutic use of diclofenac is associated with rare but sometimes fatal hepatotoxicity characterized by delayed onset of symptoms and lack of a clear dose-response relationship. The toxicity has consequently been categorized as metabolic idiosyncrasy. In fact, the acyl glucuronide of the drug has been demonstrated to be reactive and capable of covalent modification of cellular proteins, binding covalently to liver proteins in rats depending on the activity of multidrug resistance protein 2, a hepatic canalicular transporter (Tang, 2003) from a toxicodynamic point of view, both oxidative stress (caused by putative diclofenac cation radicals or nitroxide and quinone imine-related redox cycling) and mitochondrial injury (protonophoretic activity and opening of the permeability transition pore) alone or in combination, have been implicated in diclofenac toxicity. In some cases, immune-mediated liver injury is involved, as inferred from inadvertent rechallenge data and from a number of experiments demonstrating T-cell sensitization. Caspases 8 and 9 are apparently active caspases in diclofenac-induced apoptosis. In addition, an early dose-dependent increase of Bcl-X L expression parallel to the generation of reactive oxygen species in the mitochondria was found. In conclusion, the mitochondrial pathway is very likely the only pathway involved in diclofenac-induced apoptosis, which is related to CYP-mediated metabolism of diclofenac, with the highest apoptotic effect produced by the metabolite 5OH-diclofenac. Till date, cumulative damage to mitochondrial targets seems a plausible putative mechanism to explain the delayed onset of liver failure, perhaps even superimposed on an underlying silent mitochondrial abnormality (Tang, 2003). Diclofenac induces apoptosis by alteration of mitochondrial function and generation of reactive oxygen species (ROS) (Gomez-Lechon *et al.*, 2003), liver injury could not be reproduced in current animal models. Nevertheless, it is noteworthy that ultrastructural damage was found in the liver of rainbow trout exposed to various concentrations of diclofenac, thus illustrating differences in reactivity between mammals and other vertebrates (Triebkorn *et al.*, 2004).

INDOMETHACIN

Indomethacin, a methyl indole derivative was introduced in 1963. The anti-inflammatory action of indomethacin is due to inhibition of vasodilator prostaglandin E2 and prostaglandin I, synthesized from arachidonic acid through COX pathway by inhibiting COX-1 and COX-2 (Tanaka *et al.*, 2001). The inhibition

of COX, change in the mitochondrial function and free radical induced oxidative changes; all contribute to its anti-inflammatory action. Toxicity were observed in stomach pylorus and proximal duodenum. In the stomach pylorus, well defined superficial ulcers were identified of drug administration. The ulcer penetrated as far as muscularis mucosae and ulcer bed had coagulative necrosis and inflammatory cells. Stomach pylorus showed minor damage in the form of focal necrosis. Duodenum was affected less than stomach and showed villi with lost tips, tilted and distorted villi. Morphometric analysis showed changes in stomach pylorus and in duodenum. The number of mitotic figure was significantly increased in stomach pylorus. Duodenum showed insignificant to significant decrease in the height of villi. Increase in the number of goblet cells, columnar cells, and mitotic figure was also noted; which was undoubtedly part of the tissue response to an injury (Maqbool *et al.*, 2006). The incidence of liver injury associated with indomethacin appears to be low, although exact figures are not available. The hepatotoxicity usually develops within the first few months of treatment, sometimes in association with manifestations of hypersensitivity, and are fatal in rare instances. The histological findings range from an acute viral-like hepatitis to confluent or multilobular necrosis; chronic active hepatitis with cholestasis and fatty changes are also occasionally observed. The cells exposed to high concentrations of indomethacin were severely damaged, as evidenced by marked cellular necrosis, nuclear pleomorphism, margination of chromatin, swollen mitochondria, and reductions in the number of microvilli, smooth endoplasmic reticulum proliferation, and cytoplasmic vacuolation (Sorensen and Acosta, 1985).

CELECOXIB

Celecoxib, a specific COX-2 enzyme inhibitor, has been widely used to alleviate pain and inflammation in osteoarthritis and rheumatoid arthritis. It has minimal gastrointestinal, platelet, and renal side effects, but has been associated with acute hepatocellular and cholestatic injury. Gastrointestinal (GI) side effects have included nausea (3.5% to 9.09%), upper abdominal pain (7.32% to 10.4%), dyspepsia (2.8% to 8.8%), abdominal pain (1.3% to 8.5%), vomiting (less than or equal to 7.3%), diarrhea (4.9% to 10.5%), gastroesophageal reflux (4.7%), and flatulence (2.2%). Serious GI toxicity such as GI hemorrhage and ulceration have been reported and possibly linked to some deaths. Six cases of abdominal pain and swelling were also reported. Some studies have shown a decrease in gastroduodenal endoscopic ulcers with celecoxib in comparison to a traditional NSAID. These ulcers are generally asymptomatic. (Nachimuthu *et al.*, 2001). In the Celecoxib Long-term Arthritis Safety Study (CLASS) the estimated cumulative incidence of upper GI complicated and symptomatic ulcers among non-aspirin users (n equals 3105) given celecoxib 400 mg twice a day was 0.78% at nine months. Cardiovascular side effects including increased blood pressure have been reported in 3.9% to 13.4% of patients. Safety data from the Celecoxib Long-term Arthritis Safety Study (CLASS) in which patients with osteoarthritis and rheumatoid arthritis were allowed to take

concomitant low-dose aspirin (less than or equal to 325 mg/day) for cardiovascular prophylaxis indicated there were no significant differences between treatment groups (celecoxib vs. ibuprofen vs. diclofenac) in the overall incidence of serious CV thromboembolic adverse events, such as heart attack, stroke, and unstable angina (Lee *et al.*, 2007). Fatal and nonfatal cardiovascular events have been observed in a large colorectal cancer prevention trial Adenoma Prevention with Celecoxib (APC) conducted by the National Institute of Health (NIH). Hepatic side effects including abnormal liver function tests and increased transaminase have been reported in 3.9% to 13.4% of patients. Increased SGOT, SGPT, and alkaline phosphatase have been reported in less than 2% of patients. Jaundice, liver failure, hepatitis, and cholelithiasis have been reported in less than 0.1% of patients. Rarely, cholestatic hepatitis has been reported. At least once case of celecoxib-induced combined hepato-nephrotoxicity has been reported. Renal side effects have included increased blood creatinine phosphokinase (3.9% to 13.4%), increased BUN (less than 2%), and increased creatinine (less than 2%). Acute renal failure has been reported in less than 0.1% of patients. Acute interstitial nephritis (less than 0.1%), and membranous glomerulopathy have been reported during postmarketing experience. At least one case of renal papillary necrosis has been reported, in addition to a case of celecoxib-induced combined hepato-nephrotoxicity (Mukherjee *et al.*, 2001) Results of an *in vitro* study indicate that celecoxib may inhibit proliferation and induce apoptosis in human cholangiocarcinoma cells through its effect on COX-dependent mechanisms and the PGE2 pathway. Celecoxib as a chemopreventive and chemotherapeutic agent may be primarily effective for COX-2 expressing cholangiocarcinoma, but may also be effective for other tumors as well (Koki and Masferrer, 2002).

NAPROXEN

In subacute and chronic oral studies with naproxen in a variety of species, the principal pathologic effect was gastrointestinal irritation and ulceration. The lesions seen were predominantly in the small intestine and ranged from hyperemia to perforation and peritonitis. Nephropathy was seen occasionally in rats, mice and rabbits at high dose levels of naproxen. In the affected species the pathologic changes occurred in the cortex and papilla. Chronic administration of high doses of naproxen to mice appears to be associated with exacerbation of spontaneous murine nephropathy. Histopathologically the affected glands in some instances exhibited atrophic and/or hypoplastic changes characterized by decreased secretory material. (Naprosyn[®], 2008) The borderline abnormalities in one or more liver tests may occur in up to 15% of patients when treated with naproxen. The ALT test is probably the most sensitive indicator of liver dysfunction. Meaningful (3 times the normal upper limit) elevations of ALT or AST occurred in controlled clinical trials in less than 1% of patients. Severe hepatic reactions, including jaundice and cases of fatal hepatitis, have been reported with naproxen as with other NSAIDs (Giarelli *et al.*, 1987). Although such reactions are rare, if abnormal liver tests persist or worsen, and if clinical signs and

symptoms consistent with liver disease develop, or if systemic manifestations occur (e.g eosinophilia or rash), naproxen treatment should be discontinued (Mehta and Osick, 2005).

SULINDAC

Sulindac is a non-steroidal, anti-inflammatory indene derivative designated chemically as (Z)-5-fluoro-2-methyl-1-[[p - (methylsulfinyl)phenyl] methylene]-1H -indene-3-acetic acid. It is not a salicylate, pyrazolone or propionic acid derivative. Sulindac has been associated with various toxic side effects, including neurological, hematological, renal and liver damage. From a histopathological perspective, cholestatic and/or hepatocellular damage are the morphologic expressions of toxicity. 91 cases of sulindac-associated hepatic injury, among which there were four fatal outcomes (Tarazi *et al.*, 1993). The morphological features of sulindac hepatotoxicity were cholestatic hepatitis with marked anisonucleosis, cytoplasmic invaginations into the nucleus and binuclearity of hepatocytes (Wood *et al.*, 1985). Cardiovascular Thrombotic Events Clinical trials of several COX-2 selective and nonselective NSAIDs of up to three years duration have shown an increased risk of serious cardiovascular (CV) thrombotic events, myocardial infarction, and stroke, which can be fatal. All NSAIDs, both COX-2 selective and nonselective, may have a similar risk. Patients with known CV disease or risk factors for CV disease may be at greater risk. NSAIDs, including sulindac, can cause serious gastrointestinal (GI) adverse events including inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or large intestine, which can be fatal. (Clinoril[®], 2010)

PIROXICAM

Piroxicam is one of the most popular nonsteroidal anti-inflammatory drugs used for the treatment of inflammatory conditions and rheumatic disorders. It has analgesic and antipyretic activity. Some cases of liver disease have been reported in patients taking piroxicam. Liver sections appeared with inflammatory cellular infiltration, vacuolated hepatocytes, dilated sinusoids, and increased number of Kupffer cells. Kidney sections appeared with some cellular inflammations. The glomeruli were shrunk resulting in widening of the urinary space. Oedema and vacuolations were noticed in the tubular cells. Histochemical staining revealed that glycogen and protein contents had decreased in the hepatocytes. This depletion worsened gradually in liver cells after two, three, and four weeks. Similar depletion of the glycogen content was observed in kidney tissue. However, protein content appeared to be slightly decreased in the kidney tubules and glomeruli. Incensement of coarse chromatin in the nuclei of hepatocytes, Kupffer cells and most inflammatory cells were detected by Fuelgen method. Kidney tissues appeared with a severe decrease in coarse chromatin in the nuclei (Hossam Ebaid *et al.*, 2007) Isolated severe cholestatic (Poniachik *et al.*, 1998) and fatal (Rabinovitz and Van Thiel, 1996) cases have been reported in various age groups in response to piroxicam treatment. Liver morphology in these cases indicated acute hepatitis with cholestasis and confluent necrosis. Microvesicular steatosis has also been reported.

CONCLUSION

NSAIDs are effective medications that afford many veterinary patients a significant amount of relief from pain associated with both chronic disease states and acute inflammatory events. However, before initiating NSAID therapy, it is prudent for veterinarians to educate clients on signs of toxicity and to exercise sound clinical judgment in animal selection based on physical examination findings, concurrent drug therapy and preexisting diseases. Billions of doses of NSAIDs are consumed annually making them responsible for more toxic deaths than any other pharmaceutical agent. The vast majority of these deaths are from the GI toxicity from non-selective NSAIDs. COX-2 inhibitors are costly and may be less toxic than other NSAIDs. Patient education and understanding of appropriate dosing/ risk factors can prevent needless deaths and morbidity. Adjunctive methods such as weight loss and chondroprotective agents may help reduce untoward side effects of NSAIDs. During therapy, early recognition of signs of toxicity, both through routine clinicopathological monitoring and keen client based observations, are essential for safe NSAID therapy for veterinary patients.

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