EFFECT OF DIETARY FAT ON THE ORAL BIOAVAILABILITY OF TEPOXALIN, A NOVEL ANTI-INFLAMMATORY AGENT

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CHAPTER I

INTRODUCTION

Inflammation is considered a host defense mechanism that is employed to initiate the healing process of damaged tissues (Slauson, *et al*, 1990). The study of the inflammatory process over the centuries has given us the five cardinal signs of inflammation: heat, redness, pain, swelling, and loss of function (Lee, *et al*, 1992; Slauson, *et al*, 1990). The release of cytokines, the activation and migration of leukocytes, and the production of antibodies are some of the components of the inflammatory process that interact in a complex manner to produce the signs listed above. Eicosanoids are important in the chronic inflammatory process of canine osteoarthritis. The products of arachidonic acid are metabolized by either the cyclooxygenase pathway or the lipoxygenase pathway (Ringler, *et al*, 1997; Slauson, *et al*, 1990). The cyclooxygenase pathway produces prostaglandins, prostacyclin, and thromboxane necessary for vasodilatation, increased vascular permeability and platelet aggregation (Higgins, *et al*, 1984; Tizard, 1996). The lipoxygenase pathway produces the leukotrienes which serve to activate and stimulate migration of leukocytes (Lewis, *et al*, 1990). The leukotrienes also stimulate vasoconstriction of smooth muscle and increase vascular permeability at the site of inflammation. (Lewis, *et al*, 1990).

Corticosteroids are drugs which have the ability to block the release of arachidonic acid and the subsequent release of its products (Barragry, 1994). The many adverse effects of corticosteroids make their utilization too costly over a prolonged period

of time (Plumb, 1999). The non-steroidal anti-inflammatory drugs (NSAIDs) have fewer adverse effects compared to the corticosteroids, but are effective only in inhibiting the cyclooxygenase pathway (Tizard, 1996; Campbell, *et al*, 1996). The novel dual cyclooxygenase/lipoxygenase inhibitor, tepoxalin, appears to be the best drug to treat chronic inflammatory conditions in that it is able to inhibit eicosanoid production without adverse effects (Wallace, *et al*, 1991,1993). The active acidic metabolite that is produced is believed to inhibit the cyclooxygenase pathway while the parent drug, tepoxalin, inhibits the lipoxygenase pathway (Waldman, *et al*, 1996). One study has looked at the effects of a fasting diet in the absorption of tepoxalin, but no other type of diet has been investigated (Waldman, *et al*, 1996). Therefore, the objectives of this study were to determine the pharmacokinetics, in particular the absorption, of tepoxalin in the canine and to study the effect of dietary fat on drug bioavailability.

CHAPTER II

LITERATURE REVIEW

Pathophysiology of Inflammation

The study of inflammation dates back to the first century when the Roman, Cornelius Celsus, first characterized it as calor, rubor, tumor, et dolor (heat, redness, swelling, and pain) (Lee, *et al*, 1992). A fifth sign, loss of function, was later added by Rudolf Virchow in the 1800's (Slauson, *et al*, 1990). These five signs are known today as the "cardinal signs" of inflammation.

Inflammation is the reaction of the tissue of an organism to an irritant, which can be infectious, chemical, physical, immunologic, or radiant in nature (Slauson, *et al*, 1990; Lee, *et al*, 1992). The majority of complex organisms utilize the inflammatory response (Slauson, *et al*, 1990). If living organisms did not have an adequate inflammatory response to fight disease, they would not be here today (Slauson, *et al*, 1990).

The inflammatory response incorporates many different components, which in turn interact independently and with one another to create the signs seen with this response. Some processes which are initiated are chemotactic stimuli for cellular response, lysozomal enzyme release, antibody production, and the activation of the complement, coagulation, fibrinolytic, and kinin pathways (Lee, *et al*, 1992). The type of reaction, whether it is acute or chronic, can influence the participants in the inflammatory

process (Tizard, 1996). Also, the type of irritant determines which components are produced and how they interact (Tizard, 1996).

The four original classical signs of inflammation are what describe an acute response (Lee, *et al*, 1992). When a cell is damaged, it releases chemotactic and vasoactive factors into the blood stream where neutrophils and eosinophils become the first responders to the chemotactic signals of damaged tissues (Tizard, 1996). They phagocytize and destroy foreign material and in turn release more chemotactic factors to signal other specific leukocytes to the site of tissue damage (Tizard, 1996). The vasoactive factors which are released help stimulate vasodilatation and increase vascular permeability by acting on the smooth muscle of the blood vessels within the damaged area (Tizard, 1996). These vasoactive mediators can originate from the damaged tissue, the responding leukocytes, or the intermediate products of the inflammatory process.

The most common signs seen with chronic inflammation are persistent pain and swelling of the affected area, without redness or heat, which can lead to a loss of function (Lee, *et al*, 1992). Also, the composition of the cells in a chronic inflammatory response is different from an acute response. Monocytes are attracted to the area and are activated to macrophages, which in turn phagocytize any foreign material and help destroy damaged tissue (Tizard, 1996). The macrophages also release interleukin-1 (IL-1), which stimulates the formation of fibrin and collagen and allows the healing process to begin (Tizard, 1996).

The etiology of the inflammatory response determines how well the organism can respond. If the offending agent is rapidly and completely removed, then the healing process is uneventful (Tizard, 1996). If inorganic substances or certain bacterial or fungal

infections persist, then the organism forms a granuloma and tries to wall it off from the surrounding healthy tissue (Tizard, 1996). The normal inflammatory response of an organism can also lead to excessive scarring and granulation tissue formation, which in turn can lead to joint immobility (Slauson, *et al*, 1990).

The degenerative joint disease, osteoarthritis, is considered a phenomenon of aging (Rosenberg, 1999; Schiller, *et al*, 1999). Although the majority of human cases are 55 years old or older, 5% of cases are younger individuals who have had an injury to a joint, a congenital deformity of a joint, or an underlying systemic disease (Rosenberg, 1999). Osteoarthritis can afflict a number of people in any age category and is the number one cause of arthritis in the United States. (Schiller, *et al*, 1999).

There does not appear to be any initiating cause of osteoarthritis (except in young people) and it can be localized or generalized (Rosenberg, 1999). The disease is characterized by the narrowing of the joint space, increasing subchondral bone thickness with cysts, and the formation of osteophytes (Schiller, *et al*, 1999). These events are the result of the destruction of articular cartilage by chondrocytes releasing IL-1 and tissue necrosis factor alpha (TNF-α) (Rosenberg, 1999; Schiller, *et al*, 1999). It has also been discovered that the prostaglandins and interleukin-6 (IL-6) degrade the cartilage matrix which can cause joint pain following activity (Schiller, *et al*, 1999; Rosenberg, *et al*, 1999). Since the pathway for the production of prostaglandins is known, this is the area being extensively researched for the development of pain relievers for osteoarthritis and similar conditions.

The Role of Eicosanoids in Inflammation

Prostaglandins, along with leukotrienes and thromboxane, are called eicosanoids and are the metabolites of the arachidonic acid pathway (Ringler, 1997). Arachidonic acid is a 20-carbon polyunsaturated fatty acid derived from the conversion of linoleic acid or directly as a dietary source (Collins, 1999). It is found esterified to phospholipids of cell membranes, commonly as phosphatidylcholine (Slauson, *et al*, 1990; Collins, 1999). In order to produce the metabolites, arachidonic acid must first be liberated from the cell membrane by the disruption of the intact cell membrane and subsequent release of arachidonic acid, or by an antigenic or mediator stimulus (Slauson, *et al*, 1990; Ringler, 1997).

The antigenic or mediator ligand binds to a specific membrane-bound receptor, which comes into contact with a membrane-bound protein called G-protein that is then activated (Collins, 1999). The activated G-protein in turn causes the hydrolysis of phosphatidylinositol-4, 5-bisphosphate (PIP_2) to inositol-1, 4, 5-triphosphate (IP_3) and diacylglycerol (DAG) (Collins, 1999). IP_3 goes to the endoplasmic reticulum and stimulates the release of calcium ions into the cell (Collins, 1999). The increase in cytosolic calcium activates phospholipase A_2, which hydrolyzes arachidonic acid and releases it from the cell membrane where it is then metabolized by one of two pathways (Ringler 1997; Slauson, *et al*, 1990).

The pathways are named for the two enzyme systems which metabolize arachidonic acid: cyclooxygenases and lipoxygenases. The first enzyme in the cyclooxygenase pathway consists of two isoforms: cyclooxygenase-1 (COX-1) and

cyclooxygenase-2 (COX-2) (Campbell, *et al*, 1996). The form expressed depends upon the cell and the type of stimulus (Campbell, *et al*, 1996). This enzyme converts arachidonic acid to prostaglandin G_2 (PGG_2) and then converts PGG_2 to prostaglandin H_2 (PGH_2), both of which are unstable (Campbell, *et al*, 1996). Various enzymes then act on PGH_2 to produce the eicosanoids seen in the inflammatory response. Prostacyclin synthase converts PGH_2 to prostacyclin (PGI_2) (Campbell, *et al*, 1996). Isomerases convert PGH_2 to prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and prostaglandin D_2 (PGD_2) (Campbell, *et al*, 1996). Thromboxane synthase forms thromboxane A_2 (TXA_2) from PGH_2 (Campbell, *et al*, 1996).

As soon as the cyclooxygenase pathway is activated, so is the lipoxygenase pathway (Higgins, *et al*, 1984). Two different enzymes begin this pathway. The 12-lipoxygenase enzyme converts arachidonic acid to a 12-hydroperoxyeicosatetraenoic acid (HPETE), which is then converted to a 12-hydroeicosatetraenoic acid (HETE) (Higgins, *et al*, 1984). The 5-lipoxygenase enzyme acts on arachidonic acid to form 5-HPETE which is either converted to 5-HETE by various enzymes or to leukotriene A_4 (LTA_4) by LTA synthase (Higgins, *et al*, 1984). LTA hydrolase converts LTA_4 to leukotriene B_4 (LTB_4) (Higgins, *et al*, 1984). Leukotriene C_4 synthase can also convert LTA_4 to leukotriene C_4 (LTC_4), which subsequently forms leukotriene D_4 (LTD_4), leukotriene E_4 (LTE_4), and leukotriene F_4 (LTF_4) (Higgins, *et al*, 1984). These two pathways are illustrated in Figure 1.

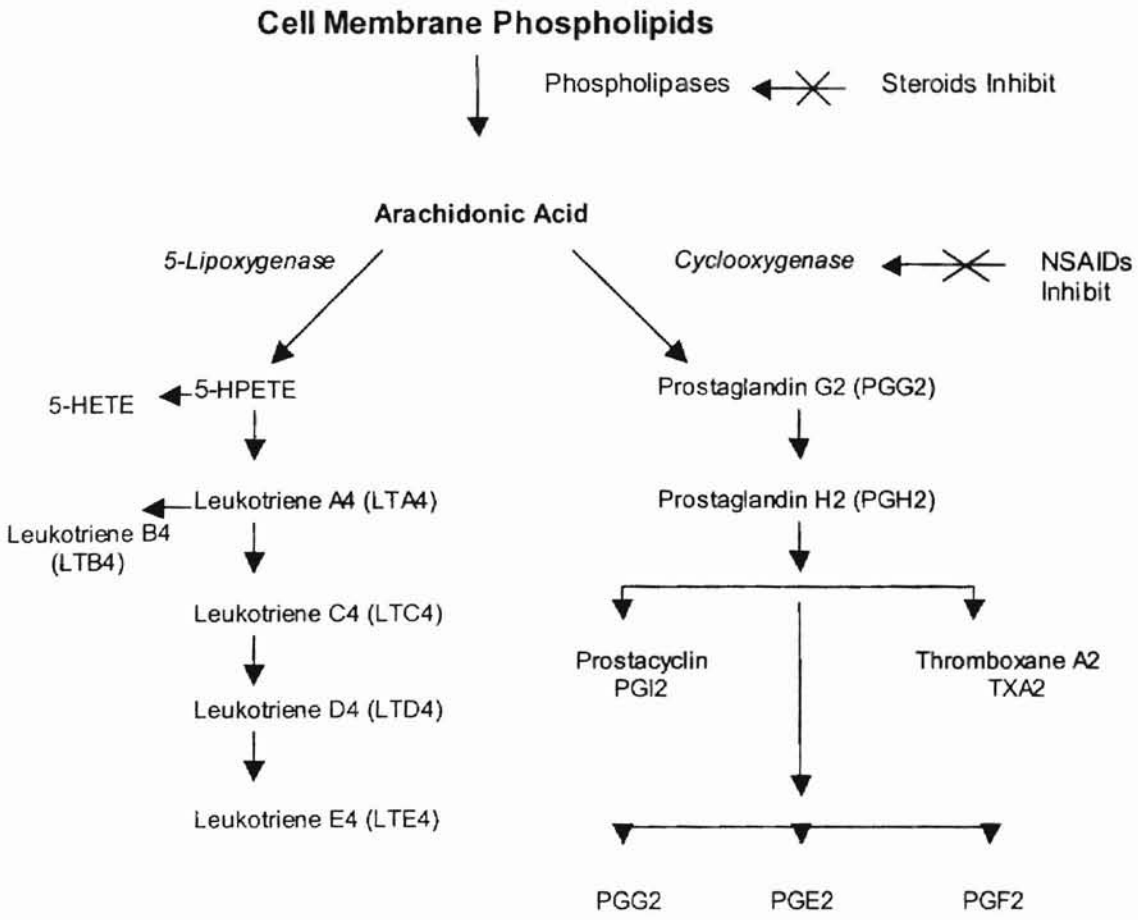

Figure 1. The Generation of arachidonic acid metabolites and their roles in inflammation (Collins, 1999).

Each of the metabolites produced by the cyclooxygenase and the lipoxygenase pathways have a role in the inflammatory response and their action depends upon the stimulus and the tissue in which they exist (Campbell, *et al*, 1996). The functions of the following eicosanoids will be discussed: PGI_2, PGE_2, $PGF_{2\alpha}$, TXA_2, and LTB_4.

Vasodilatation and increased vascular permeability is induced by PGI_2 and PGE_2 (Higgins, *et al*, 1984). These two eicosanoids are also responsible for the enhancement of pain induced by seratonin and bradykinin (Lee, *et al*, 1992). Platelet aggregation is inhibited by PGI_2 and $PGF_{2\alpha}$ while it is promoted by PGE_2 and TXA_2 (Tizard, 1996). $PGF_{2\alpha}$ and TXA_2 also stimulate smooth muscle contraction and vasoconstriction (Tizard, 1996). LTB_4 is chemotactic for neutrophils and helps them stick to endothelial cells in the affected area (Lewis, *et al*, 1990). Many of these mediators have various responses depending on their site of action, but all of them contribute to the signs seen with acute inflammation (heat, redness, swelling, and pain). Because of this, it is difficult to develop one drug that can relieve all of these signs.

The Effects of Corticosteroids on Inflammation

Steroids have been called the "silver bullets" when it comes to treating the symptoms of inflammation. They are able to suppress the redness, heat, and swelling of an acute inflammatory response and also reduce any fever if present (Davis, *et al*, 1992). Glucocorticoids act at the level of arachidonic acid release from the cell membrane by inhibiting phospholipase A_2 (PLA_2) activity (Barragry, 1994).

Corticosteroids can bind with a specific glucocorticosteroid receptor (GR) on the cell membrane or diffuse through the membrane and bind with a GR in the cytoplasm of the cell (Ferguson, *et al*, 1995). GR's within the cell are inactive and are bound to heat-shock proteins (HSP) –70 and –90, and immunophilin (Schimmer, *et al*, 1996). When the steroid binds, the proteins are released and the GR translocates the steroid to the nucleus (Ferguson, *et al*, 1995). In the nucleus, the steroid-receptor complex binds to glucocorticoid response elements (GRE's) present on certain genes of the DNA and alters their products (Davis, *et al*, 1992). The new proteins that result serve to enhance or inhibit specific cellular functions (Davis, *et al*, 1992).

One such protein that is produced is lipocortin (Ferguson, *et al*, 1995). It serves to inhibit PLA_2 activity and both the cyclooxygenase and lipoxygenase pathways (Ferguson, *et al*, 1995). The production of prostaglandins, leukotrienes, and thromboxane is inhibited and their activity on various cells can no longer occur in the inflammatory response.

The corticosteroids can also have direct effects on the cells of the inflammatory response. They decrease the number of lymphocytes in the circulation by sequestering them in lymphoid tissue and bone marrow (Winkelstein, 1992). Monocytes, eosinophils, and basophils are redistributed while neutrophils are rapidly released from the bone marrow into the circulation, but their functions are inhibited at the site of inflammation (Winkelstein, 1992). Interleukin-1 synthesis from monocytes is inhibited and monocytes cannot be activated to macrophages (Winkelstein, 1992). These are just a few of the many changes that occur in response to inflammation.

The structures of the corticosteroids lend themselves to easy modification to enhance their rate of absorption by various routes (Davis, *et al*, 1992). Corticosteroids can be absorbed from the gastrointestinal (GI) tract, through the skin, or given intravenously or intramuscularly depending on how they are formulated (Davis, *et al*, 1992). Glucocorticoids are metabolized by the liver and excreted by the kidneys (Davis, *et al*, 1992).

Along with their ability to inhibit many characteristics of inflammation, corticosteroids also have many adverse effects on the organism. These effects are common when the drugs are given over an extended period of time. Plumb (1999) lists the adverse effects seen in dogs in his Veterinary Drug Handbook as: "dull, dry hair coat, weight gain, panting, vomiting, diarrhea, elevated liver enzymes, pancreatitis, GI ulceration, lipidemias, activation or worsening of diabetes mellitus, muscle wasting and behavioral changes". Polyuria, polydipsia, and polyphagia are the more frequent signs that dog owners report seeing (Plumb, 1999). Another problem with corticosteroids is that they have to be tapered off slowly so the pituitary-hypothalamic pathway of the animal, which produces steroids in the body, can resume its normal production of glucocorticosteroids. (Plumb, 1999). If this does not happen, the excess steroid injected into the dog will suppress the production of adrenocorticotrophic hormone (ACTH), which in turn leads to the deficiency of cortisol secretion from the adrenal glands and hypoadrenocorticism (Plumb, 1999).

Other drugs, such as the NSAIDs, have been developed that do not have as many adverse effects as corticosteroids yet give similar results. The NSAIDs can be divided into two groups: the prostaglandin synthetase inhibitors (PSIs) and the non-prostaglandin synthetase inhibitors (non-PSIs) (Lee, *et al*, 1992).

The PSIs consist of those drugs which are derived from carboxylic acid or enolic acid compounds and are considered weak organic acids (Lee, *et al*, 1992). The PSIs act by inhibiting the cyclooxygenase enzyme and, therefore, the production of prostaglandins and thromboxane (Insel, 1996). The non-PSIs, on the other hand, are grouped according to their effects on the symptoms expressed and, therefore, are unrelated compounds (Lee, *et al*, 1992). Some examples are gold (an antirheumatoid arthritis agent) and allopurinol (an antigout agent) (Lee, *et al*, 1992).

The mechanism of action of aspirin, the most popular NSAID and PSI, is to acetylate serine 530 of COX-1 or serine 516 of COX-2, which results in an irreversible inhibition of the cyclooxygenase enzyme (Insel, 1996). The duration of the effect of aspirin on the enzyme is determined by how quickly more cyclooxygenase enzyme is produced (Insel, 1996). Even though NSAIDs like aspirin can provide relief to the symptoms of inflammation, they do nothing to inhibit the pathologic process of the disease itself (Ferguson, *et al*, 1995).

Many NSAIDs which are indicated for the treatment of osteoarthritis or a musculoskeletal disorder are given orally and are readily absorbed from the stomach and small intestine (Lee, *et al*, 1992). Flunixin meglumine is one NSAID which is injectable

(Lee, *et al*, 1992). The different formulations of NSAIDs allow for a choice of routes of administration and the ability to control possible adverse reactions based on the animal's state of health.

The adverse reactions seen with NSAIDs typically involve the gastrointestinal system (Insel, 1996). Irritation of the stomach or small intestine can lead to vomiting and diarrhea with possible ulceration and blood loss (Insel, 1996). This can be alleviated by giving the drug in a buffered form or reducing the dosage or duration (Insel, 1996).

Another adverse effect is on the hemopoietic system. With the inhibition of thromboxane, platelet function is ceased and this leads to an increase in the bleeding time (Insel, 1996). This can cause serious problems if the animal has an extensive laceration.

Depending on the drug, hepatic and renal adverse effects can also occur (Insel, 1996). Hypersensitivity reactions have occurred with aspirin or its derivatives, but this is less common (Plumb, 1999).

The majority of these adverse effects occur because the NSAID has to be given at a high dose over an extended period of time (Plumb, 1999). Decreasing the dose or shortening the duration will lower the efficacy of the drug. Newer drugs are being developed that have the desirable action of corticosteroids with fewer adverse effects like the non-steroidal anti-inflammatory drugs.

The Potential Role of Tepoxalin in the Treatment of Inflammation

Tepoxalin is a novel dual cyclooxygenase/lipoxygenase (CO /LO) inhibitor which has been studied extensively at the R. W. Johnson Pharmaceutical Research Institute (Argentieri, *et al*, 1994). Although another dual inhibitor, BW755C, was studied extensively at the research institute, tepoxalin was found to be 3.5 times more potent (Anderson, *et al*, 1990). Tepoxalin inhibits both cytokine production and leukocyte activation and migration seen in the inflammatory response. It has the potential to be a potent inhibitor of inflammation in many aspects of osteoarthritis, without the side effects seen with the NSAIDs

Two functional groups included in the drug structure have been shown to have their effects in the cyclooxygenase pathway. The cyclooxygenase activity of prostaglandin-H synthase-1 (PGHS1) is believed to be inhibited by the pyrazole group while the peroxidation of PGG_2 to PGH_2 is blocked by the hydroxamic acid group, which is an iron chelator (Tam, *et al*, 1995). Tepoxalin is a noncompetitive inhibitor, although the exact binding site on PGHS1 is unknown (Tam, *et al*, 1995). Tam, *et al*, have studied tepoxalin and believe it binds close to tyrosine 384 of PGHS1 and alters its conformation to elicit the hydrolysis of arachidonic acid while the hydroxamic acid of tepoxalin chelates the heme group of tyrosine 385 and inactivates peroxidase (1995).

The 5-lipoxygenase (5-LO) pathway is thought to be inhibited by the binding of tepoxalin to the 5-LO activating protein to prevent its activation, but the specifics of this interaction have not been worked out (Rainsford, *et al*, 1996). The mechanism of action

tepoxalin preventing the decay and subsequent release of IκB (which inactivates NFκB and keeps it in the cytoplasm) from NFκB (Kazmi, *et al*, 1995; Beg, *et al*, 1993).

One of the effects of this inactivation of NFκB is the inhibition of leukocytes. The cell adhesion molecules CD62E, CD11b/CD18, and CD106 are inhibited through the inactivation of NFκB while intracellular adhesion molecule-1 (ICAM-1) expression is blocked through the inhibition of IFN-γ by tepoxalin (Lee, *et al*, 1996; Zhou, *et al*, 1996). These molecules allow for the adhesion of leukocytes to vessel walls. The migration of neutrophils is blocked by the inhibition of Mac-1 and E-selectin indirectly through NFκB (Zhou, *et al*, 1996).

Tepoxalin is rapidly metabolized within the body. It is subjected to the first-pass effect whereby the parent drug is inactivated when it passes through the liver by hepatic enzymes (Benet, *et al*, 1996). In this way, significant amounts of the drug will not appear in the general circulation (Benet, *et al*, 1996). This leads to the formation of an active acidic metabolite that has been observed in many individual studies (Knight, *et al*, 1996; Depre, *et al*, 1996; Waldman, *et al*, 1996). The plasma levels of the metabolite compared to the active drug rise much higher and remain high for 24 hours while the active drug shows a rapid decline in this time period (Knight, *et al*, 1996; Depre, *et al*, 1996; Waldman, *et al*, 1996). This metabolite was shown to inhibit cyclooxygenase while the parent drug, tepoxalin, inhibited lipoxygenase in a time-dependent and dose-dependent fashion (Waldman, *et al*, 1996).

Although the metabolism of the drug has been studied, the factors affecting the absorption of the drug have not been as extensively studied. One experiment looked at the effect of diet on absorption of the drug from the gastrointestinal tract. Waldman, *et*

al, concluded that tepoxalin was readily absorbed after oral administration of 20 healthy male volunteers in the fasting state (1996). No studies have been done to date to determine how diet affects absorption in dogs. More experiments need to be conducted to investigate the pharmacokinetics of tepoxalin. The fat content of the diet may have a more profound effect on the absorption of tepoxalin from the gastrointestinal tract than a fasting state.

CHAPTER III

EXPERIMENTAL OBJECTIVES

The hypothesis upon which this research was based is that tepoxalin exhibits a pharmacokinetic disposition suitable for oral therapy in dogs and that dietary fat content does not adversely affect oral bioavailability. This hypothesis was tested by conducting a pharmacokinetic study designed to compare the oral bioavailability of tepoxalin and its pharmacologically active acid metabolite in fasted dogs and dogs fed either a low-fat or high-fat commercial diet. The specific objectives were:

(1) to determine the oral bioavailability of a novel rapid-dissolution formulation of tepoxalin; and

(2) to investigate the effects of fasting and dietary fat content on bioavailability of tepoxalin and its acid metabolite.

CHAPTER IV

MATERIAL AND METHODS

Animals

The animals used in this study were purpose-bred Beagles purchased from a breeding facility. The three males and three females were one-year-old and weighed approximately 7.5 – 9.7 kg. Upon arrival, the dogs were given a physical examination by a veterinarian. The veterinarian's assessment, along with the CBC and vaccination history that accompanied the animals, concluded that all were healthy, heartworm negative, and had no underlying medical problems. Fenbendazole was administered as a prophylactic anthelmintic. The dogs were housed in separate dog runs for the duration of the study. Animal use was approved by the OSU Institutional Animal Care and Use Committee (Protocol No. 689).

Experimental Design

The dogs were administered tepoxalin intravenously (IV) or orally (PO) with 3 different diets. The treatments were given in such a way that one male and one female received the same treatment and diet per treatment period. The fasted (Fast) diet consisted of no food 12 hours prior to drug administration until 4 hours after drug administration. The low fat (Lfat) diet consisted of dry feed (Canine Maintenance

Science Diet, 15.4% fat as a proportion of dry matter) fed 30 minutes prior to drug administration. The high fat (Hfat) diet was a canned feed (Canine/Feline a/d Prescription Diet 28.7% fat as a proportion of dry matter) fed 30 minutes prior to drug administration. These diets were only given on the days Tepoxalin was administered. The dogs received Canine Maintenance Science Diet dry feed for the other days of the study.

The experimental design is described in Table 1:

TABLE 1 – Assignment of treatments to individual animals

Treatment Period	**Dog Identification**					
	#3308-Male	#3309- Male	#3310- Male	#3311-Female	#3312-Female	#3313-Female
1	PO/Fast	PO/Lfat	PO/Hfat	PO/Hfat	PO/Lfat	PO/Fast
2	PO/Lfat	PO/Hfat	PO/Fast	PO/Fast	PO/Hfat	PO/Lfat
3	PO/Hfat	PO/Fast	PO/Lfat	PO/Lfat	PO/Fast	PO/Hfat
4	IV	IV	IV	IV	IV	IV

Ten days between treatment periods were allowed as washout periods. The complete eliminations of tepoxalin and its metabolite were confirmed be collecting blood 10 days after the first dose of tepoxalin was administered and assaying it for the drug and its metabolite.

Drug Administration and Sample Collection

For oral administration, Schering Plough Animal Health Research supplied tepoxalin as Tepoxalin Zydis 50 mg tablets. The tablets were administered by placing on the dorsum of the tongue, closing the mouth, and then stroking the throat for 10 seconds to stimulate the swallowing reflex. The dosage given was 10 mg/kg. This was accomplished by cutting the tablets when necessary using a sharp scalpel blade applied along the diameter of the tablet to give approximately two equal pieces to dose to the closest 25 mg increment. Doses administered are listed in Table 2:

TABLE 2 – Doses administered to individual dogs.

Dog ID	Starting weight (kg)	PO/Fast	PO/Lfat	PO/Hfat	IV (ml)
#3308	8.2	1 + 1/2 tabs	1 + 1/2 tabs	2 tabs	2.0
#3309	8.2	2 tabs	1 + 1/2 tabs	2 tabs	2.0
#3310	9.7	2 tabs	2 tabs	2 tabs	2.2
#3311	7.6	1 + 1/2 tabs	2 tabs	1 + 1/2 tabs	1.9
#3312	7.9	1 + 1/2 tabs	1 + 1/2 tabs	1 + 1/2 tabs	1.6
#3313	7.5	1 + 1/2 tabs	1 + 1/2 tabs	1 + 1/2 tabs	1.6

For intravenous administration, Schering Plough Animal Health Research supplied the tepoxalin as Tepoxalin Injectable solution 50 mg/ml. The calculated IV dose was identical to the PO dose used in the previous treatment period and was administered as a bolus dose via a catheter into the cephalic vein over a period of 60 seconds.

Blood samples were collected into heparinized tubes by cephalic venipuncture before drug administration and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 32, and 48 hours

after PO administration and at 0.083, 0.17, 0.33, 1, 2, 3, 4, 6, 8, 12, 16, 24, 32, and 48 hours after IV administration.

Drug Assay

The blood samples were centrifuged and the plasma was harvested within 24 hours of sample collection. The plasma was then refrigerated at 4 C until it was assayed within 72 hours of collecting it. The plasma samples were then subjected to high performance liquid chromatography using a modification of a procedure developed by the R. W. Johnson Pharmaceutical Research Institute to determine the concentrations of tepoxalin and its active acidic metabolite.

The drug and its metabolite were first extracted from the plasma sample. A glass test tube was used to combine 0.5 ml of plasma, 20 *u*l of internal standard (a derivative of tepoxalin), 1 M phosphate buffer (pH = 6), and 5 ml of methyl-t-butyl ether. The tube was capped, vortexed for 30 seconds and then centrifuged until the organic layer was separated from the aqueous layer. The organic layer was removed, placed in another glass test tube, and then evaporated under a N_2 stream in a water bath. The precipitate was dissolved in 200 *u*l of mobile phase, which was a mixture of 43% buffer (1.1 g 1-octane, sulfonic acid, sodium salt dissolved in 970 ml deionized water and 20 ml 99% triethylamine, pH = 5), 20% methanol, 20 % tetrahydrofuran, and 17% acetonitrile.

Concentration standards were prepared using plasma from untreated dogs and adding the correct amounts of tepoxalin and its acid metabolite prepared in methanol to generate 0, 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 *u*g/ml for oral dosing and 0, 0.125, 0.25, 0.5,

1.0, 2.0, 4.0, 8.0, and 16 *u*g/ml for IV dosing. The standards were subjected to the same extraction procedure as described earlier and were made fresh every day.

All extracted samples were filtered through 0.45 *u*m filters and 50 *u*l aliquots were then injected into a high performance liquid chromatography system consisting of a gradient programmer (Model 2360, Isco, Inc), pump (Model 2350, Isco, Inc), variable wavelength absorbance detector (Model V_4, Isco, Inc) set at 254 nm, and an integrator (Model SP 4290, Spectra-Physics). A 150 x 4.6 mm C_{18} column (Nucleosil 5*u*m, Phenomenex) was also used. The flow rate of the mobile phase was 0.9 ml/minute and all chromatography was done at room temperature.

The relationships between standard concentration and ratios of tepoxalin or acid metabolite and internal standard peak heights were best described using second-order polynomial equations (R^2>0.9). The limit of quantitation (LOQ) of the assay was 0.125 *u*g/ml. The limit of detection (LOD) was defined by the peak threshold setting of the integrator: peak heights that were not recorded because they were lower than this threshold were considered to be below the LOD. All estimates of intra-assay precision were within acceptable limits (≤5.57%) at the 0.25 *u*g/ml and at 2 *u*g/ml concentration levels. Accuracy was ≤12.40% at the 0.25 *u*g/ml and at 2 *u*g/ml concentration levels, except for at 0.25 *u*g/ml tepoxalin (19.08%). Acceptable limits for both the intra-assay precision and the accuracy are <15%. Samples frozen at 20 C for 2 weeks showed no significant loss of stability.

Data Analysis

The pharmacokinetic values, area under the curve (AUC), area under the first moment curve (AUMC), mean residence time (MRT), time to peak concentration (T_{max}), and peak concentration (C_{max}) were calculated using a model-independent approach with the use of a computer program (Bourne, 1989). Areas under the tepoxalin and acid metabolite concentration-time curves were calculated from time-zero to the last sampling time, using the trapezoidal method. Terminal rates of elimination, necessary for calculation of elimination half-lives, were estimated by fitting concentration-time data to biexponential equations using a computer program for iterative least-squares regression analysis (Brown and Manno, 1978). Oral bioavailability of tepoxalin was estimated by calculating the individual ratios of areas under the PO and IV curves.

The effects of diet on the bioavailability of tepoxalin and acid metabolite were tested by comparing the fraction of drug absorbed (F) (for tepoxalin) and C_{max} and T_{max} (for tepoxalin and acid metabolite) using the general linear model and separation of means by Scheffe's test. F values were log-transformed prior to analysis. Means were considered significant at the $P < 0.05$ level.

CHAPTER V

RESULTS

Concentrations of tepoxalin and acid metabolite in plasma after oral or intravenous administration of tepoxalin are presented in Tables 3 - 6 and in Figures 2 & 3. The concentration of tepoxalin declined rapidly in the first 6 hours after intravenous administration when a dose of 10 mg/kg was given. It then demonstrated a more gradual decline over the next 10 hours and was still detected in the plasma 16 hours after administration. These results suggest that tepoxalin is rapidly metabolized after absorption, which is consistent with the observation that concentrations of acid metabolite were consistently higher than corresponding concentrations of the parent tepoxalin, indicating that tepoxalin is subject to a substantial first-pass effect.

Pharmacokinetic parameters describing bioavailability of tepoxalin and its acid metabolite are listed in Tables 7 & 8. Feeding significantly increased C_{max} values for tepoxalin. Although similar trends were suspected for F values for tepoxalin and C_{max} values for the acid metabolite, the variabilities of these estimates were too high for significance to be demonstrated. Nevertheless, it is clear that if feeding affects oral absorption of tepoxalin administered to patients, it is likely to improve bioavailability. Closer examination of individual concentration-time profiles revealed considerable variation in bioavailability that was probably related to both oral absorption of tepoxalin and conversion of parent tepoxalin to acid metabolite. Whether dogs were fasted or fed

diets varying in fat concentration, the mean concentration of acid metabolite was still >0.4 μg/ml at 48 hours after administration of tepoxalin.

With the exception of defecation after administration of the IV dose of tepoxalin, no overt and significant adverse effects were observed. However, identification of adverse effects was not included as an experimental objective of this study and comprehensive physical or other examinations were not conducted after drug administration.

A few data points were excluded from the pharmacokinetic analysis (see footnotes to Tables 3 and 5) because the numbers did not correlate with the trend seen in the individual animal. This was attributed to human error in handling the sample and possible contamination of the sample with tepoxalin.

TABLE 3 – Plasma concentrations of tepoxalin after oral administration of 10 mg/kg to fasted dogs and dogs fed a low fat meal. (Units in *u*g/ml)

PO/Fasted:								
Time (hr)	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		
0.25	0.19	0.14	<LOQ	-	0.13	0.14	0.15	0.02
0.5	0.13	0.31	0.35	<LOQ	0.16	0.21	0.23	0.09
1	0.27	0.55	0.65	<LOD	0.36	0.57	0.48	0.16
2	0.36	0.65	0.58	<LOQ	0.65	0.56	0.56	0.12
3	0.49	0.39	0.65	<LOQ	0.42	0.33	0.46	0.12
4	0.49	0.22	0.53	<LOD	0.28	0.25	0.36	0.14
6	0.26	0.19	0.34	<LOQ	0.13	0.17	0.22	0.08
8	0.20	0.21	0.32	<LOQ	<LOQ	0.15	0.22	0.07
12	0.13	0.20	0.27	0.14	0.12	0.13	0.17	0.06
16	0.14	0.24	0.26	<LOQ	<LOQ	0.30	0.24	0.07
24	<LOQ	0.21	0.32	<LOD	<LOQ	0.12	0.22	0.10
32	0.16	<LOD	0.33	<LOQ	<LOQ	<LOD		
48	0.15	<LOD	0.76*	<LOQ	<LOQ	<LOD		
PO/Low Fat:								
Time (hr)	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		
0.25	<LOD	<LOQ	0.20	0.24	0.35	0.25	0.26	0.06
0.5	0.89	<LOQ	0.74	0.38	1.13	0.53	0.74	0.29
1	0.72	0.39	1.45	0.38	1.25	1.03	0.87	0.45
2	0.53	0.46	1.02	0.42	0.56	1.06	0.67	0.29
3	0.57	0.36	0.58	0.65	0.30	0.75	0.54	0.17
4	0.34	0.33	0.30	1.38	0.24	0.67	0.54	0.44
6	0.29	0.28	0.25	1.07	0.23	0.41	0.42	0.33
8	0.26	0.26	<LOD	0.61	<LOD	0.32	0.36	0.17
12	0.30	0.16	<LOD	0.39	<LOD	0.28	0.28	0.10
16	0.33	0.13	<LOD	0.12	<LOD	0.23	0.20	0.10
24	0.63*	<LOD	<LOD	<LOD	<LOD	0.23		
32	0.34	<LOQ	<LOD	<LOD	<LOQ	0.29		
48	0.22	<LOQ	<LOD	<LOD	<LOQ	0.23		

* Data points excluded from pharmacokinetic analyses.

TABLE 4 – Plasma concentrations of tepoxalin after oral administration of 10 mg/kg to dogs fed a high fat meal and after intravenous administration. (Units in *u*g/ml)

PO/High Fat:

Time (hr)	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		
0.25	0.19	0.48	<LOQ	<LOQ	0.25	<LOQ	0.31	0.16
0.5	0.87	0.30	<LOQ	<LOQ	0.26	<LOQ	0.48	0.34
1	0.82	0.48	0.59	0.84	0.41	0.56	0.61	0.18
2	1.42	0.65	1.24	1.24	0.79	1.33	1.11	0.31
3	1.24	0.62	1.01	0.94	1.10	1.47	1.07	0.29
4	0.47	0.48	0.72	0.48	1.12	0.89	0.69	0.27
6	0.33	0.60	0.34	0.24	0.36	0.25	0.35	0.13
8	0.26	0.34	0.21	0.12	0.45	0.13	0.25	0.13
12	0.23	0.31	0.15	0.11	0.27	<LOQ	0.22	0.08
16	0.21	0.53	<LOQ	<LOD	0.26	<LOQ	0.34	0.17
24	0.22	0.34	<LOQ	<LOD	0.31	<LOD	0.29	0.06
32	<LOD	0.44	<LOQ	<LOD	0.57	<LOD		
48	<LOD	0.30	<LOQ	<LOD	0.50	<LOD		

IV

Time (hr)	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		
0.083	11.74	16.31	12.16	15.69	10.83	11.51	13.04	2.34
0.17	9.15	7.69	9.41	10.48	7.27	10.77	9.13	1.42
0.33	6.60	5.68	7.17	9.22	3.34	8.54	6.76	2.11
0.5	5.24	3.70	6.88	6.81	4.06	3.08	4.96	1.62
1	2.65	3.07	3.69	4.22	2.89	4.65	3.53	0.80
2	1.90	1.26	1.72	1.57	0.69	1.78	1.49	0.45
3	0.71	0.47	0.76	0.57	0.33	0.96	0.63	0.22
4	0.20	0.32	0.47	0.28	0.18	0.61	0.34	0.17
6	0.16	0.14	0.26	<LOQ	<LOQ	0.33	0.22	0.09
8	0.24	<LOQ	0.24	<LOQ	<LOQ	0.34	0.27	0.06
12	0.11	<LOD	0.22	<LOQ	<LOQ	0.24	0.19	0.07
16	<LOD	0.14	0.19	<LOQ	<LOD	0.22	0.18	0.04
24	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD		
32	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD		
48	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD		

TABLE 5 – Plasma concentrations of acid metabolite after oral administration of 10 mg/kg tepoxalin to fasted dogs and dogs fed a low fat meal. (Units in *u*g/ml)

PO/Fasted:								
Time (hr)	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		
0.25	<LOQ	<LOQ	<LOD	-	<LOQ	<LOQ		
0.5	<LOQ	0.46	0.30	0.27	0.44	0.21	0.33	0.11
1	0.37	0.73	1.82	0.63	1.48	0.95	1.00	0.55
2	1.37	0.87	3.37	0.92	2.94	1.82	1.88	1.06
3	2.43	0.60	3.92	0.74	2.85	1.97	2.08	1.27
4	3.26	0.47	3.81	0.94	2.45	1.98	2.15	1.29
6	2.53	0.21	2.20	0.76	1.14	1.25	1.35	0.87
8	1.71	0.16	1.75	0.62	0.88	0.79	0.98	0.63
12	1.05	0.21	1.08	0.66	1.65	0.56	0.87	0.50
16	0.64	0.22	0.88	0.45	1.66	0.46	0.72	0.51
24	0.33	0.24	0.79	0.31	1.37	0.27	0.55	0.45
32	0.17	<LOD	0.43	0.26	0.94	<LOQ	0.45	0.34
48	0.23	<LOD	0.16	0.25	1.63*	<LOQ	0.57	0.71
PO/Low Fat:								
Time (hr)	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		
0.25	0.15	<LOQ	0.55	0.28	0.32	0.39	0.33	0.15
0.5	1.15	<LOQ	1.67	0.72	1.38	0.88	1.16	0.38
1	1.65	0.28	4.26	1.28	2.73	1.84	2.01	1.36
2	1.62	0.32	6.25	1.70	2.60	2.89	2.56	2.02
3	2.06	0.22	5.56	2.70	1.92	2.62	2.51	1.74
4	2.86	0.24	4.31	5.07	1.38	2.77	2.77	1.79
6	1.98	0.28	3.53	8.72	1.00	2.25	2.96	3.03
8	1.90	0.47	3.00	9.44	0.79	2.34	2.99	3.30
12	0.96	0.42	3.35	8.42	0.62	2.24	2.67	3.03
16	0.71	0.36	3.73	4.44	0.76	2.15	2.02	1.73
24	0.79	0.28	2.19	3.25	0.49	2.03	1.50	1.17
32	0.30	<LOQ	0.76	2.13	0.19	0.65	0.81	0.78
48	0.19	<LOQ	0.62	0.67	0.66	0.26	0.48	0.24

* Data point excluded from pharmacokinetic analysis.

TABLE 6 – Plasma concentrations of acid metabolite after oral administration of 10 mg/kg tepoxalin to dogs fed a high fat meal and after intravenous administration of tepoxalin. (Units in *u*g/ml)

PO/High Fat:

Time (hr)	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		
0.25	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD		
0.5	0.46	0.14	<LOQ	<LOQ	<LOQ	<LOQ		
1	1.74	0.20	0.77	1.71	0.45	0.56	0.91	0.66
2	3.87	0.34	3.79	5.24	2.03	2.33	2.93	1.72
3	5.74	0.48	5.10	7.31	2.68	3.15	4.08	2.45
4	4.30	0.45	4.87	5.20	2.70	3.87	3.56	1.76
6	4.10	0.68	3.99	4.06	2.09	2.86	2.96	1.38
8	4.90	0.65	4.48	3.64	3.36	2.57	3.27	1.53
12	3.04	0.45	3.34	2.22	2.03	1.95	2.17	1.02
16	2.91	0.50	3.61	2.21	1.47	1.68	2.06	1.10
24	2.18	0.53	2.88	1.31	1.10	1.41	1.57	0.83
32	1.19	0.13	1.98	0.77	0.78	0.79	0.94	0.61
48	0.46	<LOQ	0.42	0.48	0.76	0.39	0.43	0.22

IV

Time (hr)	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		
0.083	1.24	0.43	0.72	1.42	1.29	0.83	0.99	0.39
0.17	2.40	0.82	2.75	3.21	3.15	1.62	2.33	0.94
0.33	4.23	1.33	5.39	4.93	3.80	2.88	3.76	1.48
0.5	5.51	1.58	7.79	6.46	5.10	1.82	4.71	2.51
1	8.17	1.87	9.42	9.77	5.55	5.75	6.76	2.98
2	9.02	1.01	11.27	10.72	6.18	6.10	7.38	3.81
3	6.80	0.54	8.91	8.12	4.51	5.09	5.66	3.02
4	5.62	0.37	6.93	5.50	3.17	3.91	4.25	2.32
6	3.64	0.20	4.10	3.96	1.94	2.43	2.71	1.50
8	2.73	0.16	3.30	2.59	1.63	1.88	2.05	1.10
12	1.62	0.14	1.72	1.90	1.28	1.12	1.30	0.64
16	1.18	0.13	1.53	1.29	0.90	0.82	0.98	0.49
24	0.84	<LOQ	1.15	1.10	<LOD	0.78	0.97	0.18
32	0.40	<LOQ	1.03	0.42	0.71	0.20	0.55	0.32
48	0.19	<LOQ	0.64	0.23	0.97	<LOQ	0.51	0.37

TABLE 7 – Pharmacokinetic parameters describing disposition of tepoxalin after oral or intravenous administration (10 mg/kg).

PO/Fasted:								
Value	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
AUC (μg·hr/ml)	7.48	6.86	10.80	1.09	2.59	5.88	5.78	3.51
AUMC	152.01	84.55	160.20	12.76	11.87	67.81	81.53	64.71
MRT (hr)	20.31	12.32	14.83	11.71	4.58	11.54	12.55	5.11
$t_{1/2}$ (hr)	3190.71	18.99	30.28	-	4.43	13.39	651.56	1419.46
C_{max} (μg/ml)	0.49	0.65	0.65	0.14	0.65	0.57	0.53[a]	0.20
T_{max} (hr)	3.50	2.00	1.00	12.00	2.00	1.00	3.58	4.22
F	62.67	56.98	70.08	7.89	32.71	35.85	44.36	23.22
PO/Low Fat:								
Value	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
AUC (μg·hr/ml)	17.90	4.37	3.97	9.88	3.13	15.29	9.09	6.34
AUMC	397.32	34.03	9.82	69.16	7.39	301.24	136.49	169.07
MRT (hr)	22.19	7.79	2.48	7.00	2.36	19.70	10.25	8.62
$t_{1/2}$ (hr)	57.97	8.01	1.84	6.07	2.13	23.49	16.59	21.78
C_{max} (μg/ml)	0.89	0.46	1.45	1.38	1.25	1.06	1.08[b]	0.37
T_{max} (hr)	0.50	2.00	1.00	4.00	1.00	2.00	1.75	1.25
F	149.93	36.26	25.72	71.54	39.48	93.26	69.36	46.82
PO/High Fat:								
Value	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
AUC (μg·hr/ml)	9.73	19.62	5.68	4.81	22.07	5.45	11.23	7.69
AUMC	93.81	437.05	26.59	19.85	535.41	18.95	188.61	234.30
MRT (hr)	9.64	22.27	4.68	4.13	24.26	3.48	11.41	9.46
$t_{1/2}$ (hr)	8.97	79.12	3.24	2.69	65.57	2.36	26.99	35.47
C_{max} (μg/ml)	1.42	0.65	1.24	1.24	1.12	1.47	1.19 [b]	0.29
T_{max} (hr)	2.00	2.00	2.00	2.00	4.00	3.00	2.50	0.84
F	81.51	162.90	36.86	34.84	278.43	33.22	104.62	98.69
IV:								
Value	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
AUC (μg·hr/ml)	11.94	12.05	15.42	13.81	7.93	16.39	12.92	3.02
AUMC	23.59	24.48	50.81	11.67	6.71	59.64	29.48	21.26
MRT (hr)	1.98	2.03	3.30	0.85	0.85	3.64	2.11	1.18
$t_{1/2}$ (hr)	2.17	3.14	4.11	0.73	0.78	3.76	2.45	1.47

[a,b] Means with different superscripts are significantly different.

TABLE 8 – Pharmacokinetic parameters describing disposition of the acid metabolite of tepoxalin after administration of tepoxalin (10 mg/kg).

PO/Fasted:								
Value	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
AUC (μg·hr/ml)	33.72	7.88	46.49	19.96	37.70	19.07	27.47	14.26
AUMC	430.07	90.63	625.74	364.46	435.20	178.36	354.08	193.15
MRT (hr)	12.76	11.51	13.46	18.26	11.54	9.35	12.81	3.01
$t_{1/2}$ (hr)	10.47	12.78	10.32	21.86	26.27	7.21	14.82	7.51
C_{max} (μg/ml)	3.26	0.87	3.92	0.94	2.94	1.98	2.32	1.26
T_{max} (hr)	4.00	2.00	3.00	4.00	2.00	4.00	3.17	0.98
PO/Low Fat:								
Value	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
AUC (μg·hr/ml)	38.88	9.16	105.67	176.31	32.13	70.97	72.19	61.07
AUMC	551.48	126.07	1642.22	2903.83	575.73	1168.11	1161.24	1005.41
MRT (hr)	14.19	13.76	15.54	16.47	17.92	16.46	15.72	1.56
$t_{1/2}$ (hr)	11.65	22.06	14.05	10.60	18.31	14.15	15.14	4.31
C_{max} (μg/ml)	2.86	0.47	6.25	9.44	2.73	2.89	4.11	3.20
T_{max} (hr)	4.00	8.00	2.00	8.00	1.00	2.00	4.17	3.13
PO/High Fat:								
Value	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
AUC (μg·hr/ml)	105.46	16.25	123.37	86.37	64.54	66.90	77.15	37.41
AUMC	1696.39	280.76	132.03	1251.21	1175.48	1112.47	941.39	606.89
MRT (hr)	16.09	17.28	1.07	14.49	18.21	16.63	13.96	6.44
$t_{1/2}$ (hr)	13.21	9.65	14.29	11.97	20.70	14.23	14.01	3.71
C_{max} (μg/ml)	5.74	0.68	5.10	7.31	2.70	3.87	4.23	2.35
T_{max} (hr)	3.00	6.00	3.00	3.00	4.00	4.00	3.83	1.17
IV:								
Value	#3308	#3309	#3310	#3311	#3312	#3313	Mean	SD
AUC (μg·hr/ml)	75.39	6.61	102.38	84.62	58.01	51.26	63.05	33.21
AUMC	767.58	33.06	1319.50	873.08	905.57	489.06	731.31	434.51
MRT (hr)	10.18	5.00	12.89	10.32	15.61	9.54	10.59	3.55
$t_{1/2}$ (hr)	8.31	3.88	11.23	8.46	16.71	6.83	9.24	4.37
C_{max} (μg/ml)	9.02	1.87	11.27	10.72	6.18	6.10	7.53	3.53
T_{max} (hr)	2.00	1.00	2.00	2.00	2.00	2.00	1.83	0.41

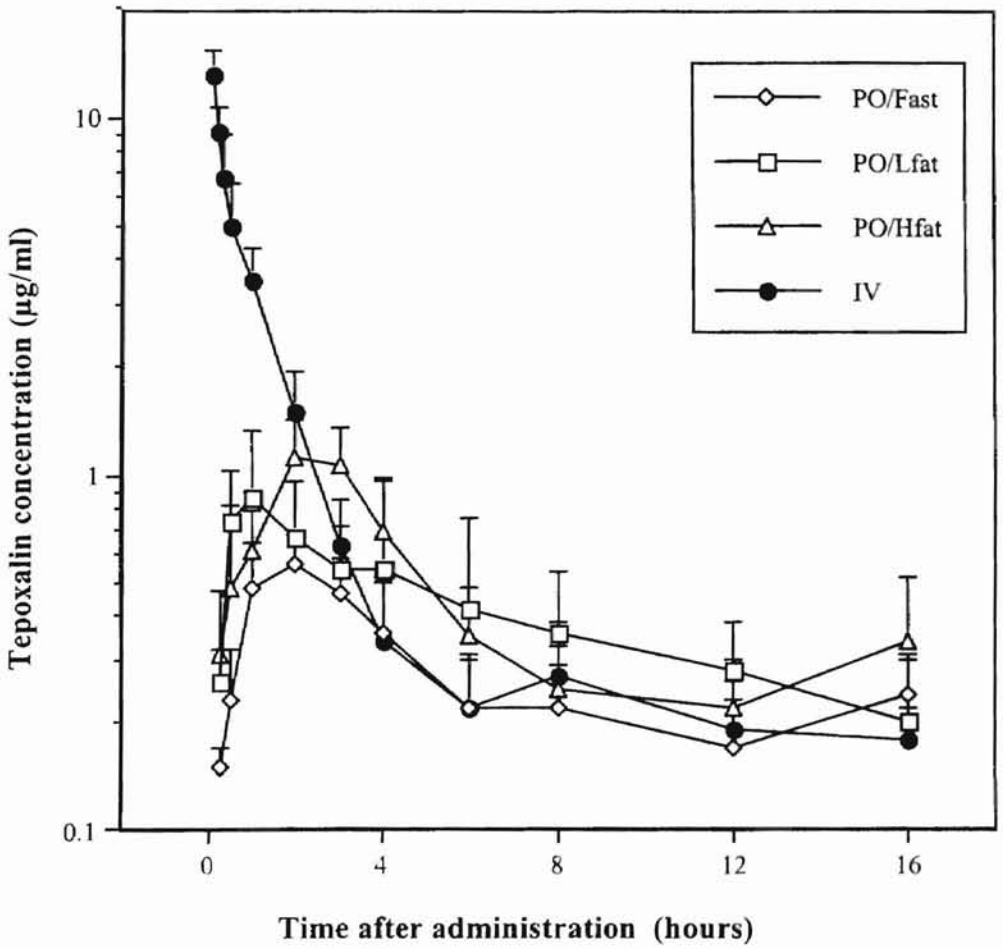

Figure 2 – Mean tepoxalin concentrations after intravenous (IV) or oral (PO) administration to fasted (FAST) dogs or dogs fed low fat (Lfat) or high fat (Hfat) diets (n = 6)

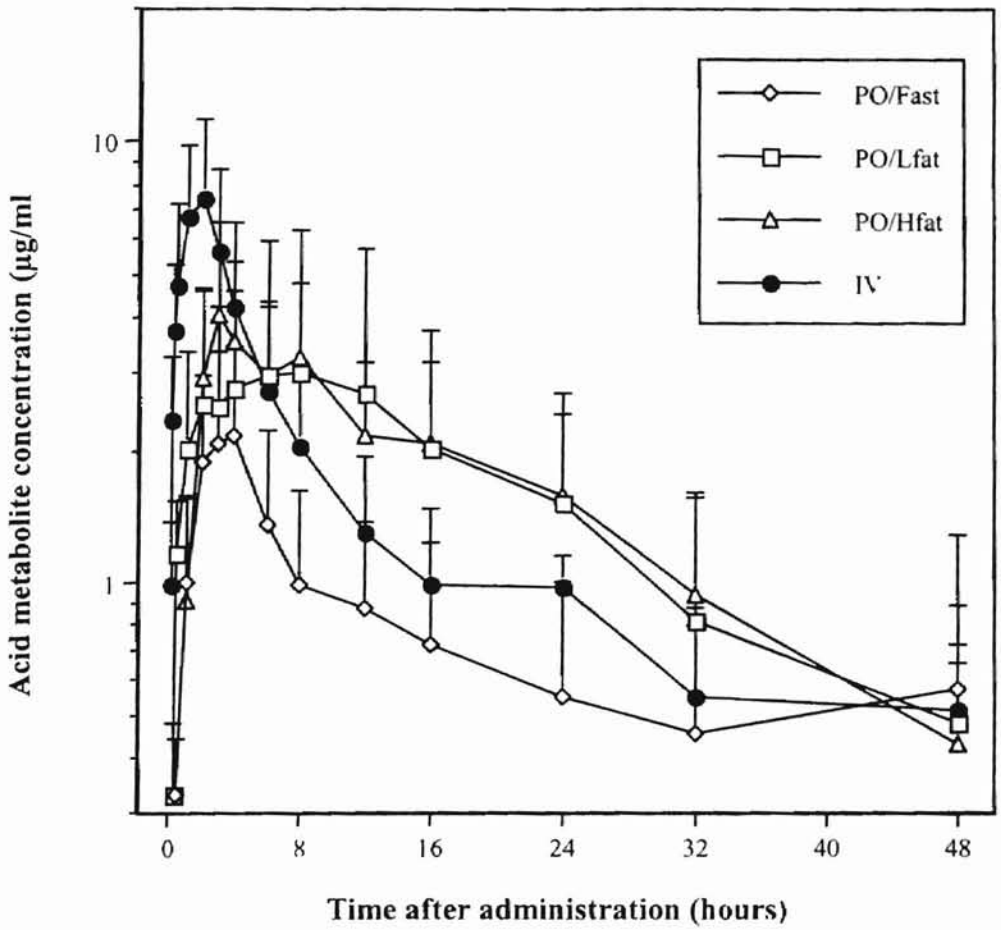

Figure 3 – Mean tepoxalin acid metabolite concentrations after intravenous (IV) or oral (PO) administration to fasted (FAST) dogs or dogs fed low fat (Lfat) or high fat (Hfat) diets (n = 6)

CHAPTER VI

DISCUSSION & CONCLUSIONS

Although it is widely known that corticosteroids and NSAIDs can help alleviate the symptoms of canine osteoarthritis, both groups of drugs have undesirable side effects. Researchers are continually looking for new drugs that can control the inflammatory process of osteoarthritis with fewer adverse side effects. Tepoxalin appears to be one such discovery. This study was conducted to describe the pharmacokinetics of tepoxalin both orally and intravenously and to investigate absorption rates of the drug when the fat content of the diet was varied.

When given intravenously, it was noted that the parent drug, tepoxalin, was rapidly metabolized and formed an acid metabolite. Tepoxalin undergoes a first-pass effect through the liver and this accounts for the rapid decrease in tepoxalin in plasma while the acid metabolite concentration increases and remains higher than the concentration of the parent drug over the 48 hour sampling period. This same pattern can be seen when the fat content of the diet is varied at the time of oral drug administration.

The oral bioavailability of tepoxalin is also affected by the diet at the time of drug administration. Several studies have looked at the effects of diet and drug bioavailability. Some of the factors considered are gastric motility and the physical interaction of the food with the drug (Toothaker, *et al*, 1980).

When food is ingested, it delays gastric emptying due to feedback mechanisms of certain chemical receptors in the small intestine (Toothaker, *et al*, 1980). Also, many

drugs are absorbed in the small intestine and a delay in stomach emptying leads to a delay in reaching the small intestine and the optimal site of absorption (Toothaker, *et* al, 1980). The food itself may act as a physical barrier preventing the drug from being absorbed from the mucosal surface or it could complex with the drug (Toothaker, *et al*, 1980). Food intake is known to decrease the oral bioavailability of tetracycline, penicillin V, penicillin G, cephalexin, erythromycin stearate, and aspirin (Toothaker, *et al*, 1980).

Tepoxalin was administered in a rapid-dissolution form to the dogs, which allowed for a faster absorption from the mucosal surface of the stomach. The structure of tepoxalin, as noted by Tam, *et al*, is composed of a pyrazole group and a hydroxamic acid group (1995). These groups give tepoxalin the ability to be lipid soluble and thus easily pass through the phospholipid bilayer of the cell membrane. The results of the research suggest that the fat in the diet allows the drug to more easily cross the GIT mucosal barrier and thus be more effective. Although a fatty diet contains micelles and some drugs may extend their efficacy by being transported in them resulting in protection from metabolic enzymes, this appears not to be the case with tepoxalin. As mentioned previously, the active acid metabolite is formed within 8 hours and for this to occur, the hepatic enzymes need to have access to the parent drug to break it down and form the metabolite.

In conclusion, this study has shown that diet has an affect on the oral bioavailability of tepoxalin and the pharmacokinetic parameters calculated have demonstrated that aspect. The ability to administer the drug orally, the increased half-life demonstrated by the drug, and the few adverse side effects make tepoxalin a good candidate as an anti-inflammatory agent in the treatment of osteoarthritis in dogs. Further

research needs to be done on the acid metabolite of tepoxalin to determine its role in the prevention of inflammation.

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