CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-903

PHARMACOLOGY REVIEW(S)

NDA 21-903

PHARMACOLOGY REVIEW OF ORIGINAL 505(b)(2) APPLICATION

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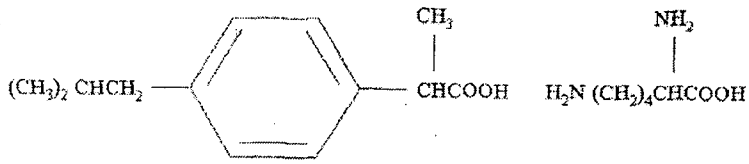
DRUG PRODUCT: NeoProfen IV Solution

REFERENCE LISTED DRUG PRODUCT: None (literature-based application)

DRUG SUBSTANCE: ibuprofen-l-lysinate



Molecular Formula: $C_{10}H_{32}N_2O_4$
Molecular Weight: 352.48
CAS No. 57469-77-9
Pharmacologic Category: nonsteroidal anti-inflammatory agent

PROPOSED INDICATION: [redacted]

FORMULATION AND ROUTE OF ADMINISTRATION: Sterile solution for intravenous infusion. Each milliliter contains 17.1 mg of ibuprofen-l-lysinate, equivalent to 10 mg of (±) ibuprofen, in Water for Injection, USP.

PROPOSED DOSAGE REGIMEN: An initial dose of 10 mg/kg is followed by two doses of 5 mg/kg each, after 24 and 48 hours. Second and third doses not to be given if anuria or marked oliguria is evident. If the ductus arteriosus is not significantly reduced in size after completion of the first course of NeoProfen or if during continued medical management the ductus arteriosus reopens, a second course of 3 doses may be given.

RELATED APPLICATION: Farmacon-IL, LLC IND 59,778 for ibuprofen lysinate in the treatment of patent ductus arteriosus in premature neonates.

NONCLINICAL PHARMACOLOGY/TOXICOLOGY DATA: Ibuprofen is a marketed drug and the need for a new drug application for the sponsor's product is dictated by the new indication, the new salt and the new route of administration. Published literature is cited, and provided, to document the non-selective inhibition by ibuprofen of cyclo-oxygenase, resulting in reduced synthesis of prostaglandins, considered to be the mechanism responsible, or mainly responsible, for the closure of the patent ductus by ibuprofen. The tone and patency of the ductus have been reported as being maintained by prostaglandins, principally PGE2.

Information provided on the potential toxicity of the l-lysine salt of ibuprofen comes from a thirteen-week oral toxicity study of L-lysine HCl in rats in which exposure to the amino acid was via the feed at dietary concentrations of up to 5% and in which there were no adverse effects reported in males or females. L-lysine is an essential amino acid for humans and widely available as a dietary supplement.

A repeated dose iv toxicity study of ibuprofen-l-lysine in beagle neonates (previously reviewed in May of 2004 under the sponsor's IND 59778) and a study exploring the replacement of bilirubin with ibuprofen on albumin receptor sites in premature infants were the only studies conducted by the sponsor. The remainder of the non-clinical part of the NDA was, according to the sponsor, collected from published peer reviewed journals.

The NDA contains numerous publications bearing on the pharmacokinetics, pharmacodynamics and general toxicity of ibuprofen administered orally to various species, including summaries of carcinogenicity and reproductive toxicity studies. In many cases there is insufficient detail regarding results and methodology to permit independent review. However, considering that the Agency has previously found this drug to be safe and effective for chronic oral use as an analgesic-antiinflammatory agent, such information is not needed to support the safety of the short-term iv treatment of premature neonates.

There are several papers comparing ibuprofen with indomethacin, another NSAID and the only drug currently approved for the PDA indication, and a number of papers that describe genetic toxicology studies performed with ibuprofen.

Pharmacology

The following text was extracted, with minor modifications, from the sponsor's "Pharmacology and Toxicology Summary."

Background

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) that possesses analgesic and antipyretic activity. Its mode of action in closing an open ductus arteriosus, like that of other NSAIDs is not completely understood, but it may be related to prostaglandin synthetase inhibition.

The sponsor, Farmacon-IL, LLC, has not conducted pharmacological studies in animals with R,S-ibuprofen 1-lysine for the closure of patent ductus arteriosus (PDA) in premature animals since the potential of ibuprofen to close the open ductus in premature infants had been shown several years prior to the development of Neoprofen. (Aranda, et al, 1997, Van Overmeire, et al, 2000).

Farmacon-IL, LLC did conduct an ibuprofen replacement of bilirubin on albumin receptor sites study in premature infants (Van Overmeire, et al, 2005) in order to support the safety evaluation of ibuprofen lysine iv for the treatment of PDA in very premature infants. They also conducted a toxicology study in neonatal dogs. The remainder of the non-clinical part of the NDA has been collected from published peer reviewed journals.

The literature references cited were published from 1969 to date. While earlier literature may be lacking methodology and details as compared with more recent publications, the older studies have very similar results to the more recently published research. The more than three decades of clinical experience gave the applicant further assurance as to ibuprofen's safety profile.

Published pharmacological studies, conducted in animals and relevant to the proposed indication, were reviewed and their results were judged valid and acceptable. Ibuprofen is a non-selective inhibitor of cyclo-oxygenase (COX-1 and COX-2), leading to reduced synthesis of prostaglandins. Since prostaglandins are involved in the persistence of keeping the ductus arteriosus (PDA) patent after birth, this mode of action is postulated to be the main mechanism of action of ibuprofen for this indication. In the three studies submitted by the applicant in NDA # 21-903, the closure rate of the ductus with the 10-5- 5mg/kg ibuprofen lysinate iv 24-hours apart treatment regimen were 65.8%, 69.1%, and 85.6%, respectively.

Prostaglandins Role to Maintain Ductus Arteriosus Patent

The three studies of the applicant and a meta-analysis of the published clinical studies (R.L. Thomas, et al, 2005), suggested that ibuprofen is efficacious and closes the open ductus. This supports the theory that ibuprofen is a prostaglandin synthetase inhibitor which plays a significant part in keeping the ductus open. The ductus arteriosus has an intrinsic tone and patency which is maintained by prostaglandins, mainly PGE2, which is the most potent ductus relaxing agent known (Coceani, 1994, Smith, et al, 1994). Although PGI2 is about 1000 times less potent than PGE2, the ductus arteriosus also relaxes in response to high concentrations of PGI2; in addition, there is functional evidence to suggest a dilator role for PGI2. While native prostaglandins are all agonists at other types of prostaglandin receptors, albeit at least an order of magnitude less potent, PGI2 is not effecting relaxation via the PGE2 receptor. The presence of PGI2 receptors in the DA has been demonstrated and, as in other blood vessels, PGI2 is the main arachidonic acid product in the DA; approximately 10 times as much PGI2 as PGE2 is synthesized (Coceani et al, 1980). It appears therefore that PGI2 also plays a physiological role in the maintenance of the patency of the DA.

Studies in genetically engineered cyclooxygenase-deficient mice suggest that patency can be maintained in the fetus by maternally synthesized prostaglandins (Loftin et al, 2001). Further,

administration of various NSAIDs in late pregnancy is known to cause premature closure of the DA in many species, including humans (Van den Veyver et al, 1993). Studies in pregnant rats have demonstrated dose-related constriction of the DA as a general property of acidic NSAIDs, with indomethacin and ibuprofen being amongst the most potent (Momma et al, 1983, Momma et al, 1984, Momma et al, 1990). Comparing clinical doses, ibuprofen was more potent than indomethacin, although the effect of indomethacin was more prolonged, possibly because its plasma half-life in the rat is longer than that of ibuprofen (Adams et al, 1969).

Ibuprofen Effect on Prostaglandins

Ibuprofen's pharmacologically specific activity for the proposed indication, i.e., closing of the open ductus, was first studied in lambs by Coceani et al, 1979. Although indomethacin did not induce closure of the DA in pre-term rat fetuses, there appear to be species differences (Momma et al, 1983, Sharpe et al, 1974). In lambs, the prostaglandin mechanism responsible for maintenance of DA patency becomes functional earlier in gestation. Ibuprofen has been shown to contract fetal lamb DA *in vitro* over the last trimester of gestation and constriction of fetal DA by ibuprofen was reported to be stronger in tissue from pre-term than in that from full-term fetuses (Coceani et al, 1979, Friedman et al, 1976, Heyman et al, 1976). Ibuprofen was shown to close the DA in lambs (Coceani et al, 1979) but it did not affect basal CBF, cerebral metabolic rate, intestinal or renal hemodynamics in animal models, in contrast to indomethacin.

The PGE2 receptor exists as four distinct subtypes denoted EP 1 to EP 4 (Coleman et al, 1994). The receptor subtypes functioning in the DA have been investigated in a number of animal species but it is not clear whether inter-species differences exist (Segi et al, 1998).
A study in fetal rabbit tissue suggests that dilation of the DA is mediated via the EP 4 receptor; the inability of indomethacin to effect closure of the DA in EP 4 deficient mice supports this, but as these mice survive *in utero* an alternative mechanism must also exist, possibly involving other subtypes (or perhaps the PGI2 receptor) (Nguyen et al, 1997, Segi et al, 1998). Agonists for EP I and EP 3 receptors also contract fetal rabbit DA *in vitro* but fetal piglet DA has been shown to express EP receptors 2, 3 and 4 in equivalent density, but no EP 1 receptor (Bhattacharya et al, 1999, Smith et al, 1995).

In the newborn piglet the density of the EP 2 receptors is maintained but there is a complete lack of the EP3 and EP 4 receptors, resulting in a reduction of total PGE2 binding capacity, which must contribute to the reduced responsiveness of the DA to prostaglandins after birth, even if other factors are also involved. It also suggests that in the newborn piglet dilation of the DA by prostaglandins is mediated via the EP 2 receptor (Bhattacharya et al, 1999).

Mechanism of the Closure of PDA

After birth, the closure of the DA occurs in two steps. During the first step, which is a functional closure, the DA becomes less responsive to oxygen and PGE2; the changes that affect this must include the down-regulation of EP receptors that causes the constriction of the medial muscle layer and usually occurs in the first few hours after term-birth. Ischemia of the medial muscle (resulting from reduced blood flow) appears to be the stimulus for the second step (Bhattacharya, et al, 1999, Clyman, et al, 1983, Clyman, et al, 1999), the anatomical closure of the DA,

involving infolding of the epithelium and disruption of the subintimal layers, which is usually completed by the second week of life. The process of closure of the DA after birth is similar in all mammalian species, although there are differences in closure rate and morphology (Clyman et al, 1983, Clyman et al, 1999, Hornblad et al, 1967).

The mechanism underlying the closure of the DA at birth is complex and is probably not yet completely elucidated (Coceani et al, 1980). The sudden increase in blood oxygen tension produced by the first breath and the drop in circulating prostaglandin levels that occurs after parturition have long been considered to be implicated in the closing of the ductus arteriosus (Hammerman et al, 2001).

Maintenance of the ischemic state is required for completion of anatomical remodeling and the longer the DA remains constricted the less it is able to respond to the dilation stimulus of prostaglandins. Therefore, when the DA remains patent in the newborn, constriction by any means followed by maintenance of the ischemic state should allow anatomical remodeling, thus closure of the ductus.

Effects of Inhibiting Prostaglandin Synthesis

It has been shown that, despite the fact that in the newborn pig COX 2 and not COX 1 is expressed in the DA, it is the circulating prostaglandin produced systemically by COX I and not local prostaglandin synthesized by COX 2 that maintains the patency of the DA (Guerguerian et al, 1998). In the fetal pig the DA contains COX 1 almost exclusively The selective COX 1 inhibitor, valeryl salicylate, and the nonspecific cyclooxygenase inhibitor, indomethacin, have been shown to cause ductal constriction and decreased PGE2 concentrations in the newborn pig, both in the plasma and in the DA. In contrast, COX 2 inhibitors, DuP697 and NS-398, reduced PGE2 levels in the DA but did not cause ductal constriction and did not affect circulating PGE2 concentrations. Therefore, although there has been no actual demonstration in newborn animals that ibuprofen will constrict the DA, it can also be expected to do so. The extended plasma half-life of ibuprofen in the human neonate will also contribute to the maintenance of constriction that is required for anatomical remodeling of the ductus arteriosus to take place.

The biological activities of the prostanoids (the autocoids generated via the cyclooxygenase pathway) are extremely diverse. They produce their effects by acting on specific receptors and the specific biological activity results from local production of the specific prostanoids and the tissue distribution of the various types of receptors (Coleman et al, 1994). Inhibition of prostaglandin synthesis may therefore interfere with these processes and result in unwanted side effects.

The adverse effects resulting from inhibition of prostaglandin synthesis after oral administration of NSAIDs are well known. The most common, the occurrence of gastro-intestinal bleeding, is usually a consequence of long-term administration; the incidence in humans is much lower for ibuprofen than for any other NSAID (Langman et al, 1994, Henry et al, 1996, Henry et al, 1999). Potential for renal toxicity is inherent in all NSAIDs as a result of inhibition of the synthesis of PGE2 and PGI2 which are involved in the maintenance of renal blood dynamics, but usually toxicity only occurs when renal perfusion is impaired (Whelton et al, 1991). The risk for adverse

effects in children aged eight months has been shown to be low after oral administration of ibuprofen (Lesko et al, 1995).

Although ibuprofen inhibits thromboxane (TxA2) biosynthesis and causes a dose-related inhibition of *ex vivo* aggregation of rat platelets after oral administration, it has little anti-coagulative activity (Evans et al, 1991, Rainsford et al, 1999). Its anti-thrombotic effect *in vivo* is weak; after i.p. administration in rabbits it was about 450 times less effective than indomethacin (DiPasquale et al, 1997, Royer et al, 1985). Ibuprofen is also a weak inhibitor of platelet aggregation in man.

Prostaglandins are involved in the physiological regulation of vascular tone, usually exerting a vasodilatory effect. However, in adult animals the effects of intravenous administration of ibuprofen on blood-flow are limited. Administered intravenously to adult baboons, ibuprofen 50 mg/kg, (either as four bolus doses of 12.5 mg/kg at 6 hourly intervals or as a continuous infusion over 24 hours) produced only mild or insignificant changes in hemodynamic parameters (mean arterial pressure, central venous pressure and heart rate) (Rao et al, 1994).

In adult mongrel dogs, however, ibuprofen, 10 mg/kg i.v, increased aortic blood pressure by 20% and caused a slight, although significant reduction of the renal blood flow (Feigen et al, 1981). The reference drug indomethacin, 2.5 mg/kg, produced a similar effect on aortic and renal flow but, whereas this dose of ibuprofen had no significant effect on mesenteric blood flow, indomethacin rapidly reduced it by 50%. Both drugs similarly attenuated the responses to arachidonic acid (i.e. inhibited prostaglandin synthesis) at the doses used, but, unlike the effects on renal vascular resistance, the ability of indomethacin to elicit mesenteric vasoconstriction was shown to be independent of its inhibition of prostaglandin synthesis.

The Effects of Ibuprofen versus Indomethacin on Blood Flow

In premature human neonates with PDA, there is a redistribution of systemic blood flow, which can lead to impaired perfusion of some organ systems, with the gastrointestinal tract, kidneys and brain being especially vulnerable (Hammerman et al, 2001). The effects of intravenous ibuprofen on regional blood flow to these organs have been investigated in newborn rabbits (Chama et al, 2000).

Ibuprofen, 0.02 and 0.2 mg/kg i.v. produced a dose-related increase in renal vascular resistance (RVR) with a consequent decrease in renal blood flow (RBF) and glomerular filtration rate (GFR) in newborn rabbits (Chama et al, 2000). Urinary volume, unaffected at the lower dose, was decreased at 0.2 mg/kg. A ten-fold increase in dose to 2.0 mg/kg had little further effect. The investigators concluded that the renal effects of ibuprofen in the newborn rabbit are at least as great as those of indomethacin, but no reference group was included in this study and the data for indomethacin, quoted from an earlier study, are for a single dose, 2.0 mg/kg only. Although the values for changes in RVR, RBF, GFR and urinary volume are comparable with those at the same dose of ibuprofen, there is no data for lower doses of indomethacin and consequently no information on the dose at which maximal response is achieved.

In a study in newborn piglets the doses of ibuprofen and indomethacin effective in closing the PDA in premature infants were compared directly (Speziale et al, 1999). A much higher dose of ibuprofen (20 mg/kg i.v.) than in the previous study increased vascular resistance in the renal cortex and medulla (by 40 and 50 % respectively) at 90 and 120 minutes after administration. After a dose of indomethacin of 0.3 mg/kg (much lower than that quoted in the previous study), RVR increased to a greater extent (66 and 70 % respectively) at 40 minutes after injection and the blood flow was significantly decreased. Consequently, the potential for ibuprofen to affect renal blood flow appears to be no greater than that of indomethacin. In the same study, ibuprofen showed no effect on gastrointestinal blood-flow in the newborn piglet, whereas indomethacin rapidly almost doubled vascular resistance in duodenum/jejunum, ileum and colon (Speziale et al, 1999).

Ibuprofen was shown in the newborn piglet not to affect cerebral vascular resistance (CVR) nor to have any significant effects on total or regional cerebral blood flow (CBF) either at a dose comparable to that used clinically for closure of the ductus arteriosus (20 mg/kg i.v.) or at a higher dose (30 mg/kg) at which prostaglandin synthesis was markedly decreased (Pellicer et al, 1999, Speziale et al, 1999). In contrast, indomethacin exerted a significant vasoconstrictive effect in the brain even at a dose that only minimally decreased cerebral prostaglandin synthesis (18-32% in 60 min.) with CBF decreased almost the same extent after both low (3mg/kg) and high (10 mg/kg) doses of indomethacin. The data suggest that PG may not play a critical role in the regulation of basal CBF in the newborn animal (Chemtob et al, 1991).

Although prostanoids may not play a critical role in the regulation of basal CBF in the newborn, they do however play a role in setting the blood pressure limits within which autoregulation of cerebral blood flow can be maintained in the newborn piglet (Chemtob et al, 1990, Chemtob et al, 1991). In newborn piglets, although global and regional blood flow was maintained at a constant proportion of mean blood flow over a blood pressure range of 50-90 mm Hg in the control group, it varied markedly outside these limits. However, in a group treated with ibuprofen (30 mg/kg i.v.) the blood pressure range over which cerebral blood flow did not vary significantly was extended to 37-117 mm Hg. In addition, at the lowest blood pressure investigated (30 mm Hg), the decrease in cerebral blood flow was significantly less in the ibuprofen-treated group than in the control group (30% compared to 75%, $p<0.001$). Thus, ibuprofen has no deleterious effect on cerebral blood flow in the newborn, but where blood flow is reduced it may assist in its maintenance.

Under artificial ventilation, frequently a necessity in premature human infants, cardiac output is decreased (Malcolm et al, 1993). When cardiac output falls, the decrease in blood flow is not distributed equally among the different vascular beds; blood flow to the brain is maintained while that to the kidney and gut is being compromised. In artificially ventilated, newborn piglets, cardiac output decreased with increasing airways pressure. Blood flow to colon, ileum and kidney decreased with increasing pressure but cerebral flow was maintained. As prostaglandin levels were unaffected they do not appear to be involved in the underlying mechanism and intravenous administration of ibuprofen, 40 mg/kg, did not influence the effects of artificial ventilation on regional blood flow; contrary to indomethacin, which at the dose of 0.3 mg/kg, further reduced blood flow to the ileum and brain.

In the lung, the low vascular tone of both arterial and vascular vessels (characteristic of the adult pulmonary circulation) is maintained by basal production of vasodilatory prostaglandins, mainly PGI2, and the adult pulmonary circulation shows little or no response to physiological or pharmacological stimulation (Abman et al, 1989).

The sudden and dramatic decrease in pulmonary vascular resistance (PVR) that occurs at birth is essential for normal transition from fetal to neonatal circulation, with the pulmonary circulation being required to dilate in order to accommodate an eight- to ten-fold increase in blood flow through the lungs. Several physiological stimuli contribute to the fall in PVR, among them, rhythmic distension of the lung and increased oxygen tension as well as the hemodynamic stress resulting from constriction of the DA. The final mediator of stress-induced vasodilation is nitric oxide (NO), which appears to play a greater part in the regulation of PVR in the perinatal pulmonary circulation than PGI2 and it has been shown that PGI2 exert its effects mainly via release of NO (Storme et al, 1999).

Retinopathy of Prematurity (ROP)

Free radicals have been implicated in the development of injury to the immature retina. Asphyxia increases the levels of both free radicals and prostaglandins and using newborn piglets, a species with retinal characteristics similar to those of the human, ibuprofen has been used to demonstrate that the cyclooxygenase pathway is probably the source of free radicals after asphyxia. Administration of a cyclooxygenase inhibitor may therefore protect the retina in the premature newborn when blood flow is compromised. Indomethacin may not afford the same overall advantage as it has been shown to impair retinal haemodynamics in the newborn piglet by a mechanism unrelated to inhibition of prostaglandin synthesis whereas ibuprofen enhances retinal and choroidal blood flow autoregulation in newborn piglets (Chemtob et al, 1991, Parys-Van Ginderdeuren et al, 1992).

Cytoprotective Effect

Ibuprofen appears to exert a cytoprotective effect on the intestinal mucosa of rats. In an experimental model of bowel ischemia in rats, animals treated with ibuprofen had a significantly lower incidence of intestinal necrosis than rats treated with indomethacin. (Grosfeld et al, 1983). It is suggested that the protective effect of ibuprofen results from inhibition of synthesis of thromboxane, which is a vasoconstrictor.

Effects of Ibuprofen on Free Bilirubin Levels

Like all the carboxylic acid NSAID's, ibuprofen binds to serum albumin at the same site as bilirubin (Cooper-Peel et al, 1996). In an *in vitro* study using infant blood serum, ibuprofen, at a concentration close to that seen in clinical use for closure of the PDA (750 nnol/1, i.e. 154.7 pg/ml), increased the free bilirubin fraction fourfold at a bilirubin to albumin molar ratio (B:A) of 1:2. No absolute values for free bilirubin concentration are quoted in the publication and it is not clear whether the free bilirubin concentration reaches a level at which a premature infant would be at risk for bilirubin kernicterus. A later study (Ma et al, 2002), using a different method

with albumin solutions at varying concentrations, reports that significant displacement of bilirubin does not occur until the B:A ratio reaches 2:1 (no effect at 1.5:1).

Since concerns have been raised about the safety of administering ibuprofen to this vulnerable group of newborn infants typically suffering from respiratory distress syndrome and jaundice, Van Overmeire, et al, 2005 ("Unbound Bilirubin in Newborn Receiving Ibuprofen", Publication Draft) undertook a study determining the effect of standard doses of ibuprofen on unbound bilirubin by measuring plasma free bilirubin levels before and after the administration of ibuprofen. The investigators found that the levels of free bilirubin are comparable with those earlier reported (Van Overmeire, et al, 2001). At the effective dose of ibuprofen for closing the PDA, bilirubin concentrations did not reach dangerous levels at which kernicterus may develop.

Additional Pharmacology Safety Studies

After many years of use, the risks for adverse effects on major physiological systems after oral administration of ibuprofen are well characterized and are always related to the primary effect of inhibition of prostaglandin synthesis. The effects of intravenously administered ibuprofen on physiological systems, which may be compromised in the presence of a PDA have been investigated in newborn animals. Safety studies in nearly one thousand premature infants did not raise any potential safety problems.

Pharmacology References

Abman SH. Accurso FJ. Acute effects of partial compression of ductus arteriosus on fetal pulmonary circulation. Am J Physiol 1989; 257:H626-H634.

Adams SS, McCullough KF, Nicholson JS. The pharmacological properties of ibuprofen, an anti- inflammatory, analgesic and antipyretic agent. Arch Int Pharmacodyn. 1969; 178:115-129.

Bhattacharya M, Asselin P, Hardy P, Guerguerian A-M, Shichi H, Hou X et al. Developmental changes in prostaglandin E2 receptor subtypes in porcine ductus arteriosus. Circulation, 1999; 100:1751-1756.

Chamaa NS, Mosig D, Drukker A, Guignard J-P. The renal haemodynamic effects of ibuprofen in the newborn rabbit. Pediatr Res 2000; 48:600-605.

Chemtob S, Beharry K., Rex J, Varma SR, Aranda N. Prostanoids determine the range of cerebral blood flow autoregulation of newborn piglets. Stroke 1990; 21:777-784.

Chemtob S, Beharry K, Barna T, Varma DR, Aranda N. Differences in the effects in the newborn piglet of various nonsteroidal anti-inflammatory drugs on cerebral blood flow but not on cerebrovascular prostaglandins. Pediatr Res 1991; 30:106-111.

Chemtob S, Beharry K, Rex J, Chatterjee T, Varma DR et al. Ibuprofen enhances retinal and choroidal blood flow autoregulation in newborn piglets. Invest Ophthalmol Vis Sci 1991; 32: 1799-1807.

Clyman RI, Chan CY, Mauray F, Chen YQ, COX W et al. Permanent atomic closure of the ductus arteriosus in newborn baboons: The roles of postnatal constriction, hypoxia and gestation. Pediatric Res. 1999; 45:19-29.

Clyman RI, Mauray F, Roman C, Heyman MA, Payne B. Factors determining the loss of ductus arteriosus responsiveness to prostaglandin E. Circulation, 1983; 68:433-436.

Coceani F. Control of the ductus arteriosus- a new function for cytochrome P450, endothelin and nitric oxide. Biochemical Pharmacology, 1994; 48:1315-1318.

Coceani F & Olley PM. Role of prostaglandins, prostacyclin and thromboxanes in the control of prenatal patency and postnatal closure of the ductus arteriosus. Seminars in Perinatology, I980; 4:109-113.

Coceani F, White E, Bodach E, Olley PM. Age-dependent changes in the lamb ductus arteriosus to oxygen and ibuprofen. Can J Physiol Pharmacol, 1979; 57:825-831.

Coleman RA, Smith WI, Narumiya S. VIII. International Union of Pharmacology classification of prostanoid receptors: properties, distribution and structure of the receptors and their subtypes. Pharmacological reviews 1994; 46:205-229.

Cooper-Peel C, Brodersen R, Robertson A. Does ibuprofen affect bilirubin-albumin binding in newborn infant serum? Pharmacol Toxicol 1996; 79: 297-299.

Davies EF, Avery, GS. Ibuprofen: A Review of its pharmacological properties and therapeutic efficacy in rheumatic disorders. Drugs, 1971; 2:416-446.

DiPasquale G, Mellace D. Inhibition of arachidonic acid-induced mortality in rabbits with several non-steroidal anti-inflammatory drugs. Agents and Actions, 1997; 7:481-485.

Elliott, GA, Purmalis, A, VanderMeer, DA, Denlinger, RH. The propionic acids. Gastrointestinal toxicity in various species. Toxicol Pathol 1988; 16:245-250.

Evans AM, Nation RL, Sansom LN, Somogyi AA. Effect of racemic ibuprofen dose on the magnitude and duration of platelet cyclo-oxygenase inhibition: relationship between inhibition on thromboxane production and the plasma unbound concentration of (S)-(+)-ibuprofen. Brit J Clin Pharmacol 1991 ; 31:131-138.

Feigen LP, King LW, Ray J, Beckett W, Kadowitz PI. Differential effects of ibuprofen and indomethacin in the regional circulation of the dog. J Pharmacol Exp Ther 1981; 219:679-684.

Friedman WF, Hirschlau MI, Printz MP, Pitlick PT, Kirkpatrick SE. Pharmacologic closure of patent ductus arteriosus in the premature infant. N Engl J Med, 1976; 295:526-529.

Grosfeld JL, Kamman K, Gross K, Cikrit D, Ross D et al. Comparative effects of indomethacin, Prostaglandin E1 and ibuprofen on bowel ischaemia. J Pediatr Surg 1983; 18:738-742.

Guerguerian A-M, Hardy P, Bhattacharya M, Olley P, Clyman RI et al. Expression of cyclooxygenases in ductus arteriosus of fetal and newborn pigs. Am J Obstet Gynecol 1998; 179: 1618-1626.

Hammerman C, Kaplan M. Comparative tolerability of pharmacological treatments for patent ductus arteriosus. Drug Safety 2001; 24:537-551.

Henry D, Drew A. Beuzeville S. Gastrointestinal adverse drug reactions attributed to ibuprofen. In: Ibuprofen a critical bibliographic review. Rainsford KD, ed. Philadelphia: Taylor & Francis, Chapter 9, pp 431-458, 1999.

Henry D, Lim LL- Y, Garcia Rodriguez LA, Gutthann SF, Carson JL et al. Variability in risk of gastrointestinal complications with individual non- steroidal anti-inflammatory drugs: results of a collaborative meta- analysis. Brit Med J 1996; 312:1563-1566.

Heyman MA, Rudolph AM, Silverman NH. Closure of the ductus arteriosus in premature infants by inhibition of prostaglandin synthesis. N Engl J Med, 1976; 295:530-533.

Homblad PY. Studies on closure of the ductus arteriosus III. Species differences in closure rate and morphology. Cardiologia 1967; 51:262-282.

Langman MJS, Weil J, Wainwright P, Lawson DH, Rawlins MD et al. Risks of bleeding peptic ulcer associated with individual non-steroidal anti-inflammatory drugs. Lancet 1994; 343:1075-1078.

Lesko SM, Mitchell AA. An assessment of the safety of paediatric ibuprofen. JAMA 1995; 273: 929-933.

Loftin CD, Trivedi DB, Tiano HF, Clark JA, Lee CA et al. Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyc1ooxygenase-1 and cyclooxygenase-2. Proc Natl Acad Sci USA 2001; 98:1059-1064.

Ma T, Ambat C, Ostrea EM, Aranda N. Influence of ibuprofen L-Iysinate (IBU) on bilirubin albumin binding as determined by the saturation index and horseradish peroxidase tests. Pediatric Academic Societies Abstract 2002; 2075.

Malcolm DO, Segar JL, Robillard JE, Chemtob S. Indomethacin compromises haemodynamics during positive-pressure ventilation, independently of prostanoids. J Appl Physiol 1993; 74: 1672-1678.

Momma K, Hagiwara H, Konishi T. Constriction of fetal ductus arteriosus by non-steroidal anti-inflammatory drugs: study of additional 34 drugs. Prostaglandins 1984; 28:527-536.

Momma K, Takao A. Transplacental effects of four popular analgesics in rats. Am J Obstet Gynaecol. 1990; 162:1304-1310.

Momma K. Takeuchi H. Constriction of fetal ductus arteriosus by non-steroidal anti-inflammatory drugs. Prostaglandins 1983; 26: 631-643.

Nguyen M. Camenisch T, Snouwaert JN, Hicks E, Coffman TM et al. The prostaglandin receptor EP4 triggers remodeling of the cardiovascular system at birth. Nature 1997; 390:78-81.

Parys-Van Ginderdeuren R, Malcolm D, Varma DR, Aranda JV, Chemrob S. Dissociation between prostaglandin levels and blood flow to the retina and choroid in the newborn pig following nonsteroidal anti-inflammatory drugs. Invest Ophthalmol Vis Sci 1992; 33:3378-3384.

Pellicer A., Aparicio M., Cabanas F, Valverde E, Quero J et al. Effect of the cyclo-oxygenase blocker ibuprofen on cerebral blood volume and cerebral blood flow during normocarbia and hypercarbia in newborn piglets. Acta Paediatr1999; 88:82-88.

Rainsford KD. Pharmacology and toxicology of ibuprofen. In: Ibuprofen a critical bibliographic review. Chapter 5 pp 143-277. Ed. Rainsford KD; pub. Taylor & Francis, 1999.

Rao PS, Cavanagh D, Dietz JR. Renal effects of intermittent versus continuous infusion of ibuprofen in the primate. Prostaglandins, Leukotrienes and essential Fatty Acids 1994; 51:249-256.

Royer GL, Seckman CE, Schwarz JH, Bennett KP. Effects of ibuprofen on normal subjects: clinical and routine and special laboratory assessments. Curr Thera Res 1985; 37:412-426.

Segi E, Sugimoto Y, Yamasaki A, Aze Y, Oida H et al. Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. Biochem Biopys Res Comm 1998; 246:7-12.

Sharpe GL, Larsson KS, Thalme B. Studies on closure of the ductus arteriosus. XII. In utero effect of indomethacin and sodium salicylate in rats and rabbits. Prostaglandins, 1974; 9:585-596.

Sharpe GL, Thalme B, Larsson KS. Studies on closure of the ductus arteriosus. XI. Ductal closure in utero by a prostaglandin synthetase inhibitor. Prostaglandins, 1974; 8:363-368.

Smith GCS, Coleman RA, McGrath JC. Characterization of dilator prostanoid receptors in the fetal rabbit ductus arteriosus. J Pharmacol Exp Thera 1994; 271: 390-396.

Smith GC, McGrath JC. Contractile effects of prostanoids on fetal rabbit ductus arteriosus. J Cardiovasc Pharmacol 1995; 25: 113-118.

Speziale MY, Allen RG, Henderson CR, Barrington KJ, Finer NN. Effects of ibuprofen and indomethacin on the circulation in newborn piglets. Biol Neonate 1999; 76:242-252.

Storme L., Rairigh RL, Parker TA, Kinsella JP, Abman SH. In vivo evidence for a myogenic response in the fetal pulmonary circulation. Pediatr Res 1999; 45:425-431.

Thomas RL, Parker GC, Van Overmeire B, Aranda N. A meta-analysis of ibuprofen versus indomethacin for closure of patent ductus arteriosus. Eur J Pediatr, 2005; 164:135-140.

Van den Veyver IB, Moise KJ. Prostaglandin inhibitors in pregnancy. Obstet Gynaecol Survey 1993; 48:493-502.

Van Overmeire B, Smets K, Lecoutere D, Van de Broek II, Weyler J et al. A comparison of ibuprofen and indomethacin for closure of patent ductus arteriosus. New Eng J Med 2000; 343: 674-681.

Van Overmeire B, Touw D, Schepens PJC, Kearns GL, van den Anker JN. Ibuprofen pharmacokinetics in preterm infants with patent ductus arteriosus. Clin Pharmacol Therap 2001: 70: 336-343.

Van Overmeire B, Vanhagendoren S, Schepens PJ, Ahlfors CE. Unbound bilirubin in newborns receiving ibuprofen. (DRAFT - 2005).

Whelton A, Hamilton CW. Nonsteroidal anti-inflammatory drugs: Effects on kidney function. J Clin Pharmacol 1991; 31:588-598.

Toxicology

As ibuprofen is a marketed drug, currently available OTC in generic oral formulations intended for chronic administration, we will restrict this section of the review to studies pertinent to the proposed new indication and route of administration, i.e., studies conducted by the intravenous route in neonatal animals, studies in which fetuses were exposed to the drug during late gestation, and genetic toxicology studies. The only study to address the effects of intravenous administration in neonates is the previously reviewed dog study, originally scheduled to run for two weeks but terminated at 8 days after most of the treated animals had died. A no-effect dose for lethality could not be estimated from this study. For details see our May 2004 review of sponsor's IND 59778. Other information pertinent to the proposed route and indication comes from published literature provided by the sponsor.

Developmental Toxicity

SS Adams et al, Absorption, Distribution and Toxicity of Ibuprofen. Toxicology and Applied Pharmacology 15, 310-330 (1969)

This publication includes a summary of a study in which ibuprofen was administered orally to rats throughout pregnancy and parturition and the young examined 3 weeks after delivery. There were 15 control liters and 6 and 10 litters that had been exposed *in utero* to maternal doses of 7.5 and 20 mg/kg/day, respectively. Viability index at weaning and weaning weight were not

significantly affected. The only finding even mildly suggestive of a teratogenic response to ibuprofen was a single fetus at the high dose with anophthalmia (apparently within accepted range of normal variation for the strain). Ninety minutes after 21 day-pregnant rats (same Wistar strain) received 20 mg/kg p.o. ^{14}C -ibuprofen, concentrations of radioactivity, expressed as ^{14}C – ibuprofen, were 55.3 and 55.4 mcg/g wet weight in maternal and fetal plasma, respectively, indicating that ibuprofen and its metabolites passed freely across the placenta.

Genetic Toxicity

Sister-chromatid exchange: second report of the Gene-Tox program, J.D. Tucker et al; Mutation Research, 297 (1993) 101-180

This is a literature review in which each published study was evaluated for adequacy and on the basis of study results, individual compounds classified as negative, as meeting only minimal criteria for a positive response ($p<0.05$ at any dose), as inducing a doubling of the SCE frequency above it's appropriate negative control but lacking a 3-point monotonic increasing dose response (in some cases because only one or two doses were evaluated), as inducing a 3-point monotonic increasing dose response (with $p<0.001$ at least at the highest dose but not a doubling above its appropriate negative control, or a doubling of the SCE frequency as well as a 3-point monotonic increase (with $p<0.001$ at least at the highest dose). The article does not place ibuprofen in any of these categories. It appears that it was submitted by the sponsor only as background for interpretation of the results reported in other publications.

Comparative mutagenic and genotoxic effects of three propionic acid derivatives: ibuprofen, ketoprofen and naproxen, B. Philipose et al; Mutation Research 393 (1997) 123-131

The three drugs (each dissolved in DMSO) were evaluated in an Ames Test (salmonella typhimurium strains TA97a, TA100 and TA102 with and without S9 fraction of rat liver homogenate from phenobarbital-treated rats). Positive controls for this plate incorporation assay in the absence of S9 were 4-nitro-*o*-phenylenediamine (NPD) for TA97a, sodium azide for TA100 and methylmethane sulphonate (MMS) for TA102. Positive controls for the assay in the presence of S9 were 2-AAF for both TA97a and TA100; no positive control for TA102. Only naproxen could be evaluated with each of the tester strains at a dose as high as 5000 mcg/plate both in the absence and presence of S9. With TA97a, Ibuprofen and ketoprofen could be evaluated at doses of up to only 1000 mcg/plate with or without S9 (toxic at 5000 mcg/plate). With TA100, ibuprofen could be evaluated at doses of up to only 1000 mcg/plate with or without S9 (toxic at 5000 mcg/plate). Although there were occasional statistically significant differences (increases) from the DMSO control in revertant frequencies, none of the revertant counts were at least 50% higher than control. All three compounds are considered to have tested negative in this assay.

The doses selected for *in vivo* sister chromatid exchange (SCE) assays were said to have been based on po and ip LD50s reported in the literature, the highest dose in each case (the only dose in the case of the po assay) being approximately one-third of the mouse LD50 for ibuprofen. For the ip assays, paraffin-coated 5-bromodeoxyuridine (BrdU) tablets were implanted subcutaneously in the flanks of mice under ether anesthesia and the test chemicals administered

as single injections one hour after the tablet implantation. Three doses (25, 50 & 100 mg/kg) of each drug were injected in DMSO to different groups of 5 animals each. Solvent control mice received DMSO while positive control mice received mitomycin C. For SCE analysis, colchicine was injected (ip) 22 hours after Brdu tablet implantation and two hours later bone marrow was expelled with 0.075M KCl . After the hypotonic treatment, cells were fixed three times with methanol/acetic acid (31). Slides were prepared and chromosomes differentially stained with fluorescence-plus-Giemsa technique. For the single dose oral study, all the drugs were gavaged with distilled water in 2% gum acacia at a dose of 270 mg/kg 0.5 hours after tablet implantation to different groups of 5 animals each. Positive control mice were gavaged with cyclophosphamide in distilled water, whereas negative controls were gavaged with 2% gum acacia in distilled water. The rest of the procedure was the same as described above. Slides were coded and 30 second-division metaphase cells (40+/-2 chromosomes) per animal were scored for SCE frequencies, i.e. a total of 150 cells were scored per dose tested. Randomly selected metaphase cells (100/animal) were scored for replicative indices (RI) analysis by their staining pattern (first, second and third division metaphases). A significant increase in SCE was observed at 50 and 100 mg/kg doses for both ibuprofen and ketoprofen, while for naproxen, a significant increase was observed only at the highest dose tested (100 mg/kg). There was a significant dose-response trend in the ip study for all three drugs. A single oral dose of ibuprofen or naproxen (270 mg/kg) also gave a significant, albeit weak, increase in SCE when compared with control. However, in both the po and ip studies, none of the SCE/cell means exceeded the solvent control mean by as much as 50%. No significant changes in replicative indices were observed in any of the treated series for these drugs. The positive control compounds produced very high frequencies of SCE over those of control and treated groups with both ip and oral administration.

Do non-steroidal anti-inflammatory drugs induce sister chromatid exchanges in T lymphocytes? Y. Ozkul et al, J Int Med Res, 1996;24:84-87

A total of 48 patients (27 women and 21 men), aged 17-24 years, with a diagnoses of soft-tissue injury, were divided into six groups of 8 patients and treated with ibuprofen (800 mg/day), indomethacin (75 mg/day), naproxen (1000 mg/day), ketoprofen (150 mg/day), diclofenac (100 mg/day) or acetylsalicylic acid (1500 mg/day). Heparinized blood samples were collected for peripheral lymphocyte culture before the start of treatment and after 2 weeks treatment. Chromosome harvesting, treatment with hypotonic solution, fixation and staining were carried out according to standard methods. Twenty metaphase cells per patient were analyzed, under a blind protocol, for the presence of sister chromatid exchanges (SCEs). There were no significant differences in the mean numbers of SCEs/cell in the peripheral lymphocytes before and after treatment with any of the tested compounds ($p>0.05$, Student's t-test for paired observations).

Investigations of the influence of nonsteroidal antirheumatic drugs on the rates of sister-chromatid exchange, W. Kullich and G. Klein, Mutation Resech, 174 (1986):131-134

Only non-smoking patients with degenerative rheumatic diseases included in this study. These subjects received diclofenac (100 mg/day), flurbiprofen (200 mg/day), ibuprofen (1200 mg/day), indomethacin (75 mg/day), isoxicam (200 mg/day), ketoprofen (150 mg/day), piroxicam (20 mg/day), pirprofen (800 mg/day) or tiaprofenic acid (600 mg/day). All drug-treated groups consisted of both males and females (total of 6-11 patients per group). Those patients ranged in

age from 22 to 61 years. Mean age by group ranged from 36 to 52 years. The control group consisted of 24 healthy, untreated, non-smoking female volunteers aged 22-30 (mean age 24 years). Mitomycin C (50 mg/ml) served as a positive control substance *in vitro*. One blood sample was taken prior to commencing therapy and a second after a two-week treatment period. Sister-chromatid exchanges in lymphocytes were evaluated under light microscopy. Twenty metaphases which contained complete well-spread chromosome sets were counted per slide. There were no significant differences in sister-chromatid exchange rates between the samples taken before treatment and the samples taken after the two weeks of treatment for any of the test substances. The largest increase was observed with diclofenac (mean increase of about 11%). The positive control produced a 7-fold increase in SCEs.

Mutagenicity Testing of Selected Analgesics in Ames Salmonella Strains. J.W. Oldham et al, J. Applied Toxicology, 6(4):237-243 (1986)

Acetaminophen, aspirin, phenacetin and ibuprofen were tested for mutagenic activity in the Ames Salmonella plate incorporation assay using strains TA98, TA100, TA1535, TA1537 and TA1538. Each of these analgesics was tested in four separate tests: without metabolic activation, and in the presence of a rat, hamster or mouse liver post-mitochondrial supernatant (S9, Aroclor 1254-induced). All were diluted in DMSO which served as the negative control substance and as the vehicle for 9-aminoacridine HCl, the positive control for strain TA1537 without S9; Na azide, the positive control for strains TA100 and TA1535 without S9; and 2-anthramine, the positive control for all strains with rat, hamster or mouse S9. Sterile water was the vehicle for dexon, the positive control for strains TA98 and TA1538 without S9. Acetaminophen and phenacetin were tested at levels of up to 5000 mcg/plate with no evidence of toxicity. Aspirin was studied at levels of up to 500 mcg/plate with toxicity observed at 250 or more mcg/plate and ibuprofen was studied at levels of up to 1000 mcg/plate with toxicity observed 750 or more mcg/plate under all 4 metabolic conditions. A dose-related increase in revertant colony counts, reaching levels twice the negative control values, was seen with phenacetin at doses of 500 or more mcg/plate in strain TA100 in the presence of (only) hamster S9. None of the other test compounds showed any mutagenic potential in any of the tester strains.

Sister Chromatid Exchange in Patients Treated with Nonsteroidal Anti-Inflammatory Drugs, Semra Sardas et al, Drug Safety 6 (5):390-392, 1991

Male and female Patients (nonsmokers 26-50 years of age) with confirmed diagnoses of degenerative rheumatic diseases and intervertebral disc disorders, who had not received previous therapy before, were treated with diclofenac (200 mg/day), ibuprofen (1200 mg/day) or indomethacin (75 mg/day). One blood sample was taken before commencing therapy and a second after a 20-week treatment with one of the antirheumatic agents. The samples were incubated (in duplicate) in ^{199}Technetium medium supplemented with fetal calf serum, reconstituted phytohaemaglutinin, penicillin, streptomycin and 5-bromodeoxyuridine at a final concentration of 10^{-5} mol/L. The lymphocytes were allowed to divide for 2 cell cycles. Colchicine was added to the cultures for the last 3 hours of incubation. The cells were centrifuged and resuspended in hypotonic solution, fixed and washed in a 3:1 mix of methanol and acetic acid. Chromosomal preparations were stained using a fluorescent dye and Giemsa starin. An average of 30 metaphase plates with 46 intact chromosomes and well differentiated

sister chromatid exchanges (SCEs) were scored from each subject to calculate the mean SCE/cell, on coded slides, by a single observer. There were no significant differences in mean SCEs/cell between lymphocytes obtained before and after therapy with any of the drugs (12-15 subjects/test compound). Mean values after treatment in all cases did not exceed pretreatment means by as much as 5%. The smallest increase (0.66%) was obtained with ibuprofen.

LABELING: The PRECAUTIONS section of the proposed package insert omits the "Carcinogenesis, Mutagenesis, Impairment of Fertility," "Pregnancy," "Nursing Mothers" and "Labor and Delivery" subsections generally required under 21CFR201.57. Considering the patient population and the limited exposure, none of this needs to be addressed in the package insert for neoprofen, with the possible exception of [redacted]. We note that the labeling for Merck's Indocin (indomethacin for injection), the only drug currently approved for closing a patent ductus arteriosis in premature infants, is also bereft of these subsections. The Indocin labeling, however, does provide information on the effects of fetal exposure to that drug when administered orally to pregnant rats and mice during the last trimester of pregnancy. *(The Indocin package insert notes an increased incidence of neuronal necrosis in the diencephalon in live born rodent fetuses (not clear from label whether the finding applies to both rat and mouse; evaluation conducted in both species) exposed to the drug during the last 3 days of gestation when compared to fetuses not exposed to the drug and further notes that oral administration of the drug during the first 3 days of life did not result in a similar increase. That indomethacin was responsible for the increase is questionable, however, considering another statement in the PI to the effect that similar exposures of rat fetuses to indomethacin during the last trimester of gestation did not result in a similar finding, although the longer exposure apparently resulted in offspring whose pulmonary blood vessels were both reduced in number and excessively muscularized, findings similar to those observed in the syndrome of persistent pulmonary hypertension of the neonate.)*

EVALUATION: Ibuprofen is a marketed drug and the need for a new drug application for the sponsor's product is dictated by the new indication, the new salt and the new route of administration. Published literature is cited, and provided, to document the non-selective inhibition by ibuprofen of cyclo-oxygenase, resulting in reduced synthesis of prostaglandins, which is considered to be the mechanism responsible, or mainly responsible, for the closure of the patent ductus by ibuprofen. The tone and patency of the ductus have been reported as being maintained by prostaglandins, principally PGE2.

Information relevant to the safety of the l-lysine salt of ibuprofen comes from a thirteen-week oral toxicity study of L-lysine HCl in rats in which exposure to the amino acid was via the feed at dietary concentrations of up to 5% and in which there were no adverse effects reported in males or females. The animals in this study were observed for clinical signs, effects on body weight, food and water consumption. Ophthalmologic, clinical pathologic, gross and histo-pathologic examinations were also conducted. L-lysine is an essential amino acid for humans and widely available as a dietary supplement.

Regarding documentation of the safety of iv ibuprofen in neonatal animals, premature or term, there is only one study presented in the application, the only toxicology study conducted by or

for the sponsor. That is a repeated dose iv toxicity study of ibuprofen-l-lysine in neonatal beagle dogs, a study that was previously reviewed in May of 2004 under the sponsor's IND 59778. The study protocol called for two weeks of daily exposure but deaths of most dogs led to termination of treatment on the 8th day. The most common lesions in treated dogs that died were hemorrhage, inflammation and/or necrosis at the injection site. Also seen were renal nephrosis and emboli in pulmonary vessels. The study pathologist attributed at least some of the deaths to sepsis and peritonitis associated with inflammation at the injection site. Pulmonary emboli were considered to be the result of septic emboli from the injection site. The lower of the two doses evaluated in this study, 80 mg/kg/day, is equivalent to about 55 mg/kg in the human neonate, which is only about 5 times the maximum dose anticipated for administration to the human neonate (10 mg/kg). A no-effect dose for lethality was not demonstrated in this study. The study findings cannot be considered evidence of probable safety when the drug is employed as intended in human premature neonates.

Another study, one not conducted by or for the sponsor but briefly summarized in a publication submitted with the application, provides information relevant to the delayed effects of *in utero* exposure to ibuprofen on neonatal development. In this study, ibuprofen was administered orally to rats throughout pregnancy and parturition with the offspring examined 3 weeks after delivery. The study has relevance for the premature human neonate. Although there was evidence that ibuprofen and its metabolites passed freely across the placenta, information on oral bioavailability in the rat or plasma levels in human neonates was not provided and, thus, we are unable to estimate the degree to which systemic exposure in these animals achieved the levels attained by iv administration in the premature infants for whom the drug is intended. In addition, although the summary indicates that there was no evidence of a teratogenic effect of the drug and that viability index at weaning and weaning weight were not significantly affected, there was no information provided regarding in *in utero* growth (i.e., birth weights) and survival.

The NDA contains numerous publications bearing on the pharmacokinetics, pharmacodynamics and toxicity of ibuprofen administered orally to various species, including summaries of carcinogenicity, reproductive toxicity and genetic toxicity studies. Few, if any, of the toxicology studies include sufficient detail regarding results and methodology to permit independent review. However, considering that the Agency has previously found this drug to be safe and effective for chronic oral use as an analgesic and anti-inflammatory agent, such information is not needed to support the safety of the short-term iv treatment of premature neonates.

There are several papers comparing ibuprofen with indomethacin, another NSAID and the only drug currently approved for the PDA indication, and a number of papers that describe genetic toxicology studies performed with ibuprofen. (The drug appears to test negative for mutagenicity in the Ames test and for sister chromatid exchanges in human lymphocytes.) However there are no other reports or descriptions of studies dealing with our only real concern with this product, long term effects on growth and development of children born prematurely and exposed at birth or shortly thereafter to ibuprofen lysinate.

RECOMMENDATIONS: The sponsor should monitor the growth and development of the drug-treated infants that had been entered into their clinical trials or agree to a prospective evaluation [redacted] Because of the expected difficulty in successfully following up the human neonate beyond the first few years of life, it is suggested that the sponsor attempt to identify or develop an animal model that will allow exploration of the long-term effects of short-term exposure of the premature neonate to NeoProfen. We do not consider the latter recommendation to constitute an approvability issue.

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Wednesday, March 01, 2006

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/s/

Charles Resnick
3/1/2006 11:50:26 AM
PHARMACOLOGIST

IND 59778

REVIEW AND EVALUATION OF A TOXICOLOGY STUDY IN NEONATAL DOGS

C.A. Resnick, Ph.D.
DCRDP (HFD-110)

SUBMISSION (NO32IT) DATED: 12 May 2004
CENTER RECEIPT DATE: 14 May 2004

SPONSOR: Farmacon-IL, LLC
Westport, Connecticut

DRUG: R,S-Ibuprofen-L-Lysine

FORMULATION: IV Solution

INVESTIGATIONAL USE: To facilitate early closure of patent ductus arteriosus (PDA) in very low birth weight infants.

14 Consecutive Twice Daily Intravenous Administrations of R,S-Ibuprofen-L-Lysine (Ibuprofen) to Neonatal Dogs

Testing Facility: [redacted]

Study Number: 1150-100 (contract lab study number)

Study Dates: Dosing initiated 14 March 2000 (main study)

GLP Compliance: Contract Lab study report includes quality assurance statement attesting to the conduct of quality assurance inspections of the study and review of the final report according to the standard operating procedures of the Quality Assurance Unit and according to the requirements of the FDA Good Laboratory Practice Standards as stated in 21 CFD Part 58.

Animals: Neonatal Beagles (15 males and 15 females) were randomized to 1 male and 1 female control group and 2 male and 2 female treatment groups. The neonates were housed with and received milk via the mothers. [redacted] was provided twice daily and tap water was provided ad libitum to the dams. Mean weights of the neonates at time of initiation of treatment were about 300g for each of the male groups and about 345g for each of the female groups. The pups were 2 days old when received from [redacted] Age at initiation of treatment was not provided.

Mode of Administration of Test Agent: Dosing formulations were prepared daily by dissolving the drug in 0.9% saline, and administering the resulting solution by slow intravenous injection into the jugular or cephalic vein twice daily (at least 6 hours apart) at a dose volume of 1 mL/kg,

adjusted every other day. Rate of injection not provided. Drug substance lot number also not provided. The protocol called for 2 weeks of exposure to ibuprofen, followed by a 2 week recovery period.

Dose Levels: Groups of 5 animals/sex received vehicle, 80 or 200 mg/kg/day (doses expressed in terms of active moiety, ibuprofen). Because of treatment-related mortality, dose levels were gradually decreased beginning on study day 4 and dosing was discontinued on study day 8.

Basis for Dose Selection: Doses were chosen based on the results of a rising dose tolerance study (conducted by the same laboratory) in 2 male neonates. Doses in that study started at 30 mg/kg/day (study days 1&2), increased to 60 mg/kg/day (study days 3&4), 90 mg/kg/day (study days 5&6), 150 mg/kg/day (study days 7&8) and, finally, 180 mg/kg/day (study days 9&10). An additional male dosed with 200 mg/kg b.i.d. died after one day of dosing. An additional female dosed once with 150 mg/kg also died. According to the study summary, "as no untoward toxicity was seen at dose levels up to 180 mg/kg/day, dose levels of 0, 80 and 200 mg/kg/day were selected for the main study, to be administered, divided into two daily doses." [This study summary statement makes no sense as a female did die after a single 150 mg/kg dose.]

Observations/Measurements: All animals were observed for mortality and moribundity twice daily. Detailed clinical observations and body weights were recorded every other day. Blood was collected from each animal from a jugular vein approximately 30 and 60 minutes and 18 hours after dosing on study day 1 for determination of plasma drug levels (analysis by sponsor), and prior to scheduled necropsy for hematology and blood chemistry (analyses conducted by [redacted] Plasma level data was not provided in the IND submission. On study day 21, all surviving animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. All animals (survivors and decedents) were necropsied and bone marrow smears prepared. The necropsies included examination of the external surface of the body, all orifices and the cranial, thoracic and abdominal cavities and their contents. The following organs from animals surviving to scheduled necropsy were weighed: liver (without gallbladder), adrenals, testes (with epididymides), ovaries, heart, kidneys, brain and spleen. The following tissues from each animal were preserved in 10% neutral buffered formalin:

adrenals	aorta (thoracic)
bone marrow (sternum)	brain
esophagus	eye
gallbladder	heart
large intestine (cecum, colon, rectum) with associated lymphoid tissue	kidneys
	liver
small intestine (duodenum, jejunum, ileum) with associated lymphoid tissue	lungs with mainstem bronchi
	mammary gland
	lymph node (mesenteric and submandibular)
ovary (female)	pituitary
pancreas	salivary gland
prostate (male)	skeletal muscle (thigh)
sciatic nerve (peripheral)	spinal cord (cervical, lumbar and midthoracic)
skin	
spleen	testes with epididymides (male)
stomach	thyroids and parathyroids
thymus	urinary bladder
trachea	vagina (female)
uterus with cervix (female)	animal identification
abnormalities/gross lesions	

Tissues from all animals found dead or sacrificed as moribund and from all animals in the control and 200 mg/kg/day ibuprofen groups from the recovery sacrifice were embedded in paraffin, stained with hematoxylin and eosin and examined microscopically. Necropsies were conducted by [redacted] personnel under the supervision of an [redacted] pathologist. Processing of the fixed tissues and light microscopic examinations were conducted at [redacted]

Results

Mortality:	Group 1 Control	Group 2 80 mg/kg/day	Group 3 200 mg/kg/day
	1♂ SD2	1 ♂ SD 3 1 ♂ SD 5 1 ♂ SD 7 1 ♂ SD 10 1 ♀ SD 6 1 ♀ SD 7	1 ♂ SD 3 2 ♂ SD 4 1 ♂ SD 5 1 ♂ SD 6 1 ♀ SD 2 1 ♀ SD 5 1 ♀ SD 8

Above numbers come from appendix 1 and differ from the numbers in the Results section of the report (which omits the death that occurred on day 10) and the Discussion section of the report (which indicates that nine animals died at the 200 mg/kg dose level rather than 8). These differences are not critical to the evaluation of the study as they do not alter the fact that the drug was poorly tolerated, even lethal to most of these neonatal dogs at doses as low as 80 mg/kg/day, the lowest dose evaluated. It should be noted that when the relationship of treatment to poor survival became apparent, the dose levels were lowered (day 4) and treatment was discontinued altogether (day 8).

Clinical Observations: Signs seen prior to death included swelling and discoloration at the injection site for a group 2 female and a distended abdomen in a group 3 female. Persistent ulceration at the injection site and a distended abdomen was also observed in a group 3 female that survived to termination.

Body Weights: No significant differences for in-life body weights were reported. However, at necropsy, body weights of surviving group 3 females (n=2) were significantly lower than body weights of surviving control females (n=5).

Clinical Pathology: A high leukocyte count was reported in one surviving group 3 female (36.5 vs 5.1-12.3 for concurrent control females). With one exception (for which findings were normal), clinical pathology data was not collected for decedents.

Anatomic Pathology: The most common lesions in treated dogs that died during the study were hemorrhage, inflammation and/or necrosis at the injection site. Renal nephrosis was observed in three Group 2 and four Group 3 males, and one Group 2 and four Group 3 females, and was

claimed to be a known effect of Ibuprofen and consistent with earlier observations of Poortinga and Hungerford in dogs (Prev Vet Med 35(2): 115-24, 1998). Another notable finding, observed in one Group 2 male, one Group 3 male and one Group 3 female dog was emboli in pulmonary vessels. The emboli were described as organized thrombi containing inflammatory cells. Occasional organized thrombi were found attached to the inner wall of larger vessels. They were considered to have resulted from septic emboli from the injection site. Extensive inflammatory lesions were reported in the abdominal organs and at the injection site, including, in some cases, sepsis and peritonitis (the latter two a probable cause of death for several of the dogs), findings said to be consistent with those reported by Adeyeye et al in rats (Pharm Res. 13(5): 784-93, 1996) and by Elliott et al in various species (Toxicol Pathol 16 (2): 245-50). The Elliott et al publication is further cited for its observation that propionic acids, of which Ibuprofen is one, produce gastrointestinal damage in most species with lesions varying from erythema, hemorrhage and erosion to ulceration and peritonitis, with rat being less tolerant than monkey and dog less tolerant than rat. [*We note that in the ibuprofen study, reported GI damage was limited to chronic active inflammation of the cecum, colon and ileum, and hemorrhage in the rectum of one of the high dose male decedents, and hemorrhage and chronic active inflammation of the rectum of one low dose female decedent.*] That paper further notes that factors other than local irritation likely play a role in development of the GI damage, as gastrointestinal lesions have been observed following both parenteral and oral administration.

EVALUATION

Ibuprofen, a drug approved for oral administration as an analgesic/antipyretic, is being investigated for efficacy in the treatment of patent ductus arteriosis (PDA) in the premature newborn (highest daily dose appears to be 10 mg/kg and maximum duration of (intravenous) treatment appears to be several days). The pediatric dog study designed by the sponsor and summarized above did not provide for a scheduled sacrifice at or shortly after the termination of treatment; necropsies were to be conducted only after a two week recovery period. However, deaths occurring in both of the drug-treated groups during the treatment phase of the study (5 of 10 dogs at 80 mg/kg/day and 8 of 10 dogs at 200 mg/kg/day) provided ample opportunity to necropsy these animals when effects of treatment would be most apparent.

Regarding the histopathological examination, the veterinary pathologist at [redacted] who authored the pathology report sums things up as follows: *"The acute and/or chronic/active inflammation at the injection site with accompanying sepsis and peritonitis appear to have been the cause of death of several of the dogs that died on study. The inoculum of test material may have been injected outside the vein, or some material may have leaked into adjacent tissues following the injection. In any event, the test material appears to be quite irritating to soft tissues at and surrounding the injection site, resulting in necrosis and acute and chronic/active inflammation of the subcutaneous tissue, skeletal muscle and, in a few cases, inflammation and thrombosis of the jugular vein. The accompanying inflammation of the mesentery (peritonitis) may have been the result of sepsis from the injection site. The pulmonary emboli in three dogs are considered to be the result of septic emboli from the injection site."* He goes on to observe that nephrosis of the kidneys that was observed in some of the treated animals, although subtle, could be separated from control kidneys when read blinded, and that there was no histologic evidence of a depressed immune system.

Although an evaluation of the acute or subchronic toxicity of the drug when administered by the iv route to neonatal animals is useful, the study should have been designed to also evaluate the potential for adverse effects on physical, behavioral and sexual development. If the intent of the sponsor was to evaluate general toxicity in a pediatric population, they should have provided for a sacrifice shortly after termination of treatment, rather than only after a two week recovery period. (Because of the large number of deaths in the ibuprofen-treated groups, much of the information that would have been provided by an early scheduled sacrifice was available anyway.) If the study was intended, as it should have been, to evaluate effects on growth and development of the neonate, it should have included an evaluation of the potential for adverse effects on physical, behavioral and sexual development. It did not. Whereas follow-up of drug-treated human neonates till physical maturity is reached is not very feasible, follow-up of drug-treated neonatal laboratory animals till physical maturity is not only feasible, but is routinely performed as part of the non-clinical toxicology workup of new drugs.

Getting back to the study that was done, it provides no support whatsoever for a conclusion that the current clinical investigation is a reasonably safe one. Half of the animals receiving 80 mg/kg/day for up to 3 days, followed by progressively lower doses, failed to survive 3-7 days of treatment. The human equivalent of 80 mg/kg in the neonatal dog is about 55 mg/kg[•], about 5 times the maximum dose to be administered to the preterm human neonate. A no-effect dose for lethality was not demonstrated.

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[•] Estimate of the human equivalent dose (HED) is based on the study day 1 neonatal dog weight of about 0.33kg and an assumed preterm human infant weight of 1kg. The following equation was employed: HED in mg/kg = dog dose in mg/kg x [dog wt in kg/human wt in kg]$^{0.333}$.

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