ELSEVIER

International Journal of Pharmaceutics 125 (1995) 309–313

international journal of pharmaceutics

Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats

Gary G. Liversidge [a,*], Phil Conzentino [b]

[a] *NanoSystems, 1250 S. Collegeville Rd. Collegeville, PA 19426–0900, USA*
[b] *Sanofi Research, 1250 S. Collegeville Rd. Collegeville, PA 19426–0900, USA*

Abstract

Gastric mucosal damage, the most common event following oral administration of NSAIDs, is due to a combination of a systemic effect and a high local drug concentration effect. It has been demonstrated in rats that the gastric irritation induced following oral administration of naproxen was decreased by reducing drug particle size from 20–30 μm to 270 nm and stabilizing the particles in suspension with pluronic F-68. The reduction in irritation is attributed to a decrease in the local high and prolonged concentration of naproxen attributable to reduced crystal size. Further the amount of irritation observed with the orally administered nanoparticle formulation is similar to that following intravenous administration of naproxen. The size reduction of naproxen was also associated with an apparent increase in the rate of absorption by approx. 4-fold. The increase in the rate of absorption is attributed to an increase in surface area for dissolution for the NanoCrystal formulation.

Keywords: Naproxen; Absorption rate; Gastric irritation; Rat; Nanocrystal

1. Introduction

Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) used in painful and inflammatory rheumatic and certain nonrheumatic conditions that has significant gastric irritation and shows a slow onset of analgesic action (t_{max} 2–4 h).

It has been demonstrated that gastric irritation induced by NSAIDs can be influenced by the route of administration (Cioli et al., 1979). The primary mechanism of gastric mucosal damage is mediated by the inhibition of endogenous prostaglandin biosynthesis (Soll et al., 1989). The mechanism by which NSAIDs cause gastric mucosal injury include: an acid-mediated direct topical effect, a breakdown in the gastric mucosal defense by destruction of the mucus barrier, the back diffusion of hydrogen ions into the mucosa and loss of intracellular ions, and acid entering the epithelial and subepithelial layers resulting in mucosal ischemia and necrosis (Earnest, 1990). Prostaglandins may also regulate gastric blood flow and inhibition of prostaglandins may result in local vasoconstriction and mucosal ischemia. A second mechanism which has been addressed in this study is through direct or topical mucosal irritation. Gastric irritation induced by NSAIDs may be dependent on two different mechanisms: a local action exerted by contact with the gastric

* Corresponding author. Tel. 610-983-7993; Fax 610-983-6929.

0378-5173/95/$09.50
SSDI 0378-5173(95)00148-4

mucosa and a generalized/centrally mediated (systemic) action, taking place following oral administration. As a consequence, certain NSAIDs are more irritating via the oral route than parenteral (Cioli et al., 1979; O'Laughlin et al., 1980; Beck et al., 1990).

It is hypothesized that formulating NSAIDs to decrease the local contact mediated irritation should result in a decrease in gastric irritation. A reduction in gastric irritation following administration of multiparticulate microsphere dosage forms has been reported (Bodmeier and Chen, 1980) which is supportive of this hypothesis. A potential approach for reducing the local gastric irritation induced by NSAIDs is to decrease the drug crystal particle size such that there is no longer a large enough crystal lodged against the mucosa to produce a sufficiently local high and prolonged concentration of drug to induce irritation.

The present study was designed to evaluate in rats the application of nanoparticle technology to naproxen to determine if reducing the crystal size of naproxen decreased the gastric irritation on the mucosa of the stomach following oral administration. Naproxen, like most NSAIDs, is soluble at intestinal pH but has low solubility at gastric pH and is thus not anticipated to show an benefits in reducing intestinal irritation when formulated as nanoparticles.

Decreasing the particle size of naproxen and stabilizing the particles to prevent agglomeration in gastric fluid is anticipated to increase the dissolution rate and vis-a-vis the absorption rate and t_{max}. The impact of particle size reduction on naproxen's oral pharmacokinetic parameters in rats was determined.

2. Materials and methods

2.1. Materials

Naproxen was purchased from Sigma. Pluronic F-68 was obtained from BASF Fine Chemicals. Zirconium oxide (Zirbeads) grinding spheres having a nominal diameter of 1 mm were purchased from Zircoa Inc.

2.2. Preparation of formulations

A nanoparticulate naproxen dispersion was prepared in a roller mill as follows. A 250 ml glass jar was charged with 120 ml of 1.0 mm diameter pre-cleaned zirconium oxide beads, 60 g of an aqueous slurry containing 3 g (5% by weight) naproxen, and 1.8 g (3% by weight) Pluronic F-68. The beads were pre-cleaned by rinsing in 1 N sulfuric acid overnight followed by several rinses with deionized water. The batch was rolled at 92 rpm for a total of 120 h.

A control formulation of naproxen was prepared by adding 5% w/v unmilled naproxen to 3% Pluronic F-68. The suspension was vortexed and sized.

The concentration of naproxen in both formulations was 50 mg/ml. Both formulations were diluted with 3% Pluronic F-68 to a dosing concentration of 10 mg/ml for oral administration. The intravenous nanoparticulate naproxen was administered with a dosing concentration of 50 mg/ml.

2.3. Gastric irritation and bioavailability studies

Animal experiments were conducted in accordance with the National Institutes of Health, Guide for the Care and Use of Laboratory Animals and Institutional Animal Care and Use Committee.

2.3.1. Animal experimental protocol

Male Sprague-Dawley rat (250–350 g) were anesthetized with a 55 mg/kg intraperitoneal injection on sodium pentobarbital. The external jugular veins were chronically cannulated to facilitate the removal of blood samples. The rats were allowed to recover for 24 h with water ad libitum. After the recovery period the rats were lightly anesthetized with Metofane, orally gavaged or intravenously injected with formulation and placed in a Bollman cage. For oral administration blood samples (100 μl) were obtained via the jugular vein at 0 (pre-administration), 5, 10, 15, 30, 45, 60, 75, 90, 120, 180 and 240 min following administration of naproxen and collected in heparinized tubes. Plasma (50 μl) was obtained im-

mediately and placed on ice. For intravenous administration the nanoparticle formulation was administered via the jugular vein over a 10–15 s interval. Two additional sampling times were added (2.5 and 150 min) to those noted above and utilized the same collection and processing procedures. After 240 min the rats were killed by intravenous bolus injection of sodium pentobarbital via the jugular vein. The stomachs were removed and cut along the line of greater curvature from the duodenum to the pyloric sphincter. The stomachs were then spread flat and pinned out on dissecting dishes, washed with cold 0.9% sodium chloride and scored for irritation.

Each formulation was administered to six rats. The formulations were administered in groups according to the formulation at a dose of 50 mg/kg naproxen with the exception of the sham oral dose. The sham oral dose was a 3% Pluronic F-68 formulation administered at the same volume as naproxen formulations.

2.3.2. Stomach irritancy scoring system

A blinded evaluation and counting of stomach irritations (erosion/lesion/ulcer) were conducted by a modification of two published scoring systems (Cioli et al., 1979; Soll et al., 1989; Beck et al., 1990; Earnest, 1990), correcting for various degrees of severity. Differences in the severity index have often been associated with the gastropathology present on the stomach following oral administration of NSAIDs (Lanza et al., 1990; Balaa, 1991).

Each stomach irritation was measured in length (or diameter) using a 10 mm surgical ruler. The measurable length of the irritations ranged from 0.25 mm to 10.0 mm. Irritations less than 0.25 mm were classified as pinpoint. Length point values (in parentheses) were assigned to each measured irritation as indicated: 10 mm (13); 9 mm (12); 8 mm (11); 7 mm (10); 6 mm (9); 5 mm (8); 4 mm (7); 3 mm (6); 2 mm (5); 1 mm (4); 0.5 mm (3); 0.25 mm (2); pinpoint (1). The irritations were categorized by color as an evaluation of severity. Irritations red in appearance were rated as mild and assigned a severity value of 1. Brown irritations were rated as moderately severe and assigned a value of 2. Irritations which were black were rated as the most severe and given a severity value of 3. A score for each irritation was determined by multiplying the length value and the point severity value. The sum total for all irritations on a given stomach was identified as the total irritation score.

2.4. Bioanalytical methods

Plasma samples (50 μl) were mixed with 130 μl of acetonitrile and 20 μl of internal standard solution (indomethacin, 20 μg/ml) and vortexed. Samples were then centrifuged and the supernates were removed and placed in WISP vials.

Samples were analyzed by HPLC using a slightly modified published method (Street, 1989). The separation of naproxen was carried out on a Waters Novopack C18 (15 cm $\times$ 4 mm, 5 μm) analytical column: the mobile phase was A: 60% acetonitrile, 40% acetate buffer (pH 4.2) and B: 70% acetate buffer, 30% distilled water, with a linear gradient from 45% A to 100% A over 8 min, held at 100% A for 2 min and returned to initial conditions. The flow rate was 1.5 ml/min with a run time of 15 min. Retention times were 6.6 and 8.3 min for naproxen and indomethacin, respectively. Detection was by UV at 240 nm. The minimum quantifiable level of naproxen was 250 ng/ml.

3. Results and discussion

The nanoparticle dispersion was sized by photon correlation spectroscopy and found to have a weight average particle size of 270 nm with no particles above 400 nm. The formulation was physically and chemically stable when stored at 4°C for up to 4 weeks. The formulation did not agglomerate or flocculate when added to simulated gastric and intestinal fluids. The use of charged stabilizer, e.g., sodium lauryl sulfate, resulted in nanoparticle formulations that agglomerated or flocculated in either simulated gastric or intestinal fluids. Such formulations were judged unsatisfactory since the aggregates or flocculates would behave a pseudo large particles thereby negating any benefits of particle size reduction.

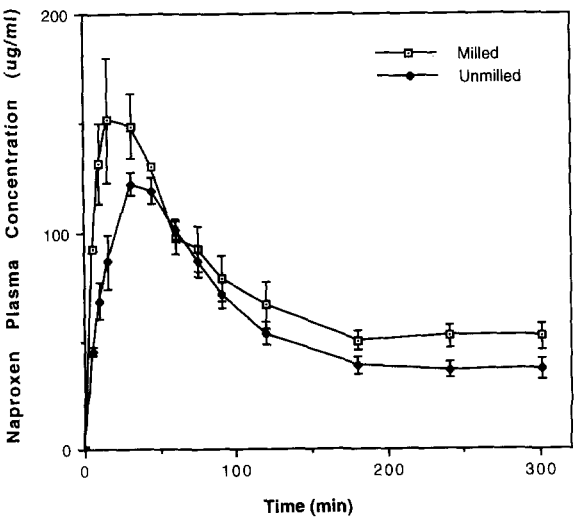

Fig. 1. Comparison of mean (±SE) plasma concentrations of naproxen following oral administration of two formulations to rats ($n = 6$)

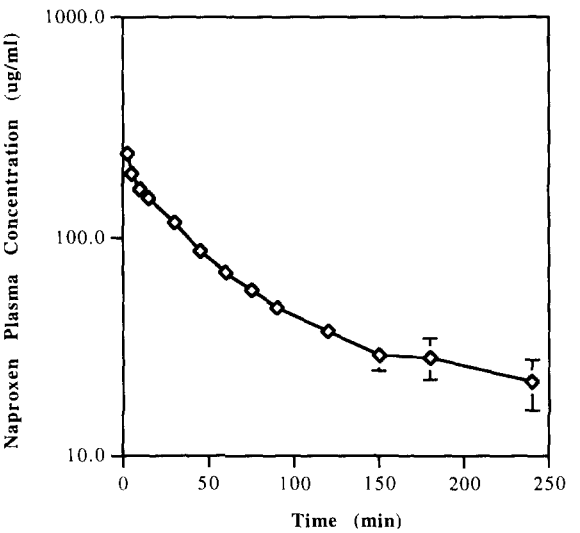

Fig. 2. Mean (±SE) plasma concentrations of naproxen following intravenous administration of a nanoparticle formulation of naproxen to rats ($n = 6$).

The mean pharmacokinetic parameters C_{max}, t_{max} and $AUC_{(0-240\ min)}$ and stomach irritation scores for the nanoparticle naproxen and unmilled naproxen are shown in Table 1 and are calculated from the parameters of individual animals. The unmilled naproxen had significantly higher irritation scores (t-test, $p = 0.01$) than the milled naproxen formulation when administered orally. There was no significant difference in stomach irritation following administration of nanoparticle naproxen comparing the oral and intravenous routes. Thus, particle size reduction of naproxen from 20 μm to 270 nm and stabilizing the formulation against agglomeration in gastric fluid has effectively eliminated any local irritant effect induced following oral administration.

The mean plasma profiles of naproxen following oral and intravenous administration (50 mg/kg) of naproxen formulations are presented in Figs. 1 and 2, respectively. The $AUC_{(0-240\ min)}$ for the oral nanoparticle formulation was significantly higher than for the unmilled suspension ($p = 0.03$). The C_{max} for the nanosuspension was significantly higher than for the unmilled suspension ($p = 0.02$). Interestingly, the $AUC_{(0-240\ min)}$ for the intravenously administered nanoparticle formulation was significantly lower than those for oral administration. It is speculated that the intravenously administered nanoparticles of naproxen are cleared quickly via the phagocytotic mechanism of the RES. The resultant pharma-

Table 1
Pharmacokinetic parameters and gastric irritancy scores following oral and intravenous administration of naproxen formulations (50 mg/kg) to rats ($n = 6$)

Formulation	C_{max} (μg/ml)	t_{max} (min)	AUC (μg h ml^{-1})	Gastric irritancy score
Unmilled naproxen	126 ± 4	33.5 ± 2.9	15 228 ± 994	163 ± 30
Nanoparticle naproxen	187 ± 18	23.7 ± 5.1	19 062 ± 573	107 ± 16
Sham oral formulation				24 ± 4
Nanoparticle naproxen i.v.			13 370 ± 814	95 ± 32

cokinetics may not accurately reflect those for a formulation in which naproxen is presented to the blood in molecular form not subject to such a clearance mechanism. Consequently, the $AUC_{(0-240\ min)}$ for intravenously administered nanoparticle naproxen may underestimate the $AUC_{(0-240\ min)}$ for free molecules of naproxen present in the blood.

The T_{max} for the nanoparticle formulation (23.7 min) was less ($p = 0.15$) than that for the unmilled suspension (33.5 min) reflecting the trend towards separation of T_{max}. In 8 min the nanoparticle formulation reached an equivalent mean plasma concentration to the C_{max} for the unmilled suspension. This data suggests that the nanoparticle formulation appears to be absorbed approx. 4-fold faster than the unmilled formulation. The increase in absorption rate for the nanoparticle formulation is attributed to the increased dissolution and surface area of the nanoparticles compared to the micron particles of the unmilled suspension of naproxen.

Particle size reduction of naproxen from 20–30 μm to 270 nm and stabilizing the formulation against agglomeration in gastric fluid has effectively eliminated any local irritant effect induced following oral administration and has increased the absorption rate in rats.

References

Balaa, M., Nonsteroidal anti-inflammatory gastropathy: From theory to practice. *Am. J. Med. Sci.*, 301 (1991) 272–276.

Beck, E.W., Schneider, H., Dietzel, K., Nuernberg, B. and Brune, K., Gastrointestinal ulceration induced by anti-inflammatory drugs in rats. *Arch. Toxicol.*, 64 (1990) 210–217.

Bodmeier, R. and Chen, H., Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen and ketoprofen. *J. Controlled Release*, 10 (1980) 167–175.

Cioli, V., Putzolu, S., Rossi, V., Barcellona, P. and Corradino, C., The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats. *Toxicol. Appl. Pharmacol.*, 50 (1979) 283–289.

Earnest, D.L., NSAID-induced gastric injury: Its pathogenesis and management. *Semin. Arthritis Rheum.*, 19 (Suppl. 2) (1990) 6–10.

Lanza, F., Graham, D., Davis, R. and Rack, M., Endoscopic comparison of cimetadine and sucralfate for prevention of naproxen-induced acute gastroduodenal injury. *Digest. Dis. Sci.*, 35 (1990) 1494–1499.

O'Laughlin, J., Hoftiezer, J. and Ivey, K., Effect of aspirin on the human stomach in normals: Endoscopic comparison of damage produced one hour, 24 hours and 2 weeks after administration. *Scand. J. Gastroenterol.*, 16 (1980) 211.

Soll, A.H., Kurata, J. and McGuigan, J.E., Ulcers, Nonsteroidal Antiinflammatory Drugs, and Related Matters. *Gastroenterology*, 96 (1989) 561–568.

Street, P., Rapid high performance liquid chromatographic methods for the determination of overdose concentrations of some nonsteroidal anti-inflammatory drugs in plasma and serum. *J. Chromatogr.*, 495 (1989) 179–193.