Dependency of Gastrointestinal Toxicity on Release Rate of Tiaprofenic Acid: A Novel Pharmacokinetic-Pharmacodynamic Model

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Purpose. To test the hypothesis that modification of release pattern of nonsteroidal anti-inflammatory drugs (NSAIDs) formulations shifts gastrointestinal (GI) toxicity of the drugs from the upper GI region to the distal intestine.

Methods. We assessed tiaprofenic acid (TA)-induced upper and lower increased GI permeability (a surrogate marker of toxicity) after administration of 20 mg and 40 mg/kg regular release (powder) and modified release formulations [sustained release (SR) beads and diethyl-β-cyclodextrin (DCD):TA inclusion complex (INC)]. Urinary excretion of oral doses of GI permeability probes sucrose and ^{51}Cr-EDTA was determined as measures of gastroduodenal and distal intestine, respectively. Pharmacokinetics of TA enantiomers were also studied following administration of a single 20 mg/kg dose of racemic TA as oral SR beads and iv solution. For powder and INC, previously reported pharmacokinetic data were used.

Results. Regular powder significantly increased the permeability at the gastroduodenal level. Modified-release formulations, on the other hand, did not cause damage in the gastroduodenum but produced significant increase in the permeability of the lower intestine. Consequently, to assess the pharmacokinetic-pharmacodynamic relationship, a new model was developed in which contribution of toxicity resulted from direct exposure to the drug was considered.

Conclusions. Since the observed site of GI damage corresponds to the site of release and absorption of NSAID from the formulation, the possibility of a shift in the site of damage must be considered for the modified release formulations. A parallel evaluation of upper and lower GI toxicity is essential for a complete assessment of NSAID-induced GI damage.

KEY WORDS: gastrointestinal toxicity; pharmacokinetic-pharmacodynamic model; NSAIDs; tiaprofenic acid; sustained release formulations.

INTRODUCTION

The nonsteroidal anti-inflammatory drugs (NSAIDs) commonly believed to cause upper gastrointestinal (GI) tract side effects (1–4). More recent reports, however, indicate that the side effects of NSAIDs are not limited to the gastroduodenal mucosa but may involve both the large and small intestine (2). The asymptomatic nature of NSAID-induced intestinal damage, however, renders diagnosis difficult (2). An increase in the GI permeability prior to mucosal inflammation and more serious complications such as ulceration has been reported (1–4). Sucrose (1,5,6) and ^{51}Cr-EDTA (2,7,8) have been suggested to be suitable markers for the NSAID-induced upper and lower GI permeability, respectively.

NSAIDs may cause their GI side effects by inhibiting cyclo-oxygenase presystemically during absorption and systemically (9). Under the assumption that the damage of NSAIDs is only limited to the upper GI tract prompted investigators to develop dosage forms of NSAIDs that do not release their active ingredient in the stomach. The consequence of altering the NSAID delivery system is the exposure of the small intestine to high concentration of the drug. This increase in the intestinal drug concentration may result in shifting of the damage to the more distal segments of the intestine (10–12).

In order to examine the formulation dependency of the GI site effects caused by tiaprofenic acid, we delineated the pharmacokinetics and GI toxicity of this drug after administration of iv and various regular and modified release oral dosage forms to the rat. In addition, attempts were made to obtain a meaningful relationship between drug concentration and GI side-effects. Since following oral administration of NSAIDs, drug molecules can enter site of toxicity (intestinal epithelium) prior to entering the systemic circulation, the linked pharmacokinetic-pharmacodynamic (PK-PD) model (13) was found to be unsuitable for the modified release formulations. We, therefore, developed a modified PK-PD model suitable for predicting and establishing relationship between the plasma concentration and GI toxicity of NSAIDs.

METHODS

Dosage Forms

The SR formulation (Surgam SR^R 300 mg capsules, lot# 0591 FGY) were beads contained in capsules. Diethyl-β-cyclodextrin (DCD):TA inclusion complex (INC)] was prepared by ethylating β-Cyclodextrin at the 2- and 6- hydroxyl positions using diethyl sulfate as an alkylating reagent (14). Particle size range of <100 mesh were administered (14). Intravenous doses (100–150 μL) were TA in PEG 400.

Animals

Adult male Sprague-Dawley rats with body weight of 300–350 g were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Pharmacokinetic Study

All rats were catheterized in the right jugular vein for sample collection and iv dosing (14). They were fasted overnight with free access to water. Single doses of 20 mg/kg tiaprofenic acid were administered iv (n = 10) or various oral (solid particles via gastric tubing) doses (n = 5). Food was allowed 2 h post-dose. Blood samples were withdrawn from the jugular vein cannula at 0, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8,

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and 24 h post-dose except for SR formulation for which an additional blood sample was collected at 12 h. All specimens were kept at −20°C until analysis. Pharmacokinetics data following oral administration of regular-release powder and INC were previously reported (14).

Permeability Studies

GI permeability was assessed following oral administration of the probes. (5,7). Animals were fasted overnight with free access to water. Food was allowed 2 h post-dose. Twenty four h after assessment of base-line permeability, single 20 mg/kg doses of oral of TA as INC, SR, regular release powder or iv dose were administered and permeability tests were carried out. The doses were corrected based on the absolute bioavailabilities (F) to give the same systemic drug levels.

Permeability of 1g sucrose in 1 mL water was assessed 1 h post TA oral dose (n = 3/group). Permeability of 0.5 mL of an aqueous solution containing 10 μCi/mL of ^{51}Cr-EDTA was assessed following all formulations 1, 2, 3, 4, 6, 8, 12, 24, 48 h post TA dose (n = 5/point). The maximum increase in the permeability occurred 1 h and 3 h post-dose for sucrose and ^{51}Cr-EDTA, respectively. To demonstrate dose dependency of changes in the intestinal permeability, doses of 20 and 40 mg/kg TA as INC (n = 5) and powder (n = 5) were tested. The urinary excretion of ^{51}Cr-EDTA was also measured following oral administration of DCD and physical mixture of DCD:TA (1:1 molar ratio, equivalent to amount present in a single dose of INC).

After oral administration of the markers, urine was collected for 24 h. Sucrose was measured using Trinder reagent (5). The urinary radioactivities following administration of ^{51}Cr-EDTA were counted directly using a multisample counter (7). Changes in the permeability following TA administration was expressed as percent increase from baseline in the 0–24 h urinary excretion of the ^{51}Cr-EDTA.

Tiaprofenic Acid Assay

The concentration of TA enantiomers in the plasma was determined using a reversed-phase stereospecific HPLC assay involving acid extraction and formation of diastereomers using trichloroethyl chloroformate and L-leucinamide (15).

Treatment of Data

The elimination rate constants (β) were estimated from the terminal portion of the fitted plasma concentration-time curves using unweighted data. The area under the plasma TA concentration-time curves from 0–24 h (AUC_{0-24}) was calculated using the linear trapezoidal rule. The total area under the plasma TA concentration-time curve ($AUC_{0-\infty}$) was the sum of AUC_{0-24} and extrapolated area (C*/β). Where C* is the last concentration quantified. The systemic clearance after iv administration was estimated as CL = Dose/$AUC_{0-\infty}$. The apparent volume of distribution was calculated using V_d = Dose/β • $AUC_{0-\infty}$. Peak plasma concentration (C_{max}) and time of its attainment (T_{max}) were estimated from our experimental data points. The pharmacokinetic model best describing time-courses of the enantiomers and total drug (R-TA + S-TA) were determined using PCNONLIN version 4.1 (16).

Permeability data are presented as the percent dose of the surrogate marker found in urine. The increases following TA administration are percent elevation from baseline. AUC_{PD} is the area under the curve of increased permeability measured using the trapezoidal rule from 0 to 24 h.

PK-PD Analysis

A previously described conventional link PK-PD (13) was used (Fig. 1A). In addition, a modified version of the above link PK-PD model was developed with a direct input into the effect compartment (DINP) and used for analysis of the plasma concentration *vs* time data and the time course of intestinal permeability (Fig. 1B). The quality of fit was assessed using Akaike (18) and Schwartz (19) methods.

For the PK-PD analysis, the plasma concentration-time curves of individual rats were fitted to generate PK rate constants. The mean PK parameters and mean permeability data were used to perform PK-PD modeling.

Statistical Analysis

Differences between two means ($p < 0.05$) were assessed using the Student's t-test for paired and unpaired data where appropriate. The differences between more than two means were evaluated using one-way ANOVA followed by Duncan's multiple range test. Unless stated otherwise, data are presented as mean ± S.D.

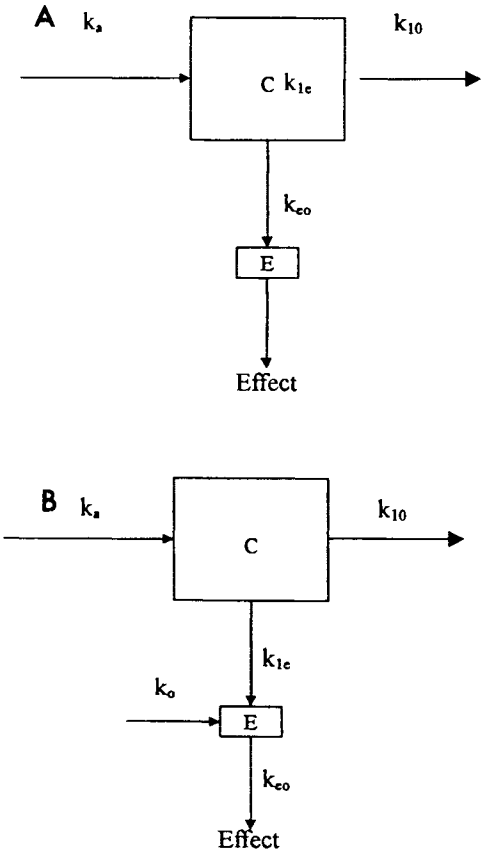

Fig. 1. Link pharmacokinetic-pharmacodynamic (PK-PD) model with a small effect compartment (A) and modified PK-PD model with a direct input to the effect compartment (B).

Table 1. Pharmacokinetic Indices Following 20 mg/kg iv Bolus of Racemic Tiaprofenic Acid

	$t_{1/2}$ (h)		CL (mL/h)		V_d (mL)	
	R-TA	S-TA	R-TA	S-TA	R-TA	S-TA
Mean	4.64	4.30	8.84	4.55[a]	55.9	27.2[a]
S.D.	1.24	0.95	2.49	1.50	10.7	7.31

[a] S-TA significantly different from R-TA.

RESULTS

Pharmacokinetic Study

Plasma concentration-time profiles of TA were stereoselective (S > R) regardless of the route of administration or formulation used. The systemic CL and V_d of R-TA, calculated from i.v. data, were significantly greater than those observed for S-TA but the $t_{1/2}$ values were not significantly different (Table 1).

Similar to what has been previously reported for INC (14), SR formulation of TA, yielded plasma concentration-time curves that indicated a continuous pattern of absorption. Tmax was achieved in 1.54 and 2.11 h for R and S-TA, respectively. The decline in concentration was substantially slower than that reported following administration of TA powder (14). The absolute bioavailability (F) of SR formulation was approximately 30% which was close to that of INC but approximately half of the powder (Table 2).

Pharmacodynamic Study

Permeability of gastroduodenal tract measured as % increased in sucrose urinary excretion exhibited substantial variability, and was significantly higher than the baseline after administration of powder but not after SR or INC (Fig. 2).

Elevation of permeability of the distal intestine measured as % increased urinary excretion of ^{51}Cr-EDTA was dependent on TA-dose. Intestinal permeability measured at the maximum effect time (3 h post-dose) following administration of powder and INC elevated proportionally when the dose was increased from 20 mg/kg (90 ± 38 and 170 ± 98%, respectively) to 40 mg/kg (171 ± 49 and 340 ± 136%, respectively). Oral administration of DCD did not increase 3 h post-TA permeability from baseline. Increased intestinal permeability following oral doses of physical mixture of DCD and TA (75 ± 125%) was comparable to that observed after oral administration of the powder (92 ± 30%).

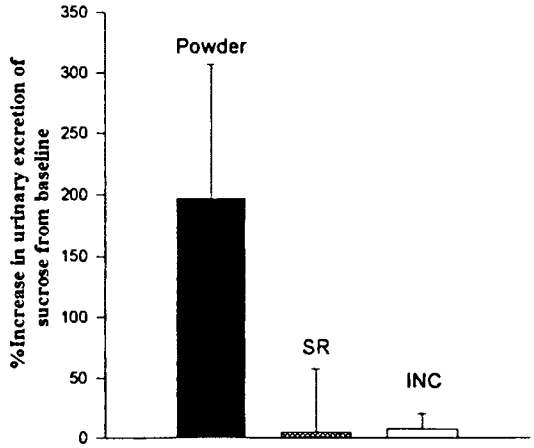

Fig. 2. Percent increase in the upper GI permeability (sucrose) from the baseline 1 h after administration of tiaprofenic acid as regular release powder, inclusion complex (INC) or sustained-release (SR) formulations. Only powder was significantly different from baseline.

AUC_{PD} or the area under % increased urinary excretion of ^{51}Cr-EDTA of both SR (2360 ± 789%·h) and INC (1510 ± 713%·h) were greater than powder (630.7 ± 90%·h) (Fig. 3), or the iv dose (227.6 ± 400%·h). The numerical difference in AUC_{PD} between SR and INC was not significant.

Plots of % increase in the urinary excretion of ^{51}Cr-EDTA versus plasma concentration of both the anti-inflammatory enantiomer (S-TA) (Fig. 4) and R + S-TA resulted in anti-clockwise hystereses for powder and INC. The anti-clockwise hysteresis was not apparent after SR.

Using the effect coempartment model (concentration in the effect compartment, C_e, *VS* effect) (13), the observed anti-clockwise hystereses collapsed (k_{eo}:0.7 h^{-1}) to curves with three

Table 2. Bioavailability Data Following 20 mg/kg of Racemic Tiaprofenic Acid Administered as i.v., Regular Release Powder, Inclusion Complex (INC), or Sustained-Release (SR) Formulations

	T_{max} (h)		C_{max} (mg/L)		$AUC_{0-\infty}$ (mg L^{-1}h)		Ratio of AUCs
Formulation	R-TA	S-TA	R-TA	S-TA	R-TA	S-TA	S-TA/R-TA
i.v.	nd	nd	nd	nd	342	668[a]	0.51
	nd	nd	nd	nd	(58.9)	(107)	
Powder	0.83	2.05[a]	45.4	57.3[a]	222[b]	354[a,b]	0.41
	(0.16)	(0.26)	(6.77)	(6.99)	(33.4)	(55.3)	
INC	1.85[c]	2.45	21.4[c]	26.6[a,c]	104[b]	148[a,b]	0.70
	(0.41)	(0.52)	(2.81)	(4.25)	(22.7)	(33.9)	
SR	1.54[c]	2.11	14.40[c]	22.2[a,c]	109.0[b]	178[a,b]	0.61
	(0.82)	(0.89)	(4.58)	(6.32)	(26.9)	(25.1)	

Note: Mean ± (S.D.); Powder and INC data previously reported (13); nd, not determined.
[a] S-TA significantly different from R-TA.
[b] Significantly different from iv.
[c] Significantly different from powder.

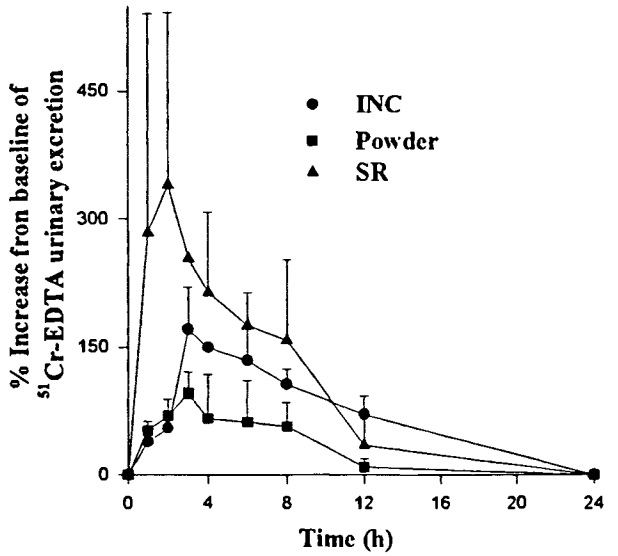

Fig. 3. The time courses of percent increase in the intestinal permeability (^{51}Cr-EDTA) from baseline after administration of tiaprofenic acid as regular release powder, inclusion complex (INC) or sustained-release (SR) formulations. SR was significantly more potent than powder at all times except for zero and 24 h. INC was significantly different from powder 3–12 h post-dose. SR was significantly more potent than INC at 1 and 3 h only.

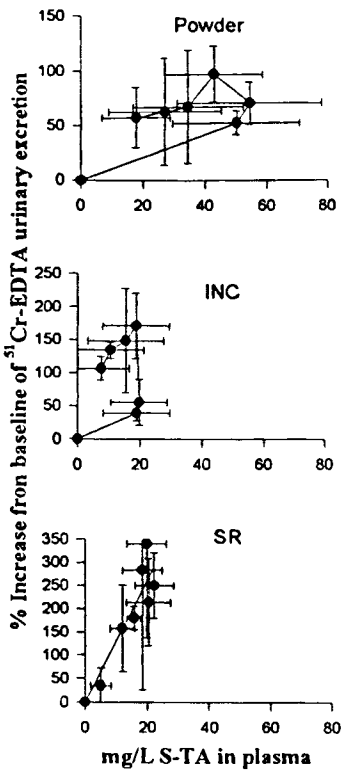

Fig. 4. Counterclockwise hystereses resulted from plotting of percent increased intestinal permeability from the base-line *vs* plasma S-TA concentration following administration of regular release powder and inclusion complex (INC) but not after sustained-release (SR) formulation. The data points are connected in a temporal order commencing from zero.

different patterns (Fig. 5A). Employing DINP model (Fig. 5B), on the other hand, resulted in similar sigmoidal C_E *vs* permeability relationships for all formulations.

Following fitting the C_E and observed GI toxicity data to a sigmoid E_{max} model, the relevant pharmacodynamic parameters were estimated for both active S-TA and total drug (R + S) (Table 3). Narrower confidence intervals, smaller standard errors, sum of weighted squared residuals, Akaike, and Schwartz (19) values were obtained for total drug as compared to those obtained for S-TA.

DISCUSSION

The modified release formulations of NSAIDs (i.e. sustained release and enteric coated) are generally believed to be relatively safe due to their ability to bypass the stomach and duodenum and deliver their active ingredient to more distal portion of GI tract. This can only be true if one assumes that the GI toxicity of NSAIDs is limited to the upper GI tract. Although enteric coated and SR formulations may potentially be useful in the reduction of the observed upper GI damage, a shift in the side of toxicity from the proximal to the more distal GI tract may occur (11,12,20–22).

The observed differences in the release characteristics of three examined dosage forms of TA enabled us to assess the influence of formulations on the GI toxicity. The powder was directly delivered into stomach, ICN releases TA on a pH-dependent fashion in the small intestine (14) and SR formulation is designed to deliver its active gradient gradually throughout the GI tract. Our previously published data (14) and pharmacokinetic observation presented here (Table 2) confirm the release pattern of these formulations.

After administration of TA powder, INC and SR, only the powder caused a significant increased sucrose permeability (Fig. 2). This indicates the possibility of damage due to direct exposure of the upper GI to TA. Neither INC nor SR substantially release TA in the upper GI, hence, it is not surprising that formulations did not cause significant elevation of sucrose permeability.

Despite safer upper GI tract profile for INC and SR as compared with immediate release powder, these formulations caused greater % increased permeability of ^{51}Cr-EDTA (Fig. 3) indicating higher effect on the distal intestine, the main site of drug release. The rapid absorption of TA from the more proximal site of the GI following administration of powder seems to spare the distal intestine from direct exposure to the noxious agent. The effect of an immediate release TA formulation on the lower intestine was, therefore, limited (Fig. 3). Despite the observed marked formulation effect for TA-induced GI toxicity, involvement of systemic GI effect cannot be ruled out since iv administration of TA also significantly increases the intestinal permeability although marginally. Following i.v. administration, AUC_{PD} was only 1/3 of that of the powder, 1/6 of INC and 1/10 of SR.

The observed shift in GI toxicity of TA is similar to that recently reported for flurbiprofen in the rat (22) and diclofenac in humans (23) using permeability tests as surrogate markers. This observation, which is an indicative of the direct effect of NSAIDs at the site of absorption, should not be extrapolated to all NSAIDs due to the heterogeneous nature of these drugs. For example, our preliminary data on ibuprofen (24) indicate systemic effect only. For ibuprofen, therefore, modification of the release pattern should not affect the site of GI toxicity.

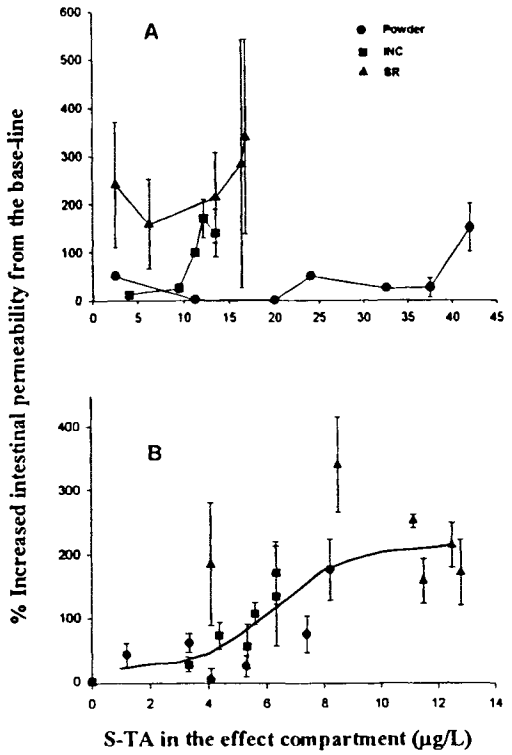

Fig. 5. Percent increase in the intestinal permeability from the base-line *vs* S-TA concentration in the effect compartment estimated using the effect compartment model (A) and the modified model with a direct input to the effect compartment (B). Curve B is the best-fit line through all data points.

Following administration of TA as immediate release powder and INC, anti-clockwise hystereses in the relationship between TA plasma concentrations and the intestinal toxicity were observed (Fig. 4). This may be explained by a lag time between the concentration of drug in the plasma (central compartment) and increased intestinal permeability. An immediate-release TA formulation is absorbed quickly and, perhaps, mainly from upper GI tract. The effect on the distal intestine, therefore, is expected to be through the systemic distribution of the drug rather than a local effect. This may explain the lag effect time. After SR, on the other hand, little or no hysteresis was evident. This may be due to the continuous absorption of the drug from SR, and thereby shortening of the time difference between concentration and effect curves. Interestingly for flurbiprofen, an opposite observation has been reported (22). Hysteresis has been observed for the SR and not for the powder. Lack of hysteresis after the flurbiprofen powder may be due to a very short onset of the increased permeability (22). The presence of hystereis following administration of the SR flurbiprofen, on the other hand, may be explained by the persistent effect of the formulation on the intestinal permeability resulting in a lag time between concentration and effect. Increased permeability was significant until 12 and 72 h following SR formulations of TA (Fig. 4) and flurbiprofen (22), respectively.

Even though the conventional effect compartment model (13) (Fig. 1A) was useful in collapsing the observed anti-clockwise hystereses, the relationship between TA concentration in the effect compartment was still formulation dependent (Fig. 5A). This indicates that the model does not adequately describe underlying relationship between the concentration and TA-induced GI damage. The SR and INC products are designed to release their content in the more distal part of the intestine, consequently, releasing the drug directly at the site of toxicity (effect compartment). NSAIDs cause both pre-systemic and systemic damage. The net observed toxicity is the combination of both systemic (after absorption) and pre-systemic (during absorption). The contribution of these two pathways is dependent on the drug and formulation (7,22). Hence conventional deep effect-compartment model does not hold valid for these types of dosage forms. Using the modified model (Fig. 1B), which accounts for the direct input of TA to the intestinal epithelial cells following oral administration of the modified release formulations, we were able to obtain a better relationship between the concentration of the drug in the effect compartment and the increase in the intestinal permeability (Fig. 5B). The curve depicting concentration in effect compartment versus intestinal effect was sigmoidal and independent of formulation used (Table 3). This was possible due to the fact that the direct exposure of the GI tract to the drug (k_0 Fig. 1B) was considered in the new model and further supports the notion of the formulation-dependent TA-induced intestinal damage.

The presence of an inactive or less active enantiomer may substantially affect the net observed toxicological response by either ameliorating or aggravating the clinical outcome (25). Interestingly, we have obtained better estimates of PD parameters such as E_{max} and EC_{50} when total concentration (R + S) of TA is used instead of only the concentration of the active S-TA (Table 3). This suggests that the R enantiomer may have contributed to the observed TA-induced intestinal damage as well. The unequivocal proof of this, however, requires further study using the individual enantiomers of TA.

The possibility of formulation dependent GI toxicity should be considered in developing and subsequent post-market evaluation of NSAID formulations, with particular attention to the absorption kinetics and its relation to the site of toxicity. Since both upper and lower GI side effects of NSAIDs are clinically important, it appears that the entire tract should be examined when toxicity of these drugs is evaluated.

Table 3. Pharmacodynamic Indices for the Intestinal Damage Caused by Tiaprofenic Acid Estimated Using the Modified Model with a Direct Input to the Effect Compartment

	E_{max}	EC_{50} (μg/L)	γ
Using total concentration (R + S) in the effect compartment			
Estimated value	186	12.3	11
S.E.	22	0.54	5.0
95% confidence interval	138–234	11–13	7.2–28
Using concentration of S-TA in the effect compartment			
Estimated value	214	6.27	3.0
S.E.	65	1.63	1.6
95% confidence interval	74–353	2.7–9.9	−0.41–6.5

APPENDIX

Derivation of Equation for PK-PD Model with Direct Entry into the Effect Compartment

Systemic CL, V_d, and k_{10} (elimination rate constant from central compartment) were estimated from iv data. Absorption

rate constant (k_a) and k_{eo} (elimination rate constant from effect compartment) were estimated after administration of regular release powder. Assuming a small effect compartment with no discernible influence on the concentration of drug in the central compartment, k_{1e} (central to effect compartment rate constant) was arbitrarily set to be 1000 time smaller than the estimated value for k_{eo}. The aforementioned values with no change were utilized in the estimation of the optimum k_o (the rate constant for entry into the effect compartment) for modified release formulations (INC and SR). Considering rapid absorption of the drug in the upper GI tract after regular release powder, the direct exposure of the distal intestine to TA was assumed negligible. Therefore, k_o (only functional during the time that the drug is in the intestine) was set to be zero after oral administration of TA as a regular release powder. After oral doses of INC and SR formulations, the assigned k_0 for S-TA are 0.00035 and 0.00047 mg/h, respectively.

We also assumed that since the GI tract has a large surface area and only a small fraction of administered dose accumulates in the site of toxicity, the concentration of drug in the effect compartment does not have discernible effect on the concentration of the drug in the central compartment (negligible return from the effect to the central compartment); 2) Input to the effect compartment (GI epithelium) is not concentration-dependent due to the excessive concentration available at the site of absorption; k_{1e} was set 1000 fold smaller than k_{eo}. Equation 1 was formulated:

$$\frac{dX_E}{dt} = k_o + k_{1e}X_c - k_{eo}X_E \tag{1}$$

Equation 1 was formulated:

$$\bar{X}_E = \frac{k_o(S + k_a)(S + k_{10}) + k_{1e}k_aF \cdot D}{S(S + k_{eo})(S + k_{10})(S + k_a)} \tag{2}$$

Where D and F are dose and fraction of dose absorbed, respectively.

Taking anti-Laplace of equation 2 using partial fraction theorem will yield:

$$X_E = \frac{k_ok_ak_{10} + k_{1e}k_aF \cdot D}{k_{eo}k_{10}k_a} - \frac{k_{1e}k_aF \cdot D}{k_a(k_{10} - k_a)(k_{eo} - k_a)} e^{-k_at} - \frac{k_{1e}k_aF \cdot D}{k_{10}(k_a - k_{10})(k_{eo} - k_{10})} e^{-k_{10}t} - \frac{k_o(k_a - k_{eo})(k_{10} - k_{eo}) + k_{1e}k_aF \cdot D}{k_{eo}(k_a - k_{eo})(k_{10} - k_{eo})} e^{-k_{eo}t} \tag{3}$$

Where X_E is the amount of drug in the effect compartment. Rearranging and simplifying equation 3.

$$X_E = k_{1e}k_aF \cdot D \cdot \left[\frac{k_ok_{10} + k_{1e}F \cdot D}{k_{eo}k_{10}k_ak_{1e}F \cdot D} - \frac{1}{k_a(k_{10} - k_a)(k_{eo} - k_a)} e^{-k_at} - \frac{1}{k_{10}(k_a - k_{10})(k_{eo} - k_{10})} e^{-k_{10}t} - \frac{k_o(k_a - k_{eo})(k_{10} - k_{eo}) + k_{1e}k_aF \cdot D}{k_{eo}(k_a - k_{eo})(k_{10} - k_{eo})k_{1e}k_aF \cdot D} e^{-k_{eo}t}\right] \tag{4}$$

Considering assumption 2 ($k_{1e} = k_{eo}/1000$), one can further simplify equation 4.

$$X_E = k_{1e}k_aF \cdot D \cdot \left[\frac{1000k_ok_{10} + keoF \cdot D}{k_{eo}k_{10}k_ak_{1e}F \cdot D} - \frac{1}{k_a(k_{10} - k_a)(k_{eo} - k_a)} e^{-k_at} - \frac{1}{k_{10}(k_a - k_{10})(k_{eo} - k_{10})} e^{-k_{10}t} - \frac{1000k_o(k_a - k_{eo})(k_{10} - k_{eo}) + k_{eo}k_aF \cdot D}{k_{eo}^2(k_a - k_{eo})(k_{10} - k_{eo})k_aF \cdot D} e^{-k_{eo}t}\right] \tag{5}$$

Setting

$$M = \frac{1000 \cdot k_Ok_{10} + k_{eo}F \cdot D}{k_{eo}^2k_{10}k_aF \cdot D},$$

$$N = \frac{1}{k_a(k_{10} - k_a)(k_{eo} - k_a)},$$

$$L = \frac{1}{k_{10}(k_a - k_{10})(k_{eo} - k_{10})} \text{ and}$$

$$Q = \frac{1000 \cdot k_o(k_a - k_{eo})(k_{10} - k_{eo}) + k_ak_{eo}F \cdot D}{k_{eo}^2(k_a - k_{eo})(k_{10} - k_{eo})k_aF \cdot D},$$

the concentration-time profile of drug in the effect compartment can be described by equation 6.

$$C_E = \frac{k_{1e}k_aF \cdot D}{V_E}[M - Ne^{-k_at} - Le^{-k_{10}t} - Qe^{-k_{eo}t}] \tag{6}$$

Where C_E is the concentration of the drug in the effect compartment. Following equilibrium between the central and effect compartments, the net transfer of drug in and out of both compartments are equal:

$$k_{1e} \cdot X_c = k_{eo} \cdot X_E \tag{7}$$

$$k_{1e} \cdot V_d \cdot C = k_{eo} \cdot V_E \cdot C_E \tag{8}$$

Where V_d and V_E are the volumes of the central and effect compartments. C is the concentration of the drug.

$$V_E = \frac{k_{1e} \cdot V_dC}{k_{eo}C_E} \tag{9}$$

At equilibrium partition coefficient, k_p, is equal to C_E/C

$$V_E = \frac{k_{1e} \cdot V_d}{k_{eo}k_p} \tag{10}$$

Substituting equation 10 in equation 6.

$$C_E = \frac{k_{eo}k_ak_pF \cdot D}{V_d}[M - Ne^{-k_at} - Le^{-k_{10}t} - Qe^{-k_{eo}t}] \tag{11}$$

The relationship between TA concentration in the effect compartment and the intestinal toxicity can best be described using sigmoid Emax model:

$$E = \frac{E_{max} \cdot C^{\gamma}}{EC_{50} + C^{\gamma}} \tag{12}$$

Where E, E_{max}, EC_{50}, and γ are the observed effect, the observed maximum effect, the concentration corresponding to 50% of the maximum effect, and a number influencing the slope of the concentration-effect curve, respectively. At equilibrium $C = C_E/k_p$

$$E = \frac{E_{max} \cdot (C_E^{\gamma}/k_p)}{EC_{50} + (C_E^{\gamma}/k_p)} \tag{13}$$

Substituting equation 11 in equation 13:

$$E = \frac{E_{max} \cdot \left[\frac{k_{eo}k_a F \cdot D}{V_d}(M - Ne^{-k_a t} - Le^{-k_{10}t} - Qe^{-k_{eo}t})\right]^{\gamma}}{EC_{50} + \left[\frac{k_{eo}k_a F \cdot D}{V_d}(M - Ne^{-k_a t} - Le^{-k_{10}t} - Qe^{-k_{eo}t})\right]^{\gamma}} \tag{14}$$

REFERENCES

1. J. B. Meddings, L. R. Sutherland, N. I. Byles, and J. Wallace. Sucrose: A novel permeability marker for gastroduodenal disease. *Gastroenterology* **104**:1619–1626 (1993).
2. I. Bjarnason, J. Hayllar, A. J. Macpherson, and A. S. Russell. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology* **104**:1832–1847 (1993).
3. L. Aabakken, S. Larsen, and M. Osnes. Visual analogue scales for endoscopic evaluation of nonsteroidal anti-inflammatory drug-induced mucosal damage in the stomach and duodenum. *Scan. J. Gastroenterol.* **25**:443–448 (1990).
4. I. Bjarnason, G. Zanelli, T. Smith, P. Smethurst A. B. Price, M. J. Gumpel, and A. J. Levi. The pathogenesis and consequence of non-steroidal anti-inflammatory drug induced small intestinal inflammation. *Scand. J. Rheumatol.* Suppl. **64**:55–62 (1987).
5. N. Davies, B. W. Corrigan, and F. Jamali. NSAID-induced upper gastoduodenal permeability as measured by sucrose: Suitability of the rat as a model for the human. *Pharm. Res.* **12**:1733–1736 (1995).
6. L. Sutherland, M. Verhoef, J. L. Wallace, G. V. Rosendaal, R. Crutcher, and J. B. Meddings. A simple non-invasive marker of gastric damage: sucrose permeability. *Lancet* **343**:998–1000 (1994).
7. N. M. Davies, M. R. Wright, and F. Jamali. Antiinflammatory drug-induced small intestinal permeability: The rat is a suitable model. *Pharm. Res.* **11**:1652–1656 (1994).
8. J. Ford, S. W. Martin, and J. B. Houston. Assessment of intestinal permeability changes induced by nonsteroidal antiinflammatory drugs in the rat *J. Pharmacol. Toxicol. Met.* **34**:9–16 (1995).
9. K. D. Rainsford. NSAID gastropathy: Novel Physicochemical approach for reducing gastric mucosal injury by drug complexation with cyclodextrins. *Drug Invest. Suppl.* **2**:3–10 (1990).
10. L. Aabakken and M. Osens. Non-steroidal anti-inflammatory drug-induced disease in the distal ileum and large bowel. *Scan. J. Gastroenterol.* Suppl. **24**:48–55 (1989).
11. F. Halter, B. Weber, T. Huber, F. Eigenmann, M. P. Frey, and C. Ruchti. Diaphragm disease of the ascending colon. Association with sustained-release diclofenac. *J. Clin. Gastroenterol.* **16**: 74–80 (1993).
12. A. Figueras, D. Capella, J. M. Castel, J. R. Laorte. Spontaneous reporting of adverse drug reactions to non-steroidal anti-inflammatory drugs. A report from the Spanish System of Pharmacovigilance, including an early analysis of topical and enteric-coated formulations. *Eur. J. Clin. Pharmacol.* **47**:297–303 (1994).
13. N. H. G. Holford and Lewis B. Sheiner. Understanding the dose-effect relationship: Clinical application of pharmacokinetic-pharmacodynamic models. *Clin. Pharmacokin.* **6**:429–453 (1981).
14. M. Vakily, F. M. Pasutto, M. Daneshtalab, and F. Jamali. Inclusion complexation of heptakis-(2,6-di-o-ehtyl)-β-cyclodextrin with tiaprofenic acid: Pharmacokinetic consequences of a pH-dependent release and stereoselective dissolution. *J. Pharm. Sci.* **84**:1014–9 (1995).
15. R. Mehvar, F. Jamali, and F. M. Pasutto. Rapid and sensitive high-performance liquid chromatographic assay of tiaprofenic acid enantiomers in human plasma and urine. *J. Chromatogr.* **425**:135–142 (1988).
16. C. M. Metzler. Nonlin, *A computer program for parameter estimation in non-linear situations*. Upjohn CO Biostatistics Dept, Michigan, 1969.
17. L. B. Sheiner, D. R. Stanski, S. Vozeh, R. D. Miller, and J. Ham. Simultaneous modeling of pharmacokinetics and pharmacodynamics: Application to *d*-tubocurarine. *Clin. Pharmacol. Ther.* **25**:358–371 (1979).
18. A. Akaike. Posterior probabilities for choosing a regression model. *Annal. Inst. Math. Stat.* **30**:A9–14 (1978).
19. G. Schwartz. Estimating the dimension of a model. *Annal. Stat.* **6**:461–464 (1978).
20. I. Bjarnason, P. Prouse, T. Smith M. J. Gumpel, G. Zanelli, P. Smethurst, S. Levi, and A. J. Levi. Blood and protein loss via small-intestinal inflammation induced by non-steroidal anti-inflammatory drugs. *Lancet* **I**:711–714 (1987).
21. I. Bjarnason, A. B. Price, G. Zanelli, P. Smethurst, M. Bruke, M. J. Gumpel, and A. J. Levi. Clinicopathological features of nonsteroidal anti-inflammatory drug-induced small intestinal stricture. *Gastroenterology* **94**:1070–1074 (1988).
22. N. M. Davies, F. Jamali. Influence of dosage form on the gastroenteropathy of flurbiprofen in the rat: evidence of shift in the toxicity site. *Pharm. Res.* **14**:1597–1600 (1997).
23. V. M. Choi, J. E. Coates, J. Chooi, A. B. Thomson, A. S. Russell. Small bowel permeability—a variable effect of NSAIDS. *Clin. Invest. Med.* **18**:357–361 (1995).
24. T. Khazaeinia and F. Jamali. Gastrointestinal toxicity of ibuprofen assessed using GI permeability as a surrogate marker in the rat: Effects of dose, formulation and route of administration. *Pharm. Res.* **13**:S-417 (1996).
25. F. Jamali, R. Mehvar, and F. M. Pasutto. Enantioselective aspects of drug action and disposition: Therapeutic pitfalls. *J. Pharm. Sci.* **78**:695–715 (1989).