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International Journal of Pharmaceutics 312 (2006) 196–199
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Note

Comparative assessment of two indices of drug induced permeability changes in the perfused rat intestine

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Received 23 November 2005; received in revised form 4 January 2006; accepted 9 January 2006
Available online 14 February 2006

Abstract

In the present study, two indices of acute intestinal permeability changes were investigated as measurements of drug induced intestinal damage. The first method was based on ^{14}C-polyethylene glycol (PEG) 4000 permeability assessment and the second was based on histological evaluation of the intestine. The test compounds were ibuprofen, ketoprofen and naproxen and the alanine, glycine and phenylalanine amide derivatives of ibuprofen. Perfusion studies were carried out using a rat model. Post-perfusion, the gut was fixed and tissue changes were assessed and scored. Ibuprofen, ketoprofen and naproxen altered the barrier properties of the intestine to PEG 4000 with significantly higher scores ($p < 0.05$) for gastrointestinal toxicity relative to blank buffer. For ketoprofen, PEG 4000 permeability and intestinal damage scores increased with increasing ketoprofen concentration. Ibuprofen amide derivatives did not induce significant histological damage or PEG 4000 permeability when compared with ibuprofen. A correlation coefficient of 0.91 is obtained when intestinal damage scores are plotted against PEG 4000 permeability for all compounds. Both indices are proposed as rapid and useful measures of drug induced acute intestinal damage.

Keywords: Intestine; Permeability; Histology; Non-steroidal anti-inflammatory drugs

1. Introduction

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is frequently associated with side effects such as nausea, gastrointestinal bleeding and ulceration. Assessment of these adverse effects includes measurement of gastrointestinal ulceration (Beck et al., 1990), gastrointestinal blood loss (Bonney et al., 1987) and mucosal arachidonic acid metabolites such as prostaglandin E2 (Arakawa et al., 1989). Ulceration and gastrointestinal blood loss may be considered as relatively insensitive markers of intestinal permeability changes as they reflect the end point of a complex process. Several NSAIDs have been reported to increase intestinal permeability to hydrophilic molecules such as ^{51}Cr-EDTA (Bjarnason et al., 1985). Measurement of early intestinal permeability changes has previously been shown to be a valid alternative to other measurements of NSAID gut toxicity (Ford et al., 1994; Ford and Houston, 1995).

In the present study, two indices of acute intestinal permeability changes were investigated as measurements of drug induced gut damage after perfusion of a range of compounds in a perfused rat model. The first method is based on ^{14}C-polyethylene glycol (PEG) 4000 permeability assessment and the second is based on histological evaluation of the intestine after NSAID administration. The compounds selected for investigation included NSAIDs known to induce changes in intestinal permeability and amide derivatives of ibuprofen which are known to exhibit less gastrointestinal toxicity than ibuprofen (Shanbhag et al., 1992).

2. Materials and methods

Ibuprofen, ketoprofen and naproxen were obtained from Sigma. The alanine, glycine and phenylalanine conjugates of ibuprofen were synthesised and characterised as previously reported (Lane et al., 2003). The single pass perfusion model was used to study permeability changes (Komiya et al., 1980). Permeability studies were carried out in Sørenson's phosphate buffer, pH 6.8 (isotonic). 40 μl of ^{14}C-PEG 4000 solution

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doi:10.1016/j.ijpharm.2006.01.009

(0.50 μCi ml^{-1}) and 8.4 mg of PEG 4000 was included in each ml of perfusion solution as a marker of intestinal water flux. All compounds were perfused for 120 min. The fraction of PEG 4000 unabsorbed was converted to a permeability coefficient using steady-state data and Eq. (1) (Komiya et al., 1980)

$$P_{app} = -\frac{Q}{2\pi rl} \ln \frac{(C_l)}{(C_0)} \quad (1)$$

where C_0 is the input perfusate ^{14}C-PEG 4000 concentration, C_l the outlet perfusate concentration, r the effective lumen radius (0.18 cm), Q the perfusate flow rate (ml s^{-1}), and l is the length of intestinal segment (33.3 cm). The fraction unabsorbed (C_l/C_0) was corrected for water flux by a gravimetric method (Peréz et al., 2002).

Post-perfusion intestinal tissues were fixed in situ according to the method reported by Swenson et al. (1994). After perfusion with 10% phosphate buffered formalin (20 ml perfused at a flow rate of 0.2 ml min^{-1}) the intestinal segment was removed and immersed in the same fixative. Cross-sections were serially sectioned at 5 μm, stained with haematoxylin–eosin and mounted on glass slides. The histological evaluation was based on the method reported by Swenson et al. (1994). Various measures of histological abnormality were quantitated on a scale of 0–3 with 0 indicating no effect and 3 indicating an extensive effect. This evaluation was carried out by an experienced pathologist. Samples were assayed for ^{14}C-PEG 4000 using a Tri-Carb liquid scintillation counter (Packard). Linear regression and correlation coefficient calculations were carried out using the statistical analysis package Minitab; p values less than 0.05 were considered to be statistically significant.

Table 1
PEG 4000 P_{app} values when perfused with ibuprofen, ketoprofen and naproxen (1 mg ml^{-1}) and in PBS 6.8

NSAID	PEG 4000 P_{app} ($\times 10^6$ cm s^{-1})	n
PBS 6.8	0.64 ± 0.47	8
Ibuprofen	2.68 ± 0.74*	6
Ketoprofen	5.25 ± 1.22*	4
Naproxen	4.34 ± 1.19*	6

n: number of animals studied. Data represent mean values ± S.E.M.
* Significantly different from PBS 6.8, $p < 0.05$.

Table 2
Histological evaluation score of rat small intestinal mucosa post-perfusion with 1 mg ml^{-1} solutions of ibuprofen, ketoprofen, naproxen and PBS 6.8

Histology	Score			
	PBS 6.8	Ibuprofen	Ketoprofen	Naproxen
Mucus/debris	0.92 ± 0.29	1.75 ± 0.89	1.50 ± 0.53	1.75 ± 0.46
Villous shortening	0	1.50 ± 1.20	1.50 ± 0.76	1.25 ± 0.46
Erosion	0	0.75 ± 0.46	0.88 ± 0.35	1.50 ± 0.53
Swollen epithelial cells	0	0	0	0
Flat epithelial cells	0	0.75 ± 0.46	0.88 ± 0.64	0.88 ± 0.35
Goblet cells	0	0	0.13 ± 0.05	0
Total score	0.92 ± 0.29	4.75 ± 1.16*	4.89 ± 1.13*	5.38 ± 1.06*

Data represent mean values ± S.E.M.
* Significantly different from PBS 6.8, $p < 0.05$.

Table 3
PEG 4000 P_{app} values when perfused with ketoprofen (0.5–2.0 mg ml^{-1}) and PBS 6.8

Ketoprofen concentration (mg ml^{-1})	PEG 4000 P_{app} ($\times 10^6$ cm s^{-1})	n
PBS 6.8	0.64 ± 0.47	8
0.5	3.33 ± 1.66*	5
1.0	5.25 ± 1.22*	4
2.0	5.71 ± 1.52*	7

n: number of animals studied. Data represent mean values ± S.E.M.
* Significantly different from PBS 6.8, $p < 0.05$.

Table 5
PEG 4000 P_{app} values when perfused with ibuprofen alanine, glycine and phenylalanine derivatives

NSAID	PEG 4000 P_{app} ($\times 10^6$ cm s^{-1})	n
PBS 6.8	0.64 ± 0.47	8
Ibuprofen	2.68 ± 0.74*	6
Ibuprofen-alanine	1.19 ± 0.64	8
Ibuprofen-glycine	1.55 ± 0.86	7
Ibuprofen-phenylalanine	1.22 ± 0.39	7

Ibuprofen and PBS 6.8 data included for comparison. n: number of animals studied. Data represent mean values ± S.E.M.
* Significantly different from PBS 6.8, $p < 0.05$.

Table 4
Histological evaluation score of rat small intestinal mucosa post-perfusion with ketoprofen (0.5–2.0 mg ml^{-1}) and PBS 6.8

Histology	Score			
	PBS 6.8	Ketoprofen 0.5 mg ml^{-1}	Ketoprofen 1.0 mg ml^{-1}	Ketoprofen 2.0 mg ml^{-1}
Mucus/debris	0.92 ± 0.29	1	1.50 ± 0.53	2.17 ± 0.41
Villous shortening	0	0.83 ± 0.75	1.50 ± 0.76	2.83 ± 0.41
Erosion	0	0.83 ± 0.75	0.88 ± 0.35	1.33 ± 0.52
Swollen epithelial cells	0	0	0	0
Flat epithelial cells	0	0.17 ± 0.14	0.88 ± 0.44	1.50 ± 0.55
Goblet cells	0	0	0.13 ± 0.05	0
Total score	0.92 ± 0.29	2.83 ± 0.75*	4.89 ± 1.13*	7.83 ± 0.75*

Data represent mean values ± S.E.M.
* Significantly different from PBS 6.8, $p < 0.05$.

Table 6
Histological evaluation of the rat small intestinal mucosa after perfusion with amide derivatives of ibuprofen and PBS 6.8

Histology	Score (S.E.M.)				
	PBS 6.8	Ibuprofen	Ibuprofen-alanine	Ibuprofen-glycine	Ibuprofen-phenylalanine
Mucus/debris	0.92 ± 0.29	1.75 ± 0.89	1.00	1.00 ± 0.53	1.17 ± 0.53
Villous shortening	0	1.50 ± 1.20	0.60 ± 0.39	0.80 ± 0.45	0.17 ± 0.11
Erosion	0	0.75 ± 0.46	0.40 ± 0.35	0.2 ± 0.15	0.67 ± 0.32
Swollen epithelial cells	0	0	0	0	0
Flat epithelial cells	0	0.75 ± 0.46	0	0	0.17 ± 0.41
Goblet cells	0	0	2.00	2.00	0
Total score	0.92 ± 0.29	4.75 ± 1.16*	2.00 ± 0.10	2.00 ± 0.71	2.18 ± 0.75

Data represent mean values ± S.E.M.
* Significantly different from blank, $p < 0.05$.

3. Results and discussion

Initial studies were carried out using solutions of ibuprofen, ketoprofen and naproxen (1 mg ml^{-1}). PEG 4000 P_{app} values for all NSAID systems were significantly different from the control P_{app} ($p < 0.05$), confirming that ibuprofen, ketoprofen and naproxen enhanced the intestinal absorption of PEG 4000 (Table 1). The histological scores for the intestinal tissue exposed to all NSAIDs were all significantly higher than the blank (Table 2, $p < 0.05$).

Three concentrations of ketoprofen were further investigated (0.5, 1.0 and 2.0 mg ml^{-1}) to assess the effects of concentration of NSAID on the intestinal epithelium. PEG 4000 permeability values for all concentrations were significantly different from the blank ($p < 0.05$; Table 3). Histological assessment confirmed that mucosal damage appears to increase as ketoprofen concentration in perfusate increases as reflected by the increase in total scores (Table 4).

Amide derivatives of ibuprofen exhibit less gastrointestinal side effects than ibuprofen itself (Shanbhag et al., 1992), which has been ascribed to the absence of the free carboxyl group that is present in the parent NSAID. Permeability values for PEG 4000 are not significantly different from the blank ($p > 0.05$; Table 5) for the amide derivatives, in contrast to the value for ibuprofen. The histological data similarly did not differ significantly from the control for the amide derivatives (Table 6, $p < 0.05$). When histological scores are plotted against PEG 4000 permeability for all compounds (Fig. 1) a correlation coefficient of 0.91 is obtained.

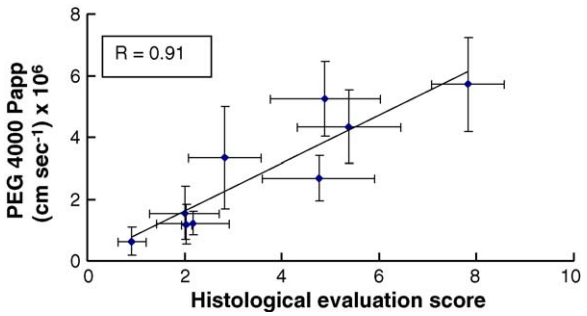

Fig. 1. Histological evaluation scores plotted against PEG 4000 P_{app}.

Ibuprofen, ketoprofen and naproxen altered the rat intestinal barrier properties to PEG 4000. For ketoprofen, PEG 4000 permeability and intestinal damage scores increased with increasing concentration. Amide derivatives of ibuprofen induced significantly lower histological damage and less PEG 4000 permeability than ibuprofen, in line with previous observations (Shanbhag et al., 1992). The intestinal permeability of PEG 4000 generally reflected the scores for histological evaluation of intestinal tissue and a linear relationship ($r = 0.91$, $p < 0.05$) was found over the range of compounds studied.

In summary NSAID-induced intestinal permeation enhancement and acute epithelial damage are correlated. Both methods should distinguish between changes in permeability caused by abnormal cell function and changes caused by gross disturbance of the structure of the mucosal surface. These two indices of acute intestinal permeability changes may offer useful alternatives to more time consuming models of chronic intestinal permeability changes such as measurement of gastrointestinal ulceration or gastric blood loss.

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