ELSEVIER

International Journal of Pharmaceutics 229 (2001) 155–162

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Citric acid as excipient in multiple-unit enteric-coated tablets for targeting drugs on the colon

P. Nykänen [a,*], S. Lempää [a], M.-L. Aaltonen [a], H. Jürjenson [b], P. Veski [b], M. Marvola [a]

[a] *Division of Biopharmaceutics and Pharmacokinetics, Department of Pharmacy, University of Helsinki, PO Box 56, FIN-00014 Helsinki, Finland*

[b] *Institute of Pharmacy, University of Tartu, EE-2400 Tartu, Estonia*

Received 4 June 2001; accepted 31 July 2001

Abstract

Delivery of drugs to the large bowel has been extensively investigated during the last decade. The aim of this study was to investigate whether enteric-coated tablets could be made from enteric-coated matrix granules and drug release targeted to the colon. Whether in vitro drug release rate and in vivo absorption could be delayed by adding citric acid to the granules and/or to the tablet matrix was also studied. Ibuprofen was used as model drug because it is absorbed throughout the gastrointestinal tract. Eudragit™ S and Aqoat™ AS-HF were used as enteric polymers. Drug release rates were studied at different pH levels and drug absorption was studied in bioavailability tests. The conclusion was that citric acid retarded in vitro drug release when used in multiple-unit tablets. In vivo absorption of ibuprofen was markedly delayed when citric acid was included in both granules and tablet matrix. Further studies are needed to determine the optimal amount of citric acid in formulations.

Keywords: Colon-specific drug delivery; Multiple-unit tablet; Enteric polymer; Ibuprofen; Citric acid

1. Introduction

Delivery of drugs to the colon has been extensively investigated during the last decade. A number of diseases, e.g. Crohn's disease, ulcerative colitis and the irritable bowel syndrome, can be treated most efficiently by local delivery of drugs (Kinget et al., 1998). Site-specific systems might also reduce systemic absorption and side effects (Rubinstein et al., 1992). It has also been suggested that colonic delivery of orally administered protein and peptide drugs might be possible, because enzyme activity is low in the colon (Rubinstein, 1995). Analgesic peptides, oral vaccines, growth hormone and insulin are candidates for use of the colon as a site for absorption (Saffran et al., 1986). Various diseases that exhibit diurnal rhythms might also be treatable using colon-specific formulations (Ashford et al., 1993a).

Targeting of drugs to the large bowel can be achieved in several ways. Enteric coating has tra-

* Corresponding author. Tel.: +358-970-859156; fax: +358-970-859580.

0378-5173/01/$ - see front matter
PII: S0378-5173(01)00839-0

ditionally been used to prevent drug release in the upper gastrointestinal tract. The solubilities of enteric polymers are pH-dependent. They are insoluble at low pH values but soluble at high pH values. Ashford et al. (1993b) used Eudragit™ S to protect tablets in the stomach and upper small bowel. Aqoat™ AS-HF-coated pellets have been used for drug delivery to the distal intestine and the proximal colon (Marvola et al., 1999).

The aim of a previous study by us (Nykänen et al., 1999) was to investigate whether drug release rates from enteric matrix granules were influenced by organic acids. It was found that inclusion of succinic acid or citric acid in granules retarded drug release, and that there was a correlation between dissolution rate and amount of organic acid in the granules. However, in vivo delays in drug absorption were less significant, perhaps because granules disintegrated too rapidly in the gastrointestinal tract under the osmotic pressure caused by the water soluble organic acid.

In the study reported now we investigated whether it was possible to make enteric-coated tablets from the granules mentioned above, and whether drug absorption might be further influenced by adding different amounts of citric acid to the tablet matrix. Ibuprofen was used as a model drug because it is absorbed throughout the gastrointestinal tract (Wilson et al., 1989).

2. Materials and methods

2.1. Substances

The enteric polymers used were the methacrylate copolymer Eudragit S (Röhm Pharma, Germany) and hydroxypropylmethylcellulose acetate succinate Aqoat AS-HF (Shin-Etsu Chemical, Japan). The polymers dissolve at pH 7 (Friend, 1991). The additives used in the granules were calcium phosphate ($CaHPO_4$ $2H_2O$) (Ph.Eur.), citric acid (Ph. Eur.), triethyl citrate (Pfizer & Co, USA) and magnesium stearate (Ph.Eur.). Microcrystalline cellulose (Emcocel LP 200, Mendell, USA) and talc (Ph. Eur.) were also used as additives in the tablets. Ibuprofen (Ph.Eur.) was used as model drug. Ibuprofen is a weak acid, with a pK_a value of 5.3 (Herzfeldt and Kümmel, 1983).

2.2. Preparation of matrix granules

A 20% solution in ethanol (Oy Primalco Ab, Finland) of the methacrylate polymer Eudragit S was prepared. Ibuprofen (60%) was mixed with two combinations of diluents (30%). The ratios of citric acid to calcium phosphate in the combinations were 0:3 (granule 0) and 1:2 (granule 10). Powder masses (200 g) were moistened with binder solution in a mortar and sieved manually through a 2.0-mm sieve. The granules were dried overnight at room temperature. The fraction 1.18–1.68 mm was separated by sieving and subjected to film coating. The amount of enteric polymer in the dried granules was 10% on the basis of chemical assay of the active ingredient (spectrophotometric measurement at 221 nm).

2.3. Coating procedure

Coating was performed in a fluidized-bed coater (Aeromatic Strea-1, Aeromatic AG, Switzerland). One hundred grams of granules were coated in each case. The coating solution contained 10% of Aqoat AS-HF, 3.5% of triethyl citrate, 3% of magnesium stearate and 83.5% of demineralized water. The coating solution was prepared according to the instructions of the manufacturer of the polymer and passed through a 0.3-mm sieve before use.

The coating solution was kept in an ice-bath while coating was in progress. Granules were preheated for 5 min at 40 ± 5 °C outlet temperature. The spraying pressure used during coating was 1 bar, the air flow rate 70 m^3/h, and the outlet temperature 40 ± 5 °C. The spraying rate was 5 g/min. Coating was continued until a theoretical weight increase of 20% had been achieved. The granules were dried after coating at the same temperature for 5 min. After the coating, the granules were kept on trays overnight.

2.4. Preparation of tablets

Six tablet formulations were prepared (Table 1). The amount of model drug in each case was 100 mg. Masses were prepared just before tableting. Coated granules, microcrystalline cellulose and

citric acid (if used) were mixed in a Turbula mixer (W. A. Bachofen, Switzerland) for 5 min. Magnesium stearate and talc were added and mixed for an additional 2 min. Tablets were compressed using concave 11-mm punches in a Korch EK-O single-punch press (Erweka Apparatebau GmbH, Germany). The weight of each tablet was 412 mg. Compression forces were chosen so that the hardness of the tablets was 60–70 N, measured using a Schleuniger 2-E/205 tablet-hardness tester (Dr K. Schleuniger and Co., Switzerland).

Tablets were enteric-coated with Aqoat AS-HF, using the method mentioned above, except that air flow rate was 100 m^3/h. One hundred grams of tablets were coated in each case. Coating was continued until a theoretical weight increase of 12% had been achieved.

A code system was developed to make it easier to process results (Table 1). The first number in the code indicates whether granules were of type 0 or type 10. The second number indicates the amount of citric acid in the tablet matrix. When tablets were enteric-coated the letter E was added to the code.

2.5. Dissolution tests

Drug release from uncoated and enteric-coated tablets was studied using the basket method described in USP 23 (apparatus: Dissolutest 07, Prolabo, France). The dissolution media (500 ml at 37 ± 0.5 °C) were pH 5.8, 6.8 and 7.4 phosphate buffers (USP 23). Dissolution studies were carried out for all uncoated and enteric-coated formulations in all three phosphate buffers. The total number of dissolution studies was 36. The speed of rotation of the basket was 100 min^{-1}. The dissolution apparatus was connected to a flow-through spectrophotometer (Ultrospect II, LKB Biochrom, UK) via a peristaltic pump. Absorbance at 221 nm was recorded automatically. Absorbances were monitored by means of a computer running tablet-dissolution software (TDS™, LKB Biochrom, UK).

2.6. Bioavailability studies

Two groups of eight healthy volunteers of both sexes participated in randomized cross-over single-dose studies, carried out in accordance with the recommendations of the Declaration of Helsinki (World Medical Assembly, 1964) as revised in Tokyo (1975). The ages of the volunteers varied from 22 to 39 years and their weights from 45 to 87 kg. Before the studies the participants underwent physical examination, routine haematological testing (Hb, ESR, S-Alat, S-Asat, S-GT, S-Crea) and ECG examination. The volunteers were informed about possible risks and adverse effects of taking the drug, and written consent was obtained from each. The study protocol had been approved by the ethical committee of the University Hospital of Tartu.

The amount of ibuprofen in each tablet was 100 mg. Three ibuprofen tablets were administered to each subject with 200 ml of water following an overnight fast for at least 10 hours. Washout periods were at least one week. A standard lunch was provided 4 hours after drug administration. Blood samples (10 ml) were collected from a forearm vein into heparinized tubes. Plasma was separated after collection and stored at -20 ° until analysed.

Table 1
Compositions of uncoated tablets (%)

	0–0	0–5	0–10	10–0	10–5	10–10
Granule 0	48.5	48.5	48.5	–	–	–
Granule 10	–	–	–	48.5	48.5	48.5
Emcocel LP 200	49.5	44.5	39.5	49.5	44.5	39.5
Citric acid	–	5	10	–	5	10
Magnesium stearate	1	1	1	1	1	1
Talc	1	1	1	1	1	1

2.7. Plasma assay

Ibuprofen plasma concentrations were determined by means of high-performance liquid chromatography (HPLC) using the method described by Avgerinos and Hutt (1986), with slight modifications. The system was equipped with a Waters Model 501 piston pump, a Waters Model 700 Intelligent Sample Processor, a Waters Model 486 Tunable Absorbance detector operated at 222 nm, and a Waters Model 2.10 Millennium workstation. Sample separation was carried out on a μBondapak C_{18} reverse-phase silica column (3.9×300 mm). The isocratic mobile phase was acetonitrile and 0.1 M sodium acetate (35:65), the pH of which was adjusted to 6.2 with glacial acetic acid. The flow rate was 2 ml min^{-1}. The standard curve was found to be linear over the concentration range 0.3–30 mg l^{-1} ($r^2 > 0.9989$). Accuracy, precision, limit of quantitation, specificity and reproducibility were investigated as recommended by Shah et al. (1992).

2.8. Pharmacokinetic parameters

The pharmacokinetic parameters assessed using the Siphar™ pharmacokinetic data analysis program (Simed, France) were lag-time in commencement of drug absorption (t_{lag}), maximum plasma concentration (C_{max}), time to peak concentration (t_{max}) and area under the concentration-time curve ($AUC_{0-12\ h}$ or $AUC_{0-14\ h}$). Rate of absorption phase was also evaluated by means of the ratio C_{max}/AUC. C_{max} and t_{max} values were used as measured. AUC values were calculated using the trapezoidal method, without logarithmic transformation. Statistical analyses were carried out using Student's paired t-test and the Wilcoxon nonparametric test (for t_{max} values).

3. Results and discussion

3.1. In vitro characteristics of uncoated tablets

The effect of addition of citric acid on drug release rate at different pH values was initially investigated with uncoated tablets (Fig. 1). Drug release was slowest, as expected, at pH 5.8 for all formulations. Percentages released by 8 h ranged only from 10 to 20 (Fig. 1 top). The enteric polymers used in the granules are insoluble at pH 5.8. The model drug probably diffused slowly

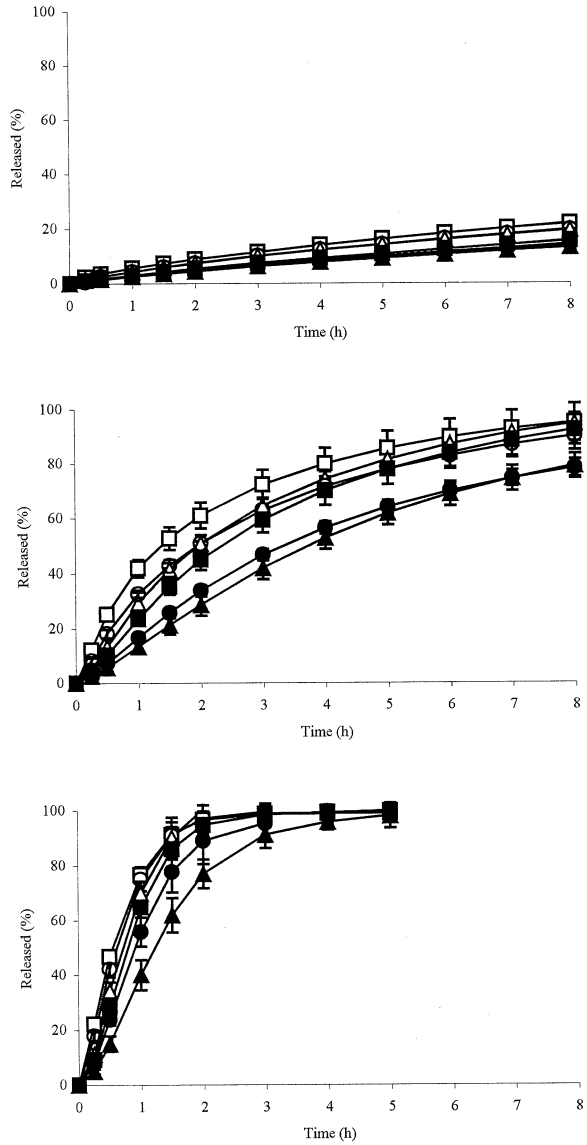

Fig. 1. Dissolution of ibuprofen from uncoated multiple-unit tablets containing different amounts of citric acid in the granule and/or in the matrix. Formulations: □ = 0–0, ○ = 0–5, △ = 0–10, ■ = 10–0, ● = 10–5, ▲ = 10–10. pH values of dissolution media: 5.8 (top), 6.8 (middle) or 7.4 (bottom), means ± S.D., $n = 6$.

from the granules through the pores in the film coat. Drug release rate was fastest at pH 7.4. At this pH the enteric polymers used dissolve and the solubility of ibuprofen is higher than at lower pH levels (Fig. 1 bottom).

Drug release was fastest from uncoated tablets containing no citric acid (code 0–0), at all pH levels (Fig. 1). At pH 6.8, 5 or 10% citric acid in the granules or in tablet matrices (0–5, 0–10, 10–0) retarded release of the model drug. Drug release rates were lowest when citric acid was included in both granules and tablets (10–5, 10–10). $T_{50\%}$ values for the formulation containing no citric acid were 1.4 h (pH 6.8) and 0.6 h (pH 7.4). For the formulation containing 10% citric acid in both granules and the tablet matrix $t_{50\%}$ values were 4.1 h (pH 6.8) and 1.1 h (pH 7.4).

As far as uncoated tablets were concerned, inclusion of citric acid had no marked retardant effect on drug release when the acid was included in the tablet matrix only. Uncoated tablets disintegrated fairly rapidly and the effect of citric acid was also less marked when it was located in the tablet matrix only (0–5, 0–10). Citric acid, which is water-soluble, could also accelerate disintegration of uncoated tablets. When uncoated multiple-unit tablets were prepared from the granules dissolution rate was retarded only when granules containing citric acid were used, and when citric acid (10%) was also included in the tablet matrix. $T_{50\%}$ values for the formulations were 3.3 h (granule 10, Nykänen et al., 1999) and 4.1 h (10–10).

4. In vitro characteristics of coated tablets

Multiple-unit tablets were next enteric-coated. Mean dissolution profiles are shown in Fig. 2. As a rule, drug release rate was markedly retarded by coating. The effect was most marked at pH 6.8, where the $t_{50\%}$ value for uncoated tablets containing no citric acid (0–0) was 1.4 h, that for enteric-coated tablets (0–0E) 2.2 h. When the formulation contained citric acid the effect of coating was more marked. $T_{50\%}$ value for the formulation 0–10 was 2.0 h, that for formulation 0–10E 4.6 h. The $t_{50\%}$ values for 10–10 and 10–10E were 4.1 h and 6.0 h, respectively. Lag-times of ~30 min (pH 7.4) and 30–60 min (pH 6.8) were seen in the dissolution curve when enteric-coated tablets contained 10% or more citric acid (Fig. 2).

The rate of release of ibuprofen was progressively retarded as amount of citric acid increased from none (0–0E, 10–0E) to 5% (0–5E, 10–5E), and from 5 to 10% (0–10E, 10–10E) in the tablet

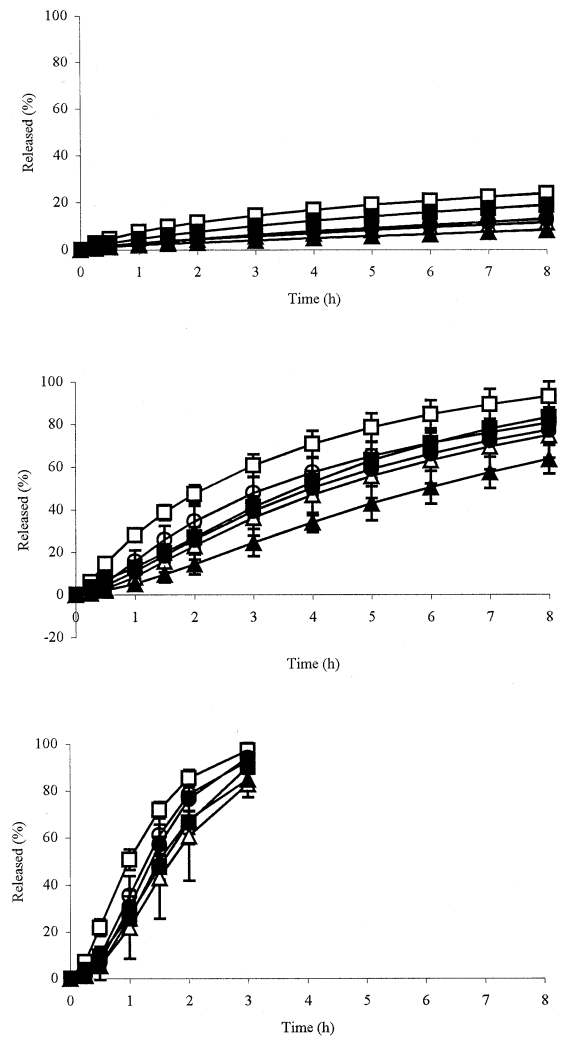

Fig. 2. Dissolution of ibuprofen from enteric-coated multiple-unit tablets containing different amount of citric acid in the granule and/or in the matrix. Formulations: □ = 0–0E, ○ = 0–5E, △ = 0–10E, ■ = 10–0E, ● = 10–5E, ▲ = 10–10E. pH values of dissolution media: 5.8 (top), 6.8 (middle) or 7.4 (bottom), means ± S.D., $n = 6$.

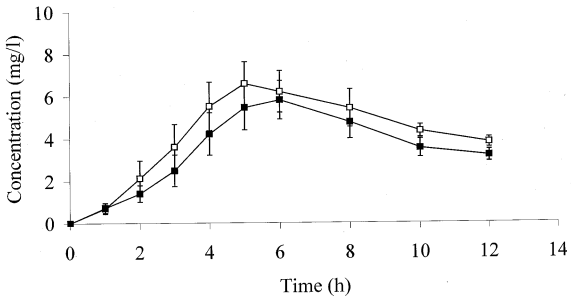

Fig. 3. Effect of addition of acid to granule cores on bioavailability of ibuprofen from enteric-coated multiple-unit tablets. Formulations: □ = 0–0E, ■ = 10–0E. Mean ± SEM, $n = 8$.

matrix. As regards the effect of including citric acid in the coated formulation, the retardant effect on drug release was greatest when both the granules and tablet matrix contained the acid (formulation 10–10E). However, in second place came a formulation containing no citric acid in the granules but 10% in the tablet matrix (formulation 0–10E). The overall conclusion is that in such enteric-coated multiple-unit tablets it is very important for the acidic excipients to be situated in the tablet matrix. Including an acid in the granules too may enhance the effect.

4.1. Bioavailability of enteric-coated tablets

Initially the effect of including citric acid in granules only was studied. Eight volunteers received multiple-unit enteric-coated tablets containing no citric acid or 10% citric acid in the granule cores (formulations 0–0E or 10–0E). Results of in vitro studies at pH 6.8 suggest that absorption would be slower if some of the calcium phosphate in the granules were replaced with citric acid (Fig. 2 middle). Absorption from film-coated tablets containing 10% citric acid in the granules (10–0E) was a little slower than from tablets containing no acid (0–0E, Fig. 3), but differences were not statistically significant (Table 2). Fig. 4 shows individual concentration/time curves for both formulations. This figure also shows no marked difference between the two tablet formulations. Replacing some of the calcium phosphate in the granules with citric acid did not change the in vivo behaviour of the enteric-coated tablets.

Table 2
Pharmacokinetic parameters of ibuprofen given as tablets containing no citric acid or 10% citric acid as excipient in granules only (means ± S.D., $n = 8$)

Parameter	0–0E	10–0E	Statistic
t_{lag} (h)	0.5 ± 0.3	0.7 ± 0.3	n.s.
t_{max} (h)	6.8 ± 3.0	6.4 ± 2.3	n.s.
C_{max} (mg l^{-1})	7.2 ± 2.9	6.4 ± 3.1	n.s.
AUC_{0-12h} (mg l^{-1} h)	51.4 ± 19.5	50.6 ± 17.1	n.s.
C_{max}/AUC_{0-12h} (h^{-1})	0.14 ± 0.01	0.15 ± 0.01	n.s.

In a further step the effect of adding citric acid to the tablet matrix was investigated. The formulations studied contained 10% citric acid in the tablet matrix, no citric acid or 10% citric acid in

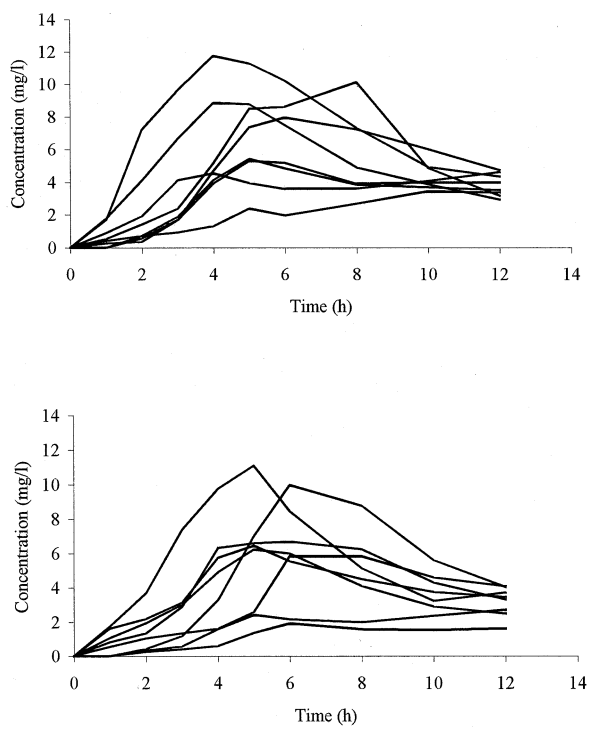

Fig. 4. Individual plasma concentration curves of ibuprofen after administration of enteric-coated multiple-unit tablets containing no citric acid in the tablet matrix and no citric acid (top) or 10% citric acid (bottom) in the granules.

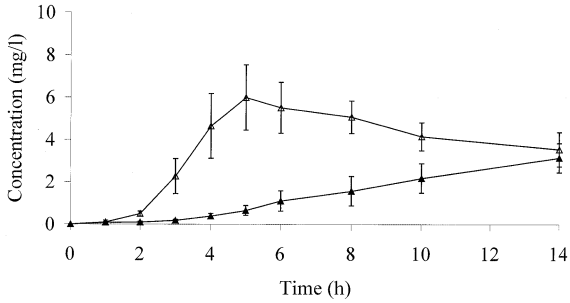

Fig. 5. Effect of addition of acid to tablet matrix on bioavavailability of ibuprofen from enteric-coated multiple-unit tablets. Formulations: △ = 0–10E, ▲ = 10–10E. Means ± SEM, $n = 8$.

the granule cores (tablets 0–10E or 10–10E). Absorption from tablets containing citric acid in both tablet matrix and granules was retarded (Fig. 5). The effect was so marked that it was impossible to calculate all pharmacokinetic parameters in the usual way because C_{max} values had not been achieved by 14 h (Table 3). A lag time of two to five hours was observed for both formulations containing acid in tablet matrices (Fig. 6).

In a previous study (Nykänen et al., 1999) the bioavailability of ibuprofen from plain granules analogous to granules 0 and 10 in the study reported here was investigated. Having compared the pharmacokinetic results obtained previously with the results reported here we conclude that manufacture of enteric-coated tablets of the kinds described here from enteric-coated granules allows retardation of drug absorption. T_{max} values for enteric-coated tablets were higher than the t_{max} value for plain granules (0), especially when citric acid was incorporated in the tablet matrix. The

Table 3
Pharmacokinetic parameters of ibuprofen given as tablets containing no citric acid or 10% citric acid as excipient in granules and 10% citric acid as excipient in tablets (means ± S.D., n = 8)

Parameter	0–10E	10–10E
t_{max} (h)	7.4 ± 3.5	–
C_{max} (mg l^{-1})	7.4 ± 3.6	–
AUC_{0-14h} (mg l^{-1} h)	51.6 ± 21.7	19.1 ± 16.2

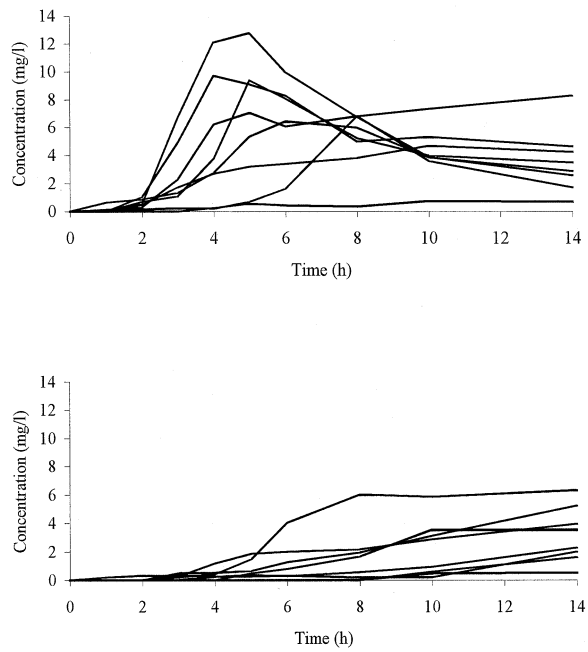

Fig. 6. Individual plasma concentration curves of ibuprofen after administration of enteric-coated multiple-unit tablets containing 10% citric acid in tablet matrix and no citric acid (top) or 10% citric acid (bottom) in the granules.

t_{max} value for the plain granules (0) was 6.1 ± 1.1 h (mean ± S.D., Nykänen et al. 1999). For formulation 0–0E it was 6.8 ± 3.0 h. For formulation 0–10E it was 7.4 ± 3.5 h.

Preparation of a multiple-unit enteric-coated tablet from enteric-coated matrix granules in such a way, that the tablet matrix contains an acid, seems to be promising way in which to prepare colon-specific formulations. Inclusion of an acidic component in granule cores would be an additional way to control the site of drug liberation. However, the percentage of citric acid (10) in formulation 10–10E was obviously too high.

In our earlier study (Marvola et al. 1999) it was found that absorption of ibuprofen from uncoated matrix granules started even in the stomach, although the solubility of ibuprofen is low in acidic environments. The lengthy lag time in relation to commencement of ibuprofen absorption in the study reported here was obviously not a consequence of low solubility of ibuprofen at low pH resulting from the incorporation of citric acid but

rather of markedly delayed disintegration of the formulation as a whole. Final verification of the in vivo disintegration site will require use of gammascintigraphy or some similar imagining method.

5. Conclusions

Previous studies by us have shown that enteric-coated matrix granules, in which the binder is also an enteric polymer, can be used to target drugs to the ileum and proximal colon. The results of study reported here show that if such granules are formulated into multiple-unit enteric-coated tablets the site of drug liberation in vivo could be advanced towards the colon. Addition of an acidic component (e.g. citric acid) to the tablet also delays drug release. The effect of adding an acid is especially marked if the acid is in the tablet matrix. The effect is weaker if the acid is in granule cores. Formulations can be optimized by varying amounts of acid and its division between granule cores and the tablet matrix. The Finnish patent application concerning these formulations has been left (FI 20010978).

Acknowledgements

This work was financially supported by the Pharmacal Research Foundation, Finland.

References

Ashford, M., Fell, J.T., Attwood, D., Woodhead, P.J., 1993a. An in vitro investigation into the suitability of pH-dependent polymers for colonic targeting. Int. J. Pharm. 91, 241–245.

Ashford, M., Fell, J.T., Attwood, D., Sharma, H., Woodhead, P.J., 1993b. An in vivo investigation into the suitability of pH dependent polymers for colonic targeting. Int. J. Pharm. 95, 193–199.

Avgerinos, A., Hutt, A.J., 1986. High-performance liquid chromatographic determination of ibuprofen in human plasma and urine in direct injection. J. Chromatogr. 380, 468–471.

Friend, D.R., 1991. Colon-specific drug delivery. Adv. Drug Deliv. Rev. 7, 149–199.

Herzfeldt, C.G., Kümmel, R., 1983. Dissociation constants, solubilities and dissolution rates of some selected nonsteroidal anti-inflammatories. Drug Dev. Ind. Pharm. 9, 767–793.

Kinget, R., Kalala, W., Vervoort, L., van den Mooter, G., 1998. Colonic drug delivery. J. Drug Target 6, 129–149.

Marvola, M., Nykänen, P., Rautio, S., Isonen, N., Autere, A.-M., 1999. Enteric polymers as binders and coating materials in multiple-unit site-specific drug delivery systems. Eur. J. Pharm. Sci. 7, 259–267.

Nykänen, P., Krogars, K., Säkkinen, M., Heinämäki, J., Jürjenson, H., Veski, P., Marvola, M., 1999. Organic acids as excipients in matrix granules for colon-specific drug delivery. Int. J. Pharm. 184, 251–261.

Rubinstein, A., 1995. Approaches and opportunities in colon-specific drug delivery. Critic. Rev. Therap. Drug Carr. Syst. 12, 101–149.

Rubinstein, A., Nakar, D., Sintov, A., 1992. Chondroitin sulfate: A potential biodegradable carrier for colon-specific drug delivery. Int. J. Pharm. 84, 141–150.

Saffran, M., Kumar, G.S., Neckers, D.G., Williams, F., Field, J.B., Jones, R.H., Panepinto, L., 1986. New approaches to the oral administration of peptide drugs. Pharm. Week Sci. Ed. 10, 43–44.

Shah, V.P., Midha, K.K., Dighe, S., McGilveray, I.J., Skelly, J.P., Yacobi, A., Layloff, T., Viswanathan, C.T., Cook, C.E., McDowall, R.D., Pittman, K.A., Spector, S., 1992. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. J. Pharm. Sci. 81, 309–312.

Wilson, C., Washington, N., Greaves, I., Kamali, F., Rees, J., Sempik, A., Lampard, J., 1989. Bimodal release of ibuprofen in a sustained-release formulation: a scintigraphic and pharmacokinetic open study in healthy volunteers under different conditions of food intake. Int. J. Pharm. 50, 155–161.