J. Pharm. Pharmacol. 1995, 47: 725–730
Received August 15, 1994
Accepted February 9, 1995



# The Influence of Uptake from the Gastrointestinal Tract and First-pass Effect on Oral Bioavailability of (Z)-alkyloxyimino Penicillins

LINDA MIZEN, VALERIE BERRY AND GARY WOODNUTT

*Microbial Infectivity Department, SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ, UK*

## Abstract

We have investigated the contribution of uptake from the gastrointestinal tract and first-pass effect to the poor oral bioavailability of a series of (Z) -alkyloxyimino penicillins in mice.

Investigative studies in gut sacs and perfused small intestine demonstrated that these penicillins were able to pass across the mucosal epithelium although to a lesser extent than amoxycillin and cyclacillin, both of which exhibit excellent oral bioavailability in man and animals. In the jejunal gut sacs the mucosal to serosal flux for BRL 44154 was approximately half that of amoxycillin and four times less than that of cyclacillin, and for all, uptake was pH dependent. The serosal to mucosal fluxes were however similar for these compounds and significantly lower than mucosal to serosal fluxes, suggesting involvement of carrier mechanisms in uptake from the mucosal surface. The order of results for the alkyloxyimino penicillins paralleled that observed for oral bioavailability in the mouse.

For the alkyloxyimino penicillins, between 5·5 and 9·9% was taken up from the perfused intestine, values which were significantly less than those for amoxycillin (13·2%) and cyclacillin (33·3%). However, uptake was concentration-dependent for BRL 44154 as it was for amoxycillin, thus confirming the possible use of carrier mechanisms in absorption.

These observations suggest that the poor peripheral blood concentrations of the alkyloxyimino penicillins achieved after oral dosing were not a consequence of the inability of the compounds to cross the mucosal epithelium. The biliary clearance of the alkyloxyimino penicillins was, however, considerably greater than for amoxycillin and cyclacillin, a finding which may well have been a contributory factor to the comparatively low peripheral concentrations of BRL 44154 and its analogues achieved after oral administration.

The antibiotic concentrations obtained in peripheral blood after administration by the oral route are influenced not only by the rate and extent of uptake from the gut lumen, but also the metabolism and elimination of the compounds during transition from the lumen via the liver to the general circulation, the first-pass effect (Benet & Massoud 1984; Gregus & Klaassen 1987). The physicochemical characteristics and chemical and metabolic stability throughout the gut lumen also influence concentrations achieved in blood. Alkyloxyimino penicillins, including the β-lactamase-stable penicillin BRL 44154 (Brown et al 1991; Hill & Merrikin 1991), were found to be stable in the pH range likely to be encountered in the gut lumen, but when administered orally to rats and mice, concentrations were barely detectable in peripheral blood.

This report describes studies to investigate the oral absorption of this series of penicillins which, in addition to bioavailability studies in mice, included estimation of uptake from the rat gut lumen and first-pass effects using an intestinal gut sac technique and intestinal perfusion in-situ. Results were compared with those for amoxycillin and cyclacillin, which are reported to be well absorbed by the oral route in both animals and man (Mizen & Woodnutt 1988; Lambert & O'Grady 1992).

Correspondence: L. Mizen, Microbial Infectivity Department, SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ, UK.

## Materials and Methods

### *Chemicals*

The series of (Z) 2-alkyloxyimino-2-(2-aminothiazol-4-yl) acetamidopenicillins were prepared at SmithKline Beecham Pharmaceuticals Research Division. The structures are shown in Fig. 1. Amoxycillin sodium was obtained from SmithKline Beecham Pharmaceuticals, Worthing, cephaloridine (Ceporin) and cyclacillin (Calthor) were commercial preparations.

### *Animals*

Albino male mice (MF1, OLAC), 18–22 g, and albino male rats (Sprague-Dawley, Charles River), 250–350 g, were used.

### *Bioavailability studies in mice*

The penicillins were administered as solutions of their sodium salts in phosphate-buffered saline pH 7·2 for subcutaneous dosing and in water for oral administration by gavage. Blood was removed at death from the cut axilla, and groups of five mice were used at each time interval. Samples were taken at times ranging from 5 to 120 min after dosing. Results are expressed as the maximum concentration observed, and area under blood level curve (AUC) over 120 min; the trapezoidal rule was used to calculate this parameter. Urinary recovery of the penicillins after oral and subcutaneous dosing was assessed over 24 h. Groups of

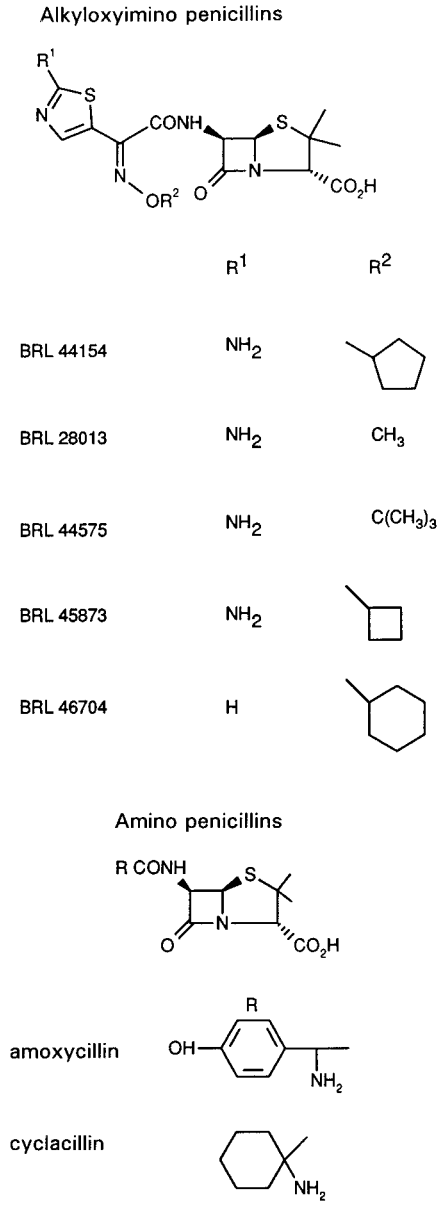


FIG. 1. Chemical structures of alkyloxyimino penicillins and amoxycillin and cyclacillin.

five mice were used and a pooled urine sample collected over Cardice from 0–6 h and 6–24 h. Results were expressed as total recovery of dose and the absorption index was calculated as the total recovery after oral administration/total recovery after subcutaneous dosing.

*Uptake studies in intestinal gut sacs*

Tissues were obtained from rats, and the method for preparing everted and non-everted intestinal sacs was based on that described by Dixon & Mizen (1977). Groups of four rats were used for each experiment. Animals were anaesthetized ($N_2O:O_2$, 1 : 1; 4% halothane) and laparotomy was performed. The small intestine was washed through with 0·9% sodium chloride (saline) in-situ, ligated and removed between the ligament of Treitz and the proximal ileum. For measuring the mucosal to serosal fluxes, the intestine was everted over a glass rod and submerged in oxygenated saline at ambient temperature (21°C). Segments of 7·5 cm were used to prepare the sacs which were filled with 1 mL Krebs bicarbonate buffer at pH 7·5. Each sac was suspended in 30 mL Krebs phosphate buffer at pH 6·5. For measuring the serosal to mucosal fluxes, the intestine was not everted, and sacs were filled with 1 mL Krebs phosphate buffer at pH 6·5 and suspended in the pH 7·5 Krebs bicarbonate buffer.

For measuring mucosal to serosal fluxes, compounds were added to the solution bathing the mucosal surface at 1000 $\mu g\,mL^{-1}$ and for the measurement of back flux they were added at the same concentration to the buffer bathing the serosal surface of the non-everted sacs.

The sacs were selected at random and uptake measured over 30 min of incubation at 37°C. The bathing solutions were aerated with 95% $O_2$–5% $CO_2$ throughout. At the end of incubation, the fluid from within the sacs was removed for analysis. Fluid transfer was measured by calculating the internal volume change of the sac. The wall tissue was homogenized in 4 vols ice-cold Krebs phosphate buffer using an Ultra Turrax (Janke and Kunkel, IKA Labortechnic).

*Uptake from isolated perfused intestine*

The perfusion method used was based on that of Sinko et al (1987). Following an overnight fast, rats were anaesthetized using $N_2O:O_2$ (1:1) and halothane (4% for induction and reduced to 1·5% for maintainance). The carotid artery was cannulated for the collection of peripheral blood. A mid-line incision was made into the peritoneum and the bile duct cannulated. A small incision in the intestine was made just below the ligament of Treitz and distally at a distance of 20 cm. The segment was gently flushed with 20 mL Krebs phosphate buffer (pH 6·5). Glass cannulae were inserted at either end of the segment and secured with silk ligatures. For perfusion the cannulae were clamped in place and attached to silicone rubber tubing passing into a temperature-controlled glass reservoir at 37°C. The intestinal segment remained within the abdominal cavity and was kept moist with pads soaked in warmed saline and the body temperature was maintained using a heated pad.

A volume of 50 mL Krebs phosphate buffer (pH 6·5) containing the antibiotic under test was placed in the reservoir and circulated at a flow rate of 2 $mL\,min^{-1}$ using a peristaltic perfusion pump. A perfusate concentration of 1000 $\mu g\,mL^{-1}$ was used for all agents but the uptake of BRL 44154 and amoxycillin was also measured over a concentration range of 100 to 2000 $\mu g\,mL^{-1}$. Perfusion continued for 2 h and samples of peripheral blood were removed every 30 min. A portal venous blood sample was taken at the end of the perfusion period. All blood samples were centrifuged at 2000 *g* at 5°C and plasma removed for assay. Bile samples were collected at 30-min intervals over the infusion period.

At the end of the study, the perfusion system was drained and the volume measured to monitor water uptake, the tubing and intestinal segment were then flushed with buffer and the washings were collected for analysis. The segment was removed, weighed and homogenized in 4 vols ice-cold buffer using an Ultra-Turrax homogenizer (Janke & Kunkel, IKA Laboratories).

Table 1. The pharmacokinetics of alkyloxyimino penicillins compared with amoxycillin and cyclacillin in mice.

| Compound (50 mg kg$^{-1}$) | Route | $C_{max}$[a] ($\mu$g mL$^{-1}$) | AUC ($\mu$g min mL$^{-1}$) | Urine (% recovered) 0–24 h | Absorption index[b] |
|---|---|---|---|---|---|
| BRL 44154 | subcutaneous | 23·8 | 647·4 | 13 | |
| | oral | 0·03 | 2·42 | 0·4 | 0·03 |
| BRL 44575 | subcutaneous | 26·9 | 605 | 22·8 | |
| | oral | – | – | 0·7 | 0·03 |
| BRL 28013 | subcutaneous | 31·0 | 770 | 95·4 | |
| | oral | – | – | 4·5 | 0·05 |
| BRL 45873 | subcutaneous | 37·4 | 364 | 37 | |
| | oral | 0·27 | 4·5 | 5·6 | 0·15 |
| BRL 46704 | subcutaneous | – | – | 14·8 | |
| | oral | 1·3 | – | 1·5 | 0·10 |
| Amoxycillin | subcutaneous | 26·2 | 770·5 | 43 | |
| | oral | 5·1 | 308 | 18 | 0·42 |
| Cyclacillin | subcutaneous | 30 | 1063·8 | 83·2 | |
| | oral | 20·6 | 1083 | 89·8 | ~1·0 |

[a] Maximum concentration observed over 5–120 min. [b] Ratio of urinary recovery from oral and subcutaneous experiments.

The percentage of compound that was absorbed was calculated as:

$$\frac{(C_s \times V_s) - (C_e \times V_e) - W - H}{(C_s \times V_s)}$$

where $C_s$ and $C_e$ are the concentrations in the reservoir at the start and end of the test and $V_s$ and $V_e$ are the initial and final volumes of the perfusate. W is the amount of compound in the washings and H the amount in the homogenate. The mean decrease in perfusate volume for all studies was 8·5 ± 2·7% which was within the range reported by others using this perfusion system (Sinko et al 1987).

*Measurement of concentrations*

Samples from all studies were kept at 4°C until assay within 4 h; no loss of activity was observed under these conditions. The large-plate microbiological assay method was used with *Staphylococcus saprophyticus* NCTC 8340 as the assay organism. Previous studies had indicated that the assay specificity would not be influenced by the presence of bioactive metabolites. Standard solutions were prepared in the appropriate diluents; mouse blood was used for all blood samples, rat plasma for peripheral and hepatic portal plasma samples, and rat bile concentrations were assayed against standards prepared in Krebs phosphate buffer. For the assay of the other samples, including tissue homogenates, standards were prepared in the appropriate buffer solution.

*Statistical methods*

Statistical significance was determined by the unpaired Student's *t*-test.

## Results and Discussion

*Bioavailability studies*

The bioavailabilities of BRL 44154 and its analogues BRL 44575, BRL 28013, BRL 45873, and BRL 46704 were particularly low after oral administration (Table 1) as indicated by either low maximum concentrations in blood or low urinary recoveries in comparison with those after subcutaneous administration. BRL 28013, although only poorly available by the oral route, gave rise to relatively high concentrations in blood and the highest urinary recovery values for this series of alkyloxyimino penicillins when administered subcutaneously. This may be indicative of greater overall metabolic stability; however absorption indices were low and of a similar order to the other analogues. The absorption indices values for amoxycillin and cyclacillin were considerably higher than those for the alkyloxyimino penicillins. The particularly poor oral bioavailability of BRL 44154 and its analogues compared with amoxycillin and cyclacillin was also observed in the rat. Similarities in the order of absorption between species is not unknown for antibacterial agents (Mizen & Woodnutt 1988); thus the rat was used for further studies investigating the mechanisms associated with the transport of alkyloxyimino penicillins.

*Intestinal gut sacs studies*

The results of studies in jejunal gut sacs are shown in Table 2. Cephaloridine (3-(1-pyridinium methyl)-7 $\beta$-[(2-thienyl) acetamido]-ceph-3-em-4-carboxylate) was included as an example of a $\beta$-lactam antibiotic that has been reported as

Table 2. The forward and backward fluxes of BRL 44154 compared with amoxycillin, cyclacillin and cephaloridine across rat jejunal sacs.

| Compound | Forward flux mucosal to serosal[a] ($\mu$g g$^{-1}$ 30 min) | Back flux serosal to mucosal[b] ($\mu$g g$^{-1}$ 30 min) |
|---|---|---|
| BRL 44154 | 39 (5·9) | 30·2 (6·3)* |
| amoxycillin | 77·3 (14·1) | 28·4 (4·8)** |
| cyclacillin | 165 (22) | 24·1 (7·5)** |
| cephaloridine | 22·5 (5·4) | 26·6 (6·7) |

[a] Determined in everted gut sacs and antibiotics added to the surrounding mucosal solution. [b] Gut sacs were not everted and antibiotic added to the surrounding serosal solution. * $P < 0.05$, ** $P < 0.01$ compared with forward flux values. Results are expressed as the mean (± s.d.) for four animals.

Table 3. The uptake of selected alkyloxyimino penicillins relative to BRL 44154 in everted sacs of rat jejunum.

| Compound | % rate of uptake |
|---|---|
| BRL 44154 | 100 |
| BRL 44575 | 105 |
| BRL 28013 | 110 |
| BRL 45873 | 160* |
| BRL 46704 | 130** |

* $P < 0.05$, * $P < 0.01$ compared with value for BRL 44154.

having poor absorption with only 1–2% of the dose excreted in urine when administered orally to man (Kislak et al 1966). The fluxes were expressed as uptake over 30 min. The mucosal to serosal flux for BRL 44154 was approximately twice that of cephaloridine, but only half that of amoxycillin. The uptake of cyclacillin, was twice that of amoxycillin, a result in agreement with published data for these penicillins in this model and others in which it was shown that cyclacillin is absorbed via an active transport process (Dixon & Mizen 1977; Kimura et al 1978; Fel et al 1994) and that the uptake of amoxycillin is facilitated (Tsuji et al 1977; Kimura et al 1978). The back fluxes of BRL 44154, cephaloridine, amoxycillin and cyclacillin were, however, similar. This is presumably indicative of passive diffusion. It was of interest that, similar to results obtained with amoxycillin and cyclacillin, the forward flux of BRL 44154 was greater than the back flux ($P < 0.05$), whereas the forward and backward fluxes were similar for cephaloridine ($P > 0.05$). This would suggest some affinity of BRL 44154 for transport processes in the mucosal membrane.

BRL 44154 analogues were compared by expressing uptake as a percentage of the results for BRL 44154 (Table 3). The uptake of BRL 28013 and BRL 44575 was similar to that for BRL 44154 ($P > 0.05$), whereas for BRL 45873 and BRL 46704, values were significantly higher ($P < 0.01$ and $< 0.05$, respectively), results which are in keeping with the absorption indices (Table 1). Concentrations of the different antibiotics in the wall tissue of the everted gut sacs (data not shown) followed a similar order of results to that obtained in the mucosal and serosal fluids, thus indicating that results were not influenced by differential binding to tissues.

The use of a peptide carrier for transport across the enterocyte has been postulated for a number of $\beta$-lactam antibiotics, and uptake is $H^+$-gradient-dependent (Nakashima & Tsuji 1985; Okano et al 1986; Inui et al 1988; Muranushi et al 1989; Dantzig & Bergin 1990; Dantzig et al 1992; Kramer et al 1992; Fel et al 1994). Transport had been shown to be greater when the mucosal pH was between 5 and 6·5. It was of interest therefore to determine whether the uptake of BRL 44154 was influenced by pH. In the absence of a pH gradient (pH 7·4 on both the mucosal and serosal sides), uptake by the gut sac over 30 min was 54% of the rate observed when the mucosal pH was 6·5 ($P < 0.05$). Following a further decrease in pH to 5·0 on the mucosal side of the sac, a slight increase in uptake (1·13 fold) was observed compared with results for an outer pH of 6·5, but the difference was not significant. Amoxycillin showed a similar trend, and uptake increased with a decrease in pH. The uptake of amoxycillin at a mucosal pH of 7·5 was 67% of that obtained at pH 6·5 ($P < 0.05$), whereas, uptake increased at pH 5·0 and was 130% of the value at pH 6·5 ($P < 0.05$).

The results in everted gut sacs would therefore suggest that the particularly poor blood levels obtained for these compounds after oral administration were not as a direct consequence of their inability to cross the mucosal epithelium; however, uptake was less than observed for amoxycillin and cyclacillin.

*Isolated perfused intestine*

The results obtained for the perfusion studies in rat intestine shown in Table 4, support those obtained in the everted gut sac. The alkyloxyimino penicillins were taken up from the perfusate. In addition, the order of results was similar in the two systems. The percentage of BRL 44154 removed from the perfusate was approximately half that of amoxycillin and one-sixth that of cyclacillin. The absorption of BRL 44575 was similar to that for BRL 44154 and almost half of that observed for BRL 28013 and BRL 45873. Concentration was also shown to affect uptake from the gut lumen (Table 5), and for BRL 44154, although there was an increase in the absolute uptake (12·8 $\mu$g vs 55 $\mu$g), the percentage uptake from a perfusate containing 100 $\mu g\,mL^{-1}$ (12·8%) was more than twice ($P < 0.01$) that observed at 1000 $\mu g\,mL^{-1}$ (5·5%). This was also observed for amoxycillin for which the percentage uptake was similar for concentrations from 500 to 2000 $\mu g\,mL^{-1}$ with values

Table 4. Pharmacokinetics of alkyloxyimino penicillins, amoxycillin and cyclacillin after perfusion of rat jejunum in-situ.

| Compound[a] | Absorbed in 2 h (%) | Amount in bile over 2 h ($\mu$g) | Portal vein plasma concn ($\mu g\,mL^{-1}$)[b] | Carotid plasma concn ($\mu g\,mL^{-1}$)[b] | Biliary clearance ($mL\,min^{-1}$)[c] |
|---|---|---|---|---|---|
| BRL 44154 | 5·5 (0·4) | 123 (24) | 0·55 (0·03) | < 0·08 | 1·9 (0·2) |
| BRL 44575 | 5·3 (0·4) | 268 (42) | 0·21 (0·03) | < 0·08 | 10·6 (1·8) |
| BRL 28013 | 9·2 (0·6) | 230 (51) | 0·41 (0·05) | < 0·31 | 4·7 (0·7) |
| BRL 45873 | 9·9 (1·0) | 166 (23) | 0·62 (0·09) | 0·19 (0·02) | 2·2 (0·4) |
| BRL 46704 | 6·6 (0·7) | 82 (21) | < 1·6 | < 1·6 | – |
| Amoxycillin | 13·2 (1·6) | 43 (5·3) | 4·0 (0·52) | 1·7 (0·18) | 0·09 (0·01) |
| Cyclacillin | 33·3 (4·2) | 850 (74) | 27·3 (1·1) | 12·6 (0·95) | 0·26 (0·02) |

[a] Perfusation concentration was 1000 $\mu g\,mL^{-1}$. [b] Steady state concentration. [c] Rate of excretion in bile ($\mu g\,min^{-1}$)/ concentration in portal vein ($\mu g\,mL^{-1}$). Results are expressed as the mean (± s.d.) for three animals.

Table 5. Effect of concentration on the uptake of BRL 44154 and amoxycillin from perfused rat jejunum in-situ.

| Compound | Perfusate concn ($\mu g\,mL^{-1}$) | Absorbed in 2 h (%) | Amount in bile over 2 h ($\mu g$) | Portal vein plasma concn ($\mu g\,mL^{-1}$)[a] | Carotid plasma concn ($\mu g\,mL^{-1}$)[a] | Biliary clearance ($mL\,min^{-1}$) |
|---|---|---|---|---|---|---|
| BRL 44154 | 100 | 12·8 (1·5) | 18·9 (3·2) | < 0·08 | < 0·08 | ≥ 1·9 |
| | 1000 | 5·5 (0·4) | 123 (24) | 0·55 (0·03) | < 0·08 | 1·9 (0·2) |
| Amoxycillin | 100 | 32·4 (3·6) | 3·6 (0·3) | 0·52 (0·06) | 0·15 (0·01) | 0·06 (0·01) |
| | 500 | 12·1 (0·9) | 27·5 (3·0) | 1·8 (0·24) | 0·62 (0·03) | 0·13 (0·02) |
| | 1000 | 13·2 (1·6) | 42·6 (5·3) | 4·0 (0·52) | 1·7 (0·18) | 0·09 (0·01) |
| | 2000 | 11·8 (1·3) | 142·6 (12·6) | 8·9 (1·0) | 5·1 (0·73) | 0·13 (0·02) |

[a] Steady state concentrations. [b] Rate of excretion in bile ($\mu g\,min^{-1}$)/concentration in portal vein ($\mu g\,mL^{-1}$). Results are expressed as the mean (± s.d.) for three animals.

ranging from 11·8 to 13·2%, but at $100\,\mu g\,mL^{-1}$, uptake increased significantly to 32·4% ($P < 0·01$). The results for amoxycillin are in agreement with those reported by Tsuji et al (1977) in this model, and suggest that the transport of not only amoxycillin, but also BRL 44154 was facilitated by a carrier mechanism which can be saturated.

The results shown in Table 4 give an indication of the other processes which may also influence peripheral blood concentrations after oral administration. At the end of the perfusion period, all compounds, with the exception of BRL 46704 for which the sensitivity of the microbiological assay was poor, were detected in portal blood at steady state, although concentrations of the alkyloxyimino penicillins were approximately one-tenth the concentrations of amoxycillin in portal blood and almost one-hundredth those of cyclacillin. These differences were greater than observed between values for uptake from the gut lumen. This could be indicative of differences in metabolic stability within gut tissues, although concentrations in peripheral blood may also influence the steady-state level in hepatic portal blood with time.

The steady-state concentrations in peripheral blood, for all compounds were lower than in portal blood which would reflect in part, the first-pass effect and would be expected for antibiotics which are metabolized in the liver and have the potential for excretion via the biliary pathway. Thus, not only the alkyloxyimino penicillins, but also amoxycillin and cyclacillin, were subjected to first-pass effects. However, differences between arterial and portal vein concentrations for amoxycillin and cyclacillin were approximately twofold, whereas, differences for the alkyloxyimino penicillins were fivefold or greater; the lack of sensitivity of the assay precluded the calculation of exact figures. It was notable that there was marked excretion of the alkyloxyimino penicillins into bile as, although all compounds were detected in bile, the biliary clearance values for the alkyloxyimino penicillins were around ten to twenty-fold those measured for amoxycillin and cyclacillin.

In conclusion, these studies illustrate the complexities of the oral absorption process. Alkyloxyimino penicillins were shown to be taken up from the gut lumen with the possible involvement of carrier mechanisms, but first-pass effects, involving extensive biliary clearance and metabolism, were significant, and may well have contributed to the poor oral bioavailability for this series of penicillins in laboratory animals.

*Acknowledgements*

The authors wish to thank David Merrikin and Hazel Smulders for their contributions to the mouse bioavailability studies and Victoria Mendizabal and Wendy Burgess for their constructive criticism of the manuscript.

## References

Benet, L. Z., Massoud, N. (1984) Pharmacokinetics. In: Benet, L. Z., Massoud, N., Gambertoglio, J. G. (eds) Pharmacokinetic Basis for Treatment. Raven Press, New York, pp 1–28

Brown, P., Calvert, S., Chapman, P. C. A., Cosham, S. C., Eglington, A. J., Elliot, R. L., Harris, M. A., Hinks, J. D., Lowther, J., Merrikin, D. J., Pearson M. J., Ponsford, R. J., Syms, J. V. (1991) β-lactamase-stable penicillins. Synthesis and structure activity relationships of (Z)-alkyloxyimino penicillins: selection of BRL 44154. J. Chem. Soc. Perkin. Trans. 1: 881–891

Dantzig, A. H., Bergin, L. (1990) Uptake of the cephalosporin, cephalexin, by a dipeptide transport carrier in the human intestinal cell line, Caco-2. Biochim. Biophys. Acta 1027: 211–217

Dantzig, A. H., Tabas, L. B., Bergin, L. (1992) Cefaclor uptake by the proton-dependent dipeptide transport carrier of human intestinal Caco-2 cells and comparison to cephalexin uptake. Biochim. Biophys. Acta 1112: 167–173

Dixon, C., Mizen, L. (1977) Absorption of amino penicillins from everted rat intestine. J. Physiol. 269: 549–559

Fel, Y-J., Kanal, Y., Nussberger, S., Ganapathy, V., Leibach F. H., Romero, M. F., Singh, S. K., Boron, W. F., Hediger, M. A. (1994) Expression cloning of a mammalian proton-coupled oligopeptide transporter. Nature 368: 563–566

Gregus, Z., Klaassen C. D. (1987) Biliary excretion. J. Clin. Pharmacol. 27: 537–541

Hill, G. K., Merrikin, D. J. (1991) BRL 44154—a novel penicillin active against methicillin-resistant staphylococci-penicillin binding protein studies. J. Chemother. Proceedings VII Mediterranean Congress of Chemotherapy 1990. Suppl. n4: 2–4

Inui, K. I., Okano, T., Maegawa, H., Kato, M., Hori, R. (1988) $H^+$ coupled transport of p.o. cephalosporins via dipeptide carriers in the rabbit intestinal brush-border membranes: difference of transport characteristics between cefixime and cephradine. J. Pharmacol. Exp. Ther. 247: 235–241

Kimura, T., Endo, H., Yoshikawa, M., Sezaki, H. (1978) Carrier mediated transport systems for aminopenicillins in rat small intestine. J. Pharmacobiodyn. 1: 262–267

Kislak, J. W., Steinhauer, B. W., Finlan, M. (1966) Cephaloridine activity in vitro and absorption and urinary excretion in normal young men. Am. J. Med. Sci. 251: 433–448

Kramer, W., Girbig, F., Gutjahr, U., Kowalewski, S., Adam, F., Scheibler, W. (1992) Intestinal absorption of β-lactam antibiotics and oligopeptides: functional and stereospecific reconstitution of the oligopeptide transport system from rabbit small intestine. Eur. J. Biochem. 204: 923–930

Lambert, H. P., O'Grady, F. W. (1992) Penicillins. In: Lambert, H.

P., O'Grady, F. W. (eds) Antibiotics and Chemotherapy. Churchill Livingstone, pp 191–230

Mizen, L., Woodnutt, G. W. (1988) A critique of animal pharmacokinetics. J. Antimicrob. Chemother. 21: 273–278

Muranushi, N., Yoshikawa, T., Yoshida, M., Oguma, T., Hirano, K., Yamada, H. (1989) Transport characteristics of ceftibuten, a new oral cephem, in rat intestinal brush-border membrane vesicles: relationship to oligopeptide and amino β-lactam transport. Pharm. Res. 6: 308–312

Nakashima, E., Tsuji, A. (1985) Mutual effects of amino-β-lactam antibiotics and glyclglycine on the transmural potential difference in the small intestinal epithelium of rats. J. Pharmacobiodyn. 8: 623–632

Okano, T., Inui, K.-I., Takano, M., Hori, R. (1986) $H^+$ gradient-dependent transport of aminocephalosporins in rat intestinal brush-border membrane vesicles: role of dipeptide transport system. Biochem. Pharmacol. 35: 1781–1786

Sinko, P. J., Hu, M., Amidon, G. L. (1987) Carrier mediated transport of amino acids, small peptides and their drug analogues. J. Contr. Rel. 6: 115–121

Tsuji, A., Nakashima, E., Kagami I., Honjo, N., Yamana, T. (1977) Effect of dose-concentration on the absorption of amoxycillin and ampicillin from the rat intestine. J. Pharm. Pharmacol. 29: 707–708