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The evaluation of some pharmaceutically acceptable excipients as permeation enhancers for amoxicillin

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Abstract

The aim of the present study was to evaluate different pharmaceutically acceptable excipients as permeation enhancers for a low permeability drug, amoxicillin. As a model for the intestinal epithelium excised rat jejunum, mounted in side-by-side diffusion cells, was used. Amoxicillin was actively transported across the intestine in the serosal-to-mucosal direction, but only if glucose was present at the mucosal side. This effect of glucose was abolished by a multridrug resistance associated protein (MRP) inhibitor benzbromarone (0.04 mM), but not by verapamil (0.2 mM). Among the tested pharmaceutically acceptable excipients only sodium lauryl sulfate (0.2 mg/ml) increased the permeability of amoxicillin in the mucosal-to-serosal direction, which was accompanying with the abolishment of the secretory oriented transport of amoxicillin. Other excipients (0.072 mg/ml Pluronic F68, 0.2 mg/ml Lutrol F127, 0.2 mg/ml Cremophor EL or 0.2 mg/ml Carbopol 934) have no influence on the permeability of amoxicillin. The effect of sodium lauryl sulfate on the active secretion of amoxicillin was mainly attributed to the reversible cellular ATP depletion. We concluded that sodium lauryl sulfate can be considered as a relatively safe permeation enhancer for amoxicillin in drug delivery systems intended to improve oral bioavailability of this drug.
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Keywords: Amoxicillin; Permeation enhancement; Multidrug resistance associated protein (MRP); Sodium lauryl sulfate; Side-by-side diffusion cells

1. Introduction

Amoxicillin is a β-lactam antibiotic that is relatively well absorbed from the gastrointestinal tract compared to the majority compounds from this group (Zhao et al., 2001). The absorption of amoxicillin in healthy humans was shown to be dose dependent with decreased absorption at higher doses. In healthy humans the absorption decreased from 72% to 45% when the oral dose increased from 500 to 3000 mg (Paintaud et al., 1992). Similar results were obtained in patients with ileostomy, where the recovery of amoxicillin and its metabolite, penicilloic acid, in urine decreased from 70% to 23% after the dose was increased from 375 to 6000 mg (Sjövall et al., 1992). The dose dependent intestinal absorption is in accordance with the carrier-mediated absorption process of amoxicillin observed in animal studies (Margarit et al., 1991; Schoenmakers et al., 1999), although the decrease in the absorption at very high doses (>3000 mg) could also be partially a result of the incomplete dissolution of amoxicillin in the intestinal tract (Paintaud et al., 1992; Sjövall et al., 1992). Studies using rat intestinal tissue suggest the involvement of H^+/peptide carrier in the absorption of amoxicillin, but it must be also pointed out that the transport of amoxicillin across the intestinal epithelium has a significant passive diffusion component (Margarit et al., 1991; Schoenmakers et al., 1999). Additionally, it was shown that amoxicillin is transported across the rat intestine by an energy-demanding efflux system, which appears to be distinct from *P*-glycoprotein-mediated transport system and suggested that this system contributes to the restricted intestinal absorption of some β-lactam antibiotics (Saitoh et al., 1997).

Although amoxicillin is relatively well absorbed compared to other penicillins (i.e. ampicillin, benzylpenicillin, phenoxymethylpenicillin) (Zhao et al., 2001), the absorption does not exceed 90% which is a boundary between the high and low permeability drugs, thus amoxicillin can be classified as a low permeability drug according to Biopharmaceutics Classification System (BCS), which was also confirmed by the results

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obtained from a regional perfusion technique in vivo in humans (Lennernäs et al., 2002).

The permeability of a low permeable drug can be improved by co-administration of permeation enhancers. In general, these substances can promote the permeability by opening the tight junctions between the intestinal epithelial cells or by inhibiting the efflux transporters located at the apical membranes of the enterocytes (Aungst, 2000). The modulation of efflux systems is of particular interest, since many pharmaceutically acceptable excipients can inhibit secretory transporters, including *P*-glycoprotein and several multidrug resistance associated proteins (MRPs) at very low concentrations (Nerurkar et al., 1996; Redondo et al., 1998; Rege et al., 2002; Lo, 2003; Bogman et al., 2003).

In the present study, we evaluated various regulatory acceptable pharmaceutical excipients at concentrations that are likely to be achieved in the intestinal tract after application of the oral dosage form as permeation enhancers for amoxicillin, with a special stress laid on the effect of sodium lauryl sulfate. As a model for the permeation enhancement studies excised rat intestinal segments were used.

2. Materials and methods

2.1. Chemicals

Amoxicillin trihydrate was obtained from Sandoz (Kundl, Austria), verapamil hydrochloride was from Teva S.R.L. (Milano, Italia) and ranitidine hydrochloride was from Vera (Hyderabad, India). Benzbromarone was from Sigma (Deisenhofen, Germany). D-Mannitol was from Fluka (Steinheim, Germany) and D-glucose was from Merck (Darmstadt, Germany). Sodium lauryl sulfate (SLS) was from Cognis (Düsseldorf, Germany). Pluronic F68 (poloxamer 188), Lutrol F127 (Poloxamer 407) and Cremophor EL (Polyoxyl 35 Castor Oil) were from Basf (Ludwigschafen, Germany). Carbopol 934 (carbomer 934) was from Noveon (USA).

2.2. Permeability experiments

The experiments conform to the European convention for the protection of vertebrate animals used for Experimental and other Scientific Purposes (Council of Europe No 123, Strasbourg 1986).

The experiments were performed in a manner similar to that described previously (Legen and Kristl, 2004). The jejunum (25 cm distally from the ileocaecal junction) was excised from 8 to 9 weeks old male Wistar rats (250–300 g), which had been fasted for 18 h before the experiment. After decapitation the small intestine was immediately excised and placed into an ice-cold bubbled (carbogen, 95:5 O_2/CO_2) 10 mM solution of glucose in Ringer buffer for not longer than 30 min. The intestine was rinsed with ice-cold Ringer buffer to remove luminal contents, cut into 3 cm long segments, excluding visible Peyer's patches and opened along the mesenteric border. It was then mounted on a special insert and placed between two EasyMount side-by-side diffusion chambers with an exposed tissue area of 1 cm^2 (Physiologic Instruments, San Diego, USA).

During the experiment the tissue was incubated on both sides with Ringer buffer (pH 7.45 or pH 6.85) containing 10 mM glucose at the serosal and, unless otherwise stated, at the mucosal sides. The incubation medium was kept at 37 °C and continuously gassed with carbogen.

After 30 min of equilibration the substance under investigation [amoxicillin (2 mM), ranitidine hydrochloride (1.9 mM) or verapamil hydrochloride (0.7 mM)] was added to the mucosal or serosal side to study mucosal-to-serosal (m-to-s) or serosal-to-mucosal (s-to-m) transport, respectively. The concentrations of ranitidine and verapamil correspond to the usual oral dosages (150 and 80 mg, respectively), taking account 250 ml as an adequate volume of gastrointestinal fluids (Aungst, 2000).

To study the effects of mannitol, 10 mM glucose at the mucosal side was replaced with an equimolar concentration of mannitol. For studies using efflux system inhibitors (40 μM benzbromarone (BB), 200 μM verapamil (VRP) or 693.5 μM (0.2 mg/ml) sodium lauryl sulfate (SLS)), the inhibitor was added 20 min before amoxicillin to the mucosal side only and was present in the mucosal solution throughout the experiment.

To study the effect of different permeation enhancers, 0.1 or 0.2 mg/ml SLS, 0.072 mg/ml Pluronic F68, 0.2 mg/ml Lutrol F127, 0.2 mg/ml Cremophor EL or 0.2 mg/ml Carbopol 934 was added together with amoxicillin to the mucosal side of the tissue. Unless otherwise stated the excipients were present in the incubation medium throughout the experiments.

Samples of 250 μl were withdrawn from the acceptor compartment at 30 min intervals up to 210 min (unless otherwise stated) and replaced by the appropriate Ringer buffer solution with additives to maintain constant concentration of sugars and/or inhibitors in the acceptor compartment.

Tissue viability and integrity were checked by monitoring potential difference (PD), short circuit current (I_{sc}) and transepithelial electrical resistance (TEER), and additionally, in the experiments with mannitol at the mucosal side, by recording the increase of I_{sc} and PD after the addition of glucose (25 mM) to the mucosal compartment at the end of experiments to evaluate the activity of Na^+/D-glucose co-transporter. Tissue electrical parameters were monitored by computer-controlled voltage–current clamp apparatus (Scientific Instruments, Aachen, Germany). For the determination of the TEER a bipolar pulse (amplitude: 50 μA) was applied for 200 ms every 120 s.

The background potential (asymmetry of the electrodes and liquid junction potential) was compensated before mounting the tissue in the diffusion chamber system. Additionally, I_{sc} and TEER were corrected for fluid resistance.

2.3. Analytical procedures

All samples from the permeability experiments were analyzed by HPLC (HP, Series 1100, Palo Alto, CA, USA) consisting of a vacuum degasser, a quaternary pump, column thermostat, an autosampler and a diode array UV detector.

For amoxicillin, mobile phase A was phosphate buffer (10 mM, pH 6.0), while mobile phase B consisted of 60% (v/v) acetonitrile and 40% (v/v) phosphate buffer (10 mM, pH 6.0). The gradient had an initial solvent composition of 97% A and 3% B that was maintained for 1 min, then linear gradient to 75% B in 2 min was applied. The column Betasil C8 (5 μm, 50 mm × 4.6 mm) (Keystone Scientific, Bellefonte, PA, USA) was applied at 30 °C. The column equilibration time was 1 min. UV detection at 228 nm was employed. The injection volume was 50 μl and flow rate was 3 ml/min.

For verapamil, mobile phase A consisted of 90% (v/v) phosphate buffer (20 mM, pH 3.0) and 10% (v/v) acetonitrile, while mobile phase B consisted of 70% (v/v) acetonitrile and 30% (v/v) phosphate buffer (20 mM, pH 3.0). The gradient had an initial solvent composition of 60% A and 40% B that was maintained for 1 min, then linear gradient to 100% B in 1.5 min was applied and maintained for 0.5 min. The column Betabasic C18 (3 μm, 50 mm × 4.6 mm) (Keystone Scientific, Bellefonte, PA, USA) was applied at 30 °C. The column equilibration time was 1 min. UV detection at 231 nm was employed. The injection volume was 50 μl and flow rate was 3 ml/min.

For ranitidine, mobile phase A consisted of 95% (v/v) phosphate buffer (20 mM, pH 3.0) and 5% (v/v) acetonitrile, while mobile phase B consisted of 70% (v/v) acetonitrile and 30% (v/v) phosphate buffer (20 mM, pH 3.0). The gradient had an initial solvent composition of 100% A that was maintained for 0.8 min, then linear gradient to 100% B in 1 min was applied and maintained for 0.5 min. The column Betabasic C18 (3 μm, 50 mm × 4.6 mm) (Keystone Scientific, Bellefonte, PA, USA) was applied at 30 °C. The column equilibration time was 1 min. UV detection at 318 nm was employed. The injection volume was 50 μl and flow rate was 3 ml/min.

2.4. Data analysis and statistics

Apparent permeability coefficients (P_{app}) were calculated according to the equation:

$$P_{app} = \frac{dQ}{dt}\frac{1}{AC_0}\ (\text{cm/s})$$

where $\frac{dQ}{dt}$ is the steady-state appearance rate on the acceptor side of the tissue, A is the exposed area of the tissue and C_0 is the initial concentration of the drug in the donor compartment.

Results are expressed as means ± standard deviation (S.D.). Two-group comparisons were analyzed by unpaired two-tailed t-test or paired Student's two-tailed t-test. In the case of unpaired t-test, F-test for variances was first applied. If the variances were equal, the standard Student's t-test was performed otherwise Behrens–Fisher test was used.

3. Results

3.1. Evaluation of the amoxicillin permeability

The P_{app} value of amoxicillin across the excised jejunal segments is lower than the corresponding value of verapamil and comparable to the permeability of ranitidine, which is in accordance with the fraction of these compounds absorbed in humans (Table 1). These results demonstrate that amoxicillin can be classified as a low permeability drug according to BCS.

Table 1
Apparent permeability coefficients (P_{app}) of amoxicillin, ranitidine and verapamil across the rat jejunum in vitro in relation to absorption in humans

Compound	Amoxicillin	Ranitidine	Verapamil
P_{app} (10^{-6} cm/s)	6.47 ± 0.69	5.15 ± 1.52	11.5 ± 3.0
Fraction absorbed (%)[a]	72	64	100

The P_{app} were determined in the m-to-s direction at pH 6.85 with 10 mM mannitol at the mucosal and 10 mM glucose at the serosal side. P_{app} values are expressed as the mean ± S.D. of three to six experiments.

[a] Human data were obtained from the literature (Zhao et al., 2001; Paintaud et al., 1992).

3.2. Effect of pH on the permeability of amoxicillin

The transport of amoxicillin across the rat jejunum in the m-to-s direction appears to be pH dependent with higher permeability observed at lower (pH 6.85) than at higher pH (7.45), although this difference was not significant ($p = 0.18$) when mannitol was present at the mucosal side instead of glucose (Fig. 1). It must be also pointed out that the permeability of amoxicillin was significantly ($p < 0.05$) lower when glucose was present at the mucosal side compared with the presence of mannitol, at both pH values (Fig. 1). Therefore, the influence of glucose on the transport of amoxicillin was further studied.

3.3. Influence of glucose and inhibitors of efflux transporters on the transport characteristics of amoxicillin

Amoxicillin permeated much faster in the s-to-m direction than in the opposite direction when 10 mM glucose was present at the mucosal side of the tissue (Fig. 2), demonstrating active secretion of amoxicillin. The difference between the m-to-s and s-to-m permeability became smaller, although still significant, when 0.2 mM VRP was added to the mucosal side (Fig. 2). The

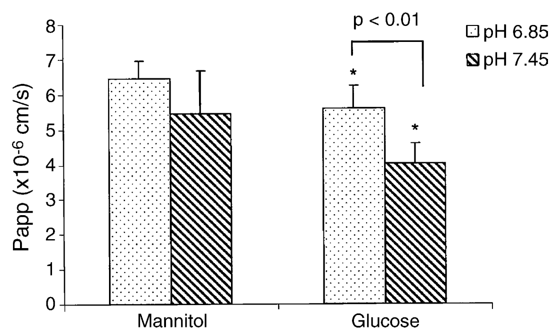

Fig. 1. Influence of pH of the incubation medium on the permeability of amoxicillin across the rat jejunum in vitro in the presence of 10 mM glucose or 10 mM mannitol at the mucosal side. The permeability experiments were performed in the m-to-s direction. Ten mM glucose was present at the serosal side in all cases. * Significantly different from the corresponding value in the presence of mannitol ($p < 0.05$). Data are expressed as the mean ± S.D. of three to 24 experiments.

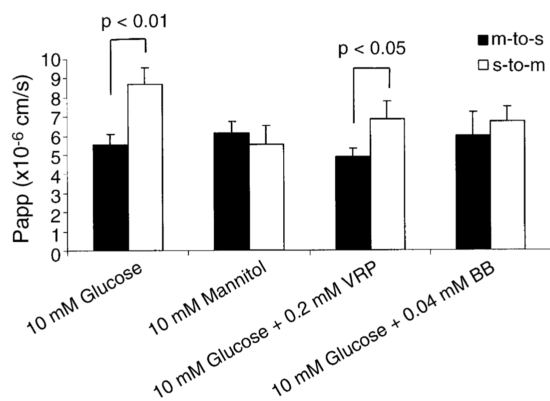

Fig. 2. Effect of different conditions at the mucosal side on the permeability of amoxicillin across the rat jejunum. Ten millimolar glucose was present at the serosal side in all cases. Samples from the acceptor compartment were withdrawn up to 150 min. Data are expressed as the mean ± S.D. of three experiments.

Table 2
Influence of some pharmaceutically acceptable excipients on the permeability of amoxicillin across the rat jejunum

	P_{app} (10^{-6} cm/s)		
	Control	Excipient	Ratio
Pluronic F68 (0.072 mg/ml)	5.13 ± 0.48	5.20 ± 0.95	1.01
Lutrol F127 (0.2 mg/ml)	5.23 ± 0.78	4.67 ± 0.50	0.89
Cremophor EL (0.2 mg/ml)	5.81 ± 0.73	5.59 ± 0.42	0.96
Carbopol 934 (0.2 mg/ml)	4.88 ± 0.23	4.73 ± 1.05	0.97
Sodium lauryl sulfate (0.2 mg/ml)	5.63 ± 0.64	9.01 ± 1.71[a]	1.60
Sodium lauryl sulfate (0.1 mg/ml)	6.12 ± 0.43	6.47 ± 1.04	1.06

All experiments were performed in the m-to-s direction with glucose at both sides of the tissue at pH 6.85. Ratio was calculated by dividing the permeability obtained in the presence of the examined excipient with the control permeability.
[a] Significantly different from the control value ($p < 0.05$). Data are expressed as the mean ± S.D. of three experiments.

asymmetry of amoxicillin transport was completely abolished when 0.04 mM BB, a non-competitive MRP inhibitor (Bakos et al., 2000), was added to the mucosal side (Fig. 2). Similarly, when glucose at the mucosal side was replaced by mannitol the difference between m-to-s and s-to-m permeability also became insignificant (Fig. 2).

3.4. Influence of some pharmaceutically accepted excipients on the permeability of amoxicillin and on the tissue electrical parameters

Among the tested excipients only SLS significantly increased the permeability of amoxicillin (Table 2). This effect of SLS was dependent on the concentration of SLS, because no effect was observed at lower (0.1 mg/ml) concentration of SLS (Table 2). Higher (0.2 mg/ml), but not lower (0.1 mg/ml), concentration of SLS also significantly decreased PD and I_{sc} (Table 3), while other excipients had no influence on the tissue electrical parameters (data not shown). SLS was not evaluated as permeation enhancer at concentrations higher than 0.2 mg/ml.

After treating the tissue with 0.2 mg/ml SLS for 20 min the PD of the SLS treated tissue dropped to significantly lower values compared to the non-treated control, but then increases, until in 160 min after the end of the SLS treatment reaches the values not significantly different from the control value (Fig. 3). These results demonstrate that the effect of SLS on the tissue electrical properties is reversible.

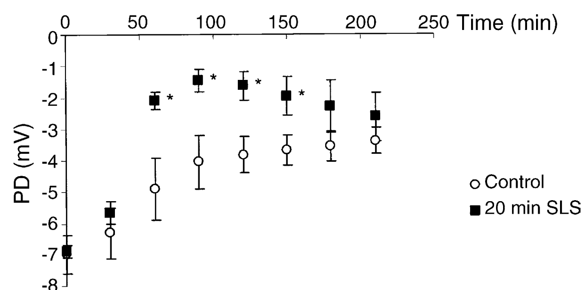

Fig. 3. Reversibility of SLS effect on the PD of the rat jejunum in vitro. SLS was added to the mucosal side of the tissue at the start of the experiments and was removed after 20 min. The experiment was performed with glucose at both sides of the tissue at pH 6.85. * Significantly different from the control value ($p < 0.05$). Data are expressed as the mean ± S.D. of three experiments.

3.5. Influence of SLS on the m-to-s and s-to-m permeability of amoxicillin

SLS added at the concentration 0.2 mg/ml on the mucosal side diminished the polarization of amoxicillin transport across the rat jejunum (Fig. 4), predominantly as a result of the increased transport in the absorptive direction, while the transport of amoxicillin in the secretory direction was only slightly decreased (Fig. 4). These results indicate that SLS inhibits the efflux system that is responsible for the asymmetry in the amoxicillin transport across the rat jejunum.

4. Discussion

The absorption of amoxicillin in humans is lower than 90%, which classifies amoxicillin among the low permeability drugs according to BCS (Lennernäs et al., 2002). This is in agreement

Table 3
Influence of SLS on the intestinal electrical parameters

	Control	SLS (0.1 mg/ml)	Ratio	Control	SLS (0.2 mg/ml)	Ratio
PD (mV)	−2.5 ± 0.2	−2.9 ± 0.2	1.16	−3.7 ± 0.2	−2.0 ± 0.3[a]	0.54
I_{sc} (μA/cm^2)	104 ± 23	136 ± 24	1.30	166 ± 47	67 ± 14[a]	0.40
TEER (Ω cm^2)	25 ± 5	21 ± 2	0.84	23 ± 5	20 ± 5	0.87

All experiments were performed in the m-to-s direction with glucose at both sides of the tissue. Ratio was calculated by dividing the value obtained in the presence of SLS with the control value. Data are expressed as the mean ± S.D. of three experiments.
[a] Significantly different from the control value ($p < 0.05$).

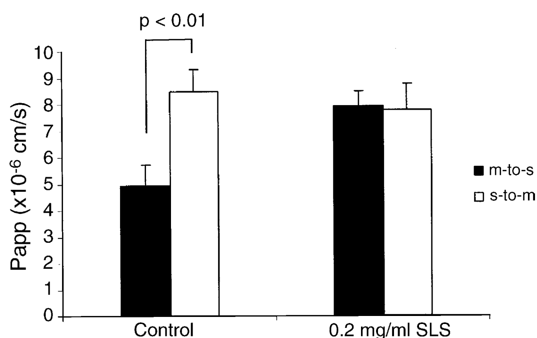

Fig. 4. Effect of SLS on the mucosal side on the transport characteristics of amoxicillin across the rat jejunum. Samples from the acceptor compartment were withdrawn up to 120 min. Data are expressed as the mean ± S.D. of three experiments.

with the permeability measurement in this study, since the P_{app} value of amoxicillin is comparable to the P_{app} value of ranitidine and much lower than P_{app} of verapamil (Table 1), which are two widely used model low and high permeability drugs (Löbenberg and Amidon, 2000).

The mechanism of amoxicillin transport across the intestinal epithelium appears to be complex and involves multiple pathways. Amoxicillin is actively absorbed by a H^+/peptide cotransporter (PepT1) (Lennernäs et al., 2002), which is also in accordance with the increased permeability of amoxicillin at lower pH of the incubation medium observed in this study (Fig. 1). It was previously shown that the decrease of the pH of the bicarbonate incubation medium (same pH was at the mucosal and serosal side) resulted in the decreased jejunal mucosal surface microclimate pH, which leads to the increased inwardly directed H^+ gradient across the apical membrane of the enterocytes (intracellular pH ~ 7.2) and consequently to the increased driving force for the PepT1 transport (Legen and Kristl, 2003).

Amoxicillin is also actively transported in the secretory direction, which was suggested to be one of the restricting factors in the intestinal absorption of β-lactam antibiotics including amoxicillin (Saitoh et al., 1997). Our results demonstrated that amoxicillin is actively transported in the secretory direction, but only if glucose was present at the mucosal side of the tissue (Fig. 2), while no asymmetry in the transport was observed when glucose was replaced by mannitol (Fig. 2). This effect of glucose on the secretory transport of amoxicillin was completely abolished by a MRP inhibitor, BB (Fig. 2) suggesting the involvement of an apical MRP transporter in the active secretion of amoxicillin across the rat jejunum. This might at least partially explain the reduced absorption of amoxicillin caused by a standard carbohydrate meal (Welling et al., 1977). Similar effect of glucose on the transport across the rat jejunum was observed previously for a MRP specific probe, fluorescein (Legen and Kristl, 2004). It was shown that this effect of glucose was not mediated only by the enrichment of energy supply of the tissue, but mostly by another mechanism (Legen and Kristl, 2004).

VRP, a well-known *P*-glycoprotein inhibitor (Woo et al., 2003), only slightly but not significantly affects glucose induced efflux of amoxicillin (Fig. 2), which is in accordance with the previous observations that β-lactam antibiotics are not secreted by *P*-glycoprotein (Saitoh et al., 1997). It should be pointed out that VRP also effectively inhibits MRP transport at concentrations higher that 100 μM (Legen and Kristl, 2004; Bandi and Kompella, 2002), thus it is somehow surprising that 200 μM VRP does not significantly affect the glucose induced polarized transport of amoxicillin (Fig. 2). One explanation could be that MRP2 transporter has multiple binding sites and that the classical substrat-inhibitor relation is not valid for this transporter (Zelcer et al., 2003).

In this study, rat small intestine was used as a model for the permeation enhancement studies. This model was shown to be more appropriate for studying the absorption enhancement of amoxicillin compared to Caco-2 cells as the alternative model for such studies (Legen et al., 2005). Five pharmaceutically acceptable excipients (SLS, Pluronic F68, Lutrol F127, Cremophor EL and Carbopol 934) were evaluated as permeation enhancers for amoxicillin. These excipients were evaluated at concentrations that are likely to be achieved in gastrointestinal fluids after application of solid dosage form of amoxicillin that contains 50 mg of the investigated excipient, taking account 250 ml as an adequate volume of the gastrointestinal fluids (Aungst, 2000). The effect of Pluronic F68 was studied at lower concentration, because 18 mg is the maximal amount allowed the in oral dosage forms according to the *Inactive Ingredients In The Food And Drug Administration* (*FDA*) *Approved Drugs* (http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm). Among the tested excipients only SLS at concentration 0.2 mg/ml significantly increased permeability of amoxicillin (Table 2).

In general, the permeation enhancers can promote the permeability in the absorptive direction by opening the tight junctions and/or by inhibiting the active efflux system (Aungst, 2000). The increase of the mucosal-to-serosal permeability of amoxicillin caused by 0.2 mg/ml SLS was accompanying with the diminished polarized transport of amoxicillin (Fig. 4), suggesting that SLS increased the absorptive transport of amoxicillin predominantly by inhibiting the MRP-mediated reduction of amoxicillin absorptive permeability. The partial increase of the absorptive permeability of amoxicillin as a result of the opening of tight junctions is also possible, because the TEER, which is related to the state of the epithelial tight junctions, was slightly, although not significantly decreased (Table 3).

At least two mechanism by which pharmaceutical excipients/surfactants inhibit the intestinal ATP dependent efflux systems, such as MRPs and P-gp, have been proposed: (a) ATP depletion, and (b) membrane fluidization that causes changes in the protein conformation (Kabanov et al., 2002; Lo, 2003). Our results indicate that SLS, which is a surfactant, diminish the MRP-mediated reduction of the absorptive transport of amoxicillin by decreasing the level of cellular ATP, although the membrane fluidization cannot be excluded. We observed that the permeability enhancement of amoxicillin by 0.2 mg/ml SLS was accompanying with the reduction of the PD and I_{sc} (Tables 2 and 3). I_{sc} is a direct measure of the active transport of ions across the intestinal epithelium and is dependent mainly on the basolateral Na^+/K^+ ATPase activity and consequently on the cellular ATP level (Söderholm et al., 1998; Legen and

Kristl, 2002). PD is a product of I_{sc} and TEER. Therefore, low values of I_{sc} and PD reflect low level of cellular ATP. On the other hand, 0.1 mg/ml SLS have no influence on the tissue electrical parameters (Table 3), and accordingly no effects on the permeability of amoxicillin were observed (Table 2). Similar ineffectiveness on the permeability of amoxicillin and on the tissue electrical parameters was observed for other tested excipients (Pluronic F68, Lutrol F127, Cremophor EL and Carbopol 934) (Table 2). It should also be pointed out that ATP depletion caused by 0.2 mg/ml SLS appears to be reversible, since PD of the tissue that was treated for 20 min with 0.2 mg/ml SLS returns to the control values in approximately 3 h after the end of the treatment (Fig. 3). These results indicate that SLS could be considered as a relatively safe excipient, since PD was shown to be related to several viability parameters of the intestinal epithelium, including cellular ATP levels (Söderholm et al., 1998).

In summary, our results show that amoxicillin is in the presence of glucose at the mucosal side actively secreted across the rat jejunum by an apical MRP transporter, resulting in the decreased absorptive permeability of amoxicillin. This secretion can be inhibited by SLS, resulting in the enhanced permeability of amoxicillin in the absorptive direction.

References

Aungst, B.J., 2000. Intestinal permeation enhancers. J. Pharm. Sci. 89, 429–442.

Bakos, É., Evers, R., Sinkó, E., Váradi, A., Borst, P., Sarkadi, B., 2000. Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. Mol. Pharmacol. 57, 760–768.

Bandi, N., Kompella, U.B., 2002. Budesonide reduces multidrug resistance-associated protein 1 expression in an airway epithelial cell line (Calu-1). Eur. J. Pharmacol. 437, 9–17.

Bogman, K., Erne-Brand, F., Alsenz, J., Drewe, J., 2003. The role of surfactants in the reversal of active transport mediated by multidrug resistance proteins. J. Pharm. Sci. 92, 1250–1261.

Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y., 2002. Pluronic block copolymers for overcoming drug resistance in cancer. Adv. Drug. Deliv. Rev. 54, 759–779.

Legen, I., Kristl, A., 2002. Ketoprofen-induced intestinal permeability changes studied in side-by-side diffusion cells. J. Pharm. Pharmacol. 54, 1419–1422.

Legen, I., Kristl, A., 2003. Factors affecting the microclimate pH of the rat jejunum in Ringer bicarbonate buffer. Biol. Pharm. Bull. 26, 886–889.

Legen, I., Kristl, A., 2004. D-glucose triggers multidrug resistance-associated protein (MRP)-mediated secretion of fluorescein across rat jejunum in vitro. Pharm. Res. 21, 635–640.

Legen, I., Salobir, M., Kerc, J., 2005. Comparison of different intestinal epithelia as models for absorption enhancement studies. Int. J. Pharm. 291, 183–188.

Lennernäs, H., Knutson, L., Knutson, T., Hussain, A., Lesko, L., Salmonson, T., Amidon, G.L., 2002. The effect of amiloride on the in vivo effective permeability of amoxicillin in human jejunum: experience from a regional perfusion technique. Eur. J. Pharm. Sci. 15, 271–277.

Lo, Y.L., 2003. Relationships between the hydrophilic–lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines. J. Control. Release. 90, 37–48.

Löbenberg, R., Amidon, G.L., 2000. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur. J. Pharm. Biopharm. 50, 3–12.

Margarit, F., Moreno-Dalmau, J., Obach, R., Peraire, C., Pla-Delfina, J.M., 1991. Intestinal absorption kinetics of a series of aminopenicillins and azidocillin. A comparative study in the rat. Eur. J. Drug. Metab. Pharmacokinet. 3, 102–107.

Nerurkar, M.M., Burton, P.S., Borchardt, R.T., 1996. The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. Pharm. Res. 13, 528–534.

Paintaud, G., Alván, G., Dahl, M.L., Grahnén, A., Sjövall, J., Svensson, J.O., 1992. Nonlinearity of amoxicillin absorption kinetics in human. Eur. J. Clin. Pharmacol. 43, 283–288.

Redondo, P.A., Alvarez, A.I., Garcia, J.L., Villaverde, C., Prieto, J.G., 1998. Influence of surfactants on oral bioavailability of albendazole based on the formation of the sulphoxide metabolites in rats. Biopharm. Drug. Dispos. 19, 65–70.

Rege, B.D., Kao, J.P., Polli, J.E., 2002. Effects of nonionic surfactants on membrane transporters in Caco-2 cell monolayers. Eur. J. Pharm. Sci. 16, 237–246.

Saitoh, H., Fujisaki, H., Aungst, B.J., Miyazaki, K., 1997. Restricted intestinal absorption of some beta-lactam antibiotics by an energy-dependent efflux system in rat intestine. Pharm. Res. 14, 645–649.

Schoenmakers, R.G., Stehouwer, M.C., Tukker, J.J., 1999. Structure-transport relationship for the intestinal small-peptide carrier: is the carbonyl group of the peptide bond relevant for transport? Pharm. Res. 16, 62–68.

Sjövall, J., Alván, G., Åkerlund, J.E., Svensson, J.O., Paintaud, G., Nord, C.E., Angelin, B., 1992. Dose-dependent absorption of amoxicillin in patients with an ileostomy. Eur. J. Clin. Pharmacol. 43, 277–281.

Söderholm, J.D., Hedman, L., Artursson, P., Franzen, L., Larsson, J., Pantzar, N., Permert, J., Olaison, G., 1998. Integrity and metabolism of human ileal mucosa in vitro in the Ussing chamber. Acta. Physiol. Scand. 162, 47–56.

Welling, P.G., Huang, H., Koch, P.A., Craig, W.A., Madsen, P.O., 1977. Bioavailability of ampicillin and amoxicillin in fasted and nonfasted subjects. J. Pharm. Sci. 66, 549–552.

Woo, J.S., Lee, C.H., Shim, C.K., Hwang, S.J., 2003. Enhanced oral bioavailability of paclitaxel by coadministration of the *P*-glycoprotein inhibitor KR30031. Pharm. Res. 20, 24–30.

Zelcer, N., Huisman, M.T., Reid, G., Wielinga, P., Breedveld, P., Kuil, A., Knipscheer, P., Schellens, J.H., Schinkel, A.H., Borst, P., 2003. Evidence for two interacting ligand binding sites in human multidrug resistance protein 2 (ATP binding cassette C2). J. Biol. Chem. 278, 23538–23544.

Zhao, Y.H., Le, J., Abraham, M.H., Hersey, A., Eddershaw, P.J., Luscombe, C.N., Butina, D., Beck, G., Sherborne, B., Cooper, I., Platts, J.A., 2001. Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure-activity relationship (QSAR) with the Abraham descriptors. J. Pharm. Sci. 90, 749–784.

http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.