Increased Intestinal Absorption of Cefixime by Nifedipine in the Rat Intestinal Perfusion Model: Evidence for a Neural Regulation

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ABSTRACT

In healthy volunteers, the simultaneous administration of nifedipine and cefixime has been shown to increase the oral absorption of the antibiotic. To investigate the pharmacological basis of this interaction, we used an in situ intestinal perfusion technique in the rat. pH 5.5 yielded optimum cefixime absorption, which was greater in segments from the duodenojejunum than in those from the jejunouleum. Cefixime absorption was similar when perfused at 0.5 and 1.0 mg/ml, suggesting transport saturation at the lower concentration. Cefixime arterial and portal blood concentrations after an intestinal perfusion of 0.5 mg/ml cefixime were significantly increased by a previous 15-min intestinal perfusion of 0.05 mg/ml nifedipine. Nifedipine did not significantly alter intestinal blood flow. At the end of the cefixime perfusion, intestinal blood flow was higher in the nifedipine group than in the control group (0.44 ± 0.12 vs. 0.26 ± 0.09 ml·min⁻¹·g of intestine wt⁻¹, respectively), although the difference did not reach statistical significance. The absorption kinetics of salicylic acid, which is strictly absorbed by passive diffusion, were unaffected by nifedipine. After 15 and 50 min of recirculation, residual salicylate levels fell from 85.1 ± 5.6% to 57.1 ± 2.8% with nifedipine compared with 87.4 ± 1.4% to 52.8 ± 1.6% without nifedipine. Thus, the improvement in cefixime absorption by nifedipine was not secondary to increased local blood flows or to induced passive diffusion mechanisms. Nifedipine did not affect intestinal motility. The action of nifedipine appears to indirect, involving a neural regulation, because any increase in cefixime absorption was prevented by tetrodotoxin and hexamethonium administration.

Cefixime is a dianionic carboxymethoxyimino-cephalosporin. Despite its poor lipophilicity and ionization at physiological pH, cefixime is significantly absorbed unchanged after oral administration (Roche, 1988). Human kinetic studies (Faulkner et al., 1988) and in vitro experiments (Tsuji et al., 1987a, 1987b) have shown that cefixime absorption is saturable. Because cephalosporin antibiotics have a chemical structure similar to that of dipeptides, transport studies have suggested common transport systems (Dantzig et al., 1992; Kimura et al., 1983; Tsuji et al., 1987b). However, simple diffusion into enterocytes (Dantzig et al., 1994) and a paracellular pathway (Inui et al., 1992) may also be involved for cefixime. Studies in healthy volunteers have shown that nifedipine, a calcium antagonist, increases both the absorption rate and the bioavailability of cefixime without modifying its distribution or elimination (Duverne et al., 1992). Similar observations have been reported by Westphal et al. (1990) with another β-lactam antibiotic, amoxicillin, which is also absorbed by the dipeptide transport system (Nakashima et al., 1984). Because cefixime and amoxicillin are not metabolized in the liver, the beneficial effect of nifedipine could be restricted to their absorption. Several mechanisms might be involved in the absorption-promoting effect of nifedipine, including a direct action on the intestinal epithelial transport of cefixime, a reduction in intestinal motility leading to an increased contact time between cefixime and the transporter and a local hemodynamic effect that could promote passive absorption. Because a direct interaction was not demonstrated on Caco-2 cells, an indirect mechanism involving a neurohormonal regulation was proposed.

The objectives of this study were to (1) analyze the absorption characteristics of cefixime, (2) reproduce the cefixime absorption-promoting effect of nifedipine and (3) investigate the possible involvement of hemodynamic or indirect neurohormonal effects of nifedipine. For these purposes, we used the well-validated in situ intestinal perfusion model in the rat (Schanker et al., 1958). To discriminate hydroelectrolytic vs. neurohormonal effects, selected drugs and chemicals with

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ABBREVIATIONS: HM, hexamethonium; PKC, protein kinase C; PYY, peptide YY; TTX, tetrodotoxin.

1 L. Harcouët, C. Carbon and R. Farinotti, unpublished observations.
defined pharmacological actions were coperefused with cefixime and nifedipine. PY, a gut hormone that is produced and released from mucosal endocrine cells of the colon and distal small bowel, was chosen to study the inhibition of intestinal and colonic motility resulting from the local inhibition of hydroelectrolytic secretions (Sheik, 1991). HM, a cholinergic and nicotinic blocker, and TTX, a nervous conduction blocker that inhibits the release of secondary neurotransmitters (Bulbring and Tomita, 1967), were used to characterize further neurohormonal regulation.

**Methods**

**Chemicals and Solutions**

Cefixime was obtained from Pharmuka (Neuilly s/Seine, France). [7-14C]Salicylic acid (2.2 GBq/mol) was from DuPont-NEN Research Products (Les Ulis, France). The nifedipine perfusate was obtained through 2-fold dilution of Adalate (0.2 mg/0.2 ml; Bayer Pharma, Puteaux, France) in distilled water. The final nifedipine solution contained 0.1 mg of nifedipine, 150 mg of 95% ethanol, 150 mg of PEG 400 and 2 ml of distilled water. HM and PYY were from Neosystem (Strasbourg, France), and TTX was from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade. For perfusion experiments, Krebs-Ringer-Tris buffer (pH 7.4) was prepared with 118 mM NaCl, 2.5 mM Tris, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4 and 9.2 mM citric acid. Isotonic Sorensen buffer (pH 7) contained 26.1 mM KH2PO4, 40.5 mM Na2HPO4 and 122 mM glucose.

**Animals**

Male Sprague-Dawley rats (Ifa Credo, L’Abresle, France) weighing 280 to 320 g were fasted for 20 hr before the experiment, with free access to water. The rats were anesthetized with urethane (1.5 g/kg i.p.) 30 min before surgery.

**Absorption Studies**

Intestinal absorption was studied with an in situ perfusion technique (Schanker et al., 1958). Briefly, the small intestine was exposed through a midline abdominal incision. The proximal end of the selected intestinal segment was cannulated with an Insite W22g cannula (Becton Dickinson, Meylan, France), and the distal end was cannulated with a polyethylene tube. It was then washed with saline for 30 min. In all experiments, the biliary duct was ligated to avoid increased cefixime absorption through enterohepatic circulation (Yamaoka et al., 1990). Rectal temperature was maintained at 37 ± 1°C with a warming blanket (Harvard Apparatus, Boston, MA). All solutions were maintained at 37°C except for the nifedipine solution, which was kept at room temperature. A peristaltic pump (Gilson Minipuls 2, Villiers Le Bel, France) was used at a flow rate of 0.6 ml/min. Perfusion rates were 0.3 mg/min with 0.5 mg/ml cefixime and 0.6 mg/min with 1 mg/ml cefixime.

**Single-perfusion technique.** To localize the cefixime absorption site, the antibiotic was perfused throughout the duodeojunum (20 cm) or jejunoumum segment (20 cm). In subsequent experiments, 40-cm duodeojunum segments were chosen. After an initial washing period, cefixime was diluted in Krebs-Ringer/Tris buffer (pH 5.5) or isotonic Sorensen buffer (pH 7.0) and perfused throughout the intestine for 1 hr. In selected experiments, nifedipine (0.05 mg/ml; i.e., 0.1 mM) or a drug-free solution was perfused 15 min before cefixime.

**Recirculation technique.** After an initial washing period, 0.05 mg/ml nifedipine or a drug-free solution was perfused for 15 min throughout the duodeojunum segment (20 cm). Then, a 0.4 mg/ml salicylic acid solution containing 1.85 KBq/ml [7-14C]salicylic acid in isotonic buffer (Schanker et al., 1958) was perfused, first in single-pass mode (10 min) and then in recirculating mode (50 min). During the recirculation period, aliquots (0.1 ml) were sampled every 10 min.

**Study of neural regulation and ionic movements.** For intravenous infusion, a catheter (Microflex 0.4 mm/G27 Vygon, Ecouen, France) was connected to an electric syringe. The following solutions were administered: (1) an intravenous bolus of HM (6.7 mg/kg) followed by intravenous perfusion (6.7 mg/kg/hr), started 30 min before and stopped at the end of the cefixime intestinal perfusion; (2) an intravenous bolus of TTX (5 mg/kg), followed by an intravenous saline infusion, started 30 min before the beginning of the cefixime intestinal perfusion and stopped at the end of the cefixime intestinal perfusion; (3) an intravenous PYY infusion of 240 pmol/kg/hr, started 15 min before and stopped at the end of the cefixime intestinal perfusion; or (4) an intravenous infusion of a control saline solution, started 30 min before the beginning of the cefixime intestinal perfusion and stopped at the end of the cefixime intestinal perfusion. The same experiments were conducted with or without a previous nifedipine (0.05 mg/ml) intestinal perfusion for 15 min started before the cefixime perfusion. Seven rats were used for each experiment.

**Regional Blood Flows**

In the cefixime and cefixime-plus-nifedipine groups, regional blood flows were determined in 6 and 7 rats, respectively, using a microsphere technique (Hadengue et al., 1988). 113Sn- or 141Ce-labeled microspheres (15 ± 3 μm; specific activity, 10 mCi/g; New England Nuclear, Boston, MA) were suspended in 0.9% NaCl containing one drop of 0.05% Tween 80 and sonicated for 10 min. A precounted aliquot of 80 μl (60,000 microspheres) was aspirated into the syringe and then flushed over 45 sec into the left ventricle with 700 μl of saline. Cardiac output was calculated according to the reference blood sample method.

Simultaneous with the microsphere injection, a reference blood sample was drawn from the femoral artery at 0.8 ml/min for 75 sec. The arterial catheter was connected to a multichannel recorder for arterial pressure and heart rate monitoring. Hemodynamic parameters were measured at the beginning of (T0) and 1 hr after (T60) cefixime perfusion throughout the small intestine. The animals were killed after 60 min, and individual organs were dissected. The radioactivity of each organ and of the reference blood sample was determined using a scintillation counter (Gamma 4000, Intertechnique, Pianisir, France).

Blood samples (600 μl) were collected 15, 30, 45 and 60 min after the beginning of the cefixime perfusion. Arterial blood samples were collected via a septum-equipped catheter inserted in the carotid artery (Insyte W22g, Becton Dickinson). Portal blood samples were collected in two ways: either (1) via a polyethylene catheter (0.3 mm external diameter) inserted directly into the portal vein and fixed with cyanoacrylate glue or (2) according to the method of Hadengue et al. (1988) (i.e., via a polyethylene catheter inserted at the junction of two small ileal veins and advanced by visual observation up to the confluence of the superior mesenteric and splenic veins). Each sample was replaced with 300 μl of 0.9% NaCl. Serum was separated by centrifugation and stored at −20°C.

**Analytical Procedures**

Cefixime concentrations were determined by reverse-phase high performance liquid chromatography with UV detection. After a deproteinization step using trichloroacetic acid, the supernatant was injected into the chromatography system. The column was an Ultra- base C18 (5-μm, 4.6 × 150 mm). The mobile phase was a mixture of acetonitrile and water (13:100 v/v) containing 1% perchloric acid and 1% triethylamine. The flow rate was 1.7 ml/min. The detection wavelength was set at 280 nm (Shimatsu SPD6A, Touzart et Matignon, France).

[7-14C]Salicylic acid concentrations were determined by measuring radioactivity: 100 μl of sample was transferred into vials containing 10 ml of scintillation fluid (Ultima Gold, Packard, Rungis, France).
France) and directly counted by scintillation (beta counter, Inter-
techique, Montigny le Bretonneux, France).

Statistical Analysis

Results are presented as mean ± S.E.M. Student’s t test for
unpaired data was used to compare treatment groups. For the intesti-
nal absorption site study, the Mann-Whitney U test was used.
Differences were considered significant at a level of P < .05.

Results

Cefixime Uptake

Mean cefixime concentrations in arterial blood after a 0.5
mg/ml perfusion into the duodenjejunal or jejunileum
segments are shown in table 1. After 30 min, cefixime ab-
sorption was significantly better in the duodenjejunal segment
than in the jejunileum segment, promoting the use of the
former in subsequent experiments.

Cefixime portal concentrations increased linearly with
time, regardless of the perfusate concentration (0.5 or 1 mg/
ml) (table 2). However, achieved cefixime concentrations did
not differ significantly between the groups receiving the two
concentrations: at 60 min, mean cefixime concentrations
were 13.6 mg/liter in the 0.5 mg/ml perfusion group com-
pared with 14.4 mg/liter in the 1.0 mg/ml group.

Cefixime absorption was also compared at perfusate pH 5.5
and 7.0. As shown in figure 1, at 30 min, mean cefixime con-
centrations in arterial blood were significantly higher
when cefixime was perfused at pH 5.5 compared with pH 7.0
(P < .05). At 60 min, cefixime concentrations in the pH 5.5
group were twice those in the pH 7.0 group (9.4 ± 1.2 vs.
4.2 ± 0.7 mg/liter, P < .01).

Interaction Studies

Effects of PYY on cefixime absorption. PYY was used
to study the influence of ionic secretion and motility decrease
on cefixime absorption in the small intestine.

Arterial concentrations of cefixime, measured during its
intestinal perfusion, were not altered by a 240 pmol/kg/hr
continuous infusion of PYY (fig. 2).

Effect of nifedipine on salicylic acid absorption. Sal-
icylic acid was used to study the effect of nifedipine on the
passive diffusion. Over 50 min, residual percentages of sali-
cylate fell from 85.1 ± 5.6% to 57.1 ± 2.8% with a previous
nifedipine perfusion compared with a decrease from 87.4 ±
1.4% to 52.8 ± 1.6% without nifedipine (table 3).

The decline in residual percentage of salicylic acid over
time was log linear (one order kinetic). Except at 20 and 30
min, values were always comparable with or without nifedi-
pine. Therefore, nifedipine did not consistently modify sali-
cylic acid absorption.

Effect of nifedipine on cefixime absorption. Cefixime
concentrations in arterial (table 4) or portal blood (fig. 3)
were compared between two groups who received 1 hr of 0.5
mg/ml cefixime perfusion with or without previous perfusion
of 0.05 mg/ml nifedipine for 15 min. Cefixime absorption was
markedly enhanced by the 15-min nifedipine perfusion. At 45
min, cefixime concentrations increased by 60% in arterial
blood and by 76% in portal blood (P < .05). Cefixime concen-
trations in arterial blood were always lower than those mea-
sured in portal blood. Because arterial concentrations are

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td>Cefixime absorption from 20-cm segments of the rat small intestine during a 1-hr single-pass mode perfusion of a 0.5 mg/ml solution of cefixime at pH 5.5</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Time (min)</td>
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<td>30</td>
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<tr>
<td>45</td>
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<tr>
<td>60</td>
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</tbody>
</table>

Values are mean ± S.E.M.

* P < .05, Mann-Whitney U test.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td>Cefixime absorption from 40-cm rat duodenjejunal segments during a 1-hr single-pass mode perfusion of 0.5 and 1 mg/ml cefixime solutions at pH 5.5 (flow rate, 0.6 ml/min)</td>
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<tr>
<td></td>
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<tr>
<td>Time (min)</td>
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<td></td>
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<td>15</td>
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<td>30</td>
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<tr>
<td>45</td>
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<tr>
<td>60</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.
TABLE 3
Effect of nifedipine on salicylic acid absorption

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>85.1 ± 5.6</td>
<td>87.4 ± 1.4</td>
</tr>
<tr>
<td>15</td>
<td>84.5 ± 1.3</td>
<td>80.3 ± 1.2</td>
</tr>
<tr>
<td>30</td>
<td>78.2 ± 2.1</td>
<td>76.8 ± 0.9</td>
</tr>
<tr>
<td>60</td>
<td>74.7 ± 2.4</td>
<td>66.2 ± 1.9</td>
</tr>
<tr>
<td>120</td>
<td>68.3 ± 2.0</td>
<td>64.0 ± 2.2</td>
</tr>
</tbody>
</table>

Salicylic acid 0.4 mg/ml with 1.85 KBq/ml [7-14C]salicylic acid was driven by a recirculating procedure after a 15-min nifedipine (nifedipine) or its adjuvant (control) perfusion.

*P < .05, difference between percentages.

TABLE 4
Effect of the duration of a nifedipine perfusion on arterial cefixime concentrations during a 1-hr single-pass mode perfusion of a 0.5 mg/ml cefixime solution at pH 5.5 in 40-cm duodenojejunum segments

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cefixime concentration (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>30</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>45</td>
<td>6.7 ± 0.8</td>
</tr>
<tr>
<td>60</td>
<td>9.4 ± 1.2</td>
</tr>
</tbody>
</table>

*P < .05 vs. group I.

Fig. 3. Effect of a nifedipine perfusion before 1-hr cefixime intestinal perfusion (0.5 mg/ml, pH 5.5) on mean cefixime portal blood concentration (mean ± S.E.M., n = 12). When applicable, nifedipine was perfused for 15 min before start of the cefixime perfusion. *P < .05.

dependent on distribution processes and portal concentrations are not, the latter more closely reflects the absorption phase. The hyperosmolarity of the nifedipine solution did not influence cefixime absorption, as preliminary experiments showed a similar time course of cefixime concentrations with or without a previous perfusion of an hyperosmolar solution for 15 min (data not shown). Thus, the absorption of cefixime appears to be the specific target of nifedipine.

The comparison of arterial blood cefixime concentrations between the groups in which cefixime perfusion was preceded by a 15- or 30-min nifedipine perfusion (table 4) indicated that the effect of nifedipine was delayed. At 15 min, cefixime concentrations were significantly higher in the group with the longest nifedipine perfusion, but later concentrations became similar regardless of the infusion duration.

At the beginning of the cefixime perfusion (i.e., immediately after nifedipine perfusion), arterial pressure was significantly lower in the nifedipine-plus-cefixime group (59.3 ± 1.4 vs. 82.2 ± 3.0 mm Hg). This most likely is due to the antihypertensive action of nifedipine.

With the exception of cardiac blood flow, all regional blood flows were significantly reduced by a 60-min cefixime perfusion (table 5). In particular, intestinal blood flow fell from 0.62 ± 0.06 at T0 to 0.26 ± 0.09 ml cremin −1 · g of intestine wt1 at T60. However, at 60 min, regional blood flows were not statistically different between the cefixime and nifedipine-plus-cefixime groups. Because at 60 min, cefixime arterial concentrations were significantly higher in the nifedipine-plus-cefixime group (table 4), the effect of nifedipine on cefixime absorption appeared to be independent of blood flow.

The effects of nervous conduction blocking agents on cefixime intestinal absorption are shown in figure 4. At 15 min, the increase in cefixime portal concentrations caused by a previous nifedipine perfusion was reduced by a concomitant IV administration of HM or TTX. During the experiments, portal concentrations of cefixime in the nifedipine-plus-intravenous HM and nifedipine-plus-intravenous TTX groups were not significantly different from those measured in controls.

Discussion

Our results clearly indicate that in rats, the absorption of cefixime is better in the duodenojejunal segment, is saturable and is improved at low intraluminal pH. Moreover, cefixime absorption is enhanced by nifedipine via a blood flow-independent mechanism. The action of nifedipine appears to be an indirect one, involving a neural regulation. The lower absorption rate of cefixime from the ileum compared with the duodenum is in agreement with results obtained by Maekawa et al. (1977), who showed that after 1 hr, the disappearance of another oral cephalosporin, cephalexin, was much higher from the proximal small intestine (56%) than from the distal small intestine (15.6%). The reason for such a discrepancy between different portions of the digestive tract is unknown; it could be due to the more basic environment of the ileum, to a lower number of carrier-mediated transport systems or to a reduced number of enterocytes per unit of surface area.

The active transport of cefixime and of other β-lactams via the same transporter as that of dipeptides has been widely...
The inhibition kinetics of cefixime uptake by glycyl-L-proline 
kinetics (active transport) (Sugawara et al., 1987a, 1987b), cefixime uptake follows a mixed-type kinetic pattern, which involves both a nonsaturable process (passive diffusion) and dose-dependent saturable Michaelis-Menten kinetics (active transport) (Sugawara et al., 1990). Indeed, the inhibition kinetics of cefixime uptake by glycyrl-L-proline and cyclacillin are consistent with a competitive type of inhibition, as shown by Tsuji et al. (1987a) in everted rat jejunums. Their model keeps the physiological characteristics of the intestinal mucosa; so does our model, which is independent of intestinal blood flow and motility. Our results were confirmed in vitro in cultured Caco-2 cells (Harcouet et al., 1995), in which cefixime absorption was inhibited, at acid pH, by glycine-L-proline and cefadroxil.

Cefixime transport is influenced by the pH of the medium; we show its improvement at low intraluminal pH 5.5. Inui et al. (1988) suggested that cefixime does not interact with the transport system of a-amino β-lactam antibiotics at neutral pH but is transported via a different peptide carrier in an acidic pH region.

We found that cefixime was better absorbed at pH 5.5 than at pH 7.0, confirming in vitro observations obtained with intestine brush border membrane vesicles from rabbits (Inui et al., 1988), rats (Sugawara et al., 1991; Tsuji et al., 1987b) and humans (Sugawara et al., 1992). Experiments carried out with an everted gut sac method (Tsuji et al., 1986, 1987a) were also consistent with these results. All these studies concluded that the acidic environment of the mucosal surface of the small intestine (Lucas, 1983; Lucas et al., 1985) maximizes the uptake of cefixime. Low intestinal pH appears to be maintained by a mucus coating (Shiau et al., 1985) or by hydrogen ion secretions (Mur et al., 1976). In the proximal and middle jejunum, hydrogen ions would be controlled by a Na⁺/H⁺ antiporter located on the luminal surface of the enterocytes. The Na⁺/H⁺-ATPase of the basolateral cell membrane would pump the Na⁺ out of the cell (Ganapathy and Leibach, 1985).

Nifedipine could increase cefixime absorption via several mechanisms: (1) by slowing the fluid movement through the gut, thus allowing more time for absorption to occur; (2) by increasing intestinal blood flow, thus favoring passive absorption (provided that transmembrane passage is not a limiting factor); (3) by increasing, directly or indirectly, the rate of uptake by epithelial carriers and (4) by causing intestinal lesions secondary to the hyperosmolarity of its solution. The last hypothesis appears to be very unlikely on the basis of the experiments of Mogard and Nylander (1982), who studied the effect of intraluminal hyperosmolarity on absorption by us-

### TABLE 5

**Hemodynamic effects of nifedipine (0.05 mg/ml) in rats before (T0) and 1 hr after (T60) a 1-hr single-pass mode cefixime (0.5 mg/ml) intestinal perfusion at pH 5.5**

<table>
<thead>
<tr>
<th></th>
<th>Cefixime group (n = 6)</th>
<th>Nifedipine + cefixime group (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T60</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>82.2 ± 3.0</td>
<td>56.5 ± 5.6*</td>
</tr>
<tr>
<td>Organ blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>0.62 ± 0.006</td>
<td>0.26 ± 0.09*</td>
</tr>
<tr>
<td>Mesentery-pancreas</td>
<td>0.36 ± 0.04</td>
<td>0.17 ± 0.06*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.50 ± 0.06</td>
<td>0.21 ± 0.05*</td>
</tr>
<tr>
<td>Heart</td>
<td>10.3 ± 0.87</td>
<td>8.32 ± 2.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ml/min/g tissue wt</td>
</tr>
<tr>
<td>Portal tributary blood flow</td>
<td>2.52 ± 0.29</td>
<td>1.14 ± 0.42*</td>
</tr>
<tr>
<td>Splanchnic tributary blood flow</td>
<td>4.00 ± 0.42</td>
<td>1.76 ± 0.54*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.

* P < .05, T60 vs. T0.

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**Fig. 4.** Effect of a previous nifedipine (N) perfusion with or without concomitant intravenous administration of HM or TTX on cefixime intestinal absorption. Saline was perfused in the duodenojejunum segment for 15 min; then, 0.1 mM nifedipine or a drug-free solution (control group) was perfused for 15 min. When applicable, at the beginning of the saline perfusion, HM was administered as an intravenous bolus (6.7 mg/kg) followed by a constant rate infusion (6.7 mg/kg/hr). Similarly, TTX was first injected as an intravenous bolus (5 μg/kg) followed by a saline infusion. Then, a 0.5 mg/ml cefixime intestinal perfusion was started. *P < .05.
ing a recirculation intestinal perfusion technique in the rat. They showed that passive absorption of iodine and active absorption of selenomethionine were not modified by intraluminal hyperosmolarity (830 mOsmol/kg water).

Calcium antagonists inhibit transmembrane calcium influx via calcium channels expressed in various cell types, including cardiac and vascular smooth muscle cells. They might also have some actions on smooth muscles of the gastrointestinal tract, such as those involved in motility or local microcirculation (De Ponti et al., 1993). Although nifedipine does not alter jejunal motility in humans (Santander et al., 1988), it reduces spike and mechanical activities in dogs (De Ponti et al., 1989). In rats, nifedipine also decreased intestinal motility by spontaneously inhibiting migratory motor complexes (Thollander et al., 1993). In our experimental conditions, the intestinal mucosa was always in contact with a constant concentration of cefixime and the perfusion rate therefore was sufficiently high to rule out the influence of an increased residence time in the intraluminal site. Indeed, we showed that the absorption rate of salicylic acid was not modified by nifedipine.

Furthermore, PYY, which delays the propagation of migratory motor complexes and inhibits spike activity in rat jejunum (Sheik, 1991), had no effect on cefixime absorption with or without nifedipine. Therefore, cefixime-absorption and nifedipine-promoting effect on the latter appears to occur independent of intestinal motility. In vitro, calcium antagonists relax the mesenteric-portal vein (Sutter, 1990), but in vivo, their hemodynamic effects are more complex. In humans, nifedipine is an effective vasodilator, especially of the splanchnic vascular bed, increasing blood flow in splanchnic territory (Gasic et al., 1987) and hepatic and portal veins (Reiss et al., 1991). Other calcium antagonists do not influence splanchnic hemodynamics (Agner et al., 1984). Nifedipine has no effect on the suprahepatic pressure gradient during portal and hypertension, in animals or humans (Combis and Vinel, 1991). We also found that nifedipine did not significantly alter small bowel blood flow. From the beginning of the cefixime perfusion, our values of organ and tributary vessel blood flow were lower than those previously reported by Hadengue et al. (1988). The use of different experimental conditions, such as anesthesia with urethane or the fact that the abdomen was kept open during the washing period, may account for these differences. At the end of the experiment, when cefixime blood concentrations were markedly different between treatment groups, blood flow was similar in the group that received a previous nifedipine perfusion and in the control group. Therefore, a hemodynamic effect of nifedipine was not responsible for its absorption-promoting effects. Nifedipine did not increase the cefixime concentration gradient between intravascular and extravascular compartments, which might have resulted in an increased passive diffusion of the antibiotic (Winne, 1980). This is also supported by the lack of any promoting effect of nifedipine on salicylic acid absorption, which has been shown to occur via passive diffusion, in an analogous in vivo model and at similar concentrations to those used in our experiments (Schanker et al., 1958).

Westphal et al. (1990) showed that in humans, the absorption kinetics of amoxicillin were enhanced by nifedipine, a result of its effect on specific electrical movements. Similarly, the effect of nifedipine on cefixime transport could be explained in terms of electrical movements. By decreasing calcium uptake by the enterocytes (Hurt et al., 1993), calcium channel blockers might Westphal (Westphal et al., 1995) stimulate the apical Na⁺/H⁺ antiporter (Donowitz, 1983), thereby setting up a proton gradient favorable to cefixime absorption by the intestinal H⁺/dipeptide cotransport system. Globally, we did not observe any effect of PYY, which is known not only to inhibit intestinal motility but also to inhibit proximal small intestinal hydroelectrolytic secretions in rats. Therefore, cefixime absorption is not modified by a decrease in hydroelectric secretions.

Additional experiments are, however, necessary to specifically address the role of electrical movements in the nifedipine-cefixime interaction, particularly the codeadministration of nifedipine with amiloride, which is known to block the effect of the Na⁺/H⁺ antiporter. Calcium antagonists, and particularly verapamil and nifedipine, inhibit the active efflux of many drugs, with a direct effect on P-glycoprotein within the cell apical membrane. P-glycoprotein expression is responsible for the multidrug resistance phenotype of tumoral cells and has been identified in human enterocytes. It works like an ATP-dependent pump, expelling drugs from the cells, probably as a detoxification mechanism or as a way to intestinal absorption. A verapamil-sensitive (P-glycoprotein) exists on the apical membrane of Caco-2 cells and is responsible for the efflux of vinblastin, vincristine and ciprofloxacin (Griffith et al., 1993; Hunter et al., 1993) This P-glycoprotein, which is pH and pHe independent (Altenberg et al., 1993) probably is not involved in the mechanism of cefixime absorption enhancement in the presence of nifedipine because in Caco-2 cells, nifedipine, verapamil and diltiazem did not modify cefixime epithelial transport at neutral and acid pH values (Harcouët, 1995).

The mechanism of the interaction appears quite different from that suggested by Westphal (Westphal et al., 1990). Rather, it seems characterized by an indirect neural effect on the intestinal epithelium of the entire system. Indeed, we showed that the interaction was abolished by a neural blockade with either HM or TTX. Intrinsic and extrinsic innervation play an important role in the regulation of electrolyte transport throughout the small intestine (Cooke and Reddix, 1994).

Numerous factors of luminal, neural, endocrine or circulatory origin may modulate the level of expression and the function of the transport within epithelial cells; the details of their complex interactions are still poorly understood. Calcium is a key regulatory element of many cellular functions, such as contraction, differentiation and secretion. Calcium antagonists—sensitive voltage-dependent channels—were identified in many cell types. Neural circuits also show a great dependence on calcium. In neurons, calcium fluxes play an important role in the regulation of neuronal excitability and the release of neurotransmitters. Many receptors of neurotransmitters and neuromodulators have been identified on intestinal epithelial cells, and a neural regulation of the dipeptide transport system has recently been suggested by Brandsch et al. (1994). These authors have shown that PKC inhibits the transport of the dipeptide glycylysarcosine across the intestine. Furthermore, PKC, which is stimulated by several gut hormones, also inhibits the Na⁺/H⁺ antiport.

Therefore, the effect of nifedipine would not be directly mediated via calcium channels of enterocyte membranes but...
would result from indirect mechanisms involving, at least in part, a neural regulation. One hypothesis would be that nifedipine modulates the activity of peripheral or central autonomic neurons (intraperiael myenteric and submucous plexus). The nervous stimulus to the enterocytes would then be modified, and one could envision that one or more neurotransmitters would be delivered in different amounts to the enterocytes. A modulation of PKC could be occurring via such a mechanism. One would have to show that in vitro PKC modulation can modulate ceftime absorption.

In conclusion, nifedipine significantly increased ceftime intestinal absorption without modifying intestinal blood flow or intestinal motility. Nifedipine could affect ceftime absorption, directly or indirectly, via a neural mechanism by increasing the activity of carrier-mediated transport systems within the intestinal epithelium. Further study of dihydropyridine-β-lactam interactions may help to better understand some of the regulatory processes involved in the control of the intestinal absorption of antibiotics.

References


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