Gastric Penetration of Amoxicillin in a Human Helicobacter pylori-Infected Xenograft Model

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The delivery of antibiotics into Helicobacter pylori-infected human stomachs is still poorly understood. Human embryonic gastric xenografts in nude mice have recently been proposed as a new model for the study of H. pylori infection. Using this model, we compared the penetration of amoxicillin, after intraperitoneal administration of a dose of 20 mg/kg of body weight, into the gastric mucosa of infected and uninfected xenografts. The concentrations of this drug in serum and superficial gastric mucosae were determined at 20 min and 1 and 3 h after injection. Ten mice with H. pylori-infected grafts (n = 5) or uninfected grafts (n = 5) were studied. Mucosal samples were obtained by cryomicrotomy. The concentrations in serum were similar to those obtained in the serum of humans after oral administration of 1 g of amoxicillin. The mean area under the tissue concentration-versus-time curve from 0 to 3 h obtained for mice with infected grafts was significantly higher than that obtained for the animals with uninfected grafts (P = 0.01). These results suggest that the penetration of amoxicillin into the superficial gastric mucosa may be substantially increased in the case of H. pylori infection. Thus, human xenografts in nude mice represent a new, well-standardized model for investigation of systemic delivery of drugs into H. pylori-infected gastric mucosa.

In vitro, Helicobacter pylori is naturally susceptible to most antibiotics. Unfortunately, when administered to a human, no single-antibiotic therapy is able to achieve a high eradication rate (1, 10, 20, 27, 32, 34). This lack of clinical efficacy may be explained by acquired resistance, poor compliance, insufficient antibiotic penetration into the site of infection, and/or a low level of drug stability at this location. The relative ineffectiveness of administration of single antibiotics has empirically led to the use of triple therapies that consist of combinations of two antibiotics (amoxicillin, clarithromycin, or imidazoles) with an antisecretory drug (proton pump inhibitor or H2-receptor antagonist) and that have been shown to be most effective (8). However, today, these recommended therapies do not result in eradication in all patients. The search for optimal H. pylori treatment was essentially based on the results of a great number of clinical trials. Until now, no pharmacological approach has been systematically used to improve existing therapeutic regimens or to search for new treatments. This may be explained by the absence of a convenient and suitable experimental model for investigation of the characteristics of local or systemic delivery of drugs to the stomach, although it may have a poor correlation to the situation in humans, particularly when systemic delivery is predominant. In such a model, it may be difficult to mimic the in vivo pharmacokinetics observed in humans. Moreover, the use of human gastric mucosa infected with H. pylori would be more appropriate for extrapolation to humans.

The severe combined immunodeficient mice, in which human fetal thymic and liver tissues have been implanted, was recently proposed as a model for determination of the in vivo effectiveness of different therapeutic agents on immunodeficiency virus infection (28). We have previously developed a new model of H. pylori infection in nude mice using human gastric xenografts which exhibit differentiated human gastric epithelium (19). The aim of this work was to investigate the usefulness of this model for the study of amoxicillin penetration into the infected human stomach after parenteral administration.

MATERIALS AND METHODS

H. pylori infection model. Ten pangetic, 6- to 8 week-old, Swiss nude mice purchased from Iffa Credo (Lyon, France) were used. They were housed in individual cages, fed a commercial rodent diet, and given water ad libitum. All animal experimentation was performed in accordance with the institutional guidelines and approval of the Service Vétérinaire de la Santé et de la Protection Animale (Direction Générale de l’Alimentation du Ministère de l’Agriculture et de la Forêt).

This model has previously been described in detail (19). Briefly, human embryonic stomachs (gestational age, 6 to 8 weeks) were obtained after legal abortion. They were stored at 4°C in a sterile isotonic glucose solution and within 4 h were grafted into mice that were under general anesthesia induced with ketamine (Ketalar; Parke-Davis, Courbevoie, France) administered intraper-
taneously (0.1 g/kg of body weight). Anesthesia could be prolonged if required by repeated administration of ketamine (one-fourth of the initial dose every 20 min). Mice were placed in a sterile environment and were subjected to surgery under aseptic and aseptic surgical conditions. The skin of the abdominal wall was opened at the midline by a xiphipubic incision and was then loosened from the underlying musculoaponeurotic layer. The anterior aponexis was opened, and the musculus rectus abdominis was detached from the epigastric vessels and the parietal peritoneum. An incision was then built on the epigastric vessels and the parietal peritoneum at the back and the abdominal muscle layer in front. The entire stomach, which measured about 3 by 2 by 1 mm, was introduced into this cavity in such a way that its back was in close contact with the epigastric vessels. The stomach and the abdominal wall were then closed with successive single-layer sutures. Fourteen stomach implants were successful.

Eighty days after implantation, mice were anesthetized as described above. The abdominal skin was disinfection and then opened. The human stomach, which measured at this time about 2 by 2 by 3 cm, was punctured, and the gastric juice was aspirated. The gastric wall was opened, and a reference biopsy speci- men was taken for histological examination (hematoxylin-eosin) to ensure that all grafts exhibited human gastric epithelium. A Silastic catheter with an outer diameter of 600 μm (Lambert Riviére, Fontenay-aux-Bois, France) was introduced into the stomach through the catheter. The catheter was slid under the thoracic skin and came out at the nape of the neck, to which it was securely attached. The observance of rigorous standards of hygiene permitted maintenance of the catheter for 3 months. Thus, gastric juice aspiration could be performed through the catheter twice a day (1 to 1.5 ml/day) during the whole experimental time to avoid fistulization.

At 1 to 3 days after catheter implantation, bacterial challenge was performed. The catheterized graft of each animal was aspirated and gastric juice was sampled for H. pylori isolation (phylOTyper Instrument, Clifton, N.J.). This permitted us to ensure that the gastric juice was acid, since the pH ranged from 1.5 to 2 for all grafts studied. Five randomly assigned grafts were inoculated, through the gastric catheter, two times at 3-day intervals with 0.6 ml of a bacterial suspension (approximately 10^8 organisms/ml in tryptose soy broth [Oxoid, Basingstoke, United Kingdom]) of H. pylori LBI, which was originally isolated from a patient with duodenal ulcer and severe gastritis (19). Three months after inoculation, each animal was anesthetized as described above. After disinfecting the skin of the abdominal wall, each graft was microsurgically opened and two biopsy specimens were taken from adjacent sites in the gastric antrum for culture and histology. Finally, the gastric and the abdominal walls were closed. One biopsy specimen was fixed in 10% (wt/vol) buffered formalin (16 to 24 h) for histological examination, and the second was immediately placed in a semisolid agar transport medium (Portagirm pylori; bioMérieux, Marcq l’Étoile, France) for culture. This sample was transferred to 0.5 ml of brucella broth (Difco, Detroit, Mich.) and was homogenized for 1 min with an Ultra Turrax grinder (Labo-Moderne, Paris, France) before inoculation onto selective and nonselective agar plates (19). For this purpose, the assay was performed by a standard method, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined for histopathological changes. All inoculated xenografts were considered infected on the basis of positive culture results. In these grafts, widened or ophrytized erythematous areas were visible on the surface of the anterior wall and were associated with minimal hemorrhagic points. No visible gastric erosions or ulcerations were seen. Histological examination of the gastric mucosa showed mild inflammation and polymorphonuclear leukocyte infiltration. Mucosal edema in the affected graft and was associated with capillary dilatation and proliferation. In contrast, in the other five un inoculated and culture-negative xenografts, macroscopic and histological examination revealed no abnormalities.

Study design and sampling. The pharmacokinetic study was performed for 5 days after the evaluation of the infection. At this time, mices with infected (mean ± standard deviation [SD] weight, 30.5 ± 3.76 g) and uninfected (mean ± SD weight, 29.8 ± 4.31 g) xenografts were anesthetized, and a catheter in Teflon was microscopically placed in the femoral artery for blood collection. The grafts were then microscopically opened as described above, and gastric juice was taken for pH determination. For the kinetic study, animals were maintained under anesthesia. Each mouse was given a single intraperitoneal dose of amoxicillin (20 mg/kg). This permitted attainment, as observed in a preliminary study (unpub- lished data), of a maximum measured concentration in serum (measured C_{max}) of 1.600 μg/liter for control mucosal samples, were 1.8, 2, 4.5, 2, 3.5, and 5%.

Drug assay. (16). All chemicals and solvents used were of high-performance liquid chromatography (HPLC) grade. Tritated powder of sodium amoxicillin (SmithKline Beecham Laboratories, Nanterre, France) was dissolved in ultrapure water to give a stock solution of 100 mg/liter. The latter was further diluted in ultrapure water and human blank pooled serum (1:9 [vol/vol]) to obtain calibration standards with concentrations of 0.1, 1, and 10 μg/ml for the deter- mination of concentrations in serum. For the determination of concentrations in the mucosa, standard solutions with concentrations of 0.1, 0.5, and 5 μg/ml were prepared in 0.1 M phosphate buffered saline (pH 7.5). After vortex mixing for 5 min, this solution was kept at 4°C for 2 h. Then, mucosal samples as well as standard solutions were processed in the same way described above for serum samples. The isocratic HPLC system consisted of a 110 A solvent delivery module (Beckman, Fullerton, Calif.), a model 210 sample injection valve equipped with a 50-μl sample loop (Beckman), and a model 160 variable-wavelength detector (Beckman). Chromatograms were processed with a Beckman recording data processor with Gold, version 6.01, software. Separations were performed on a high-speed analytical column (inner diameter, 75 by 4.6 mm) packed with 3-μm- diameter particles (UltraspHERE XL-ODS; Beckman). The mobile phase consisted of 2 M ammonium acetate-0.1 M tetrabutylammonium-acetonitrile–water (0.75:5:13:81.25 [vol/vol/vol/vol]) adjusted to pH 7.5 with sodium hydroxide. The flow rate was set at 1 ml/min, and the eluent was monitored at 227 nm.

RESULTS

As shown previously (19), the gastric juice pH was consistent with that found in H. pylori infection since it was increased in all infected xenografts (pH range, 5 to 7.5) and remained low in uninfected xenografts (pH range, 1 to 2). No macroscopic blood contamination was evidenced in mucosal or gastric juice samples thereafter.

In mice with uninfected xenografts, amoxicillin concentrations in serum decreased from 18.76 ± 5.57 μg/ml at 20 min to 5.14 ± 2.28 μg/ml at 3 h (Table 1). These concentrations were not statistically different from those observed at the same time in mice with infected xenografts. The mean measured C_{max} in the serum of mice with infected (19.03 ± 2.16 μg/ml) or uninfected (19.12 ± 5.10 μg/ml) xenografts (Table 2) were similar to those observed in humans after administration of a 1-g oral single dose of amoxicillin (19.7 ± 5.4 μg/ml) (6).
reached in serum at 20 min in all mice except for the serum of one mouse with an uninfected xenograft, which exhibited a $C_{\text{max}}$ at 1 h. Moreover, the mean AUC$_{0-3}$ observed for the serum of both groups of mice (infected mice, 30.04 ± 5.65 μg · h/ml; uninfected mice, 33.29 ± 8.85 μg · h/ml) were similar to the mean AUC$_{0-3}$ calculated from data obtained for humans after administration of the same oral dose mentioned above (AUC$_{0-3}$, 36.94 μg · h/ml) (6).

The highest concentrations of amoxicillin in superficial gastric mucosa were measured in the samples of either the uninfected or infected xenografts taken at 1 h except for one graft in each group (taken at 20 min) (Table 3). Amoxicillin concentrations in the uninfected antral mucosae were significantly lower than those observed in serum at 20 min ($P = 0.0003$) and at 1 h ($P = 0.004$), with ratios of the concentration in the mucosa to that in serum (mucosa/serum ratio) at the two times ranging from 0.07 to 0.29 and 0.15 to 0.48, respectively. In the infected grafts, the mean concentration in serum was significantly higher than the mean concentration in the mucosa at 20 min ($P = 0.005$). Mean amoxicillin concentrations in the mucosa were not statistically different ($P = 0.7$) from those observed in serum at 1 h in the infected group (range of mucosa/ serum ratios, 0.60 to 1.16) and at 3 h in both groups (infected group, $P = 0.3$ [range of mucosa/serum ratios, 0.77 to 1.50]; uninfected group, $P = 0.1$ [range of mucosa/serum ratios, 0.41 to 1.20]). In infected xenografts the mean concentrations in the mucosa were at least twofold higher than those in uninfected xenografts at each time point. The mean of the AUC$_{0-3}$ for mucosa to that for serum (mucosa/serum AUC$_{0-3}$) ratio was also significantly higher for infected xenografts than for uninfected xenografts ($P = 0.01$) (Table 3).

**DISCUSSION**

Until now only a few studies of gastric penetration of antibiotics have been performed with H. pylori-infected patients (5, 11, 33). This may be partially explained by the fact that in these studies the number and the nature of regimens that can be ethically used are limited. Other in vivo studies have always included the deep, vascularized layers of the gastric wall. In the superficial gastric mucosa, especially at the intercellular junctions. Moreover, it has been shown that H. pylori may invade epithelial cells in vivo (3, 19, 25). The superficial gastric mucosa may therefore more adequately represent this micronich. Westblom et al. (35) used scraping with a glass slide to remove this portion from the stomach of adult guinea pigs to study the intragastric penetration of clindamycin. However, the distance between the luminal surface of the gastric mucosa and the muscularis mucosa may vary in the same stomach because of gastroplication. Freezing allows better stretching of the gastric

<p>| Table 2. Pharmacokinetic parameters for serum and human mucosa from mice with uninfected and infected xenografts |
|-------------------------------------------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Graft infection status</th>
<th>Serum</th>
<th>Gastric mucosa</th>
<th>AUC$_{0-3}$ (μg · h/ml)</th>
<th>$C_{\text{max}}$ (μg/ml)</th>
<th>AUC$<em>{0-3}$ for mucosa/ AUC$</em>{0-3}$ for serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected (n = 5)</td>
<td></td>
<td></td>
<td>33.29 ± 8.85</td>
<td>19.12 ± 5.10</td>
<td>10.69 ± 3.70</td>
</tr>
<tr>
<td>Infected (n = 5)</td>
<td></td>
<td></td>
<td>30.04 ± 5.65</td>
<td>19.03 ± 2.16</td>
<td>22.58 ± 5.10</td>
</tr>
<tr>
<td>$P$ value$^b$</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>0.007-0.29</td>
<td>0.11-0.76</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Data are means ± SDs. $^b$ Determined by the paired Student’s t test.

**Table 3. Concentrations of amoxicillin in superficial gastric mucosa of uninfected and infected xenografts and mucosa/serum ratios**

<table>
<thead>
<tr>
<th>Time post-injection$^a$</th>
<th>Mean ± SD concn (μg/g) in mucosa in:</th>
<th>Mucosa/serum ratio (range) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected xenografts</td>
<td>Infected xenografts</td>
</tr>
<tr>
<td>20 min</td>
<td>2.05 ± 1.04</td>
<td>7.98 ± 5.35</td>
</tr>
<tr>
<td>1 h</td>
<td>5.06 ± 2.02</td>
<td>10.14 ± 2.90$^a$</td>
</tr>
<tr>
<td>3 h</td>
<td>2.91 ± 1.01</td>
<td>4.92 ± 2.19</td>
</tr>
</tbody>
</table>

$^a$ After intraperitoneal administration of 20 mg/kg. $^b$ Statistically significantly different from mean concentration in uninfected grafts (paired Student’s t test).
mucosa, which minimizes gastroplication. This may explain why cryomicrotomy may represent a more reproducible way to obtain gastric superficial mucosa (17). However, in guinea pigs, pharmacokinetic studies were performed with the superficial mucosa of the whole stomach, which permitted retrieval of enough material to detect even low levels of antibiotics, but one animal was killed at each time point. In our study, the pharmacokinetics of amoxicillin in the gastric mucosa have been studied at all time points with the same animal. The use of large biopsy specimens was necessary to be able to detect concentrations above the detection threshold, and so specimens from only a limited number of time points could be studied.

Antibiotics are usually given orally to eradicate *H. pylori*, but they may act after local or subsequent systemic delivery. The latter would play a role in therapeutic efficacy (18). Our results suggest that this penetration may be enhanced by *H. pylori* infection, since the concentrations of amoxicillin in the mucosa were significantly higher in mice with infected xenografts than with those in uninfected xenografts. This could also be due to contamination of mucosal samples with blood, the risk of which would be increased by the important neoangiogenesis observed in all the infected xenografts. However, this seems unlikely or at least negligible since no macroscopic blood contamination was detected at the time of sampling and since cryomicrotomy prevents any significant contamination from interstitial tissue and plasma (17). Amoxicillin is unstable at normal gastric pH (pH 1 to 2) (9). Thus, the lower concentrations observed in the superficial mucosa of uninfected grafts, in which the gastric juice pH was low, may be due to the hydrolytic degradation of amoxicillin in vivo but also ex vivo for acid-containing samples. However, all biopsy specimens were immediately rinsed with phosphate buffer (pH 7.5) and frozen. This renders any ex vivo degradation unlikely. The increased amoxicillin concentrations in the infected mucosa may be due to enhanced diffusion because of local vessel proliferation, capillary dilatation, and/or a better stability of amoxicillin at neutral pH (13, 15) and were also at least 10-fold higher than the minimal bactericidal concentration (0.1 µg/ml) for *H. pylori* at neutral pH (13, 15) and were also at least 10-fold higher than the minimal bactericidal concentration. However, as determined by Mégraud et al. (22). Some investigators (2, 22) have suggested that the bactericidal activity of amoxicillin against *H. pylori* may be concentration dependent. Thus, the high concentrations obtained at 1 h in the gastric mucosa may be sufficient to obtain a good bactericidal effect. If our results were extrapolated to humans, they would suggest that the lack of eradication observed after therapies with amoxicillin in combination with proton pump inhibitors (26) would not be explained by low levels of antibiotic penetration but would more likely be explained by amoxicillin resistance (7) or other not well known mechanisms, including the possible intracellular localization of *H. pylori* (3, 19, 25). However, as the nature of the bactericidal effect of amoxicillin against *H. pylori* is still controversial (23, 24), it remains hazardous to draw any conclusion about this point and the nature of the effect requires further studies.

Our model permits study of the gastric penetration of xenobiotic agents into infected human gastric mucosa. In this study, it has been applied to the study of the systemic delivery of amoxicillin. However, it could also be used to study the local delivery of drugs in the superficial gastric mucosa, the penetration of antibiotics into tissue, and the effects of antibiotics against *H. pylori* in vivo. Therefore, it represents a new well-standardized model for the investigation of new anti-*H. pylori* agents.

REFERENCES


