ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1995, p. 766–768 Vol. 39, No. 3
0066-4804/95/$04.00+0


# Use of Cryomicrotomy To Study Gastric Diffusion of Amoxicillin in Guinea Pigs

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Received 22 March 1994/Returned for modification 4 September 1994/Accepted 11 December 1994

**Cryomicrotomy has been used as a new technique for removing gastric mucosae from adult guinea pigs for the study of amoxicillin secretion across gastric mucosae. This method allowed a very regular thickness of the removed surface layer of mucosa to be obtained with good reproducibility. Gastric superficial mucosa concentrations and gastric juice concentrations of amoxicillin were determined 1, 2, and 4 h after intramuscular administration (50 mg/kg) in 21 guinea pigs by a microbiological method. No antibiotic was detected in gastric samples at 4 h, except for a low-level mucosal concentration in one animal, thus indicating the short time that amoxicillin is present in gastric samples.**

Gastric human biopsies may be used to study the diffusion of drugs, such as antibiotics in the stomach (1, 8). These biopsies may include deep vascularized layers of the gastric wall and therefore be inadequate for the determination of antibiotic concentrations in the superficial gastric mucosa. On the contrary, the guinea pig is a more convenient model for pharmacological studies since it permits the superficial luminal portion of the mucosa of the whole stomach, free of blood contamination, to be obtained (14). For this purpose, Westblom et al. (14) used glass slide scraping. We propose to modify this technique by using cryomicrotomy, which we have applied to the study of amoxicillin gastric diffusion after parenteral administration.

Each adult male guinea pig, after an overnight fast, was given a single intramuscular (i.m.) dose of amoxicillin (50 mg/kg) or saline (controls) in the thigh. Blood samples were collected on citrate at 1, 2, and 4 h after i.m. administration by intracardiac puncture, and plasma was obtained by centrifugation. Animals were then immediately sacrificed with chloroform and autopsied. After clamping, a total gastrectomy was performed and gastric juice was aspirated. Then the whole stomach was opened by cutting through larger and smaller curvatures and was thoroughly rinsed in citrate buffer.

The superficial gastric mucosa was removed by standardized cryomicrotomy (Cryo-microtome Leitz; Frigomat Jung, Nussloch, Germany) of the whole stomach and controlled each time by histological examination. First, tangential sections of mucosae from five guinea pigs at depths from 50 to 800 μm were obtained. A depth of 400 μm was retained for this study as it permitted the removal of the superficial mucosa. Under these conditions, the muscularis mucosa was always at least 100 μm from the section plane (Fig. 1). The weight of each superficial mucosal specimen obtained was 400 ± 50 mg, and samples were always devoid of vascular structures.

Mucosae were ground in citrate buffer. Homogenate was centrifuged at 3,000 rpm (GR 412 centrifuge; Jouan) for 10 min, and the supernatant was recovered. All specimens were stored at −80°C until assayed.

The concentrations of amoxicillin in plasma, gastric mucosae, and gastric juice were measured by a microbiological method. Gastric juice pH was determined with a pHmeter 28 (Radiometer, Copenhagen, Denmark). AMES multiple reagent strips (Bayer Diagnostics, Brussels, Belgium) were used for hemoglobin determinations (sensitivity, 150 μg/liter) of mucosal specimens and gastric juice.

Amoxicillin (sodium amoxicillin; Beecham Laboratories) was dissolved in citrate buffer (pH 6.5) to give a stock standard solution of 100 mg/liter. Reference solutions in gastric juice (0.125 to 10 mg/liter) and plasma (0.25 to 50 mg/liter) were prepared from control guinea pigs. Amoxicillin solutions in citrate buffer (pH 6.5) (0.125 to 10 mg/liter) were used for mucosal concentration measurements. Plates were prepared with antibiotic medium no. 1 (Difco Laboratories, Detroit, Mich.) and *Bacillus subtilis* (ATCC 6633) spore suspension (Difco Laboratories) as described by Rosin et al. (12). Disks (6.5-mm diameter) (Difco Laboratories) were soaked in specimens, standards, and controls and applied in triplicate to plates. All standard solutions were prepared daily and stored at 4°C until used in assays. The linear range of this assay was between 0.062 and 50 mg/liter. The between-group variance for this assay, as determined at 25, 5, and 0.25 mg/liter for plasma and at 10, 5, and 0.25 mg/liter for gastric juice and mucosae, was 2.5 to 6.4%. The lower limit of detection for the different biomatrices was 0.04 mg/liter. Statistical analyses were performed with STAT.ITCF 5 (Institut Technique des Céréales et des Fourrages, Paris, France).

Mucosal and gastric juice samples were free of any blood contamination. No macroscopic bile contamination was evident, and gastric juice pH remained low. Any animal with gastric juice contamination was not included in this study. Samples from control animals were free of any antibacterial activity as no inhibition zone was observed.

Amoxicillin concentrations in gastric juice ($P = 0.36$) and mucosal samples ($P = 0.29$) were not statistically different at 1 and 2 h (Student's $t$ test) (Table 1). No statistical differences were noted between mucosal and gastric juice samples at 1 h ($P = 0.15$) and 2 h ($P = 0.32$).

A low-level concentration of amoxicillin was found in the gastric mucosa of only one animal 4 h after i.m. administration. At that time, amoxicillin was not detected in gastric juice. Standard deviations were high, which can be explained by the fact that animals were not inbred. However, they are of the

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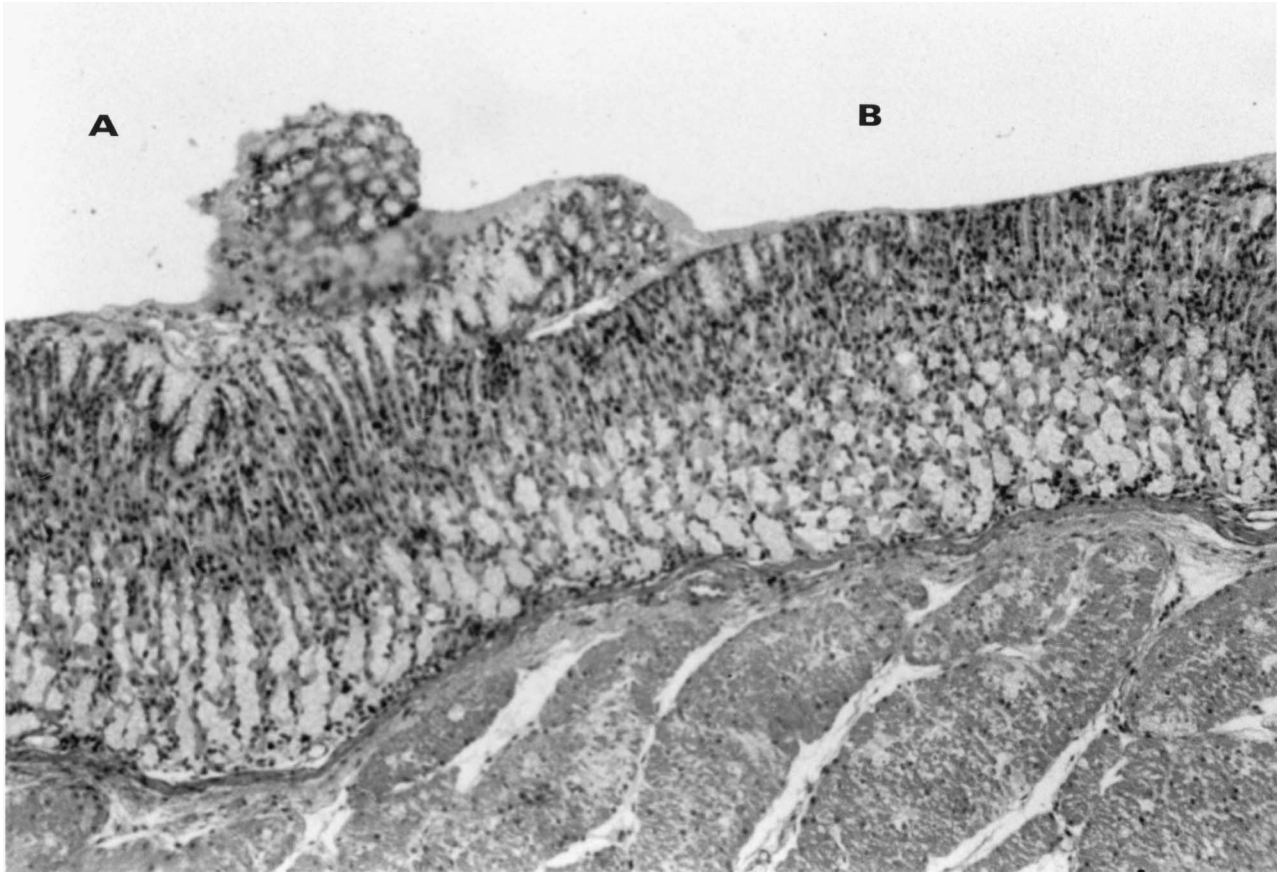


FIG. 1. Histologic section of guinea pig gastric mucosa before (A) and after (B) cryomicrotomy. The removed mucosa contained superficial mucus-producing cells. No inflammatory infiltrates were present. Wright-Giemsa stain; magnification, ×200.

same order as those in Westblom's study (14), considering the smaller number of animals in this study.

The distance between the luminal surface of the gastric mucosa and the muscularis mucosa varies, even in the same stomach, because of gastroplicature. Freezing allows better stretching of the gastric mucosa, which minimizes gastroplicature. Section standardization and microscopic control minimize the risk of a deep section. The absence of any blood contamination reinforces the quality of samples. The weights of mucosal specimens were higher and less variable in our study than those of Westblom et al. (14). This may be due to a better sampling method.

Amoxicillin is the most commonly used antibiotic against *Helicobacter pylori* infection. High eradication rates were achieved by using oral combinations of this antibiotic with bismuth subcitrate or proton pump inhibitors. However, given alone, amoxicillin provides a low eradication rate (11), despite its high activity against *H. pylori* in vitro (3, 4, 7, 9, 10) and good stability in gastric juice (5, 13).

In a guinea pig, amoxicillin is present in gastric mucosa and juice for less than 4 h. Thus, its diffusion across the gastric mucosa by circulation is short. If our results were extrapolated to humans, such a short contact time between antibiotic and *H. pylori* would not be sufficient for eradication to occur (2).

However, further studies to elucidate the role of associated drugs in the eradication of *H. pylori* in vivo by our technique in another model, such as *H. pylori*-infected mice (6), are necessary.

TABLE 1. Concentrations of amoxicillin in guinea pig plasma, gastric mucosa, and gastric juice following a 50-mg/kg i.m. injection

| Time post-injection (h) | No. of animals | Mean concentration ± SD in: | | | Mean gastric juice pH ± SD |
|---|---|---|---|---|---|
| | | Plasma (mg/liter) | Mucosa (mg/kg) | Gastric juice (mg/liter) | |
| 1 | 7 | 15.24 ± 6.02 | 4.01 ± 2.04 | 2.64 ± 0.90 | 2.03 ± 0.88 |
| 2 | 7 | 9.12 ± 4.63 | 2.87 ± 1.52 | 2.03 ± 1.29 | 1.49 ± 0.44 |
| 4 | 7 | 0.31 ± 0.10 | 0.20[a] | ND[b] | 1.14 ± 0.35 |

[a] Mean concentration and standard deviation were not computed as only one animal showed the presence of antibiotic.
[b] ND, not detected.

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