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Guinea Pig Model for Antibiotic Transport across Gastric Mucosa: Inhibitory Tissue Concentrations of Clindamycin against *Helicobacter pylori* (*Campylobacter pylori*) following Two Separate Dose Regimens

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An animal model for antibiotic secretion across gastric mucosa was developed using adult guinea pigs. Antibiotics were given intramuscularly, and levels in serum and gastric mucosa were measured by high-pressure liquid chromatography at 1, 2, 4, 6, and 8 h postinjection. Mucosal levels of the drugs were measured in the superficial luminal portion of the mucosa, which was removed by mechanical scraping. Clindamycin levels were measured after doses of 10 and 100 mg/kg of body weight. After doses of 100 mg/kg, levels in serum peaked at 15.95 μg/ml at 2 h. Gastric mucosa showed a bimodal concentration curve with peaks of 15.91 μg/g at 1 h and 25.07 μg/g at 4 h. Concentrations in mucosa remained high when levels in serum fell, showing a mucosa/serum ratio of 87.70 after 8 h. At all times, clindamycin levels in mucosa were in excess of the MIC for 90% of the *Helicobacter* (*Campylobacter*) *pylori* strains tested.

The discovery of *Campylobacter pylori* (9, 14), now renamed *Helicobacter pylori* (4), has led to the recognition that the etiology of gastritis and peptic ulcer disease may involve infectious agents (15). An effective therapy for such conditions would therefore logically include an antimicrobial agent aimed at eradicating the infective organism (3). However, the stomach represents a unique environment in the host. To extrapolate known pharmacokinetics and in vitro data for antibiotics and apply them directly to the clinical treatment of *H. pylori* infection would be speculative. Several clinical studies have shown poor outcomes from treatment with antibiotics in spite of good in vitro susceptibilities (2, 7, 11, 13). Furthermore, the presence of hydrochloric acid in the stomach would necessitate that any antibiotics used for the treatment of *H. pylori* gastritis remain active at a pH much lower than that in other parts of the body. Such antibiotics would also have to be secreted from the blood back into the stomach. Otherwise, because of the emptying of the stomach, oral antibiotics would function only as intermittent topical agents.

There is in the literature a paucity of studies examining the secretion of antibiotics into the stomach. We therefore developed an animal model to investigate antibiotic transportation across gastric mucosa. The guinea pig proved to be an appropriate size and to have a suitable physiologic acid production similar to that of humans.

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MATERIALS AND METHODS

Adult Hilltop guinea pigs were fasted overnight and then injected with either clindamycin phosphate (10 mg/kg of body weight), clindamycin phosphate (100 mg/kg), or saline (controls). All injections were given intramuscularly in the thigh. The animals were then sacrificed at fixed intervals of 1, 2, 4, 6, and 8 h by injecting intraperitoneal nembutal. At the time of sacrifice, intracardiac puncture was performed and blood was collected to determine levels of clindamycin in serum. The stomach was removed and cut on a plane going through larger and smaller curvatures. If any remaining food particles were observed, the stomach was also gently rinsed in phosphate-buffered saline. One-half of the stomach was then pinned to a wax plate. A glass slide was used to gently scrape the mucosal surface and separate the top layer from the deeper mucosal and muscularis layers. Removed mucosa was weighed and then mixed with 3 ml of phosphate-buffered saline in a glass tissue grinder. After being ground, the homogenate was centrifuged in a refrigerated ultracentrifuge (model CRU 5000; International Equipment Co., Needham Heights, Mass.) at 3,000 rpm for 10 min. The supernatant was removed and filtered through a 0.2-μm-pore-size filter. Blood from the heart puncture was centrifuged at 3,000 rpm (in the same ultracentrifuge) for 10 min after it had been allowed to clot. The serum was separated after centrifugation. All samples were stored at −70°C until they could be analyzed. Clindamycin concentrations in serum and tissue homogenates were measured by Bio-Pharmaceutical Reference Laboratory, Inc., Houston, Tex., using a model 481 spectrophotometer with a 501 high-pressure liquid chromatography pump and a U6K injector (Waters Associates, Inc., Milford, Mass.). Recordings were done on an Omni Scribe recorder (Industrial Scientific Inc., Houston, Tex.). Identification of clindamycin peaks were made by comparison of retention times with those of known standards, without distinguishing clindamycin from clindamycin phosphate. Precision and accuracy testing were done on prepared concentrations of 0.5, 1.0, 5.0, and 10.0 μg/ml. Interday serum readings showed a relative standard deviation of 3% at 0.5 μg/ml, increasing to 7.8% at 5.0 μg/ml. Intraday relative standard deviations varied between 1.9% at

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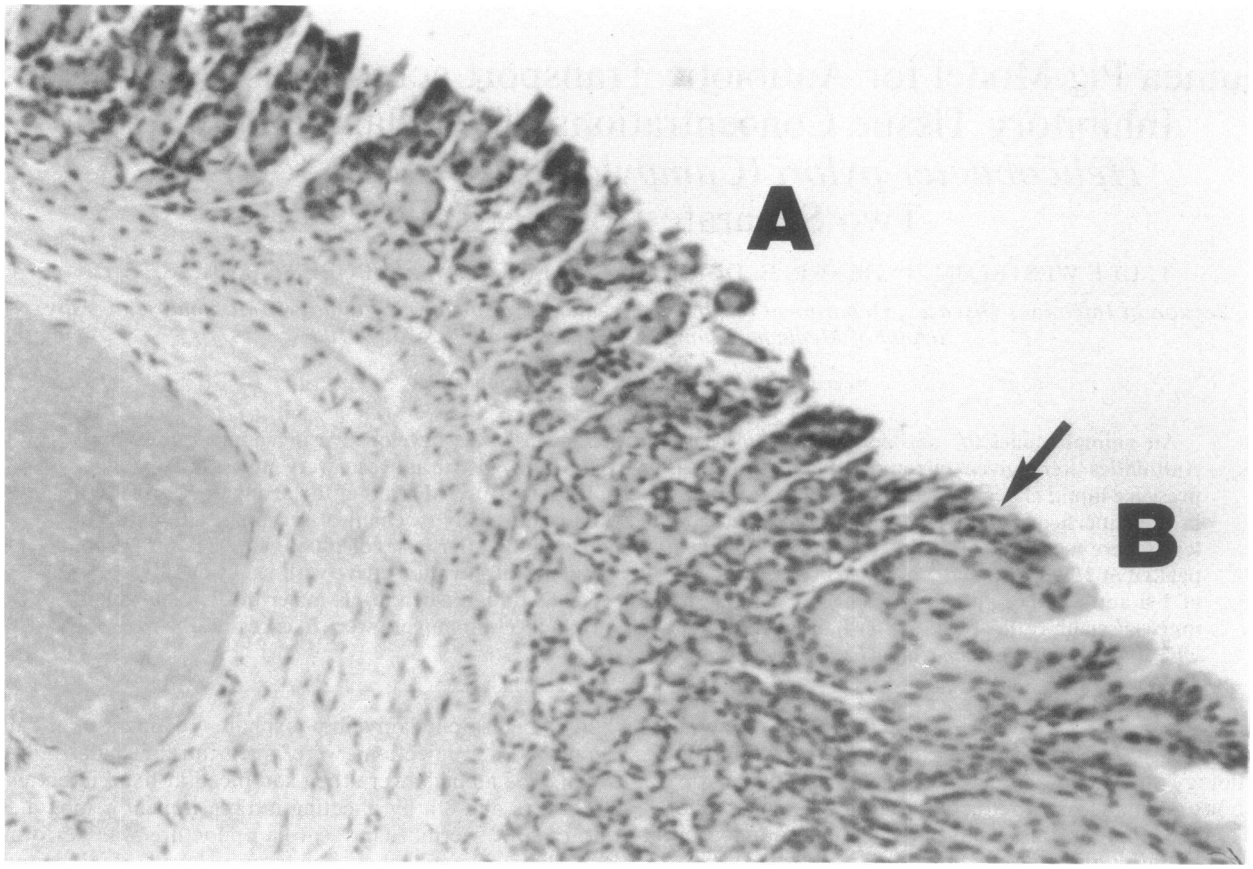

FIG. 1. Histologic section showing line of demarcation (arrow) between scraped (A) and unscraped (B) guinea pig gastric mucosa. The unscraped surface demonstrates superficial mucus-producing cells, while the scraped area shows cells with granular cytoplasm on the surface. Inflammatory infiltrates are not present in the lamina propria. Wright-Giemsa stain; magnification, ×400.

1.0 μg/ml and 5.6% at 5.0 μg/ml. Standard curves for tissue homogenate supernatants were prepared in phosphate-buffered saline. Relative standard deviations for the tissue samples varied from 3.4 to 6.6% interday and from 1.6 to 8.3% intraday. The correlation coefficients for all standard curves were greater than 0.99. Control tissue samples from animals that had not been injected with clindamycin showed no endogenous interference. Statistical analysis of data was performed using Statgraphics 3.0 (Graphics Software Systems, Inc., Rockville, Md.).

RESULTS

The scraping of the mucosa was expected to remove the very top layer in which *H. pylori* resides (6). To assess the effectiveness of our method, we examined histologic sections of scraped and unscraped mucosa. These revealed that the glass slide was able to remove the top 20% of the mucosa, including the mucus (Fig. 1). All concentrations in mucosa in our study refer to this portion of the mucosa. The pH of guinea pig mucosa was measured with a flat-surface pH probe (model 476551; Corning Glass Works, Corning, N.Y.) on unscraped portions immediately after the stomach was opened. The average pH was 1.69 (range, 1.20 to 2.06).

We investigated two different doses of clindamycin. In our first experiment, we injected all guinea pigs with a dose of 10 mg/kg. Clindamycin concentrations in serum peaked at 1 h postinjection, with an average level of 1.9 μg/ml. In spite of low levels in serum, clindamycin was readily found in the gastric mucosa, showing a bimodal concentration curve with an initial peak averaging 6.2 μg/g at 1 h followed by a second peak of 3.4 μg/g at 4 h. At all times the concentrations in mucosa exceeded the levels in serum, with the highest mucosa/serum ratio seen at 4 h.

In our second experiment, we increased the clindamycin dose to 100 mg/kg. Even though this is not a therapeutic dose in humans, it was anticipated that the concentrations of the drug in serum following such a dose would be close to what would be seen in human patients. As expected, concentrations in both serum and mucosa were higher following this dose (Table 1). Concentrations in serum peaked at 2 h, with

TABLE 1. Mean concentrations of clindamycin in guinea pig serum and gastric mucosa following 100-mg/kg intramuscular injection

Time postinjection (h)	No. of animals	Concn ± SD (variance) in:		Mucosa/serum ratio
		Serum (μg/ml)	Mucosa (μg/g)	
1	3	15.31 ± 3.93 (15.47)	15.91 ± 3.42 (11.72)	1.08
2	3	15.95 ± 10.62 (112.90)	8.09 ± 1.61 (2.58)	0.69
4	3	1.89 ± 1.16 (1.35)	25.07 ± 7.83 (61.27)	22.90
6	2	0.35 ± 0.42 (0.17)	15.75 ± 5.39 (29.03)	74.98
8	3	0.12 ± 0.12 (0.01)	10.30 ± 2.56 (6.58)	87.70

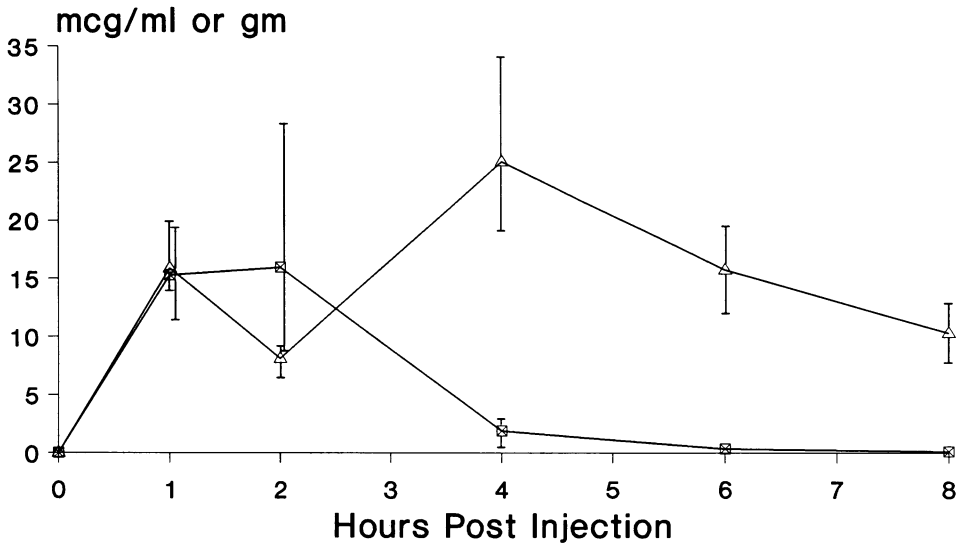

FIG. 2. Concentrations of clindamycin in serum and tissue following 100-mg/kg intramuscular injection. Symbols: ⊠, serum; △, gastric mucosa.

an average level of 15.95 μg/ml. Concentrations in mucosa once again showed a bimodal curve with an initial peak of 15.91 μg/g at 1 h and a second peak of 25.07 μg/g at 4 h (Fig. 2). There was a temporary drop in levels in tissue at 2 h, similar to what had been seen with the lower dose of 10 mg/kg. Mucosa/serum ratios increased over time and at 8 h showed almost a 100-fold concentration of the drug in the mucosa.

DISCUSSION

The first objective of our study was to develop an animal model that could provide data on antibiotic secretion into the stomach. McNulty et al. have reported on gastric antibiotic levels following oral drug intake in human volunteers (10). Compared with what can be obtained in an animal model, only small pieces of tissue can be obtained with endoscopic biopsies in humans, which increases the margin of error and the threshold of antibiotic detection. A biopsy specimen is likely to include both superficial and deeper layers of the mucosa, without any means of effectively separating the two. Furthermore, because the antibiotic to be tested is given orally, there is a risk of uneven distribution of leftover drug on the mucosal surface and biopsies are exposed to the risk of sampling errors.

The animal model offers a more suitable way of evaluating mucosal antibiotic concentrations. By using intramuscular injections, we eliminated the risk of patchy concentrations and variable drug absorption. In removing the whole stomach we obtained much more tissue than is possible using endoscopic biopsies. Most mucosal scrapings weighed between 150 and 300 mg, enough to detect even low levels of antibiotics. We chose to examine antibiotic concentrations in the surface layer of the mucosa rather than the whole stomach because of the known pattern of colonization by *H. pylori*. The organism typically will be found in the intraluminal mucus layer or close to the intracellular junctions of gastric epithelial cells (6). Invasion of the mucosa is not seen, but organisms inside epithelial cells have occasionally been observed (1). The mechanical scraping of the stomach was an easy and reproducible way of removing the surface portion of the mucosa. The antibiotic concentrations obtained in our model represent the presence of the antibiotic in the exact microniche in the stomach in which it can be expected to have the greatest therapeutic importance. We found the pH of the guinea pig stomach to be adequately low, and on histologic examination of sampled specimens, we saw no evidence of intrinsic gastric disease among the animals. This is important, since it indicates that we were performing our analysis on normal stomachs with a physiologic acid production similar to what can be seen in humans.

Our second objective was to measure the gastric secretion of clindamycin, an antibiotic which on theoretical grounds has potential for the treatment of *H. pylori* infections. In vitro studies of clindamycin have shown a MIC of 0.5 μg/ml against 50% of the *H. pylori* strains tested and a MIC of 2.0 μg/ml for 90% of the strains tested (5). It is stable at low pHs and is able to concentrate down a pH gradient (8). The closely related antibiotic lincomycin has excellent in vitro penetration into mucus when studied in hog gastric mucus. In the same studies, lincomycin remained biologically active in the mucus, in contrast to some other antibiotics, such as the tetracyclines, which were inactivated through chelation (12).

We found that clindamycin is transported into the gastric mucosa in vivo and that concentrations in tissue exceeded levels in serum. This was true for doses of the antibiotic of 10 or 100 mg/kg. The higher dose of 100 mg/kg is the one of particular interest, since it produced levels in serum which were close to what can be expected in humans taking therapeutic doses of clindamycin. Concentrations in mucosa following this dose peaked at 25.07 μg/g, almost twice the peak levels in serum. As the levels in serum fell over time, the mucosa/serum ratio increased to almost a 100-fold-higher concentration in the mucosal tissue. At all times the concentrations in the mucosa exceeded the MICs against 90% of the *H. pylori* strains tested.

We think that these results are directly applicable to clinical situations. In a previously reported pilot study of human patients receiving parenteral clindamycin, we found that gastric juice specimens contained concentrations of clindamycin which were almost twice the corresponding

levels in serum (1a). These data for humans are in agreement with the current guinea pig results and validate the animal model.

Concentration-in-tissue curves for both doses of clindamycin show a bimodal pattern with two different peaks. The first peak coincides with the peak of clindamycin in serum, while the second peak occurs as levels in serum are declining (Fig. 2). The fall in concentrations in tissue around 2 h after injection, between the two peaks, most likely reflects a true physiologic phenomenon, since it was seen in all the animals sacrificed at this time and with both doses of the drug. In preliminary results from studies of two other antibiotics, ciprofloxacin and cefuroxime, the same fall in tissue concentrations is seen at 2 h (D. E. Duriex and T. U. Westblom, unpublished data). Furthermore, statistical analysis of the concentrations at 2 h showed them to be significantly different from the concentrations at both 1 h ($P = 0.02$) and 4 h ($P = 0.02$).

The concentration curve seen for both doses of the antibiotic is unusual. The initial concentration peak in the tissues may be due to a passive transfer mechanism that closely follows levels in serum. As concentrations in serum fall, the corresponding levels in tissue decline. However, the late concentrations in tissue must be using a separate transport mechanism, which might include an active transport system since high concentrations are established. By 8 h there is a highly significant difference between levels in serum and tissue ($P = 0.002$). Clindamycin is known to reach high concentrations in bile. It is therefore possible that hepatic excretion with back flow into the stomach could have affected the late concentrations in tissue. However, we find this unlikely for several reasons. The consistent finding of these high concentrations in tissue would necessitate that all the guinea pigs be exposed to such back flow when sacrificed after 4 h or more. Furthermore, visual inspection of the mucosa as well as the pH measurements performed did not indicate the presence of bile reflux into the stomach. None of the mucosal scrapings showed visual evidence of blood contamination. Since hemoglobin concentrations in the samples were not measured, small amounts of contamination cannot be completely ruled out. However, at 6 and 8 h after injection, the levels in serum were so low that even a thorough contamination with blood would only marginally affect the levels. These late concentrations of clindamycin in tissue have important clinical implications. Since the drug continues to accumulate in gastric mucosa in the face of falling levels in serum, it seems very likely that a regular every-6-h regimen of clindamycin will lead to sustained high levels in tissue. With such levels far exceeding the known MIC of the drug against 90% of the *H. pylori* strains tested, clindamycin should be an excellent candidate for treatment of *H. pylori*-induced gastritis. These findings will now have to be further investigated in controlled clinical trials.

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