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Short Communication

Implications on Emergence of Antimicrobial Resistance as a Critical Aspect in the Design of Oral Sustained Release Delivery Systems of Antimicrobials

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Purpose. To assess the effects of the unabsorbed fraction of an orally administered antimicrobial drug which enters the colon on the emergence of resistance among the natural microflora, a phenomenon largely overlooked so far despite its clinical importance, especially when sustained release formulations are used.

Methods. Effects of an orally administered model β-lactam antibiotic (amoxicillin) on emergence of resistant bacteria were assessed using a microbiological assay for qualitative and quantitative determination of resistant bacteria in fecal samples of rats following gastric administration of the drug to rats for 4 consecutive days. Time- and site-controlled administration of a β-lactamase to the rat colon was assessed as a potential strategy for prevention the emergence of resistant bacteria following oral administration of incompletely absorbed antimicrobials.

Results. Emergence of resistant bacteria was demonstrated following oral administration of amoxicillin to rats, whereas de-activation of the β-lactam prior to entering the colon, by infusion of a β-lactamase into the lower ileum, was shown to prevent the emergence of resistant colonic bacteria.

Conclusions. This study illustrates the need to consider the emergence of antimicrobial resistance as a goal equally important to microbiological and clinical cure, when designing oral sustained-release delivery systems of antimicrobial drugs.

KEY WORDS: antimicrobial resistance; β-lactam antibiotic; colonic microflora; delayed action preparations; oral dosage form.

INTRODUCTION

The short elimination half-life of many antimicrobials, as well as attainment of pharmacodynamic targets, establish a sound rationale for compounding them in oral sustained release delivery drug systems (1–3). However, while the focus is on the antimicrobial concentration-effect relationship at the infection site (which is usually reflected by the drug concentration-time profile in blood), an orally administered antimicrobial agent has another site of action, hitherto generally overlooked: the natural microflora within the colon. This aspect of oral sustained-delivery dosage forms of antimicrobials and its possible link to the emergence of antimicrobial resistance deserves careful consideration, particularly in light of clinicians' tendency to focus on short-term outcomes of antimicrobial therapy (clinical cure), while largely ignoring longer-term consequences, such as emergence of resistance (4).

Following oral administration of drugs in a sustained-release dosage form, the drug continues through the gastrointestinal tract, with a certain unabsorbed fraction reaching the colon, where it encounters the normal microflora. As sustained-release formulations generally result in a larger portion of unabsorbed drug reaching the colon than their corresponding immediate release formulations (5), this unintended and clinically unnecessary exposure of the colonic microflora to the antimicrobial may favor the selection of resistant bacteria residing within the colon. Emergence of resistance within a single host (regardless of the causative events) may have both clinical and epidemiological consequences. A resistant strain might become pathogenic and cause superinfection in the host or persist as a colonizer to cause future infection or spread to surrounding contacts such as other patients, healthcare personnel and family members.

The present study was designed to address the potential implications of oral sustained-release delivery systems of antibiotics on the emergence of antimicrobial resistance. In

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ABBREVIATIONS: ARB, Ampicillin-resistant bacteria.

this study we focused on β-lactam antimicrobials; the frequency with which they are used, their short elimination half-life and their time dependent killing kinetics make them natural candidates for formulation of oral sustained-release delivery systems (6). However, β-lactams share a limited absorption window that is confined to the small intestine. Beyond this point absorption is negligible (7), and the unabsorbed fraction is of no clinical value whereas its effects on colonic microflora are paramount. Amoxicillin, a β-lactam commonly used for treatment of various infections, was chosen as a β-lactam model drug for this study. Amoxicillin's microbiological spectrum includes many aerobic Gram-positive microorganisms (*Enterococcus faecalis*, methicillin-susceptible *Staphylococcus* spp. (β-lactamase-negative strains only), *Streptococcus pneumoniae*, *Streptococcus* spp. (α- and β-hemolytic strains only)) and some aerobic Gram-negative microorganisms (β-lactamase-negative strains only *of Escherichia coli*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Proteus mirabilis*, *Helicobacter pylori*). Its oral bioavailability is reported to be ~90% (8); thus, at least 10% of a given dose administered as an oral immediate release formulation is not absorbed and reaches the colon. As discussed above, an oral sustained release delivery system of a β-lactam might increase the unabsorbed fraction reaching the colon beyond that of its corresponding immediate release formulations, thereby potentially increasing the disruption of the natural colonic microflora and emergence of resistant bacterial strains.

The present *in vivo* study employed a unique experimental strategy for assessment of the impact of an orally administered β-lactam on rat colonic microflora. Briefly, emergence of amoxicillin resistant bacteria in rat colon was determined quantitatively following oral administration of amoxicillin by gavage, and following de-activation of the unabsorbed fraction timed to occur after the absorption window and before reaching the colon. It has been demonstrated that the absorption window for β-lactams in rats is similar to that in humans (9), which makes the rat model suitable for this study.

Specifically, the present study was designed to address two questions:

1. Does the direct exposure of the colonic microflora to the unabsorbed fraction of orally administered amoxicillin induce selection of resistant enteric bacteria?
2. Can de-activation of the drug during its transition from the terminal ileum to the upper colon prevent the unintentional and undesired emergence of resistance?

MATERIALS AND METHODS

Animal Model

Male Wistar rats weighing 200–250 g were individually housed in metabolic cages in a specific-pathogen free (SPF) unit, with unrestricted access to drinking water and food. The research adhered to the ''Principles of Laboratory Animal Care'' (NIH publication #85-23, revised in 1985).

Assessment of Growth of Amoxicillin-Resistant Bacteria (ARB) within the Gut Following Administration of Amoxicillin by Gastric Gavage

In order to determine the effect of exposure to amoxicillin on selection of ARB within the gut flora of rats, an amoxicillin dose of 10 mg/1 ml in sterile water was administered to five rats by gastric gavage every 24 h for 3 consecutive days. Five additional rats, serving as a control group, received 1 ml of drug-free sterile water by gastric gavage. Feces was collected prior to the first dose (day 0) and then daily for 3 consecutive days (days 1–3).

Feces collected from each rat were weighed, diluted in sterile water (1 g feces in 10 ml DDW) and homogenized with a tissue homogenizer. Colony counts were performed by spreading 100 μl of each diluted sample of feces on MacConkey agar plates, an agar media for isolation and differentiation of enteric Gram negative rods, and on modified MacConkey agar plates containing ampicillin at a concentration of 64 μg/ml, which is twice the breakpoint concentration of the Clinical and Laboratory Standards Institute against Enterobacteriaceae, the dominant subgroup of enteric Gram-negative bacteria. These modified plates served as selective media for ARB.

Each plate was incubated overnight at 37°C, after which bacterial colonies were counted, and the original fecal bacterial concentration was derived accordingly. As ampicillin and amoxicillin present similar antimicrobial spectra, growth (or no growth) of bacteria on ampicillin-spiked agar is indicative of effects of exposure to amoxicillin.

Determination of Gastrum-Ileum Transit-Time in Rats

Groups of three rats each were administered charcoal spiked with 1 or 10 mg amoxicillin by gastric gavage. The charcoal served as a visual marker for transit of gastrointestinal contents. Starting 60 min after gavage groups of rats (n=3) were sacrificed at 5-min intervals and the contents of the jejunum, ileum and cecum and were visually inspected. In those groups where the charcoal was seen reaching the jejunum or beyond, jejunal, ileal and cecal contents were sampled and analyzed for amoxicillin concentrations. Amoxicillin concentrations were determined using a microbiological assay, shown to be linear within a concentration range of 2–125 mcg/ml (data not shown).

Assessment of Growth of ARB within the Gut Following Administration of Amoxicillin by Gastric Gavage and Ileal Administration of β-Lactamase

Activities of the β-lactamase (β-lactamase type 1, obtained from Bacillus cereus, Sigma, Germany) in aqueous media and in rat feces were validated *in vitro* (data not shown). Rats (n=12) were anesthetized for the duration of surgery by intra-peritoneal injection of 1 ml/kg of ketamine-xylazine solution (9%:1%, respectively) and placed on a heated surface maintained at 37°C. A polyethylene cannula was implanted in the lower ileum and exteriorized at the dorsal part of the neck, making is possible to carry out the investigation in non-anesthetized and unrestrained rats. Following surgery rats were allowed two to 3 days for

recuperation from the stress of surgery and anesthesia. Study group rats (n=6) were administered a 10 mg/1 ml dose of amoxicillin by gastric gavage. Seventy-five minutes later, infusion of 100 units of β-lactamase in 1 ml of a 100 mM Tris HCl buffer with 0.1% W/V bovine serum albumin (pH=7.0) was started at a rate of 1 ml/hr, directly into the terminal ileum via the ileal cannula. The 75 min lag time between gastric gavage of amoxicillin and intra-ileal infusion of β-lactams was experimentally found to be the transit time of amoxicillin from the gastrum to the beginning of the terminal ileum (see results section). The remaining rats (n=6), serving as a control group, were given 1 ml of sterile water by gastric gavage, followed 75 min later by a 1-h intra-ileal infusion of 1 ml of the Tris buffer alone (β-lactamase free).

These procedures were performed on all 12 rats for 4 consecutive days. Feces collection and microbiological processing were performed as described above.

Statistical Analysis

Data were analyzed statistically by the non-parametric, unpaired, two-tailed Mann–Whitney test. For all comparisons, statistical significance was set at P=0.05. Mean ARB counts were normalized to baseline (day 0) counts which were given a value of 1.

RESULTS

Assessment of Growth of ARB within the Gut Following Administration of Amoxicillin by Gastric Gavage

Mean normalized ARB counts for the amoxicillin group on days 1 (1.04×10^{1}), 2 (3.84×10^{3}) and 3 (4.52×10^{4}) were significantly (p<0.01) higher than those in the control group (9.64×10^{-1}, 1.56×10^{0} and 1.80×10^{0}, respectively), as shown in Fig. 1. By 3 days of treatment, mean ARB count has increased by a factor of 4.5×10^{4}, whereas mean ARB count of the control group showed an insignificant change.

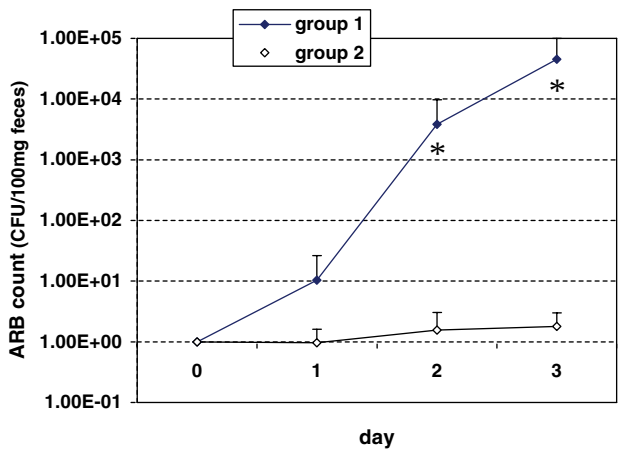

Fig. 1. Semi-logarithmic plot of mean ampicillin-resistant bacteria (ARB) counts in rat feces samples cultured on ARB-selective MacConkey agar plates. Group 1: amoxicillin by gastric gavage (n=5); group 2: sterile water by gastric gavage (n=5). *p<0.01.

Determination of Gastrum-Ileum Transit Time in Rats

The transit time of amoxicillin from the stomach to the terminal ileum was within the range of 70–75 min. Mean amoxicillin concentrations within the jejunum and ileum were found to be 27.5 μg/g and 0, respectively, 70 min post-administration, and 15.6 μg/g and 12.6 mcg/g, respectively, 75 min post administration. This indicated that amoxicillin first appears in the ileum between 70 and 75 min after oral administration. Findings were similar with both 1 and 10 mg amoxicillin doses.

Assessment of Growth of ARB within the Gut Following Administration of Amoxicillin by Gastric Gavage and Ileal Administration of β-Lactamase

The mean increase in ARB counts was significantly lower (p<0.01) in the amoxicillin-β-lactamase group as early as day 1 post-administration, compared with the amoxicillin + buffer control group as shown in Fig. 2.

DISCUSSION

The present study was designed to assess the effect of oral sustained release drug delivery systems of antimicrobials on the emergence of resistant bacteria within the colon. We found that resistant colonic bacteria were detected in feces of rats following oral administration of the β-lactam amoxicillin, but not following placebo. This finding clearly demonstrates that the unabsorbed fraction of an orally administered β-lactam reaching the colon induces emergence of resistant bacteria within the colon. A similar effect was shown following intravenous administration of a β-lactam to beagle dogs (10,11).

The present study has also demonstrated that the undesired consequences specified above may be circumvented by using a novel experimental strategy for timed

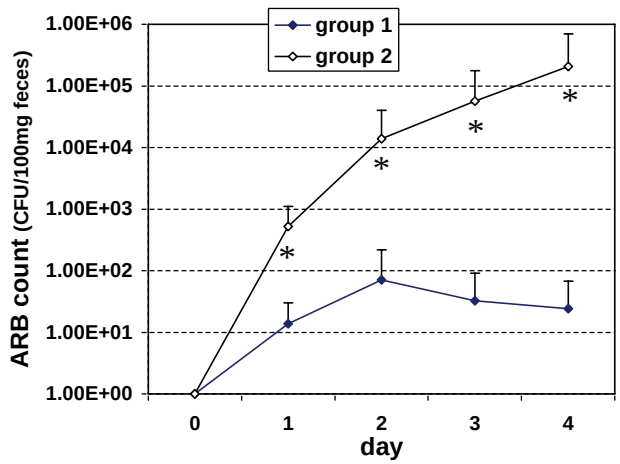

Fig. 2. Semi-logarithmic plot of the mean increase in ampicillin-resistant bacteria (ARB) counts in rat feces samples cultured on ARB-selective MacConkey agar plates. Group 1: amoxicillin by gastric gavage, **β**-lactamase infused directly into the terminal ileum (n=6); group 2: amoxicillin by gastric gavage, buffer infused directly into the terminal ileum (n=6). *p<0.01.

degradation of the excessive, unabsorbed fraction upon transit from the terminal ileum into the upper colon, prior to encountering the large mass of normal colonic microflora. Such a system serves as a specific "waste bin" for this fraction of unabsorbed drug. By inactivating this residual fraction of drug, the consequences related to its intra-colonic antimicrobial action are prevented and the natural colonic microflora remains unaffected, as shown in our study.

Presently, design of dosage forms and dosing regimens of antimicrobials is primarily focused on maximization of pathogen eradication rate (microbiological cure) and infection resolution (clinical cure), with additional goals being minimization of toxicities and costs and optimization of patient adherence, (4,5,12,13). Oral sustained release delivery systems for antimicrobial drugs also prioritize these goals, by addressing pharmacokinetic parameters (such as extent of absorption and half-life) and pharmacodynamic parameters (such as concentration-effect profile and killing kinetics).

However, in addition to the immediate benefits gained at the specific patient level, selection of an appropriate antimicrobial for a specific treatment must also consider other potential implications, such as those derived from the presence and effects of unabsorbed portions of the antimicrobial within the colon.

For the polar, peptidomimetic β-lactams, the absorption window is entirely within the small intestine, where active transport systems (specifically PEPT1) transfer the drug across the intestinal mucosa into the systemic circulation (14–16). Any fraction of an oral β-lactam dose not absorbed within the absorption window along the small intestine reaches the colon, where the PEPT1 transport system is largely absent.

Consequently, the natural abundant microflora within the colon is exposed to the excessive, unabsorbed fraction of the drug. In natural, uninterrupted circumstances, the native microflora resists colonization of potentially destructive competitors such as "non-native" resistant bacteria, a phenomenon termed "colonization resistance" (17). However, the exposure of naturally occurring colonic bacteria to the unabsorbed fraction of an orally administered antimicrobial drug results in eradication of susceptible subpopulations. At the same time, resistant bacterial subpopulations have the opportunity to thrive (by either selection or de-novo formation). In other words, the presence of active antimicrobial drug in the colon may abolish colonization resistance. Bearing in mind that the colon is the largest bacterial reservoir in human body (18), this may have significant implications at three levels.

Firstly, at the immediate host level, this drug-induced alteration of natural enteric microflora may result in deleterious events such as infections caused by resistant bacteria, and intestinal adverse effects such as diarrhea and colitis. These effects of orally administered antimicrobials of various groups (β-lactams, macrolides, lincosamides, tetracyclines, nitroimidazoles, quinolones) have been established in a large number of studies (reviewed by 19–22).

Secondly, in the event that the antimicrobial treatment induces emergence of resistant bacteria, future options for effective antimicrobial therapy for the specific patient might be limited. Should resistance to the initial antimicrobial therapy emerge, any future infection caused by these resistant bacteria may be more difficult to treat, requiring a broader-spectrum antimicrobial with greater toxicities and/or a higher cost.

Finally, as antimicrobial exposures are among the most prominent driving forces for selection of resistant bacteria and/or induction of de-novo resistance, a broader prospect should consider the more troubling epidemiological consequence for the potential spread of resistant bacteria to the host's environment, including other patients (23,24).

In the present paper we highlight the concerns associated with sustained-release oral dosage forms, which increase the unabsorbed fraction reaching the colon to a greater extent than that of their corresponding immediate-release dosage forms. Thus, from an epidemiological point of view, sustained-release oral dosage forms of antimicrobials may contribute even more to the emergence of antimicrobial resistance, an aspect of pharmaceutical design which was not considered in its full relevance, despite the fact that this problem has become a major global concern, imposing a rapidly growing heavy clinical and economical burden on health-care institutions (25). We suggest that this issue should require careful consideration when designing oral sustained-release drug delivery systems. For instance, a number of such products are currently FDA-approved based on studies demonstrating superiority over comparators in achieving at least one of these goals (26–33); however, none of them were designed to address the resistance issue outlined above.

CONCLUSIONS

Based on the study results, it is of concern that sustained-release oral dosage forms of antimicrobials might be a yet-unrecognized contributor to selection and/or emergence of resistant bacteria. This may justify consideration of emergence of resistant bacteria as an equally important goal in future designing of such dosage forms by both makers of oral antimicrobial dosage forms and regulators. Taking into account the additional goal of minimization of amount of antimicrobial drugs reaching the colon, without compromising the basic goals of antimicrobial therapy, may significantly contribute to the global efforts to combat antimicrobial resistance. Sustained-release gastroretentive dosage forms may serve as a potential pharmaceutical solution to overcome this problem (34).

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