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Impact of ciprofloxacin in the human-flora-associated (HFA) rat model: Comparison with the HFA mouse model

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Abstract

The ecological impact of different doses of ciprofloxacin was investigated in an experimental germ-free rat model into which human fecal flora was inoculated. Animals received oral doses (gavage) of 0, 0.25, 2.5, and 25 mg/kg body weight (bw) of ciprofloxacin once daily for 5 weeks. All doses of ciprofloxacin significantly reduced aerobic populations. Elimination of *Enterobacteriaceae* and reduction of bifodibacteria were noticed in the group treated with 25 mg/kg of the antibiotic. The rest of the intestinal flora was not affected. These effects were reversible after the treatment ended. The percentage of resistant enterococci increased in rats treated with 2.5 and 25 mg/kg; however, this increase was not statistically significant. There was a significant ($P < 0.05$) emergence of ciprofloxacin-resistant *Bacteroides fragilis* group with 25 mg/kg bw, which is equivalent to a human therapeutic dosage of the antibiotic. The MIC values and the percentage of resistance remained elevated 2 weeks after the end of treatment in this anaerobic population. Although sub-populations of enterococci and *Enterobacteriaceae* showed decreased susceptibility after ciprofloxacin administration, resistance was not evident. The ability of an exogenous strain of *Salmonella* to colonize the intestine of animals treated with 25 mg/kg of ciprofloxacin confirmed that the drug disrupted the colonization barrier effect of the indigenous flora at the high dose level tested. No changes in the metabolic parameters occurred during the antibiotic treatment. The results obtained in the HFA rat model were similar to those obtained in our previous study using the HFA mice model where ciprofloxacin at 0.125, 1.25, and 12.5 mg/kg bw induced a decrease of enterococci and *Enterobacteriaceae* populations. The high dose of ciprofloxacin also induced a decrease in bifidobacteria counts, an increase in levels of resistant *B. fragilis* group and a significant ($P < 0.05$) disruption of the colonization resistance of the barrier flora in HFA mice.
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1. Introduction

The use of antimicrobials in food-producing animals may result in antibiotic residues in edible products which might produce adverse effects on the human intestinal flora after ingestion. European and United States guidelines for veterinary drug registration recommend that, in the establishment of the acceptable daily intake (ADI) of a drug, the microbiological hazards from antimicrobial residues must take into account the potentially harmful effects of the residues on the human intestinal flora (CVM, 2000; JECFA, 2002). In vitro or in vivo approaches are used by animal health industries, contract laboratories, and regulatory authorities to assess the effect of ingested antimicrobial residues on human intestinal flora. The ADIs for drug residues have been determined using minimum inhibitory concentrations (MICs) values of a drug tested against pure strains isolated from intestinal flora; however, ADIs determined by this method do not take into account the interaction of intestinal microorganisms, the conditions in the gastrointestinal tract, or the metabolism of the host.

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The HFA-rat model was used to assess the effects of therapeutic and residual doses of ciprofloxacin, the main metabolite of the veterinary drug enrofloxacin, on the human intestinal flora. This was done to address questions about the appropriateness of the HFA-mouse model raised at a meeting of the Veterinary International Cooperation on Harmonization (VICH) Microbial Safety Task Force in July, 2000. The experts agreed that the HFA-rat model would probably be more appropriate than the HFA-mouse model for determining the no-observed-effect level (NOEL) of antimicrobial drug residues because (a) the HFA-rat model allows for more independent replications of a single drug dose, and (b) the rat is the more commonly used species in toxicology studies and therefore, there is an ample database concerning toxicological effects in this species. Therefore, the results of this study will be compared with the results of a previous study performed with ciprofloxacin using the HFA-mouse model.

The microbiological endpoints evaluated in this study included bacterial counts, susceptibility of the flora to ciprofloxacin, changes in metabolic activity, and disruption of the colonization barrier effect of the flora. From the above, it was concluded that a NOEL would be lower than the lowest dose used in the study. A similar conclusion was drawn from the previous study using the HFA-mouse model (Perrin-Guyomard et al., 2005).

2. Materials and methods

2.1. Test substance

Ciprofloxacin chlorohydrate, batch AATNE, and ciprofloxacin, batch R123-3, were received from the Bayer company and stored at ±4 °C. Batch AATNE powder was soluble in distilled water and batch R123-3 powder was soluble in acidified (HCl 1.2 N) distilled water. The purity of the first batch was estimated at 99.5% and of the second batch, 99.8% by Bayer company.

2.2. Experimental design

Two independent trials were performed with ciprofloxacin. The first trial evaluated the reduction or overgrowth of bacterial populations, changes in susceptibility of target organisms (as indicated by increasing counts of resistant bacteria and shifts in MIC distributions), and metabolic activity parameters (fecal short chain fatty acid [SCFA] concentrations). The second trial evaluated disruption of the colonization barrier effect of the flora. Overviews of each study design, sampling times, and parameters evaluated in each trial are detailed in Table 1.

2.2.1. Human donors

While the population sizes of different bacterial species in the intestinal flora are stable within an individual, there may be large variation in the proportions of the major species from person to person (Bertazzoni Minelli et al., 1993; Finegold et al., 1974; Gorbach et al., 1967). Human variability in the flora was considered by the VICH Microbial Safety Task Force as a major issue that should be addressed in the process of validating a model for studying effects of antimicrobial drugs on human intestinal flora. Therefore, inocula from two volunteers with marked differences in the proportion of major bacterial species were used to address the inter-individual variability while evaluating the appropriateness of the HFA-rat model.

Table 1
Overview of the study periods, sampling times, and parameters evaluated

Trials	Periods	Number of sampling times	Duration of the periods (weeks)	Parameters evaluated
First trial	Pre-treatment	3	3	Bacterial populations and metabolic parameters
	Treatment	5	5	
	Post-treatment	2[a], 3[b]	3	
Second trial	Pre-treatment		3	Barrier effect
	Treatment	10	5	
	Post-treatment	5	4	

[a] Bacterial populations.
[b] Metabolic parameters.

Three healthy human volunteers, 1 female (both trials) and 2 males (one per trial) from the laboratory staff, were involved in the study. None used any antimicrobial agent for at least 3 months prior to the investigation. They were selected according to the similarity of their flora with a "normal human intestinal flora" (Mitsuoka, 1992; Rowland et al., 1985; Salminen et al., 1995; Sherwood and Gorbach, 1993) and the susceptibility of target bacteria to ciprofloxacin (less than 10% of resistance) from 11 (first trial) and 7 (second trial) male and female volunteers.

2.2.2. HFA-rat model

Germ-free rats were acclimatized for approximately one week, inoculated intragastrically with a strain of *Bacteroides fragilis* ATCC 25285 (a non-enterotoxin-producing strain) according to our previous model (Perrin-Guyomard et al., 2001) and, two (for trial 2) or three (for trial 1) days later, inoculated with diluted feces. Rats were isolated from the bedding by a floor grid to avoid coprophagy.

2.2.2.1. First trial. Sixteen female and sixteen male germ-free rats (OFA, 3–6 weeks old) were obtained from IFFA Credo (Lyon, France). At their arrival, the rats were housed in one isolator and randomized into 8 groups of 4 animals (sex separated). The 8 groups were evenly divided among 4 separate isolators. Within each isolator, the rats were randomized again into 2 groups of 4 animals (2 males and 2 females for each inoculum). The rats were housed individually (one rat/cage) in each sterile isolator (one isolator per treatment group). In preparation for the inoculations, human flora were collected from 2 healthy donors that had been selected in a pilot study among volunteers and separately diluted a 100-fold in pre-reduced tryptone glucose yeast extract (TGY) medium. The diluted feces of each donor constituted one inoculum. The inocula were administered by gavage at a volume of about 1 ml/rat. In each isolator, one group of 2 males and 2 females were given the inoculum 1 and the other group of 2 males and 2 females, the inoculum 2.

2.2.2.2. Second trial. In the first trial, the effects of ciprofloxacin were statistically unrelated to the animal gender. Moreover, males were more easily handled. Therefore, in the second trial, only males were used. 16 male germ-free rats (Sprague Dawley, 3-6 weeks old) were obtained from IFFA Credo (Lyon, France). At arrival, rats were housed in one isolator and randomized into 4 groups of 4 animals each. Each group of 4 rats was transferred to a separate isolator and randomized again into 2 groups of 2 animals each. The rats were housed individually (one rat/cage) in each sterile isolator (one isolator per treatment group) and maintained for one week in a germ-free status. Human feces were collected from 2 healthy donors and treated as in Trial 1 to constitute each inoculum. The two inocula were then administered to the rats as in Trial 1. In each isolator, one group of 2 males were given the inoculum 1 and the other group of 2 males the inoculum 2.