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Original scientific article

Lactobacillus acidophilus: effects on the pharmacokinetics of marbofloxacin in rats

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Summary

Background: Probiotics are currently produced commercially and widely used for improving human and animal health. They modulate the gut environment through secretion and production of different molecules and enzymes. Hence, they play a major role in changing the pharmacokinetics of an orally administered drug.
Purpose: To determine the effect of *Lactobacillus acidophilus* (KCTC 3140) on the pharmacokinetics of marbofloxacin.
Materials and Methods: Five male and five female 8-week old healthy Sprague Dawley rats were treated with 10^9 CFU/ml of *L. acidophilus* daily for seven days via the intra-gastric route. Marbofloxacin (20 mg/kg) was administered orally at the beginning and the end of the experiment. The plasma concentration of marbofloxacin was measured using high performance liquid chromatography (HPLC).
Results: The area under the curve (AUC) of marbofloxacin declined from 5.78 µg.h/ml to 2.57 µg.h/ml after treatment with *L. acidophilus.* Similarly, the maximum plasma concentration (C_{max}) of the drug decreased from 2.4 µg/ml to 1.2 µg/ml and the T_{max} increased from 0.54 to 0.73 h. The elimination half-lives of marbofloxacin before and after treatment with *L. acidophilus* were 1.19 h and 0.69 h, respectively. The study was conducted by separating the male and female rats; no significant difference was observed between the sexes.
Conclusion: The treatment of rats with *L. acidophilus* decreased the plasma AUC and C_{max} after oral administration of marbofloxacin. Hence, studying the interaction of a probiotic with an antibiotic drug is essential prior to co-administration of the probiotic with the oral antibiotic.

Introduction

Probiotics are live bacterial agents present in the normal gut flora with low or no virulent activity and which are known to be beneficial to the health of the host when administered in adequate numbers (*Saavedra, 1995; Hozapfel et al., 1998*). Bacteria of the genus *Lactobacillus, Lactococcus* and *Bifidobacterium* are well known probiotics with desirable properties and documented clinical effects (*Salminen et al., 1998*). Probiotics have been widely used in food producing animals such as cattle, pigs and chickens for growth performance and prevention against pathogenic microbial infection with and without oral antibiotics. *Lactobacillus acidophilus* is a lactic acid producing, Gram-positive bacteria (*Kandler, 1983*)

which has shown a variety of pharmacological effects in rats. It is the most commercially utilized probiotic, especially in the dairy industry (*de Vos, 2011*).

Probiotics are known to balance the intestinal microbial flora. Their presence limits the pathogenic potential of another microorganism by competing for nutrients, lowering the luminal pH, and by producing and releasing antimicrobial agents; they are also involved in the modulation of the specific and innate immune system (*Shortt, 1998; Mcfarlane and Cummings, 1999; Sanders, 1999; Oelschlaeger, 2010*).

As a result of interacting with the normal microbial flora, probiotics modulate the composition and activity of the gut enzymes in addition to providing their own specific enzymatic activities. Hence, probiotics potentially affect the pharmacokinetics of drugs (*Gibson and Roberfroid, 1995*). There are some reports of probiotic effects on the pharmacokinetics of a drug. The effect varies depending upon the strain of priobiotic bacteria utilized, the host involved, type of drug used as well as the health status of the animals (*Matuskova et al. 2014; Al-Salami et al. 2008; Alvarez-Olmos and Oberhelman, 2001*).

Currently varied research findings have been reported for the effects of probiotics on the health of their hosts. However, there are only limited data on the effects of specific probiotics on the pharmacokinetics of drugs. In addition, there is no information about the effect of probiotics on the pharmacokinetics and/or bioavailability of orally administered antibiotics such as marbofloxacin which has a 100% bioavailability in some animals.

Therefore, the objective of the present work was to investigate the pharmacokinetics of marbofloxacin administered orally to Sprague Dawley rats in the absence and presence of *L. acidophilus* as a probiotic.

Materials and methods

Animals

All the procedures with animals in this study were approved by the institute of animal uses and care committee of Kyungpook National University (Approval number: KNU-2013-0088). Ten eight-week old Sprague Dawley (Crl:SD) specific pathogen free (SPF) rats (Orientbio, Sungnam, Korea) in the same condition were selected. They were provided with adequate commercial feed 5L79 (PMI Nutrition International, LLC, Brentwood, MO, USA) and filtered tap water. The rats were arranged in two groups, consisting of five males and five females, and were conditioned to the laboratory situation for one week. Rats in each group were arranged in two cages (25 cm width by 40 cm length by 20 cm height). Each of the cages contained two or three rats and was provided with sterile 100% virgin wood fiber bedding (Beta chip®, Northeastern Products Corp. NY, USA). The average body weights of the female and male rats were 201.2 and 285.2 g, respectively. Rats were housed at an average temperature of 25°C and humidity of 60%. The general health status of the rats was monitored by physical and physiological examination prior, during and at the end of the study. The room had an equal 12 h of light and darkness.

Experimental protocol and measurements

On the first and the ninth day of the experiment, rats were given 20 mg/kg of marbofloxacin intragastrically using a 22 G ball tip needle. Starting on the second day, 10^9 CFU/ml of *L. acidophilus* KCTC 3140 (obtained from the Korean collection for type culture, Daejon, South Korea) (*Park et al.*, 2006) in 200 µl volume was administered intragastrically daily for 7 days. A maximum of 300 µl of blood was collected using a microvette (Microvette® CB 300 K2E, Sarstedt, Germany) from the tail of rats at 0, 0.25, 0.5, 0.75, 1, 2, 4, 8 and 12 h after administration of marbofloxacin. Finally, the plasma was collected after centrifugation at 10000 RPM for 10 min at 4°C and stored at -20°C until analyzed using high performance liquid chromatography (HPLC).

The marbofloxacin concentration in the plasma was measured by HPLC using a Hewlett Packard Agilent 1100 series comprising an HPLC pump, HP ODS Hypersil column (250 × 4.6mm, 5µm), autoinjector and UV detector. The wavelength of the UV detector was set at 293 nm and the column temperature was 30°C. An aliquot of rat plasma was deproteinated by adding an equal amount of acetonitrile. After vortex-mixing and centrifugation at 16,000 × g for 1 min, a 20 µl aliquot of the supernatant was added to the auto-sampler vial and injected directly onto the HPLC column. The mobile phase consisting of 10% acetonitrile, 10% methanol and 80%, 20 mmol potassium phosphate (0.05 M, ACS reagent, Sigma® ≥99.0% purity, pH=2.9) buffer, pH =3 was run at a flow rate of 1 ml/min.

The standard and quality control (QC) samples were prepared using stock solutions of marbofloxacin (1 mg/ml; Fluka, Sigma-Aldrich, Germany). Drug free rat plasma samples were spiked with these solutions to prepare standard curves, determine the accuracy, precision and detection limits of the assays. The same samples were also used as QC samples for intra-assay and inter-assays. All the stock solutions were stored at 4°C until used.

Method validation was determined according to the FDA guidelines. The detection limit and quantitation limit were calculated based on the standard deviation of the response and the slope of the calibration curve.

The calibration curve was created using a high concentration of marbofloxacin (100 µg/ml) in plasma and stepwise dilutions of 50, 20, 10, 5, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.02, and 0.01 µg/ml. Analyses were based on peak areas. Three sets of calibration curves were used to validate the method. The data were validated using a standard statistical curve. The intra-assay precision was determined at 20, 4, 0.5 and 0.05 µg/ml. Accuracy was determined by comparing the measured concentrations with the calculated nominal concentrations.

A bioassay of the plasma was performed to standardize the HPLC result. Ten ml of LB agar (Difco™, BD, USA) was added to a sterile plate. *E. coli* strain BE was grown in LB broth and transferred to sterilized LB agar media at a specific dilution rate. The LB agar media containing 10^6-10^7 CFU/ml of the bacteria was added to the provided LB agar plate. Then the media were kept at 4˚C until used.

A paper disc was sterilized by autoclaving and 60 µl of plasma was applied in a biological safety cabinet. After the paper had been dried, it was transferred to the prepared agar plate and incubated overnight at 37˚C aerobically. A known concentration of marbofloxacin in distilled water and in plasma, as well as plasma alone, were used as controls for HPLC analysis.

Statistical analysis

The statistical analysis was conducted using SAS version 9.4 (SAS Institute Inc., NC, USA). The Phoenix WinNonlin (Pharsight Corp., St. Louis, MO, USA) software program was used to compute the pharmacokinetics analysis. A trapezoidal method of non-compartmental analysis was used for each plasma concentration and the data were analyzed using nonlinear least-squares regression analysis. Comparison of the mean values of the pharmacokinetic parameters before and after treatment was statistically evaluated using t-test and the P value <0.05 was considered as statistically significant.

Results

Method Calibration

The HPLC retention time for marbofloxacin was 7.8 min at a flow rate of 1 ml/min. The spiked samples showed peaks without any interference at the specified retention time (Fig 1). A linear relationship was

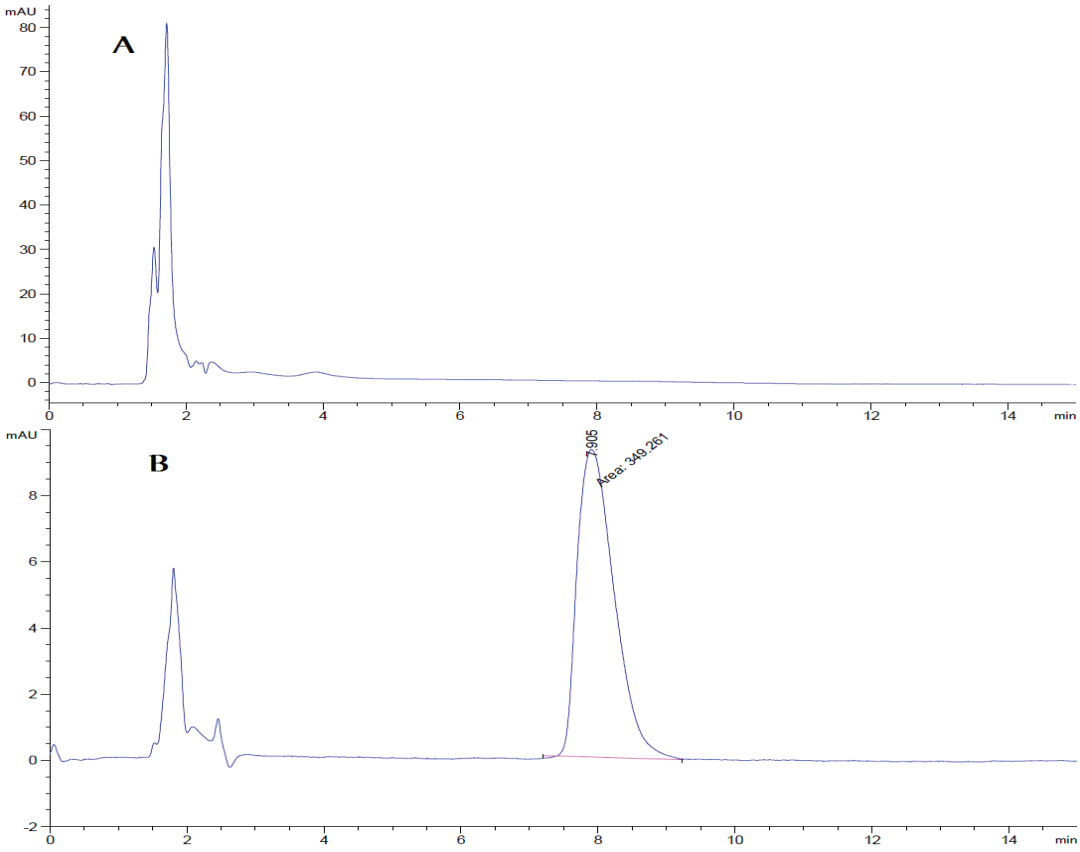

Figure 1: HLPC peaks for the control (A) and spiked (B) plasma with marbofloxacin

Table 1: Pharmacokinetics of MRB (Mean ± SE) before and after treatment with *L. acidophilus*

Parameters	Units	Before	After
AUC	µg.h/ml	5.78 ± 0.56	2.57 ± 0.13*
K_{01_HL}	H	0.17 ± 0.03	0.39 ± 0.1*
K_{10_HL}	H	1.19 ± 0.2	0.69 ± 0.18*
CL_F	ml/h/mg	699.55 ± 69.19	1560.83 ± 81.31*
Tmax	H	0.54 ± 0.06	0.73 ± 0.03
Cmax	µg/ml	2.42 ± 0.08	1.24 ± 0.02*

AUC, area under the concentration-time curve; K_{01_HL}, Half-life of absorption; K_{10_HL}, Elimination half-life; CL_F, total body clearance; T_{max}, Time taken to achieve maximum concentration; C_{max}, maximum concentration. *Statistically significant difference at $P<0.05$.

maintained for the calibration curve at both lower and higher concentrations. The linearity of the standard curve of marbofloxacin concentration in the spiked plasma was shown by the value of regression (R^2=0.9986).

Marbofloxacin was detected at a concentration of 0.01 µg/ml. Hence, the limit of detection (LOD) was 0.01 µg/ml and the limit of quantitation (LOQ) was 0.05 µg/ml. The inter-day and intra-day coefficients of variation were < 10. The overall bias of the plasma sample was 6.5%.

Pharmacokinetics

The calculated PK parameters of marbofloxacin in plasma before and after treatment of the rats with *L. acidophilus* are summarized in Table 1. The kinetics of marbofloxacin are best described by a one-compartment open model. The area under the curve (AUC) of marbofloxacin was significantly decreased from 5.78 µg.h/ml to 2.56 µg.h/ml after the rats were treated with *L. acidophilus* (Fig 2). Likewise, the C_{max} declined from 2.4 µg/ml to 1.2 µg/ml and the T_{max} increased from 0.54 to 0.73 h. In this study, no significant difference was observed between the two sexes. The elimination half-lives of marbofloxacin before and after treatment with *L. acidophilus* were 1.19 h and 0.69 h, respectively.

The linearity of the spiked plasma in the bioassay showed a logarithmic correlation of R^2 = 0.9868. The microbiological assay result showed that the maximum concentration of marbofloxacin was 7.66 µg/ml and 7.08 µg/ml which were obtained at 0.5 h and 0.25 h before and after treatment, respectively (Fig 3).

Discussion

The utilization of probiotics in human health in the recent years has become more popular. Lactic acid producing bacteria including, *Lactobacillus*, *Lactococcus* and *Bifilidobacterium* are the most widely

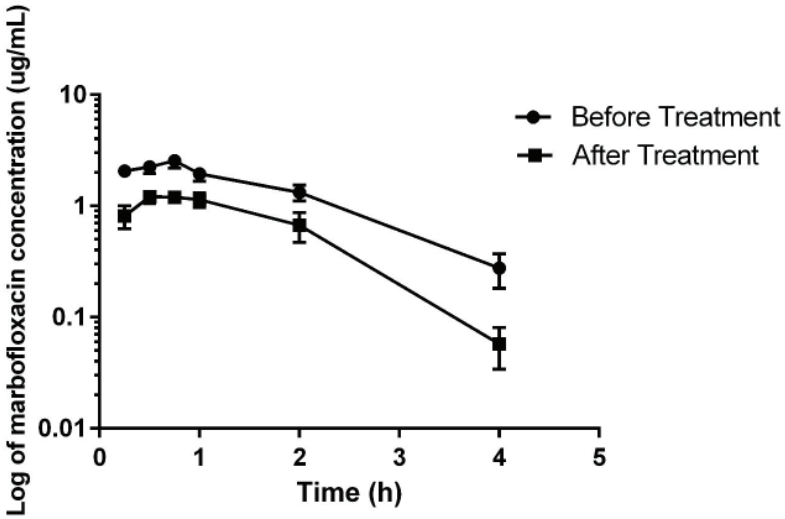

Figure 2: Semi-logarithmic plot of the serum concentration (Mean ± Std Err) of Marbofloxacin before and after treatment with *L. acidophilus.*

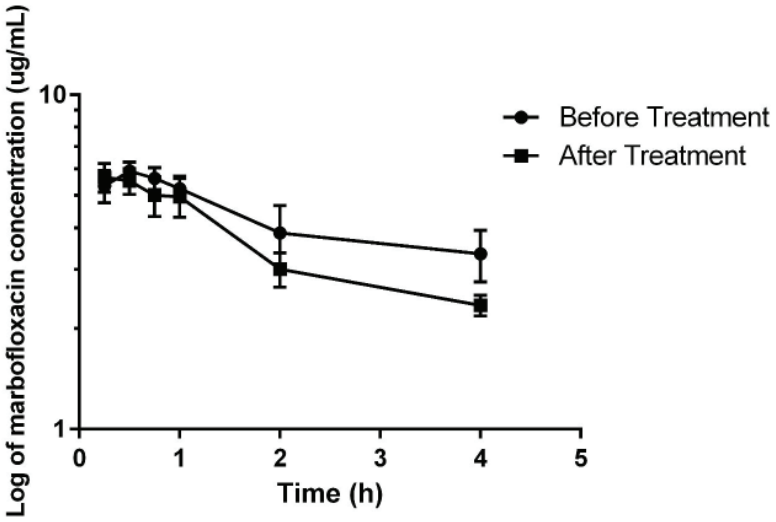

Fig 3: Semi-log of microbiological assay of MRB concentration vs. time from rats (n=10) serum. Bars shows standard error of the mean.

commersialised probiotic bacteria (*Vasiljevic and Shah, 2008; Holzapfel et al., 2001*). There is increasing scientific evidence that consumption of probiotics in adequate numbers confers health benefits to humans and animals (*Kechagia et al., 2013; Narayan et al., 2010*). These bacteria are used as an antimicrobial agent but they will also metabolise antibiotics and can be used in the treatment of acute diarrheal diseases, prevention of antibiotic-associated diarrhea, and improvement of lactose metabolism (*Kechagia et al., 2013; Wilson and Nicholson, 2009*). Furthermore, there are recent reports of the effects of probiotics on the pharmacokinetics of antimicrobial agents. However, the results vary depending on the type probiotic strains applied, the drug tested and the type of study conducted.

In this particular study, we have tried to establish the effect of *L. acidophilus* on the pharmacokinetics of marbofloxacin using a rat model. It has been shown that the plasma concentration of marbofloxacin was reduced in the rats treated with *L. acidophilus* for 7 days, indicating that the probiotic decreases the bioavailability of the antibacterial agent. This result, agrees with the reports of Al-Salami and his colleagues (2008), who showed a decreased concentration of gliclazide in healthy rats after treatment with three different probiotics. The probiotic treatment reduced gliclazide absorption and bioavailability in healthy rats. This might be attributed to the activation of the intestinal efflux drug transporter by the probiotics. Another explanation might be the formation of a 'thicker' layer of the adherent mucous, which comprises the physical barrier protecting the enterocytes. In addition, the probiotic might stimulate the presystemic metabolism of the drug (*Al-Salami et al., 2008; AlSalami et al., 2012a*).

However, the result is contrary to the findings of Matuskova *et al.* (*2014*), who reported the increased bioavailability of amidarone and altered pharmacokinetics of the drug after utilizing *E. coli* Nissle 1917 as a probiotic. This difference in response might be due to the application of different strains of probiotics with different drugs.

Probiotics affect the metabolism of drugs in the gut. They induce various cytochrome enzymes, or phase II conjugating enzymes, in the intestine responsible for drug metabolism while others like, *L. acidophilus,* upregulate intestinal electrolyte absorption while inhibiting the cellular uptake of micellar cholesterol (*Wilson and Nicholson, 2009; Raheja et al., 2010; Huang and Zheng, 2010*). *L. helveticus* R389 indirectly affects calcium metabolism through enhanced expression of the main calcium transporter in the epithelial cells of the duodenum (*Vinderola et al., 2007; Resta, 2009*). The alteration of drug bioavailability might also be affected by the expression of intestinal transporters that are involved in drug transport or by decreasing the expression of proteins which are important for the disposition of drugs (*Saksena et al., 2011; Matuskova et al., 2011; Huang et al., 2010*).

In conclusion, the treatment of rats with *L. acidophilus* in the present study significantly decreased the plasma AUC and C_{max}, but increased the clearance of orally administered marbofloxacin. The type of effect exerted by a certain probiotic strain depends on its metabolic properties, the kind of surface molecules expressed or on the secreted components. Hence,

strain identification is recommended to establish probiotic suitability and performance for commercial application since, closely related probiotic strains may have different clinical effects (*Alvarez-Olmos and Oberhelman, 2001*). This can be achieved by a combination of phenotypic and genetic identification tests (*FAO, 2001*). In addition, prior to administration of probiotics along with antibiotics, *in vivo* studies should be carried out instead of relying only on *in vitro* experiments, and the bioavailability of oral antibiotics should be determined. Interestingly, although there are clear differences in the pharmacokinetic effects of probiotics (*Al-Salami et al., 2008; Al-Salami et al., 2012a; Matuskova et al., 2014*), the relationships between pharmacokinetic changes and intestinal microbiota have not been studied. Therefore, we plan to conduct genetic studies to determine the relationship between marbofloxacin pharmacokinetics and metagenomics of intestinal microbiota.

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