

Lactobacillus plantarum IS-10506 probiotic administration increases amlodipine absorption in a rabbit model

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Abstract

Objective: Probiotics are beneficial in human health. In this study, we investigated the effect of probiotics on absorption of amlodipine, a dihydropyridine calcium antagonist used in the treatment of angina and hypertension, in a rabbit model.

Methods: *Lactobacillus plantarum* IS-10506 probiotic was administered for 14 days to male New Zealand rabbits. Blood samples were collected before and after probiotic supplementation. Amlodipine (10 mg) was then administered to all groups. Blood samples from a marginal vein were withdrawn at 5, 15, 30, 60, and 120 minutes to determine amlodipine concentrations in rabbit plasma.

Results: Amlodipine concentrations in the *L. plantarum* IS-10506 group were 4.95 ± 1.22 , 8.71 ± 0.69 , and 12.48 ± 2.53 ng/ml, and those in the control group were 1.69 ± 0.31 , 3.89 ± 1.23 , and 7.17 ± 1.85 ng/ml at 30, 60, and 120 minutes, respectively after administration of amlodipine.

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Amlodipine concentrations in the *L. plantarum* IS-10506 group were significantly higher than those in the control group at 30, 60, and 120 minutes after amlodipine administration.

Conclusion: Our results suggested that supplementation of *L. plantarum* IS-10506 significantly increases amlodipine plasma concentrations in rabbits.

Keywords

Probiotic, *Lactobacillus plantarum*, dadih, amlodipine, absorption, calcium channel blocker, hematology, gastrointestinal tract

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Introduction

Oral administration is the easiest and most convenient way of drug administration.¹ Factors that may affect the absorption rate are intestinal integrity, physiological status, gastrointestinal motility, site of drug absorption, membrane transporters, pre-systemic drug metabolism, and the effect of food or concomitant medication.² The intestine has a substantial influence on drug biotransformation because of the presence of numerous enzymes, especially those produced by gut microbiota.³

In recent years, there has been some interest in the manipulation of the composition of intestinal microbiota by probiotics and prebiotics.⁴ The World Health Organization defined probiotics as “live organisms administered in adequate amounts to confer a health benefit for the host”.⁵ Probiotics have beneficial health effects via multiple mechanisms. These mechanisms include release of antibacterial substances (bacteriocins and bacteriocin-like inhibitory substances), secretion of non-specific antimicrobial substances, induction of production of antimicrobial compounds (defensins) by the host, direct enzymatic activities of probiotics within the gut lumen, reduction in luminal pH, inhibition of bacterial adherence,

competition for nutrients, and immune responses.^{6–8} Probiotic bacteria may affect the expression and functionality of various proteins and membrane transporters of other bacteria in the gut via changing gut concentrations of certain polypeptides. These actions might be due to induction or suppression of membrane transporters or by the process of direct signaling.⁹

Previous studies have reported the potential of probiotics to enhance drug absorption.^{10,11} Supplementation of the probiotic *Lactobacillus plantarum* IS-10506 together with zinc increases the zinc status of Indonesian school children.¹⁰ Other reports have shown changes in gliclazide pharmacokinetics in diabetic rats that were pre-treated with a mixture of three probiotics (*L. acidophilus*, *L. rhamnosus*, and *Bifidobacterium lactis*) in a suspension prepared from freeze-dried probiotic powders. Probiotic treatment of diabetic rats increases gliclazide bioavailability and lowers blood glucose levels by insulin-independent mechanisms, suggesting that administration of probiotics may be a beneficial adjunct therapy in the treatment.¹¹ Therefore, we hypothesized that probiotic supplementation increases drug absorption in the gastrointestinal tract.

In this study, we investigated the potential use of *L. plantarum* IS-10506 probiotic

to enhance amlodipine concentrations in rabbit plasma. Amlodipine is a calcium channel blocking agent of a dihydropyridine derivative that is used for treating hypertension and angina.¹² Because patients depend on amlodipine, this study examined the use of probiotics, as adjuvants, to increase the effectiveness of amlodipine therapy.

Material and methods

Materials

Microencapsulated *L. plantarum* IS-10506, which is a novel probiotic that is isolated from dadih (a traditional Indonesian fermented buffalo milk), was prepared as previously described.¹⁰ Amlodipine (amlodipine besylate), the internal standard nortriptyline hydrochloride (NOR), and 4-chloro-7-nitrobenzofurazan (NBD-Cl) were purchased from Sigma Aldrich (Singapore). Methanol, acetonitrile, ethanol, sulfuric acid, hydrochloric acid, boric acid, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). All reagents that were used were analytical grade, except for methanol, which was high-performance liquid chromatography (HPLC) grade.

Stock solution of amlodipine was prepared by dissolving 10 mg of free base with 2 mL ethanol and diluting to 100 mL with water. A plasma standard with serial dilution of 2.5, 10, 40, 160, 640, and 1280 ng/mL was prepared to provide plasma calibration samples. NOR was prepared at a concentration of 100 µg/mL and diluted to obtain 50 ng/mL of working solution. NBD-Cl solution (0.08%, w/v) was freshly prepared in methanol. Teorell and Stenhagen buffer solution¹³ was composed of phosphoric acid, citric acid, 0.1 M sodium hydroxide, and 0.1 M hydrochloric acid, and adjusted to a pH of 8.6.

Rabbit experiments

Twelve male New Zealand White rabbits within the age range of 6 to 12 months old with a weight of 1.5 to 3 kg were used in this study. The rabbits were adapted to environmental conditions with individual stainless steel cages for 7 days and were clinically healthy before the study. Rabbits were allocated into two different groups: (1) pre-treated with *L. plantarum* IS-10506 at a dose of 10¹⁰ colony-forming units (CFU)/day for 14 days; and (2) a control group without pre-treatment of *L. plantarum* IS-10506. Both groups were then fed with a basal diet. A total of 10¹⁰ CFU/day for 14 days of supplementation provides good adhesion of *L. plantarum* IS-10506 to intestinal mucus.¹⁴ After 14 days, a suspension of amlodipine besylate (equivalent to 10 mg of free base) was administered to all rabbits. A total of 10 mg amlodipine was equivalent to 3 to 6 mg/kg body weight to reach a high distribution of amlodipine in the rabbits.^{15,16} Blood samples from the marginal vein were collected into EDTA tubes at 5, 15, 30, 60, and 120 minutes after amlodipine administration. The tubes were centrifuged, and the plasma samples were frozen at -80°C until HPLC analysis. The experimental protocol was approved by the Universitas Padjadjaran Ethical Committee for Health Research (Ethical Approval Number: 416/UN6.C1.3.2/KEPK/PN/2016).

Analysis of amlodipine

A total of 500 µL of plasma was transferred into a test tube, and then 100 µL IS, 100 µL NaOH 0.1 N, and 1 mL acetonitrile was added. After the solution was mixed for 1 minute on a vortex mixer and centrifuged for 15 minutes at 4500 g, 1 mL of aliquot was evaporated under a stream of nitrogen. The residue was dissolved in 100 µL methanol, followed by addition of 100 µL buffer

solution (pH, 8.6) and 100 μ L NBD-Cl solution. The solution was kept at 70°C for 20 minutes. After cooling, 100 μ L 0.1 N H₂SO₄ was added.

The chromatographic system consisted of liquid chromatography (Waters e2695; Waters Associates, Milford, MA, USA) with a fluorescence detector (Waters 2475 FLR, Waters Associates), LiChrospher RP 18 column (125 mm \times 4 mm, inner diameter; Merck, Darmstadt, Germany) and LiChrospher RP 18 guard column (4 mm \times 4 mm, inner diameter, Merck). The mobile phase used was methanol-phosphoric acid 0.01% (v/v) (65:35) with a flow rate of 1 mL/minute. Detection was at an excitation wavelength of 480 nm and emission wavelength of 535 nm. Calibration curves were constructed by analyzing a series of plasma calibration samples that were spiked with concentrations ranging from 2.5 to 1280 ng/mL. Linear regression showed that the value of the coefficient of determination was $r^2 = 0.9989$. The mean recovery was $61.68\% \pm 5.96\%$. The lower limit of quantification (LLOQ) was 2.5 ng/mL. The coefficient variation was less than 15% for quality control samples and the LLOQ, while the percentage difference for accuracy was less than 15% for quality control samples and less than 20% for the LLOQ.

Hematological analysis

Blood samples were analyzed by using the Samsung LABGEO^{HC10} Hematology Analyzer (Samsung Electronics Co.,

Suwon, Korea) for analysis of white blood cells, lymphocytes, monocytes, granulocytes, red blood cells (RBC), hemoglobin, hematocrit (HCT), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width. All of the procedures followed the manufacturer's instructions. The quality control material from the manufacturer was run on a daily basis throughout the entire study period.

Statistical analysis

Differences between groups were analyzed using independent t-tests. P values of less than 0.05 were considered significant. SPSS (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

Results

The HPLC chromatogram of amlodipine and NOR in rabbit plasma is shown in Figure 1. Amlodipine concentrations are shown in Figure 2. There was no significance difference in amlodipine concentrations between the *L. plantarum* IS-10506 supplementation group and the control group at 5 and 15 minutes after amlodipine administration. However, amlodipine concentrations in the *L. plantarum* IS-10506 group were significantly higher than those in the control group at 30, 60, and 120 minutes after amlodipine administration ($P = 0.001$).

L. plantarum IS-10506 supplementation affected some blood hematological

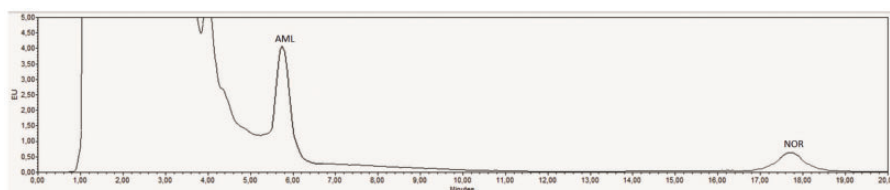


Figure 1. Chromatogram of amlodipine and nortriptyline as an internal standard in rabbit plasma AML: amlodipine; NOR: nortriptyline hydrochloride.

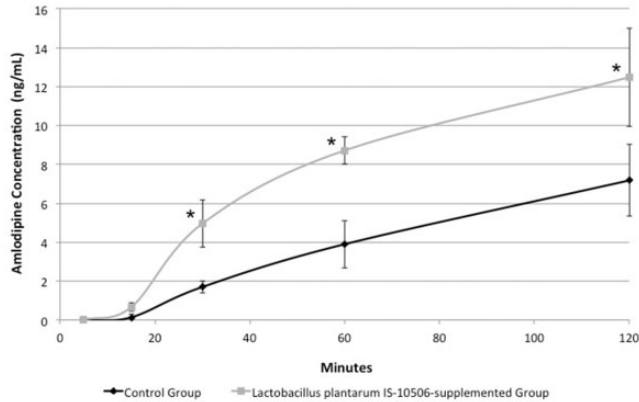


Figure 2. Amlodipine concentrations in rabbit plasma with and without *Lactobacillus plantarum* IS-10506 supplementation.

Table 1. Effect of *Lactobacillus plantarum* IS-10506 supplementation on hematological parameters

Hematological parameter	Control group (mean \pm SD)	<i>Lactobacillus</i> <i>plantarum</i> IS-10506 (mean \pm SD)	P value
White blood cell count ($10^3/\mu\text{L}$)	9.773 \pm 3.401	9.029 \pm 2.178	0.820
Lymphocytes ($10^3/\mu\text{L}$)	3.143 \pm 1.363	2.633 \pm 1.139	0.673
Monocytes ($10^3/\mu\text{L}$)	1.852 \pm 1.036	1.501 \pm 0.818	0.768
Granulocytes ($10^3/\mu\text{L}$)	4.782 \pm 2.713	4.894 \pm 1.899	0.995
Lymphocytes (%)	0.328 \pm 0.127	0.307 \pm 0.140	0.939
Monocytes (%)	0.188 \pm 0.092	0.161 \pm 0.071	0.823
Granulocytes (%)	0.484 \pm 0.207	0.533 \pm 0.117	0.830
Red blood cell count ($10^6/\mu\text{L}$)	4.657 \pm 1.192	5.677 \pm 0.358	0.040
Hemoglobin (g/dL)	11.167 \pm 1.965	12.678 \pm 0.531	0.088
Hematocrit (%)	0.277 \pm 0.056	0.329 \pm 0.016	0.021
Mean corpuscular volume (fL)	60.167 \pm 4.622	57.889 \pm 3.140	0.389
Mean corpuscular hemoglobin (pg)	24.4 \pm 2.519	22.356 \pm 0.930	0.045
Mean corpuscular hemoglobin concentration (g/dL)	40.533 \pm 1.727	38.611 \pm 1.643	0.056
Red cell distribution width (%)	0.174 \pm 0.018	0.171 \pm 0.013	0.879

A P value of less than 0.05 was considered as significant.
SD: standard deviation.

parameters (Table 1). *L. plantarum* IS-10506 supplementation led to a significantly higher RBC count and HCT compared with the control group (both $P < 0.05$). These two parameters may play roles in drug absorption.

Discussion

We performed an *in vivo* experimental study in rabbits to investigate the effect of

L. plantarum IS-10506 supplementation on plasma amlodipine concentrations. The chromatogram of amlodipine and NOR showed that there was good separation of amlodipine and NOR (Figure 1). Our finding of an increase in amlodipine concentrations in the *L. plantarum* IS-10506 group (Figure 2) is in agreement with previous studies. Al Salami and co-workers showed an increase in glimepiride bioavailability in

diabetic rats that were pre-treated with a mixture of three probiotics (*L. acidophilus*, *L. rhamnosus*, and *Bifidobacterium lactis*).¹¹ Furthermore, Matuskova and co-workers showed that concomitant supplementation with the probiotic *Escherichia coli* strain Nissle 1917 was responsible for better amiodarone absorption from the gastrointestinal tract, which led to increased bioavailability of amiodarone.¹⁷

We performed hematological analysis in the control group and *L. plantarum* IS-10506-supplemented group. We found that there was significant increase in RBC and HCT in the *L. plantarum* IS-10506-supplemented group (Table 1). RBC play roles in tissue oxygen delivery. RBC may sense tissue O₂ requirements via their degree of deoxygenation when they travel through the microcirculation and release vasodilatory compounds that enhance blood flow in hypoxic tissues;¹⁸ this vasodilatory effect indicates decreased blood flow. Furthermore, HCT is a global hematological marker of the amount of hemoglobin in blood.¹⁹

Enhancement of blood flow caused by *L. plantarum* IS-10506 supplementation may potentially increase absorption of amlodipine. This possibility is consistent with a study by Crouthamel et al.²⁰ who showed that a reduction in blood flow resulted in progressive impairment of sulfaethidole absorption. Moreover, higher levels of RBC and HCT indicate a lower sedimentation rate. Lowering the sedimentation rate leads to an increase of plasma protein. Amlodipine is a drug bound with plasma protein. Therefore, increasing plasma protein can increase the absorption of amlodipine.

Additionally, *L. plantarum* is a prokaryote that has ATP-binding cassette transporters. Prokaryotic cells are bacteria that can either be exporters or importers.²¹ Amlodipine is a calcium channel blocker that is transported via binding with ATP-

binding cassette transporters.²² The presence of *L. plantarum* provides more ATP-binding cassette transporters to transport amlodipine via the intestinal tract and causes enhancement of amlodipine absorption.

Our study has some limitations. In the current preliminary study, we did not use a hypertensive rabbit model group and non-surviving *L. plantarum* IS-10506 pre-treatment group. This is because of a lack of data on detection of *L. plantarum* IS-10506 at the end of treatment. We also did not investigate the dose response of amlodipine absorption to amlodipine administration and pre-treatment of *L. plantarum* IS-10506 or a normal reference lactic acid bacterium. These limitations are currently under investigation in a follow-up study in our laboratory. Despite these limitations, our study suggests that supplementation of *L. plantarum* IS-10506 significantly increases amlodipine plasma concentrations in rabbits. These results may be due to the ability of *L. plantarum* IS-10506 to enhance blood hematological parameters that play roles in drug absorption.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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