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MICROBIOLOGY

Lactobacillus acidophilus BIOTECH 1900 decreases the transepithelial mucosal-to-serosal transport of colchicine in an *ex vivo* non-everted gut sac model

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Abstract: Colchicine (COL) is a permeability-glycoprotein (P-gp) substrate drug used for familial Mediterranean fever, acute pericarditis, and the management of acute gout. It has a narrow therapeutic index which implies that a small change in the drug's absorption profile may lead to either toxicity or therapeutic failure. Absorption can be altered by modulating the function of P-gp via the concomitant use of drugs, herbal medicines, or food supplements such as probiotics. Here, we investigated the effect of probiotic *Lactobacillus acidophilus* BIOTECH 1900 on COL's transepithelial mucosal-to-serosal transport in the jejunum of ICR mice. A high-performance liquid chromatography-photodiode array (HPLC-PDA) method for the assay of COL was developed and validated. The HPLC-PDA method was applied in an *ex vivo* non-everted gut sac model to measure COL's cumulative mucosal-to-serosal transport and apparent permeability (P_{app}). Treatment of *L. acidophilus* BIOTECH 1900 resulted to a significantly lower COL transport and P_{app} value compared to the control group. Additionally, the activity of *L. acidophilus* BIOTECH 1900 was found to be similar to dexamethasone, a known P-gp inducer. We report that *L. acidophilus* BIOTECH 1900 decreases the transepithelial mucosal-to-serosal transport of COL, suggesting possible P-gp induction. Further studies are recommended to substantiate this transporter-based drug-probiotic interaction.

Key words: colchicine, HPLC-PDA, *Lactobacillus acidophilus*, non-everted gut sac, P-glycoprotein, probiotics.

INTRODUCTION

Gout is a rheumatological disease that occurs through the deposition of uric acid crystals in the joints as a consequence of hyperuricemia (Engel et al. 2017). An ancient medication called colchicine (COL) is used as prophylaxis and considered to be one of the first-line treatments for acute gout (Slobodnick et al. 2017). Although effective, gastrointestinal adverse effects are common which includes diarrhea and abdominal pain (Dalbeth et al. 2014). To minimize these gastrointestinal problems, probiotics are used as adjunct therapy (de Vrese & Marteau 2007). Probiotics are defined as 'live microorganisms, which when administered in adequate amounts, confer health benefits to the host' (FAO/WHO 2006). Strains belonging to *Lactobacillus acidophilus* are commonly used as probiotics (Klaenhammer & Kullen 1999). Recent studies have identified several *L. acidophilus* strains such as LAC0201 and KB27, as novel uricolytic bacteria, capable of lowering serum uric acid levels (Yang et al. 2016, Garcia-Arroyo et al. 2018). This makes *L. acidophilus* a strong candidate in the management of gout. Moreover, previous investigations among patients on concomitant COL and *L. acidophilus* INMIA 9602 Er-2 use

revealed positive clinical outcomes with respect to blood parameters and intestinal microbiome (Balayan et al. 2015, Pepoyan et al. 2017). It is important to note, however, that probiotic activities are strain specific, signifying that the activities of one strain cannot be extrapolated to another. Thus, significant complication like drug interaction is also possible with the concurrent use of COL and a strain of *L. acidophilus*. In pharmacotherapy, drug interaction is problematic especially in drugs that have narrow therapeutic index, COL for instance (Finkelstein et al. 2010), because minor alteration in the concentration of absorbed drug can result to either toxicity or therapeutic failure (Tamargo et al. 2015).

Intestinal transporters play a vital role in the absorption of drugs (Katsura & Inui 2003). An example is permeability-glycoprotein (P-gp) present in the apical side of the enterocytes. It is an ATP-dependent efflux pump that transport drug substrates, such as COL, out of the cells (Druley et al. 2001). Inhibition of P-gp function increases the concentration of drug substrate diffusing across the basolateral membrane of the enterocyte, thereby increasing the drug present in the general circulation. On the contrary, inducing the activity of P-gp increases the concentration of drug substrate that is pumped out to the intestinal mucosa causing a decrease in the bioavailability of the drug (Glaeser 2011). Sertraline (SER) is an example of P-gp inhibitor, while dexamethasone (DEX) is a well-known P-gp inducer (Wessler et al. 2013). Aside from drugs, other modulators of P-gp include herbal remedies, food supplements and probiotics (Saksena et al. 2011, Cho & Yoon 2015). Because probiotics forms a reproducible microbial population in the host's intestine, it may affect the function of intestinal P-gp. To screen articles that modulate P-gp, different experimental models were developed. One of the most commonly performed method is the *ex vivo* model utilizing gut sacs. Albeit laborious process, it offers an accurate evaluation of the P-gp function and allows an evaluation of the interrelationship between P-gp and the article under study (Gameiro et al. 2017).

In the present study, we examined a transporter-based drug-probiotic interaction between COL and *L. acidophilus* BIOTECH 1900. We developed and validated a simple high-performance liquid chromatography–photodiode array (HPLC-PDA) bioanalytical method for the assay of COL that was applied in an *ex vivo* non-everted gut sac model to determine the effect of *L. acidophilus* BIOTECH 1900 on the transepithelial mucosal-to-serosal transport of the drug.

MATERIALS AND METHODS

Chemicals and reagents

Colchicine (≥ 95%) was procured from Sigma-Aldrich, Singapore. The HPLC grade acetonitrile (ACN), water, methanol (MeOH); analytical grade chemicals for the preparation of potassium phosphate buffer and Krebs Ringer Bicarbonate Buffer (KRBB) were procured from Harnwell Chemicals Corporation, Makati City, Philippines. Standard DEX and SER were provided by the Institute of Pharmaceutical Sciences – National Institutes of Health, University of the Philippines Manila. Blood Agar, Sabouraud Dextrose Agar (SDA) and De Man, Rogosa and Sharpe (MRS) broth and agar were received as kind gifts from the Department of Medical Microbiology, College of Public Health, University of the Philippines Manila (DMM). All other chemicals and reagents were of analytical grade or equivalent.

Bioanalytical chromatographic method development

Chromatographic instrumentation

An HPLC-PDA method was developed for the quantification of COL in KRBB which simulates the jejunal fluid. Waters Alliance e2695 HPLC system and 2998 PDA detector was used, equipped with HiQsil C_{18} column (250 mm long, 4.6 mm internal diameter, 5 µm particle size; 100Å pore size). Empower® 3 software was employed for data acquisition.

Chromatographic method optimization

Chromatographic conditions were optimized for the analysis of COL in terms of mobile phase composition and pH, mode of elution, flow rate, UV detection and injection volume. Ambient temperature was used during the analysis with an isocratic mobile phase consisting of 20 mM KH_2PO_4 (pH 3.20) and ACN in 67:33 ratio at a flowrate of 1.20 mL/min. Detection of COL was performed at 350 nm for an injection volume of 10 µL. The run time for the optimized chromatographic method was 7.30 min. The responses of the analyses were evaluated based on tailing factor (*T*), and theoretical plates (*N*).

Preparation of stock solution, calibration standards and quality control samples

Primary stock solution of COL (40 µg/mL) was prepared in MeOH. Calibration standards were prepared in KRBB (7.00 g/L NaCl; 0.34 g/L KCl; 0.046 g/L $MgCl_2$; 1.8 g/L glucose; 0.1 g/L Na_2HPO_4; 0.18 g/L NaH_2PO_4; and 1.26 g/L $NaHCO_3$) by spiking with suitable stock solution dilutions of COL to obtain standard concentrations in the range of 0.29 – 25.00 µg/mL. The resulting solutions were vortexed and filtered using 0.22 µm syringe filter. Three quality control samples namely, low quality control (LQC) - 0.29 µg/mL, medium quality control (MQC) - 12 µg/mL, and high quality control (HQC) - 20 µg/mL were also prepared in the same way as the calibration standards.

Chromatographic method validation

The optimized method for the determination of COL was validated according to the Food and Drug Administration's bioanalytical method validation guide (FDA 2018). Parameters that were evaluated included, specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), carry-over effect, accuracy, and precision. The specificity of the optimized method was assessed by determining any interference of P-gp modulator drugs (DEX and SER) and *L. acidophilus* BIOTECH 1900. The linearity was identified using a calibration curve obtained in triplicate. From the calibration curve, the equation of the line and correlation coefficient were derived. The LOD and LOQ of the optimized method were calculated based on the standard deviation of the response and slope from the data obtained in linearity. For LOD and LOQ, equations 1 and 2 were respectively employed:

$$LOD = 3.3\sigma/S \quad \text{(Eq. 1)}$$

$$LOQ = 10\sigma/S \quad \text{(Eq. 2)}$$

where σ is the standard deviation of the response, while S is the slope of the calibration curve. Carryover effect was identified in triplicate by the injection of a spiked sample at the maximum considered concentration of COL (25 µg/mL) followed by a blank sample. The responses at the retention time of COL were then evaluated. The accuracy and precision of the optimized method within- and between-run were determined using five different spiked samples for each of the quality control samples. Accuracy was expressed as the measured mean concentration and percent recovery, while

precision was expressed by percent relative standard deviation (%RSD).

Transporter-based drug-probiotic interaction study

Microorganism

The probiotic *L. acidophilus* BIOTECH 1900 (Malilay et al. 2019) was procured from the National Institutes of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Laguna. The microorganism was processed and stored at DMM.

Bacterial variability in KRBB with COL

Pure colonies of *L. acidophilus* BIOTECH 1900 were inoculated in KRBB with COL and were aerobically incubated for 60 min at 37°C. The duration was based on the time *L. acidophilus* BIOTECH 1900 was exposed in KRBB in the *ex vivo* non-everted gut sac study. A loopful was obtained, streaked in MRS agar, and underwent 72 h incubation at 37°C. The colonies were characterized based on its morphological appearance, biochemical profile, and gram-staining.

Bacterial preparation

The *L. acidophilus* BIOTECH 1900 was aerobically cultured in MRS broth for 72 h at 37°C. Cells were obtained by centrifugation at 8,000 x *g* for 10 min at 4°C. The bacterial cells were washed twice with KRBB at 4°C and resuspended to a concentration of 1.2 x 10^9 colony-forming units (cfu)/mL in KRBB prior to use.

Health monitoring of experimental animals

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of the Philippines Manila with clearance code 2019-029. Healthy ICR mice were procured from the Research Institute for Tropical Medicine, Philippines. Animals were maintained under conventional conditions with food and water provided *ad libitum*. The animals were housed at the animal room of DMM. The room was assessed for the presence of fungal air contamination by placing an open SDA plate at the centre of the room for 15 min once daily throughout the animal experimentation and incubating the plate at room temperature for 2 days. The photoperiod for animals was kept at 12 h/12 h light/dark cycle. Acclimatization and health monitoring were done for 7 days prior to the *ex vivo* non-everted gut sac study. Animals were visually monitored for any signs of illness or discomfort. Faecal microbiological and endoparasitic assessments were likewise performed at days 1 and 8. For faecal microbiological assessment, faeces were collected, suspended and vortexed in sterile water. A loopful of the resulting solution was streaked in Blood Agar plates to determine any haemolytic enteric microorganisms present. The plates were incubated at 37°C for 24 h. For endoparasitic assessment, a perianal tape test was performed on mice (Parkinson et al. 2011). A cellophane tape was applied on the mouse's perineum, including the perianal area several times. A drop of mineral oil was placed on a glass slide prior to the application of cellophane tape to the slide. Another drop of mineral oil was added on the tape surface and was covered with a cover slip afterwards. The microscope slide was read using 40x objective on a light microscope. Animals that tested positive for enteric haemolytic microorganisms and endoparasites were excluded from the study.

Animal groupings and treatment

The animals were randomly divided into 4 groups and treated as (i) - control group (COL only), (ii) – P-gp inducer control group (DEX + COL), (iii) – P-gp inhibitor control group (SER + COL) and (iv) - treatment group (*L. acidophilus*

BIOTECH 1900 + COL). After acclimatization, overnight fasting was performed before euthanasia, which was the administration of Tiletamine + Zolazepam (Zoletil®) (IM; 40 mg/kg bw) followed by cervical dislocation (Close et al. 1997). Small intestines were excised for the *ex vivo* non-everted gut sac study. To maintain consistency between experimental animals, duodenum (5 cm segment distal to the pyloric sphincter) and ileum (10 cm section extending proximally from the caecum) were first removed. The remaining small intestinal tissue represents jejunum (Mateer et al. 2016).

Ex vivo non-everted gut sac study

Five cm of the jejunum was used in the *ex vivo* non-everted gut sac study, with some modifications from previous methods (Goda et al. 1993, Al-Mohizea 2010, He et al. 2011). The jejunum was washed with cold KRBB. The jejunum was tied from one end using a silk suture and was loaded with 0.3 mL COL solution (25 µg/mL), mixed with DEX (25 µg/mL), SER (25 µg/mL) or *L. acidophilus* BIOTECH 1900 (1.2×10^9 cfu/mL), in KRBB. A control was prepared containing only COL in KRBB. The other end of the jejunum was tied to close the gut, creating a sac. The sac was immersed in a beaker containing 10 mL oxygenated KRBB and maintained at 37°C in a heating block. The KRBB simulates the jejunal fluid, wherein KRBB inside the sac acts as the mucosal medium, while KRBB outside the sac serves as the serosal medium. Samples from the serosal medium (1 mL) were taken at 15, 30, 45 and 60 min. The amount of COL transported from the mucosal to the serosal side was quantified using the HPLC-PDA method developed as described above. For every 1 mL taken from the serosal medium, an equivalent amount of KRBB was replenished. The apparent permeability (P_{app}) of COL in the non-everted gut sac was calculated using the equation 3 below (Ruan et al. 2006):

$$P_{app} = dQ/dt \times (1/AC_0) \quad \text{(Eq. 3)}$$

where dQ/dt is the steady-state appearance rate of COL on the serosal medium, A is the surface area of the intestinal sac, and C_0 is the initial concentration inside the sac.

To ensure that the study was valid, the viability test of the gut sacs was performed by monitoring the glucose transport. Glucose is actively transported in the small intestines; whereby healthy and metabolically active gut sacs concentrate glucose in the serosal side. The concentrations of glucose were measured from the serosal medium using a glucometer and were reported at each time points (Lee et al. 2018).

Statistical analysis

The results were reported as mean ± standard deviation (SD). The statistical difference between groups were determined by one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test using GraphPad Prism 9. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Chromatographic optimization

The bioanalytical method developed was first optimized in terms of various chromatographic conditions to achieve the best chromatographic detection for COL. Several modification in the mode of elution (gradient and isocratic), flowrates (0.80, 1.00 and 1.20 mL/min), pH values of 20 mM KH_2PO_4 (2.88, 3.00 and 3.20), sample injection volumes (1, 5, 10, 15 and 20 µL), UV detection wavelengths (254, 305, 350 nm) and variations in the ratio of mobile phase 20 mM KH_2PO_4:ACN (75:25, 70:30, 67:33, and 65:35) were made. The best chromatographic condition for the detection of COL in KRBB is summarized

in Table I with chromatographic performances summarized in Table II.

Chromatographic method validation

The optimized chromatographic method developed was validated according to the FDA (2018) guidelines on bioanalytical method validation. The method was specific for COL in KRBB (Fig. 1). No interfering peaks were observed at the retention time of COL in the blank KRBB and KRBB spiked with DEX, SER and *L. acidophilus* BIOTECH 1900.

Linearity analysis was performed by the calculation of the regression equation and correlation coefficient from non-zero calibrator levels. A correlation coefficient value of 0.9999 was observed indicating a linear relationship between concentration of COL and the area under the curve. Using the standard deviation of the response and slope from the linearity analysis, the LOD was found to be 0.011 µg/mL, while the LOQ was 0.035 µg/mL. Potential carryover effect was also investigated and was found to be negligible for the developed method since no COL peak was detected. The accuracy and precision of within- and between-run are presented in Table III. The accuracy based on the measured mean concentration was within the allowed deviation from the nominal concentration of 15% for LQC, MQC and HQC samples. Similarly, the within- and between-run precision of the method were acceptable as the %RSD values did not exceed 15% for LQC, MQC and HQC. Hence, the developed method was accurate and precise.

Table I. Optimized chromatographic conditions as a method of detection and quantification of COL in KRBB.

PARAMETER	OPTIMIZED CONDITION
Mode of Elution	Isocratic
Flowrate	1.20 mL/min
pH Value of 20 mM KH_2PO_4	3.20
Sample Injection Volume	10 µL
UV Detection	350 nm
Mobile Phase Ratio (20 mM KH_2PO_4:ACN)	67:33

Health monitoring of experimental animals

No fungal growths were observed in the SDA plates that were exposed in the animal laboratory, indicating that the environment was acceptable in terms of fungal air contamination. Throughout the study, no signs of illness and discomfort were observed from the animals. Faecal microbial analysis showed only non-haemolytic microorganisms. Furthermore, all animals were negative for endoparasitic infestations.

Transepithelial mucosal-to-serosal transport of COL

The scheme for the transepithelial mucosal-to-serosal transport of COL is summarized in Fig. 2a with the corresponding cumulative concentration of COL for each group depicted in Fig. 2b. In the P-gp inducer group DEX, the transported COL in the serosal fluid was significantly lower compared to the P-gp inhibitor group SER and the control group. Likewise, significantly lower concentration of transported COL was also observed in the presence of *L. acidophilus* BIOTECH 1900 when compared with SER and control. Between *L. acidophilus* BIOTECH 1900 and DEX group, no statistically significant differences were observed in the cumulative COL

Table II. Performance parameters for the optimized chromatographic method.

PARAMETER	RESULTS	ACCEPTANCE CRITERIA
Retention time (min)	6.05 ± 0.03	-
Tailing factor (*T*)	0.78 ± 0.01	$T < 2.0$
Theoretical plates (*N*)	9909.63 ± 2896.02	$N > 2000$

concentration in all time points. With respect to the P_{app} values (Fig. 3), no statistically significant difference was observed between *L. acidophilus* BIOTECH 1900 and DEX. Additionally, both groups were found to have statistically significant difference with SER and control groups. In the gut sac viability study, the glucose concentrations in the serosal medium were found to increase from the 15 min reading to the final reading at 60 min in all groups (Fig. 4). No statistically significant differences were observed across all groups. The results suggest that the gut sacs were viable and the concentration of the drugs and *L. acidophilus* BIOTECH 1900 utilized were well tolerated by the jejunum.

DISCUSSION

To the best of our knowledge this is the first study dealing with a bioanalytical method for the assay of COL in the presence of P-gp modulators and probiotic applied in an *ex vivo* non-everted gut sac study. The developed method was precise, accurate and specific for COL in KRBB. No interfering peaks were observed in KRBB spiked with two P-gp modulator drugs, namely, DEX and SER. The absence of DEX and SER peaks indicate that the drugs do not absorb UV at 350 nm given the chromatographic method. In addition, the presence of *L. acidophilus* BIOTECH 1900 did not interfere on the COL peak. In the same way, COL has no effect on the viability of *L. acidophilus* BIOTECH 1900 incubated at 37°C for 60 min.

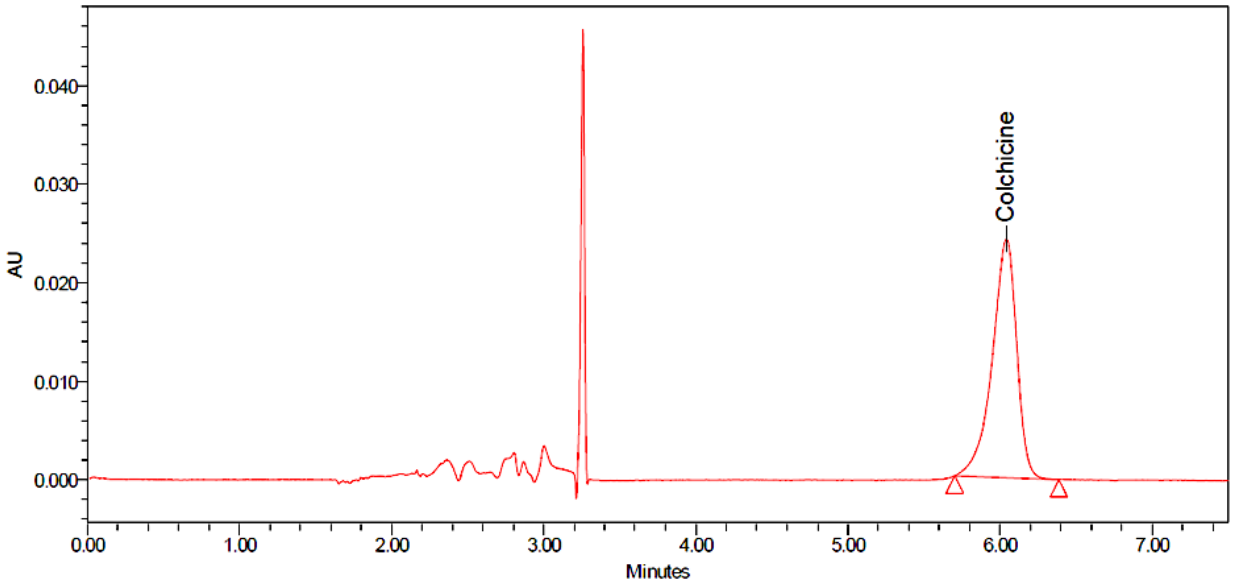

Figure 1. Representative chromatogram of COL using the developed HPLC-PDA method.

Table III. Accuracy and precision of the optimized chromatographic method.

Quality Control Sample	Nominal Concentration (µg/mL)	Within-run			Between-run		
		Measured concentration (µg/mL)	% Recovery	% RSD	Measured concentration (µg/mL)	% Recovery	% RSD
LQC	0.29	0.294	101.26	0.802	0.291	100.53	0.22
MQC	12.00	12.216	101.80	0.037	12.333	102.77	1.30
HQC	20.00	20.438	102.19	0.339	20.594	102.97	0.76

The *L. acidophilus* BIOTECH 1900 is a facultative anaerobic microorganism, allowing it to grow in the presence of oxygen and previous studies have demonstrated that COL hardly affects gut microbes (Shimizu et al. 2016) which includes *L. acidophilus*. In the present study, colonies grew as pinpoint, flat with irregular edges after incubating the MRS agar plates streaked with a loopful of the incubated COL-*L. acidophilus* BIOTECH 1900 mixture. Cells from the colonies were gram-positive bacilli, and were both catalase- and oxidase-negative. These are all prominent characteristics of *L. acidophilus*.

The P-gp is a drug efflux pump of the ATP-binding cassette transporter family that plays an important role in drug absorption (Lin & Yamazaki 2003). In humans, P-gp is encoded by *MDR1* gene and is physiologically expressed in the intestine (De Iudicibus et al. 2008, Katayama et al. 2014). Animal studies concerning P-gp are commonly performed in rodents, such as mice. Mice have two *MDR1* homologues, *mdr1a* and *mdr1b*, which possess important physiological, pharmacological, and toxicological functions, including the transportation of drugs across the cell membrane similar to human *MDR1* (Schinkel et al. 1997, Hoffmann & Kroemer 2004). Moreover, mice are used in pre-clinical studies involving gut since they show similarity with human microbiota at the genus level and are functionally alike (Wang et al. 2017). Thus, mouse is a logical representative to study human intestinal P-gp in *ex vivo* animal setting. In the study, the intestinal health of ICR mice were ensured since bacterial and parasitic infections can degrade the integrity of the intestines thereby disrupting its absorptive function (Siccardi et al. 2008, Di Genova & Tonelli 2016). The absence of haemolytic enteric bacteria and endoparasites allowed the ICR mice to be used in the *ex vivo* non-everted gut sac study, which is one of the most commonly used models in the screening of P-gp modulators (Gameiro et al. 2017). *Ex vivo* gut sac models are sensitive in the analysis of P-gp modulators with an additional advantage of specificity on the intestinal site. In addition, non-everted intestinal rodent sac model offers a means for the prediction of the human absorption of compounds (Ruan et al. 2006).

Since the absorption of drugs is partly regulated by P-gp, any alteration in the P-gp

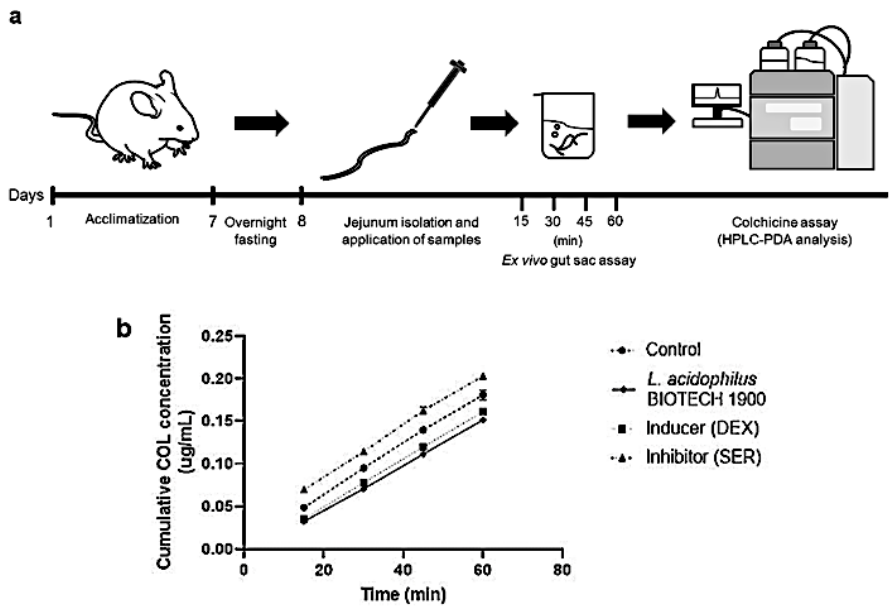

Figure 2. a. Summarized scheme for the transepithelial mucosal-to-serosal transport of COL using an *ex vivo* non-everted gut sac model and HPLC-PDA analysis. b. Cumulative COL concentrations measured from the serosal medium in the *ex vivo* non-everted gut sac model. Values are mean ± SD (n = 3).

function would eventually change the drug's bioavailability. Drugs, herbal medicines, dietary and food supplements, including probiotics are some preparations that can modulate P-gp function (Wang et al. 2012, Cho & Yoon 2015). In general, inhibiting the P-gp function causes an increase in intestinal permeability and transport of the substrate drug from the mucosal to the serosal region of the intestines (Finch & Pillans 2014). In the study, SER was used as the P-gp inhibitor control, which resulted to an increased transepithelial COL transport. Three mechanisms wherein P-gp can be inhibited were proposed, (i) competitive/non-competitive/ allosteric blocking of the drug binding site in P-gp, (ii) interference in the ATP hydrolysis, and (iii) alteration of the cellular membrane lipid integrity (Amin 2013). Conversely, inducing the function of P-gp causes more of the substrate drug to be transported back into the intestinal mucosa resulting to reduced drug concentration

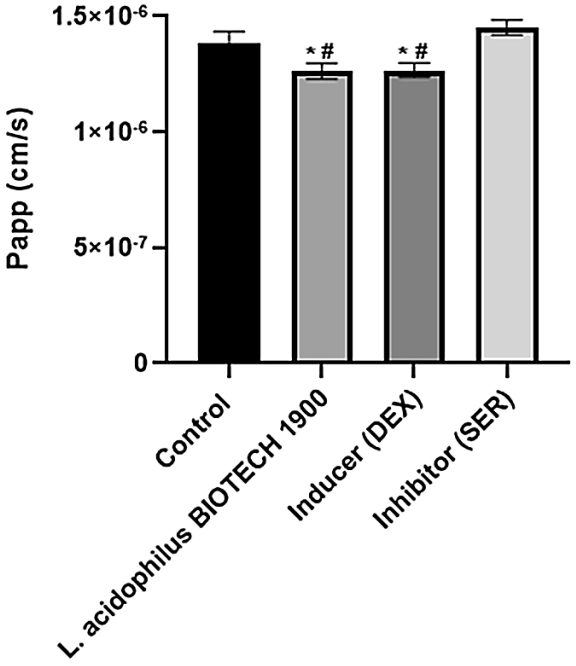

Figure 3. Calculated P_{app} values. Values are mean ± SD (n = 3). * indicate significant difference at *p < 0.05* compared to control. # indicate significant difference at *p < 0.05* compared to inhibitor control (SER).

in the serosal region (Johne et al. 1999, Hamman et al. 2001). The stimulation process reduces the intestinal permeability of the substrate. This was observed in the study's P-gp inducer group, DEX, and *L. acidophilus* BIOTECH 1900. Both groups registered a low cumulative COL concentration in the serosal medium, which is supported by their corresponding low P_{app} values. The data from the treatment group was comparable to the inducer group which may indicate that *L. acidophilus* BIOTECH 1900 also induces P-gp function. Our *ex vivo* findings corroborate with the *in vitro* study of Saksena et al. (2011), suggesting *L. acidophilus* treatment increases the activity of P-gp in Caco-2 cell lines. Although the mechanism of stimulation was not included in the present study, two pathways involved in the expression of P-gp were previously identified, namely, phosphoinositide 3-kinase and ERK1/2 MAPK (Saksena et al. 2011). As with other probiotics, *L. acidophilus* is given at a concentration of more than 10^8 cfu/day to ensure a sustainable population growth in the intestine (Andersson et al. 2010, Gomes et al. 2014). In our study, a concentration of $1.2x10^9$ cfu/mL was introduced in the jejunum to mirror the amount of probiotic that is necessary to form a viable population in the gut. The formation of a reproducible *L. acidophilus* population in the gut may induce P-gp, believed to be mediated by nuclear receptors such as pregnane X receptor and constitutive androstane receptor which are key transcriptional regulators of the efflux pump (Wu et al. 2016). The increase in the number of intestinal efflux pumps, increases the amount of drug substrate that is transported out to the mucosa, provided that ATP is available. Soluble factors secreted by the microorganisms may also have elicited the stimulatory effect on P-gp (Wang et al. 2012, Priyamvada et al. 2016). In general, *L. acidophilus* produces metabolites that are components of

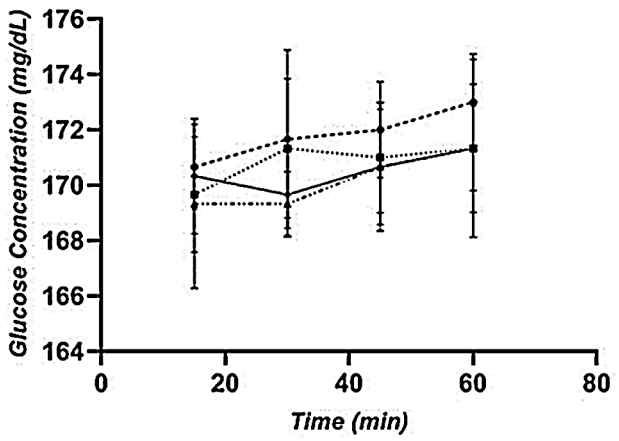

Figure 4. Glucose concentration in the serosal side over time. Values are mean ± SD (n = 3).

biochemical reactions needed in the production of ATP, such as succinic, fumaric and citric acid in the Krebs cycle (Chamberlain et al. 2019). In addition, intestinal microorganisms were found to release ATP that permeates the intestinal epithelium of the host (Proietti et al. 2019). The abundance of ATP can fuel the P-gp function, thereby facilitating the drug transporting mechanism. Further studies are needed to map the metabolites that directly or indirectly affects the action of P-gp. Nevertheless, the discovery that a reproducible amount of *L. acidophilus* BIOTECH 1900 decreases the transepithelial mucosal-to-serosal transport of COL has clinical significance in the field of public health pharmacy and public health microbiology. The extensive use of various probiotics as adjunct therapy might create interactions which may affect the bioavailability of the drug concerned. Thus, profiling of drug-probiotic interactions must be conducted because the activities of these microorganisms are strain specific. In the present study, we identified that *L. acidophilus* BIOTECH 1900 could cause a transporter-based interaction with COL, resulting to the drug's decreased intestinal absorption.

CONCLUSION

A new bioanalytical HPLC-PDA method was developed and validated for the assay of COL. By applying this method, COL concentration was quantified in simulated jejunal fluid from an *ex vivo* non-everted gut sac study. Similar to a P-gp inducer DEX, *L. acidophilus* BIOTECH 1900 decreased the permeability of COL resulting to a decline in the transepithelial transport of the drug from the mucosal to the serosal region of the jejunum. A possible transporter-based drug-microbe interaction might have occurred, which requires further studies to verify the notion and identify the mechanism involved. This kind of interaction should be identified to avoid drug toxicity or therapeutic failure in the clinical use of probiotic as an adjunct treatment.

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REFERENCES

AL-MOHIZEA AM. 2010. Influence of intestinal efflux pumps on the absorption and transport of furosemide. Saudi Pharm J 18: 97-101.

AMIN L. 2013. P-glycoprotein inhibition for optimal drug delivery. Drug Target Insights 7: 27-34.

ANDERSSON U, BRANNING C, AHRNE S, MOLIN G, ALENFALL J, ONNING G, NYMAN M & HOLM C. 2010. Probiotics lower plasma glucose in the high-fat fed C57BL/6J mouse. Benef Microbes 1: 189-196.

BALAYAN M, MANVELYAN A, MARUTYAN S, ISAJANYAN M, TSATURYAN V, PEPOYAN A, MAROTTA F & TOROK T. 2015. Impact of *Lactobacillus acidophilus* INMIA 9602 Er-2 and *Escherichia coli* M-17 on some clinical blood characteristics of familial Mediterranean fever disease patients from the Armenian cohort. Int J Probiotics Prebiotics 10: 133-144.

CHAMBERLAIN CA, HATCH M & GARRETT TJ. 2019. Metabolomic profiling of oxalate-degrading *Lactobacillus acidophilus* and *Lactobacillus gasseri*. PLoS ONE 14: e0222393.

CHO HJ & YOON IS. 2015. Pharmacokinetic interactions of herbs with cytochrome P450 and P-glycoprotein. Evid Alternat Med 2015: 736431.

CLOSE B ET AL. 1997. Recommendations for euthanasia of experimental animals: part 2. Lab Anim 31: 1-32.

DALBETH N, LAUTERIO TJ & WOLFE HR. 2014. Mechanism of action of colchicine in the treatment of gout. Clin Ther 36: 1465-1479.

DE IUDICIBUS D, DE PELLEGRIN A, STOCCO G, BARTOLI F, BUSSANI R & DECEORTI G. 2008. ABCB1 gene polymorphisms and expression of P-glycoprotein and long-term prognosis in colorectal cancer. Anticancer Res 28: 3921-3928.

DE VRESE M & MARTEAU PR. 2007. Probiotics and prebiotics: effect on diarrhea. J Nutr 137: 803-811.

DI GENOVA BM & TONELLI RR. 2016. Infection strategies of intestinal parasite pathogens and host cell responses. Front Microbiol 7: 256.

DRULEY TE, STEIN WD, RUTH A & RONINSON IB. 2001. P-glycoprotein-mediated colchicine resistance in different cell lines correlates with the effects of colchicine on P-glycoprotein conformation. Biochemistry 41: 4323-4331.

ENGEL B, JUST J, BLECKWENN M & WECKBECKER K. 2017. Treatment options for gout. Dtsch Arztebl Int 114: 215-222.

FINCH A & PILLANS P. 2014. P-glycoprotein and its role in drug-drug interactions. Aust Prescr 37: 137-139.

FINKELSTEIN Y, AKS SE, HUTSON JR, JUURLINK DN, NGUYEN P, DUBNOV-RAZ, POLLAK U, KOREN G & BENTUR Y. 2010. Colchicine poisoning: the dark side of an ancient drug. Clin Toxicol 48: 407-414.

FAO/WHO. 2006. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria [online]. Available from: http://fao.org/3/a-a0512e.pdf [accessed December 09, 2019].

FDA. 2018. Bioanalytical method validation guidance for industry [online]. Available from: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf [accessed December 16, 2019].

GAMEIRO M, SILVA R, ROCHA-PEREIRA C, CARMO H, CARVALHO F, BASTOS MDL & REMIAO F. 2017. Cellular models and in vitro assays for the screening of modulators of P-gp, MRP1 and BRCP. Molecules 22: 600.

GARCIA-ARROYO FE ET AL. 2018. Probiotic supplements prevented oxonic acid-induced hyperuricemia and renal damage. PLoS ONE 13: e0202901.

GLAESER H. 2011. Importance of P-glycoprotein for drug-drug interactions. In: FROMM M & KIM R (Eds), Drug transporters. Handbook of experimental pharmacology, Berlin: Springer, p. 285-297.

GODA T, TAKASE S & HOSOYA N. 1993. Maltitol-induced increase of transepithelial transport of calcium in rat small intestine. J Nutr Sci Vitaminol 39: 589-595.

GOMES AC, BUENO AA, DESOUZA RGM & MOTA JF. 2014. Gut microbiota, probiotics and diabetes. Nutr J 13: 60.

HAMMAN MA, BRUCE MA, HAEHNER-DANIELS BD & HALL SD. 2001. The effect of rifampin administration on the disposition of fexofenadine. Clin Pharmacol Ther 69: 114-121.

HE H, SHEN Q & LI J. 2011. Effects of borneol on the intestinal transport and absorption of two P-glycoprotein substrates in rats. Arch Pharm Res 34: 1161-1170.

HOFFMANN U & KROEMER HK. 2004. The ABC transporters MDR1 and MRP2: multiple functions in disposition of xenobiotics and drug resistance. Drug Metab Rev 36: 699-701.

JOHNE A, BROCKMOLLER J, BAUER S, MAURER A, LANGHEINRICH M & ROOTS I. 1999. Pharmacokinetic interaction of digoxin with an herbal extract from St John's wort (*Hypericum perforatum*). Clin Pharmacol Ther 66: 338-345.

KATAYAMA K, NOGUCHI K & SUGIMOTO Y. 2014. Regulations of P-glycoprotein/ABCB1/MDR1 in human cancer cells. New J Sci 2014: 1-10.

KATSURA T & INUI KI. 2003. Intestinal absorption of drugs mediated by drug transporters: mechanisms and regulation. Drug Metab Pharmacokinet 18: 1-15.

KLAENHAMMER TR & KULLEN MJ. 1999. Selection and design of probiotics. Int J Food Microbiol 50: 45-57.

LEE BH, CHOI SH, KIM HJ, PARK SD, RHIM H, KIM HC, HWANG SH & NAH SY. 2018. Gintonin absorption in intestinal model systems. J Ginseng Res 42: 35-41.

LIN JH & YAMAZAKI M. 2003. Role of P-glycoprotein in pharmacokinetics: clinical implication. Clin Pharmacokinet 42: 59-98.

MALILAY JKT, OLIVEROS MCR, BAUTISTA JAN & CASTILLO-ISRAEL KAT. 2019. Functional, safety and technological properties of *Lactobacillus acidophilus* BIOTECH 1900. Phil J Vet Anim Sci 45: 11-21.

MATEER SW, CARDONA J, MARKS E, GOGGIN BJ, HUA S & KEELY S. 2016. *Ex vivo* intestinal sacs to assess mucosal permeability in models of gastrointestinal disease. J Vis Exp 108: 53250.

PARKINSON CM, O'BRIEN A, ALBERS TM, SIMON MA, CLIFFORD CB & PRITCHETT-CORNING KR. 2011. Diagnosis of ecto- and endoparasites in laboratory rats and mice. J Vis Exp 55: e2767.

PEPOYAN AZ, BALAYAN MH, MANVELYAN AM, MAMIKONYAN V, ISAJANYAN M, TSATURYAN VV, KAMIYA S, NETREBOV V & CHIKINDAS ML. 2017. *Lactobacillus acidophilus* INMIA 9602 Er-2 strain 317/402 probiotic regulates growth of commensal *Escherichia coli* in gut microbiota of familial Mediterranean fever disease subjects. Lett Appl Microbiol 64: 254-260.

PRIYAMVADA S, ANBAZHAGAN AN, KUMAR A, SONI V, ALREFAI WA, GILL RK, DUDEJA PK & SAKSENA S. 2016. *Lactobacillus acidophilus* stimulates intestinal P-glycoprotein expression via a c-Fos/c-Jun-dependent mechanism in intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 310: G599-608.

PROIETTI M ET AL. 2019. ATP released by intestinal bacteria limits the generation of protective IgA against enteropathogens. Nat Commun 10: 250.

RUAN LP, CHEN S, YU BY, ZHU DN, CORDELL GA & QIU SX. 2006. Prediction of human absorption of natural compounds by non-everted rat intestinal sac model. Eur J Med Chem 41: 605-610.

SAKSENA S, GOYAL S, RAHEJA G, SINGH V, AKHTAR M, NAZIR TM, ALREFAI WA, GILL RK & DUDEJA PK. 2011. Upregulation of P-glycoprotein by probiotics in intestinal epithelial cells and in the dextran sulfate sodium model of colitis in mice. Am J Physiol Gastrointest Liver Physiol 300: G1115-1123.

SCHINKEL AH ET AL. 1997. Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci U S A 94: 4028-4033.

SHIMIZU J, KUBOTA T, TAKADA E, TAKAI K, FUJIWARA N, ARIMITSU N, UEDA Y, WAKISAKA S, SUZUKI T & SUZUKI N. 2016. *Bifidobacteria* abundance-featured gut microbiota compositional change in patients with Behcet's disease. PLoS ONE 11: e0153746.

SICCARDI D, MUMY KL, WALL DM, BIEN JD & MCCORMICK BA. 2008. *Salmonella enterica* serovar Typhimurium modulates P-glycoprotein in the intestinal epithelium. Am J Physiol Gastrointest Liver Physiol 294: 1392-1400.

SLOBODNICK A, SHAH B, KRASNOKUTSKY S & PILLINGER MH. 2017. Update on colchicine, 2017. Rheumatology 57: i4-i11.

TAMARGO J, LE HEUZEY JY & MABO P. 2015. Narrow therapeutic index drugs: a clinical pharmacological consideration to flecainide. Eur J Clin Pharmacol 71: 549-567.

WANG P, SHEHU AI & MA X. 2017. The opportunities of metabolomics in drug safety evaluation. Curr Pharmacol Rep 3: 10-15.

WANG Y, LIU Y, SIDHU A, MA Z, MCCLAIN C & FENG W. 2012. *Lactobacillus rhamnosus* GG culture supernatant ameliorates acute alcohol-induced intestinal permeability and liver injury. Am J Physiol Gastrointest Liver Physiol 303: 32-41.

WESSLER JD, GRIP LT, MENDELL J & GIUGLIANO RP. 2013. The P-glycoprotein transport system and cardiovascular drugs. J Am Coll Cardiol 61: 2495-2502.

WU J ET AL. 2016. Induction of P-glycoprotein expression and activity by *Aconitum* alkaloids: implication for clinical drug-drug interactions. Sci Rep 6: 25343.

YANG D, YU X, WU Y, CHEN X, WEI H, SHAH NP & XU F. 2016. Enhancing flora balance in the gastrointestinal tract of mice by lactic acid bacteria from Chinese sourdough and enzyme activities indicative of metabolism of protein, fat, and carbohydrate by the flora. J Dairy Sci 99: 7809-7820.

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