Manuscript number: BDD-13-0083

Date of Revision: February , 2014

Inhibitory effect of ciprofloxacin on β-glucuronidase-mediated deconjugation of mycophenolic acid glucuronide

Takaaki Kodawara[a.b], Satohiro Masuda[b,c*], Yoshitaka Yano[d], Kazuo Matsubara[b], Toshiaki Nakamura[a], Mikio Masada[a]

[a]Department of Pharmacy, Fukui University Hospital, Faculty of Medical Sciences Fukui University, Yoshida-gun, Fukui 910-1193, Japan

[b]Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

[d]Education and Research Center for Clinical Pharmacy, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan

[c]Present address: Department of Pharmacy, Kyushu University Hospital, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Funding: Supported in part by a Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Science, Culture, Sports, and Technology of Japan (MEXT) and by a Funding Program for Next Generation World-Leading Researchers (NEXT Program: LS073) initiated by the Council for Science and Technology Policy of the Japan Society for the Promotion of Science of Japan.

Running title: Ciprofloxacin-MPAG interaction via β-glucuronidase

Correspondence to: Satohiro Masuda, Ph.D.

Professor and Director

Department of Pharmacy

Kyushu University Hospital

Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

TEL number: +81-92-642-5918

FAX number: +81-92-642-5937

E-mail address for this manuscript: masuda@kuhp.kyoto-u.ac.jp

Present e-mail address: satomsdb@pharm.med.kyushu-u.ac.jp

Abstract

The interaction between mycophenolate (MPA) and quinolone antibiotics such as ciprofloxacin is considered to reduce the enterohepatic recycling of MPA, which is biotransformed in the intestine from MPA glucuronide (MPAG) conjugate excreted via the biliary system; however, the molecular mechanism underlying this biotransformation of MPA is still unclear. In this study, we established an in vitro system to evaluate β-glucuronidase-mediated deconjugation and examined the influence of ciprofloxacin on the enzymatic deconjugation of MPAG and MPA resynthesis. Resynthesis of MPA via deconjugation of MPAG increased in a time-dependent manner from 5 to 60 min in the presence of β-glucuronidase. Ciprofloxacin and phenolphthalein-β-D-glucuronide (PhePG), a typical β-glucuronidase substrate, significantly decreased the production of MPA from MPAG in the β-glucuronidase-mediated deconjugation system. In addition, enoxacin significantly inhibited the production of MPA from MPAG, while levofloxacin and ofloxacin had no inhibitory effect on MPA synthesis. Pharmacokinetic analysis revealed that ciprofloxacin showed a dose-dependent inhibitory effect on MPA production from MPAG via β-glucuronidase with a half-maximal inhibitory concentration (IC_{50}) value of 30.4 μM. While PhePG inhibited the β-glucuronidase-mediated production of MPA from MPAG in a competitive manner, ciprofloxacin inhibited MPA synthesis via noncompetitive inhibition. These findings suggest that reduction in the serum MPA concentration during the co-administration of ciprofloxacin is at least in part due to the decreased enterohepatic circulation of MPA because of noncompetitive inhibition of deconjugation of MPAG by intestinal β-glucuronidase.

Keywords

Ciprofloxacin, MPAG, drug interaction, β-glucuronidase

Introduction

Mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil (MMF), has been introduced into immunosuppressive protocols after solid organ transplantation and against autoimmune diseases [1, 2]. MPA interferes with the *de novo* pathway of synthesis of purine nucleic acid through the inhibition of inosine monophosphate dehydrogenase and thus inhibits T and B cell proliferation [3]. MPA is a well-established prophylactic agent when used in combination with calcineurin inhibitors after kidney and pancreas transplantation and has largely replaced azathioprine in the immunosuppressive regimens for organ transplant recipients [4, 5]. After oral administration, MMF is rapidly converted to MPA in the body and is excreted as the glucuronide conjugate (MPAG) in the urine and bile. MPAG excreted in the bile is subject to deconjugation in the small intestine, where it is converted back to MPA and therefore the resynthesized MPA is reabsorbed from the intestinal lumen (enterohepatic recycling). MPAG is thought to be cleaved by gram-negative and anaerobic gut bacteria, which express the β-glucuronidase enzyme. Enterohepatic recycling of MPA is indicated by the presence of "a second MPA peak" at approximately 6 to 8 h after dosing and by the recovery of MPAG in the bile of orthotopic liver transplant patients [6]. Antibiotics are indispensable for prophylaxis against an opportunistic infection in transplant patients who are administered immunosuppressive agents. However, the use of antibiotics can be associated with a reduction in the second peak of MPA as a result of disruption of the normal intestinal flora, which possesses β-glucuronidase activity. Schmidt *et al.* [7] reported a substantial reduction of 30 % in the area under the concentration-time

curve (AUC) of MPA in 6 liver transplant recipients who underwent selective bowel decontamination with tobramycin and cefuroxime. Multivariate analysis performed by Borrows *et al*. [8] indicated that amoxicillin/clavulanate potassium (Augmentin), ciprofloxacin, and metronidazole are responsible for the trough level of MPA in 121 kidney transplant patients. In addition, the decrease in the MPA level (1.1 mg/L) in the course of 7 days of antibiotic therapy with ciprofloxacin or amoxicillin/clavulanic acid recovered on the third day after cessation of antibiotic therapy in the stable renal transplant patients [9]. These findings and backgrounds indicate that the variation in MPA pharmacokinetics cannot be explained only by disruption of the intestinal flora with antibiotics, and the presence of some other mechanism(s) should be clarified. However, the molecular mechanisms underlying direct interaction between MPA and the antibiotics, which affect the pharmacokinetics of MPA, have not been proven thus far. The purpose of this study was to clarify the β-glucuronidase-mediated biotransformation of MPAG to MPA and explain that this mechanism may be a potential target of interaction between MPAG and antibiotics.

Materials and Methods

MPA and phenolphthalein-β-D-glucuronide (PhePG) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ciprofloxacin was kindly supplied by Mitsubishi Tanabe Pharma Co. (Tokyo, Japan). Levofloxacin and ofloxacin was a gift from Daiichi Sankyo Co. Ltd. (Tokyo, Japan). β-Glucuronidase was purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). MPAG was purchased from LC laboratories (Boston, MA). All other chemicals used were of the highest purity available.

Enzymatic assay of β-glucuronidase

Deconjugation of MPAG was performed using β-glucuronidase in a buffer solution. The composition of an incubation buffer was potassium phosphate buffer 75 mM with 1.0% (w/v) bovine serum albumin (pH 6.8) [10, 11]. The pH of the buffer was adjusted with potassium hydroxide (KOH) solution. The samples for enzyme assay experiments were preincubated with 230 μL of incubation buffer for 10 min at 25 ℃. Then, 20 μL of the buffer containing β-glucuronidase was added to the incubation buffer for 30 s, and 50 μL of MPAG buffer was added followed by incubation for specified periods at 25 ℃. Inhibitors were added to the incubation buffer during preincubation. The enzymatic reaction was stopped using methanol solution, and the sample was cooled in an ice bath for 20 min. The amounts of enzyme and temperature varied in experiments that were performed to determine the desirable conditions.

Sample analysis

The MPA and MPAG concentrations were measured using modifications to previously published assays using HPLC (model LC-10A; Shimadzu, Kyoto, Japan) [12]. Standard curves were constructed with pure MPA (Sigma) and MPAG dissolved in methanol and added to MPA-free or MPAG-free phosphate buffer. For MPA and MPAG analysis, the samples (300 µL) were 850 µL of methanol mixed with the internal standard solution (indomethacin), and the solution was vortex-mixed for 1 min. After the mixture was centrifuged for 5 min at 10,000*g*, an 800-µL aliquot of the clear supernatant was evaporated and reconstituted in the mobile phase of acetonitrile/50 mM phosphoric acid (30/70). The solution was vortex-mixed for 1 min and centrifuged for 5 min at 10,000*g*, and a 50-µL aliquot of the clear supernatant was injected onto a ZORBAX ODS column (5 µm, 4.6 × 250 mm; Agilent Technologies, USA) maintained at 40 °C. MPAG was measured at a wavelength of 254 nm with a switch to 330 nm for MPA, and the internal standard was measured at a wavelength of 254 nm. The retention time was 4.5 min for MPAG, 13.9 min for MPA, and 20 min for the internal standard. The standard curves of MPA and MPAG were linear from 0 to 120 µg/mL and 0 to 250 µg/mL, respectively. Detection limits of both MPA and MPAG in this study were 0.3 µg/mL.

Statistical analysis

The kinetic parameters of maximum velocity (Vmax) and Michaelis constant (Km), were calculated by fitting the rate data to the Michaelis–Menten equation for single enzyme using

regression curve plot (KaleidaGraph). The time course data of MPAG and MPA concentration (μM) were simultaneously fitted to a linear first-order degradation model for each of the three conditions (control, ciprofloxacin, and PhePG). The model equations are given as follows;

$$C(t)_{MPAG} = C(0)_{MPAG} \cdot \exp(-k \cdot t) \qquad \text{Eq.(1)}$$

$$C(t)_{MPA} = C(0)_{MPA} + MAX \cdot (1 - exp(-k \cdot t)) \qquad \text{Eq.(2)}$$

where *C(t)* is the concentration of MPAG or MPA (identified by subscripts) at time t, *C(0)* is the concentration at time zero, *k* is the first-order degradation rate constant from MPAG to MPA, and MAX is the maximum concentration of MPA at infinite time. The values for $C(0)_{MPAG}$, $C(0)_{MPA}$, *k* and *MAX* were estimated by a least squares method using the Microsoft Excel solver tool. Statistical significance of differences between mean values was calculated using the non-paired *t*-test. Multiple comparisons were performed using the Scheffé's *F*-test after analysis of variance (ANOVA). A *p* value less than 0.05 was statistically significant.

Results

Effect of β-glucuronidase concentration on the deconjugation of MPAG

We first examined resynthesis of MPA from MPAG dependent on the concentrations of β-glucuronidase in the potassium phosphate buffer. The MPA production gradually increased with an increase in the incubation time from 15 min to 60 min, and MPA production increased in a concentration-dependent manner of β-glucuronidase (Fig. 1). In contrast to MPA resynthesis, the residual MPAG concentration in the reaction mixture decreased in the time- and enzymatic activity-dependent manner (data not shown). Based on these results and the technical limitation of quantitation by HPLC, the desirable incubation time and enzymatic activity for deconjugation by β-glucuronidase in the present study were set at 30 min and 0.066 U/mL, respectively.

Effects of coexistence of ciprofloxacin or PhePG on the production of MPA

We examined the effect of the coexistence of ciprofloxacin or PhePG on MPA production from MPAG. MPA production increased with an increase in the incubation time of 15, 30, 45, 60, and 90 min, but it was completely inhibited in the presence of either 500 μM ciprofloxacin or PhePG (Fig. 2A). The concentration of residual MPAG decreased slightly in the presence of 500 μM ciprofloxacin or PhePG (Fig. 2B).

Influence of coexistence of levofloxacin, ofloxacin, or enoxacin on β-glucuronidase-mediated deconjugation of MPAG

Next, we examined the influence of levofloxacin, ofloxacin, or enoxacin on β-glucuronidase-mediated deconjugation of MPAG at the same concentration as that used above (500 μM). Production of MPA from MPAG increased; however, MPA production markedly inhibited in the presence of PhePG, ciprofloxacin, and enoxacin, but not in the presence of levofloxacin and ofloxacin (Fig. 3).

Effect of concentration of ciprofloxacin and PhePG on β-glucuronidase-mediated deconjugation of MPAG

Before calculating the half-maximal inhibitory concentration (IC_{50}) values of ciprofloxacin and PhePG on β-glucuronidase-mediated deconjugation activity by measuring the MPA production from MPAG, we examined whether β-glucuronidase-mediated deconjugation of MPAG follows Michaelis–Menten kinetics by measuring a wide range of concentrations. The MPA production from MPAG increased in a dose-dependent manner; Vmax, 8.3 ± 0.2 μmol/U/30 min and Km, 1494 ± 57.2 μM (mean ± standard deviation [SD] for 3 experiments; Fig. 4). Then, we measured the resynthesis of MPA from 1500 μM of MPAG in the presence of various concentrations of ciprofloxacin or PhePG. The production of MPA from MPAG markedly decreased in the presence of ciprofloxacin or PhePG in a dose-dependent manner with the IC_{50} values of 30.4 ± 4.1 μM and 218 ± 11.0 μM, respectively (Fig. 5).

Mode of inhibition of ciprofloxacin and PhePG on the resynthesis of MPA from MPAG

To further understand the inhibitory effect of ciprofloxacin and PhePG on the β-glucuronidase-mediated deconjugation of MPAG, we examined the concentration-dependent production of MPA from MPAG in the presence or absence of ciprofloxacin and PhePG. The production of MPA from MPAG significantly decreased in the presence of PhePG (200μM), and the decrease in MPA production was much more in the presence of ciprofloxacin (30μM) (Fig. 6). The Eadie–Hofstee plot after correction of the nonsaturable components is shown in the inset in Fig. 6. These results showed that PhePG inhibited the β-glucuronidase-mediated deconjugation of MPAG in a competitive manner, while ciprofloxacin inhibited it in a noncompetitive manner.

Least squares fitting by a linear model

Finally, we analyzed the time course data about the inhibitory potency of PhePG and ciprofloxacin against deconjugation of MPAG. In the model analysis, we set the parameters *C(0)* for MPAG and MPA because the concentration data at the starting time (t = 0) was not zero, and similar values were estimated for $C(0)_{MPAG}$ and $C(0)_{MPA}$ in all conditions, which were almost equal to the initial concentration of MPAG added into the experimental system. The estimated parameters were shown in Table 1. The parameter MAX for PhePG and ciprofloxacin are much greater than the observed concentration at the last sampling time point (90min), indicating that the concentration of these compound increased almost linearly within 90min. The estimated values for MAX in all conditions were lower than the initial MPAG concentration, suggesting that deconjugation of MPAG into MPA were not complete and some compounds except MPA

were suggested to be generated. The parameter *k* differs in ciprofloxacin and PhePG from control data, suggesting the deconjugation process was inhibited by these two conditions. The *k* values were around 4% (PhePG) or 3% (ciprofloxacin) of the parameter in the control condition, suggesting the deconjugation processes of the β-glucuronidase were inhibited by these inhibitors.

Discussion

MPA is frequently used in combination with calcineurin inhibitors and steroids to prevent rejection after organ transplantation. Shaw *et al*. [13] suggested that trough MPA levels between 1 and 3.5 μg/mL may reduce toxicity and acute rejection episodes after renal transplantation. If the serum MPA concentration is decreased because of interactions between some drugs, the risk of cellular rejection may also increase because of insufficient pharmacological effect. Borrows *et al*. [9] reported that the 12-h predose serum MPA concentration in renal transplant patients decreased during concomitant administration of ciprofloxacin. Because the enterohepatic recycling of MPA is initiated by the deconjugation of MPAG by endogenous bacteria in the small intestine, it is possible that decreasing the bacterial content using broad-spectrum antibiotic therapy will reduce the reuptake of MPA at the intestinal wall. β-glucuronidase is produced by gram-negative bacilli such as *Escherichia coli*, which are a part of the normal human intestinal flora [14]. Treatment with antibiotics must be able to effective against intestinal flora because *E. coli* is markedly sensitive to quinolone antibiotics such as ciprofloxacin and levofloxacin [15]. However, not every quinolone antibiotic affects the deconjugation of MPAG and enterohepatic circulation of MPA in organ transplant patients. We first created an in vitro model of intestinal bacterium-mediated deconjugation of MPAG and examined the effects of MPA production from MPAG via β-glucuronidase. In addition, we examined the effects of ciprofloxacin and PhePG on MPA production from MPAG. The MPA production by β-glucuronidase depends on the incubation time and β-glucuronidase concentration (Fig. 1), and it is inhibited in the presence of PhePG and

ciprofloxacin (Fig. 2A). Therefore, we suggested that ciprofloxacin affected the deconjugation of MPAG by direct inhibition of β-glucuronidase as well as elimination of the intestinal flora. In the in vitro deconjugation model, MPA production from MPAG was significantly inhibited in the presence of ciprofloxacin and enoxacin but not in the presence of levofloxacin and ofloxacin (Fig. 3). Levofloxacin and ofloxacin include a morpholine on a chemical structural formula and bearing a methyl group in the piperazine skelton. However, it is not observed similar features in the ciprofloxacin and enoxacin. Norfloxacin with clinical report also has a feature similar to the latter. Therefore, these structural features might relate inhibitory potency against β-glucuronidase.

The concentration of ciprofloxacin in the deconjugation model is much higher than its clinical dose used for infectious diseases and thus the results of this interaction cannot be extrapolated to a clinical scenario. Therefore, we examined the IC_{50} values of ciprofloxacin by measuring the production of MPA from MPAG to confirm whether such an interaction can occur in clinical practice. Our results showed that the IC_{50} value of ciprofloxacin was 30.4 μM (Fig. 5), and the range of intestinal ciprofloxacin concentration was considered to be sufficient compared to that in patients taking ciprofloxacin [16]. MPA is mainly metabolized by uridine diphosphate glucuronosyltransferases in the gastrointestinal tract, liver and kidney into the inactive MPAG metabolite [17]. According to data of the pharmaceutical company, in MMF given to rats, approximately its 77.3 % and 21.0 % were excreted in bile and urine, respectively. The ratio of MPAG excreted in bile is 84.0 %, but MPA excretion into bile was less then 1.0 % of dose [18].

MPA and MPAG are subject to enterohepatic circulation, which can account for up to 10 to 60% of the total dose-interval MPA area under the concentration time curve (AUC) [19]. Therefore, the inhibitory effect of ciprofloxacin on deconjugation of MPAG may influence the pharmacokinetics of MPA, because most of MPAG is supplied in gastrointestinal tract by bile flow. However, some in vivo examinations with animals and/or humans should be required to show the significance of the present results in future.

The interaction between ciprofloxacin and MPAG in a clinical setting was considered to decrease the serum concentration of MPA because of the reduction in MPAG deconjugation by intestinal *E. coli*-mediated inhibition of β-glucuronidase. In the present study, we clarified that ciprofloxacin-mediated reduction of deconjugation of MPAG was based on the direct inhibition of β-glucuronidase. Our results showed that the *k* value of β-glucuronidase-mediated deconjugation of MPAG decreased approximately 96 % in the presence of PhePG in a competitive manner (Fig. 6, Table 1). On the other hand, the *k* value decreased to approximately 97 % in the presence of ciprofloxacin in a non-competitive manner, which suggested that the binding sites of ciprofloxacin and PheG on MPAG were not the same. In the present study, the reaction time of 30 min is not suitable to further discuss about the β-glucuronidase activity for the less linearity in the time course experiment (Fig. 1). However, the parameters (*k* and MAX) obtained in data analysis may be useful in the evaluation of inhibitors within the limitation of the present experimental condition. So, the examination using optimal conditions needs to determine the inhibition mechanism.

Recently, coadministration of co-amoxiclav in patients taking MMF after renal transplantation decreased the AUC of MPA in 2 patients, and the AUC of MPA returned to normal in 1 patient several days after discontinuation of co-amoxiclav [20]. The MPAG-antibiotic interaction varied with different drugs and in different patients; thus, factors other than inhibition of β-glucuronidase are also involved in decrease in MPA production. In addition, the coadministration intravenous ciprofloxacin in a patient receiving MMF after bone marrow transplantation decreased the AUC of MPA by one-third on day 8 than that on day 2, and the patient developed severe graft-versus-host disease and died [21]. Tanimura *et al*. [22] reported that a part of ciprofloxacin is excreted in the bile from the digestive tract.

Conclusion

In conclusion, MPA pharmacokinetics may be affected in patients treated with oral or intravenous administration of ciprofloxacin by inhibiting the β-glucuronidase-mediated deconjugation of MPAG in the small intestine. In addition, some other drugs, which inhibit the intestinal deconjugation of MPAG by β-glucuronidase and thus decrease the MPA exposure, can be easily evaluated using the present in vitro system.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Science, Culture, Sports, and Technology of Japan (MEXT) and by a Funding Program for Next Generation World-Leading Researchers (NEXT Program: LS073) initiated by the Council for Science and Technology Policy of the Japan Society for the Promotion of Science of Japan.

Footnotes

Conflict of interest:

The authors declare no conflict of interest.

Abbreviations

MMF	mycophenolate mofetil
MPAG	mycophenolic acid glucuronide
MPA	mycophenolic acid
PhePG	phenolphthalein-β-D-glucuronide
E. coli	*Escherichia coli*
HPLC	high performance liquid chromatography

References

1. Simmons WD, Rayhill SC, Sollinger HW. Preliminary risk-benefit assessment of mycophenolate mofetil in transplant rejection. Drug safety : an international journal of medical toxicology and drug experience 1997; **17**: 75-92. [PubMed: 9285199]

2. Zimmer-Molsberger B, Knauf W, Thiel E. Mycophenolate mofetil for severe autoimmune haemolytic anemia. Lancet 1997; **350**: 1003-1004. [PubMed: 9329520]

3. Wu JC. Mycophenolate mofetil: Molecular mechanisms of action. Perspectives in Drug Discovery and Design 1994; **2**: 185-204.

4. Merion RM, Henry ML, Melzer JS, Sollinger HW, Sutherland DE, Taylor RJ. Randomized, prospective trial of mycophenolate mofetil versus azathioprine for prevention of acute renal allograft rejection after simultaneous kidney-pancreas transplantation. Transplantation 2000; **70**: 105-111. [PubMed: 10919583]

5. Oh JM, Wiland AM, Klassen DK, Weidle PJ, Bartlett ST. Comparison of azathioprine and mycophenolate mofetil for the prevention of acute rejection in recipients of pancreas transplantation. J Clin Pharmacol 2001; **41**: 861-869. [PubMed: 11504274]

6. Shaw LM, Nowak I. Mycophenolic acid: measurement and relationship to pharmacologic effects. Therapeutic drug monitoring 1995; **17**: 685-689. [PubMed: 8588242]

7. Schmidt LE, Rasmussen A, Norrelykke MR, Poulsen HE, Hansen BA. The effect of selective bowel decontamination on the pharmacokinetics of mycophenolate mofetil in liver transplant recipients. Liver Transplant 2001; **7**: 739-742. [PubMed: 11510021]

8. Borrows R, Chusney G, James A, Stichbury J, Van Tromp J, Cairns T, Griffith M, Hakim N, McLean A, Palmer A, Papalois V, Taube D. Determinants of mycophenolic acid levels after renal transplantation. Therapeutic drug monitoring 2005; **27**: 442-450. [PubMed: 16044100]

9. Borrows R, Chusney G, Loucaidou M, James A, Van Tromp J, Cairns T, Griffith M, Hakim N, McLean A, Palmer A, Papalois V, Taube D. The magnitude and time course of changes in mycophenolic acid 12-hour predose levels during antibiotic therapy in mycophenolate mofetil-based renal transplantation. Therapeutic drug monitoring 2007; **29**: 122-126. [PubMed: 17304160]

10. W.H. F. Methods of Enzymatic Analysis. 1974; **Vol. II. 2nd Edition. Academic Press, New York,** : pp. 930-932.

11. Kim DH, Jin YH, Jung EA, Han MJ, Kobashi K. Purification and characterization of beta-glucuronidase from Escherichia coli HGU-3, a human intestinal bacterium. Biological & pharmaceutical bulletin 1995; **18**: 1184-1188. [PubMed: 8845801]

12. Seebacher G, Weigel G, Wolner E, Mallinger R, Grimm M, Laufer G, El Menyawi I, Griesmacher A, Muller MM. A simple HPLC method for monitoring mycophenolic acid and its glucuronidated metabolite in transplant recipients. Clinical chemistry and laboratory medicine : CCLM / FESCC 1999; **37**: 409-415. [PubMed: 10369111]

13. Shaw LM, Holt DW, Oellerich M, Meiser B, van Gelder T. Current issues in therapeutic drug monitoring of mycophenolic acid: report of a roundtable discussion. Therapeutic drug monitoring 2001; **23**: 305-315. [PubMed: 11477311]

14. Sperker B, Backman JT, Kroemer HK. The role of beta-glucuronidase in drug disposition and drug targeting in humans. Clinical pharmacokinetics 1997; **33**: 18-31. [PubMed: 9250421]

15. Mitscher LA. Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents. Chem Rev 2005; **105**: 559-592. [PubMed: 15700957]

16. Gonzalez MA, Uribe F, Moisen SD, Fuster AP, Selen A, Welling PG, Painter B. Multiple-dose pharmacokinetics and safety of ciprofloxacin in normal volunteers. Antimicrob Agents Chemother 1984; **26**: 741-744. [PubMed: 6517556]

17. Bowalgaha K, Miners JO. The glucuronidation of mycophenolic acid by human liver, kidney and jejunum microsomes. British journal of clinical pharmacology 2001; **52**: 605-609. [PubMed: 11736871]

18. Westley IS, Brogan LR, Morris RG, Evans AM, Sallustio BC. Role of Mrp2 in the hepatic disposition of mycophenolic acid and its glucuronide metabolites: effect of cyclosporine. Drug metabolism and disposition: the biological fate of chemicals 2006; **34**: 261-266. [PubMed: 16272406]

19. Schutz E, Shipkova M, Armstrong VW, Wieland E, Oellerich M. Identification of a pharmacologically active metabolite of mycophenolic acid in plasma of transplant recipients treated with mycophenolate mofetil. Clinical chemistry 1999; **45**: 419-422. [PubMed: 10053049]

20. Ratna P, Mathew BS, Annapandian VM, Saravanakumar K, Basu G, Tamilarasi V, Fleming DH. Pharmacokinetic drug interaction of mycophenolate with co-amoxiclav in renal

transplant patients. Transplantation 2011; **91**: e36-38. [PubMed: 21383599]

21. Goutelle S, Mialou V, Gouraud A, Parant F, Bleyzac N. Probable drug interaction between intravenous ciprofloxacin and mycophenolate mofetil in a bone marrow transplant recipient. Pharmacotherapy 2011; **31**: 114.

22. Tanimura H, Tominaga S, Rai F, Matsumoto H. Transfer of ciprofloxacin to bile and determination of biliary metabolites in humans. Arzneimittel-Forschung 1986; **36**: 1417-1420. [PubMed: 3790196]

Figure legends

Fig. 1. Effects of mycophenolic acid resynthesis from mycophenolic acid glucuronide using different concentrations of β-glucuronidase

The buffer was incubated at 25 °C with 500 μM mycophenolic acid glucuronide (MPAG) added to 0 (*diamond*), 0.033 (*square*), 0.066 (*triangle*), and 0.1 (*circle*) U/mL β-glucuronidase. MPAG was added to the incubation buffer during preincubation and incubation periods. After incubation for 0, 15, 30, 45, and 60 min, the levels of mycophenolic acid (MPA) and MPAG were measured. Each point represents the mean ± standard error (S.E.) of 3 experiments. ***$p < 0.001$ significantly different from 0 U/mL β-glucuronidase.

Fig. 2. Effects of ciprofloxacin or phenolphthalein-β-D-glucuronide on mycophenolic acid resynthesis (A) from mycophenolic acid glucuronide (B) by β-glucuronidase

The buffer was incubated at 25 °C with 500 μM mycophenolic acid glucuronide (MPAG) in absence (*circle*) or presence of 500 μM phenolphthalein-β-D-glucuronide (PhePG) (*triangle*) or ciprofloxacin (*square*). MPAG was added to the incubation buffer during preincubation and incubation periods. After incubation for 0, 15, 30, 45, 60, and 90 min, the levels of MPA and MPAG were measured. Each point represents the mean ± standard error (S.E.) of 3 experiments. ***$p < 0.001$ significantly different from control.

Fig. 3. Effects of quinolones or phenolphthalein-β-D-glucuronide on mycophenolic acid

resynthesis by β-glucuronidase

The buffer was incubated for 30 min at 25 ℃ with 500 μM mycophenolic acid glucuronide (MPAG) in the absence or presence of β-glucuronidase substrate or quinolones (500 μM). Phenolphthalein-β-D-glucuronide (PhePG), levofloxacin, ciprofloxacin, ofloxacin, and enoxacin were added to the incubation buffer during preincubation and incubation periods. After incubation, the levels of MPA and MPAG were measured. Each column represents the mean ± standard error (S.E.) of 3 experiments. ***$p < 0.001$ significantly different from control.

Fig. 4. Effects of mycophenolic acid resynthesis from various concentrations of mycophenolic acid glucuronide by β-glucuronidase

Mycophenolic acid glucuronide (MPAG) was added to the incubation buffer during preincubation and incubation periods. Resynthesis of mycophenolic acid (MPA) from MPAG at various concentrations (1, 10, 50, 100, 500, 750, 1000, 2500, and 5000 μM) by β-glucuronidase was measured for 30 min at 25 ℃. Each point represents the mean ± standard error (S.E.) of 3 experiments.

Fig. 5. Concentration-dependent inhibition of β-glucuronidase-mediated mycophenolic acid resynthesis by ciprofloxacin and phenolphthalein-β-D-glucuronide

Mycophenolic acid glucuronide (MPAG) was added to the incubation buffer during preincubation and incubation periods. Resynthesis of MPA from 1500 μM MPAG by β-glucuronidase in the

presence of various concentrations (1, 10, 30, 100, 300, 1000, and 3000 μM) of phenolphthalein-β-D-glucuronide (PhePG) (*open circle*) and ciprofloxacin (*closed circle*) was measured for 30 min at 25 °C. Each point represents the mean ± standard error (S.E.) of 3 experiments.

Fig. 6. Effects of phenolphthalein-β-D-glucuronide or ciprofloxacin on β-glucuronidase-mediated resynthesis of mycophenolic acid using various concentrations of mycophenolic acid glucuronide

Mycophenolic acid glucuronide (MPAG) was added to the incubation buffer during preincubation and incubation periods. Resynthesis of mycophenolic acid (MPA) from MPAG at various concentrations (1, 10, 50, 100, 500, 750, 1000, 2500, and 5000 μM) by β-glucuronidase in the presence of 200 μM phenolphthalein-β-D-glucuronide (PhePG) (*triangle*) or 30 μM ciprofloxacin (*square*) was measured for 30 min at 25 °C. Each point represents the mean ± standard error (S.E.) of 3 experiments. *Inset*: Eadie–Hofstee plots of MPA after correction for the nonsaturable component; V, MPA synthesis rate (μmol/U/30 min); S, MPAG concentration (μM). The dotted line is derived from the result of the data analysis in Fig.4.

Table 1. Results of the least squares fitting by a linear model

Parameters	MPAG	MPAG + PhePG		MPAG + Ciprofloxacin	
	Estimate	Estimate	Ratio to MPAG	Estimate	Ratio to MPAG
$C(0)_{MPAG}$ (μM)	469.5	476.4	1.01	494.4	1.05
k (/min)	0.0551	0.0022	0.040	0.0015	0.027
$C(0)_{MPA}$ (μM)	17.4	10.7	0.61	5.85	0.34
MAX (μM)	356.2	212.5	0.60	235.9	0.66

The time course data of MPAG and MPA concentration (μM) were simultaneously fitted to the linear first-order degradation model for each of the three conditions (control, ciprofloxacin, and PhePG). The model equations are given as follows;

$$C(t)_{MPAG} = C(0)_{MPAG} \cdot \exp(-k \cdot t) \qquad \text{Eq.(1)}$$

$$C(t)_{MPA} = C(0)_{MPA} + MAX \cdot (1 - exp(-k \cdot t)) \qquad \text{Eq.(2)}$$

where *C(t)* is the concentration of MPAG or MPA (identified by subscripts) at time t, *C(0)* is the concentration at time zero, *k* is the first-order degradation rate constant from MPAG to MPA, and MAX is the maximum concentration of MPA at infinite time. $C(0)_{MPAG}$, $C(0)_{MPA}$, *k* and *MAX* were estimated by a least squares method using the Microsoft Excel solver tool.

Fig. 1

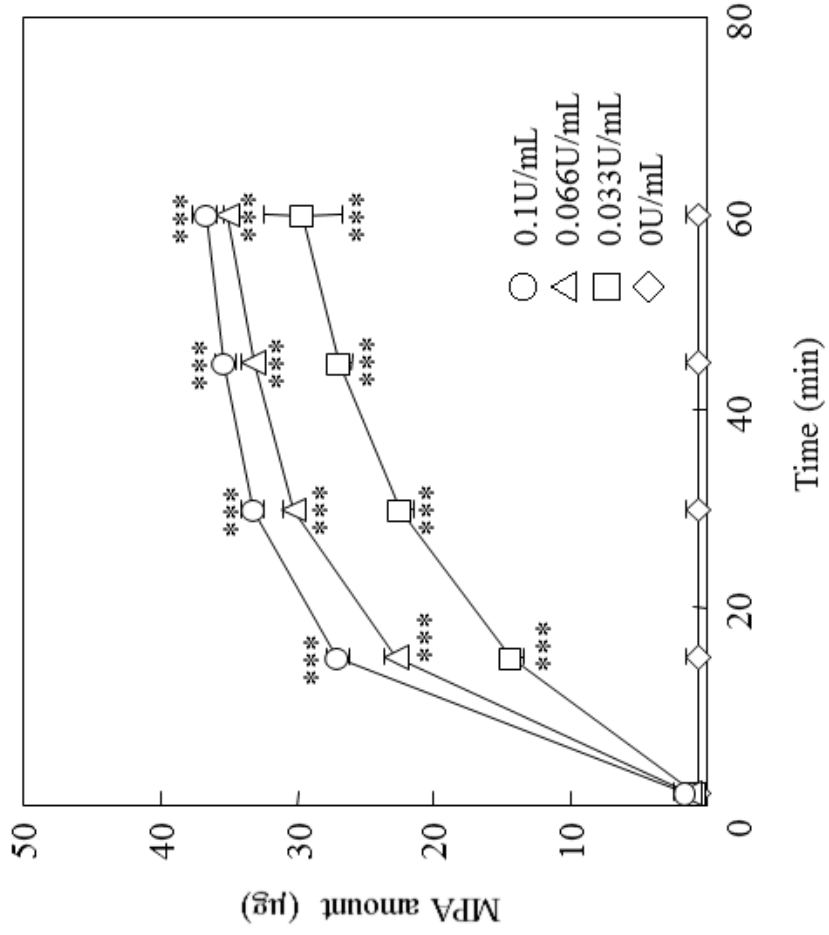

MPA amount (μg)
Time (min)
0.1U/mL
0.066U/mL
0.033U/mL
0U/mL
0
20
40
60
80
10
20
30
40
50

Fig. 2

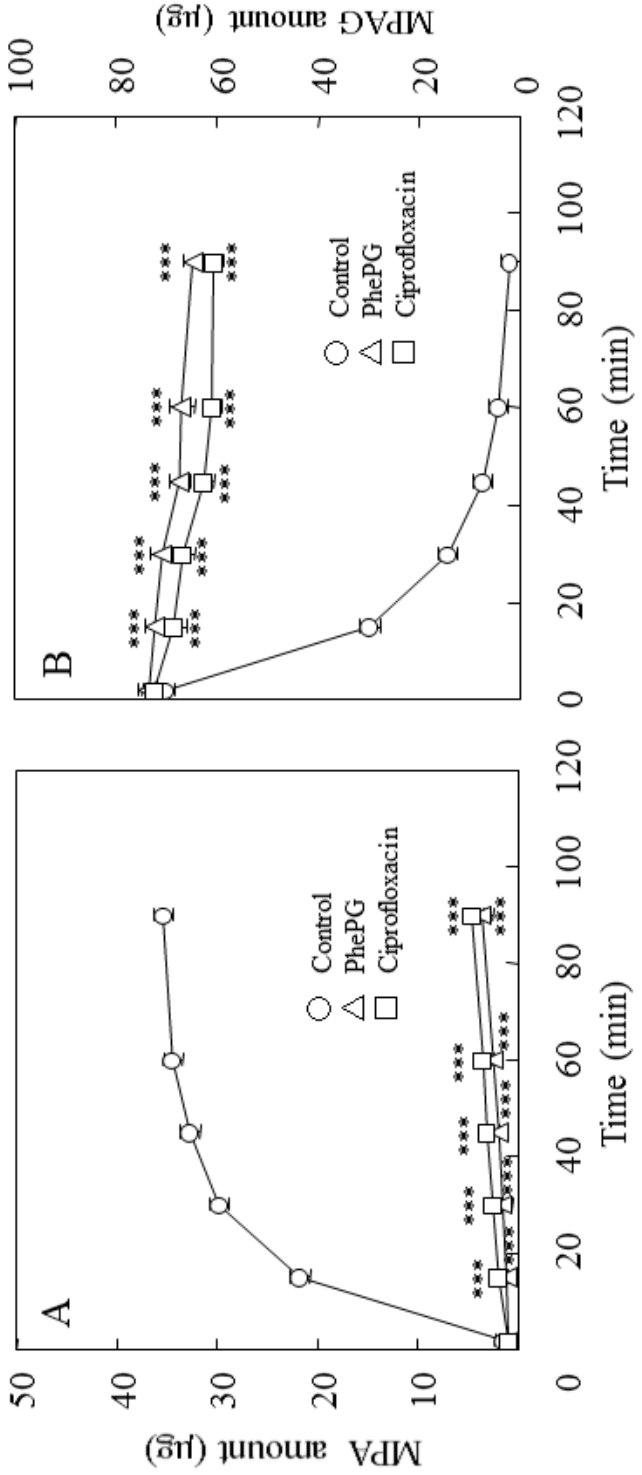

A
B
MPA amount (μg)
MPAG amount (μg)
Time (min)
Time (min)
Control
PhePG
Ciprofloxacin
***

Fig. 3

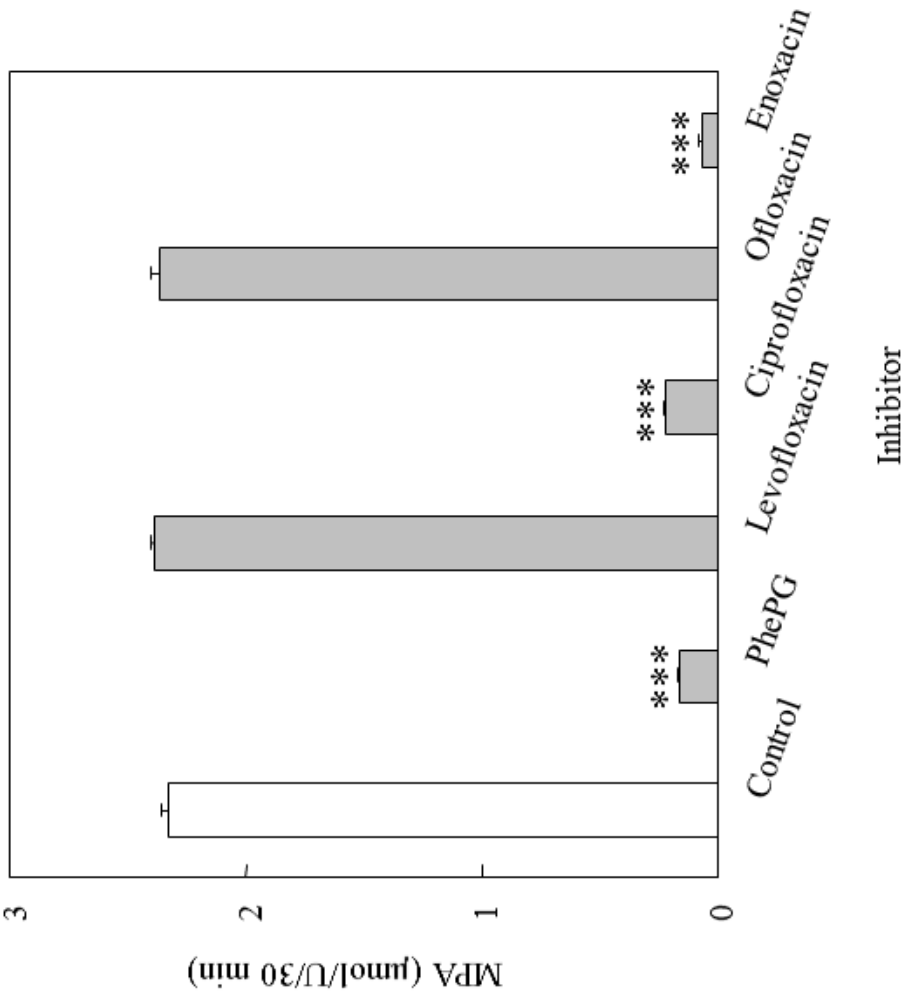

MPA (μmol/U/30 min)
0
1
2
3
***
***
***
Control
PhePG
Levofloxacin
Ciprofloxacin
Ofloxacin
Enoxacin
Inhibitor

Fig. 4

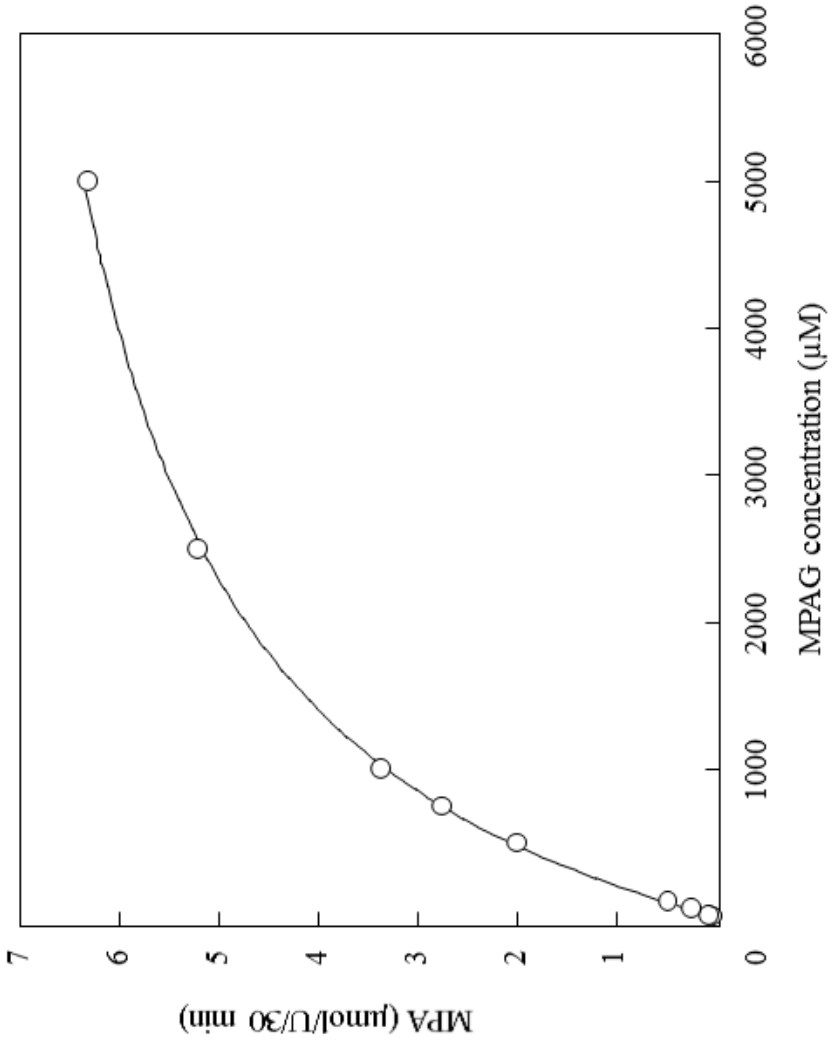

MPA (µmol/U/30 min)
0
1
2
3
4
5
6
7
0
1000
2000
3000
4000
5000
6000
MPAG concentration (µM)

Fig. 5

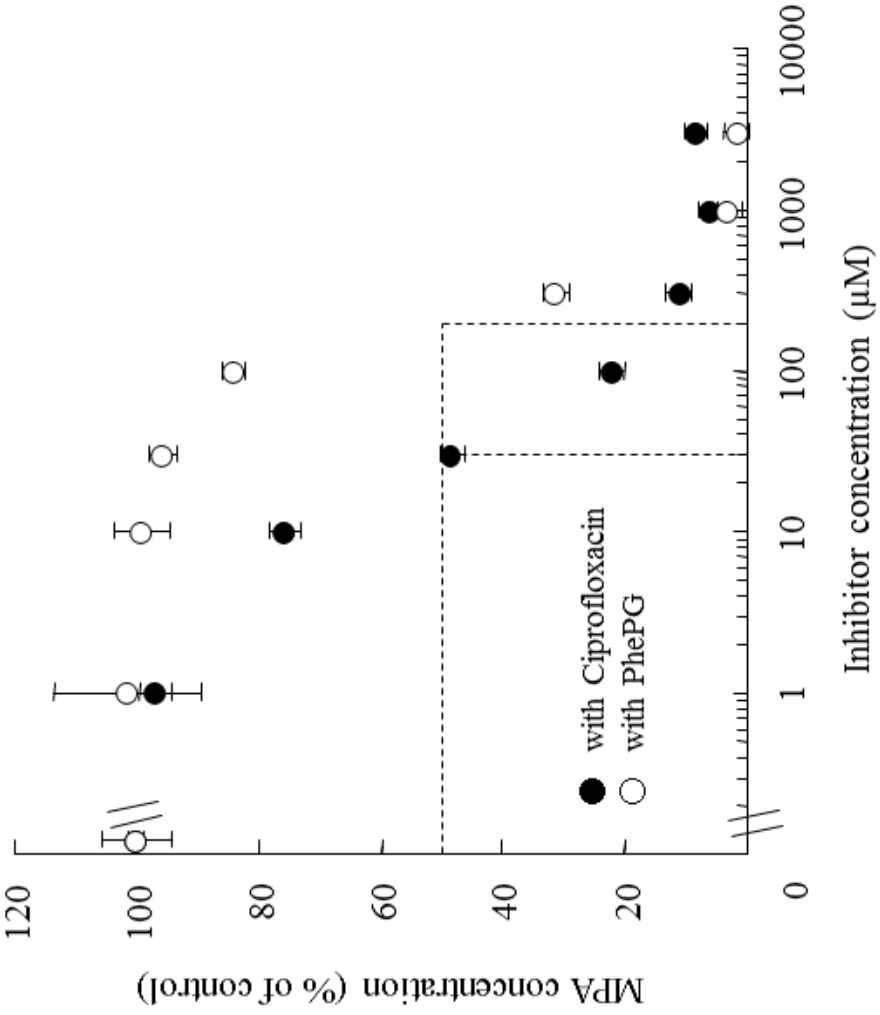
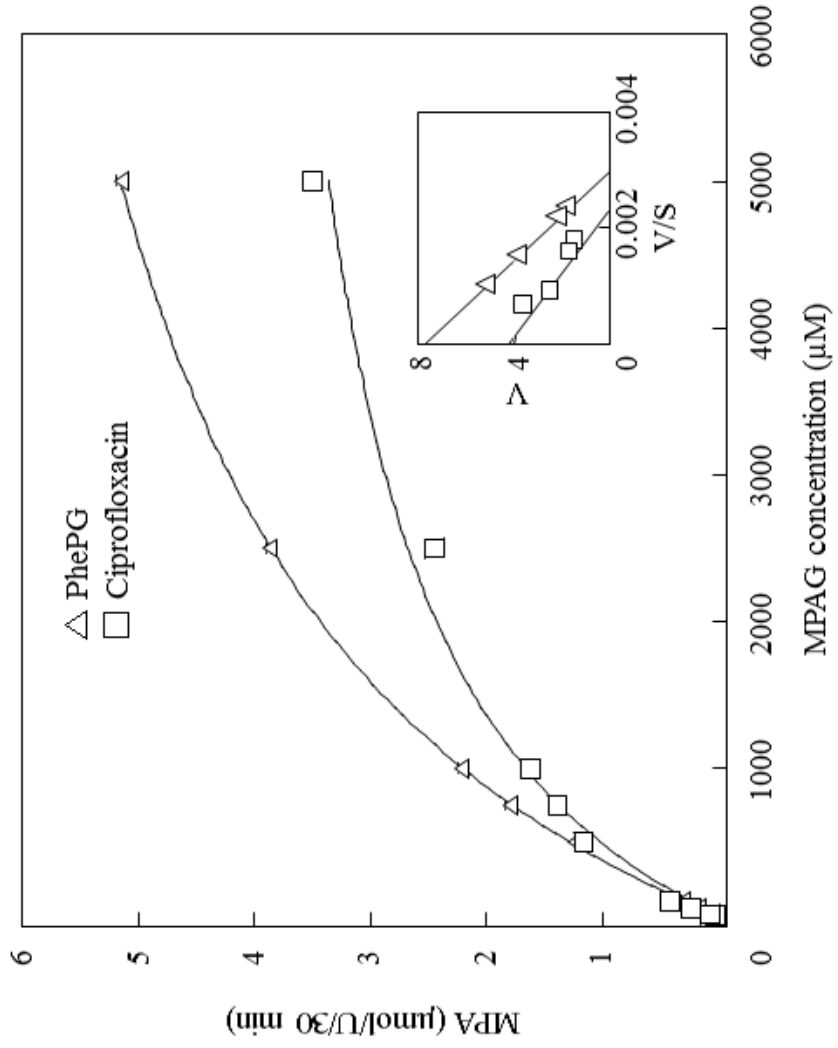

MPA concentration (% of control)
120
100
80
60
40
20
0
with Ciprofloxacin
with PhePG
1
10
100
1000
10000
Inhibitor concentration (μM)

Fig. 6


△ PhePG
□ Ciprofloxacin
MPA (μmol/U/30 min)
MPAG concentration (μM)
V/S
V
0
1000
2000
3000
4000
5000
6000
0.002
0.004
4
8