HOKKAIDO UNIVERSITY

Title	Influence of gastrointestinal activity on the absorption of nilotinib
Author(s)	Sasaki, Maaya; Aoyama, Tsuyoshi; Sugawara, Mitsuru et al.
Citation	Drug metabolism and pharmacokinetics, 35(1), 102-110 https://doi.org/10.1016/j.dmpk.2019.08.006
Issue Date	2020-02
Doc URL	https://hdl.handle.net/2115/80327
Rights	© 2019. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	Drug Metab Pharmacokinet_35_102.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Influence of gastrointestinal activity on the absorption of nilotinib

Sasaki Maaya[1], Tsuyoshi Aoyama[2], Mitsuru Sugawara[1], Yoh Takekuma[1*]

1) Faculty of Pharmaceutical Sciences, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

2) Graduate School of Life Science, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

*To whom correspondence should be addressed

Yoh Takekuma, Ph. D.,

Faculty of Pharmaceutical Sciences, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

Tel/Fax: +81-11-706-3977/ +81-11-706-4984

E-mail: y-kuma@pharm.hokudai.ac.jp

ORCID: 0000-0002-4325-0785

Abstract

Nilotinib has bioavailability (BA) of only about 25% or less. The purpose of this study was to evaluate the influence of gastrointestinal activity on the absorption of nilotinib. In order to change gastrointestinal activity, mosapride was used for enhancement and butylscopolamine was used for suppression. Experiments on oral administration of nilotinib using rats whose gastrointestinal activity was altered by mosapride or butylscopolamine were carried out. The results of oral administration of acetaminophen to rats with peristalsis movement changed showed that the effects of peristalsis and

gastric emptying rate (GER) on drug absorption could be evaluated in this experimental system. Similarly, even with nilotinib, no change in T_{max} was observed, but C_{max} increased and decreased significantly. Due to the change in gastrointestinal activity, C_{max} of nilotinib changed greatly. This showed that gastrointestinal activity affected the emulsifying action of bile and that the absorbability changed. As a result of examining the contribution to the emulsifying action, it was found that when the bile does not exist in the gastrointestinal tract, absorption of nilotinib did not change even when gastrointestinal motility was enhanced. Therefore, the results suggested that gastrointestinal activity influenced the emulsifying action of bile and the absorption of nilotinib was changed.

Keywords; tyrosine kinase inhibitor (TKI), nilotinib, peristalsis movements, gastric empty rate (GER), bile

Introduction

In the treatment of chronic myelogenous leukemia, imatinib, a tyrosine kinase inhibitor (TKI), was the first molecularly targeted drug that inhibits BCR-ABL tyrosine kinase activity, and nilotinib (Fig. 1) and dasatinib later became aveilable as second-generation TKIs. The bioavailability (BA) of imatinib is remarkably high, about 100% (in humans) [1], but the BA of nilotinib is remarkably low, about 25% or less [2]. The low BA is deeply involved in inter-individual variability in pharmacokinetics. Various factors causing the inter-individual variation in the pharmacokinetics of nilotinib can be considered, but it is important to focus on factors in the dissolution process and absorption process of nilotinib, especially with consideration of the BCS classification [3] and physicochemical properties [4].

The drug dissolution process is essential for an orally administered drug to be absorbed from the gastrointestinal tract. However, since nilotinib has high lipid solubility, its solubility is very low. Furthermore, since it is a weakly basic drug, it does not show good dissolution unless it is in a low pH environment. Through the dissolution process, the drug permeates through the small intestine, which is the main drug absorption site, and to the bloodstream. Since various problems in the dissolution process and absorption process may influence the clinical efficacy of nilotinib, it is necessary to reveal the factors responsible for the pharmacokinetic fluctuations that the affect the clinical efficacy of TKIs and to achieve better clinical outcomes by overcoming or alleviating the problems.

In previous studies including case studies, reduction of nilotinib blood levels in cases in which a combination of nilotinib and a PPI was used and in bile duct drainage cases have been reported [5], [6]. Therefore, the aim of this study was to determine which variation of the gastrointestinal environment contributes most to the absorption of nilotinib and to determine whether peristalsis movement activity causes fluctuation in the absorption of nilotinib.

2. Materials and Methods

2.1. Materials

Nilotinib monohydrochloride monohydrate, mosapride and scopolamine butylbromide were purchased from MedChem Express (New Jersey, USA), Combi-Blocks (San Diego, USA), and Towa Pharmaceutical Co., Ltd. (Osaka, Japan), respectively. Other reagents were purchased from Wako Pure Chemical unless otherwise noted. All reagents were of the highest grade available and used without further purification.

2.2. Animals

Male Wistar rats, 8-9 weeks old (210-300 g in weight), were obtained from CRER Japan Inc. (Tokyo, Japan). The housing conditions were the same as those described previously [7]. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the ‘Guide for the Care and Use of Laboratory Animals’.

2.3. Evaluation of peristaltic activity by the charcoal meal method

Charcoal meal was obtained by adding 0.25 g (10%) of arabic gum and 0.125 g (5%) of powdered activated carbon to 2.5 mL of water, followed by stirring [8], [9], [10]. Mosapride solution (0.18 mg/mL) was prepared by dissolving 3.6 mg of mosapride in 20 mL of citric acid aqueous solution adjusted to pH 2.0 by warming to about 80°C on a hot stirrer to dissolve. Butyl scopolamine solution was prepared by dissolving butyl scopolamine at 4.0 mg/kg weight per 1 mL of saline. When evaluating changes in peristalsis activity by butyl scopolamine, butyl scopolamine solution (or saline) was continuously administered from the tail vein for 1 hour and charcoal meal was administered orally by a stomach tube. When evaluating changes in peristalsis activity by mosapride, mosapride solution (or citric acid aqueous solution) was administered orally by a stomach tube and charcoal meal was administered orally by a stomach tube 15 minutes later. One hour after

administration of the charcoal meal, the rats were sacrificed, the abdomen was incised, and the distance from the tip of where the charcoal meal has reached in the small intestine to the cecum was measured. As evaluation, cases in which the charcoal meal had reached the cecum were regarded as positive, and cases in which it had not reached the cecum were regarded as negative (Fig. 2).

2.4. Measurement of acetaminophen in rat plasma

The plasma concentration of acetaminophen was measured using HPLC-UV. One hundred microliters of β-glucuronidase solution in 0.1 M sodium acetate buffer (pH 5.0) (25000 units/mL) was added to 100 μL of a rat plasma sample and incubated at 37°C for 60 min. Ten microliters of an internal standard (caffeine) in water (1000 μg/mL) and 10 μL perchloric acid (60%) were added to the solution. The solution was vortexed for 1 min, frozen at -20°C for 20 min, and centrifuged at 21500×g for 20 min. Then 20 μL of the injected into an Inertsil ODS-4 column (3.0×150 mm). Acetaminophen was eluted [mobile phase: 20 mM potassium phosphate buffer (pH 2.2)/methanol, 85:15 (vol/vol)] at a flow rate of 0.4 mL/min and was detected by UV absorbance at 245 nm [11]. The retention times of acetaminophen and caffeine were 6.65 min and 17.35 min, respectively. Peak area measurements were used for quantification and were compared with standard solutions of acetaminophen.

2.5. Sample preparation to determine the concentration of nilotinib in plasma

The plasma concentration of nilotinib was measured using UPLC-UV. Ten microliters of imatinib solution (internal solution), 10 μL of methanol and 2M Tris (30 μL) were added to 100 μL of rat plasma and the solution was vortexed for 5 seconds. Then 1.5 milliliters of butyl acetate : butanol (4: 1) and 100 microliters of water were added and the solution was vortexed for 30 seconds. After centrifugation at 21500×g for 20 min at 4°C, 1.3 milliliters of supernatant was evaporated at 45°C for 1 hour. After solvent evaporation, 100 μL of the mobile phase was added and the solution

was vortexed for 30 seconds and centrifuged at 3500 rpm for 20 min. This supernatant was used as a sample.

2.6. Determination of nilotinib concentration

Ten microliters of the sample was injected into a 1.7 μm ACQUITY UPLC® BEH C18 column (2.1×100 nm). Nilotinib were eluted [mobile phase: acetonitrile/methanol/50 mM sodium phosphate, 30:15:55 (vol/vol)] at a flow rate of 0.25 mL/min and was detected by UV absorbance at 267 nm. The retention times of imatinib and nilotinib were 1.6 min and 4.2 min, respectively. Peak area measurements were used for quantification and were compared with standard solutions of nilotinib.

2.7. Oral administration experiments in rats in which gastrointestinal motility was enhanced or suppressed

Rats with either promoted or suppressed peristalsis movement were prepared by the same method as that described in 2.3. Acetaminophen (50 mg/kg weight) or nilotinib (8.0 mg/kg weight) was orally administered at the same timing as administration of the charcoal meal. A nilotinib suspension was obtained by adding 24 mg of nilotinib to 10 mL of pure water, stirring, sonicating, adding 50 mg of CMC, and stirring. For collection of rat plasma samples, the neck of each anesthetized rat was dissected to expose the jugular vein, and 350 μL of blood was taken from the jugular vein over time and immediately mixed with a small amount of sodium heparin. The collected blood was centrifuged at 750×g for 10 min at 4°C and the supernatant was collected as a plasma sample and stored at -20°C.

2.8. Experiments on oral administration of nilotinib in combination with mosapride in rats treated with biliary duct cannulation

With the rat anesthetized, the abdomen was incised along the midline and a tube was inserted into the bile duct. The incision was sutured and used 16-20 hours later for oral administration experiments. Experiments on oral administration of nilotinib were conducted by the same method as that described in 2.6.

2.9. Determination of nilotinib solubility in bile

A nilotinib suspension (2.4 mg/mL) was obtained by the same way as 2.6. To investigate vortex time dependency, the suspension of 100 μL was added to 100 μL of bile. These were vortexed for 15-60 min at 37°C. Also, to investigate the concentration of bile dependency, the suspension of 100 μL was added to 100 μL of bile, water or equal mixture of bile and water. These were vortexed for 60 min at 37°C. After centrifugation (750×g for 10 min at 4°C), a 200-fold dilution of the supernatant was used as a sample. The control was a 6.0 μg/mL nilotinib solution in methanol.

2.10. Sample preparation to determine the concentration of nilotinib in bile

The concentration of nilotinib was measured using UPLC-UV. Ten microliters of imatinib solution (internal solution), 10 μL of methanol and 2M Tris (30 μL) were added to 100 μL of a sample and the solution was vortexed for 5 seconds. Then 1.5 milliliters of butyl acetate: butanol (4: 1) and 100 microliters of water were added, and the solution was vortexed for 30 seconds. After centrifugation at 21500×g for 20 min at 4°C, 1.3 milliliters of supernatant was evaporated at 45°C for 1 hour. After solvent evaporation, 100 μL of the mobile phase was added and the solution was vortexed for 30 seconds and centrifuged at 3500 rpm for 20 min. This supernatant was used as a sample. The concentration of nilotinib was measured using UPLC-UV in the same way as 2.6.

2.11. Bile secretion measurement at the time of butyl scopolamine administration

Rats with suppressed peristalsis movement were prepared by the same method as that

described in 2.3. At the same time as administration of butyl scopolamine, a cannulation tube was inserted into the bile duct and bile drained was collected for 6 hours. The bile flow was measured by the weight method assuming that the specific gravity of the bile was 1.

2.12. Data analysis

To analyze the pharmacokinetics of nilotinib and acetaminophen, the area under the curve (AUC) was calculated by the trapezoidal rule. Student's t-test was used to determine the significance of differences between the two group means. Data are expressed as means with standard deviation (SD). Statistical significance was defined as $p<0.05$.

3. Results

3.1. Evaluation of the effects of mosapride and butyl scopolamine on gastrointestinal activity

3.1.1. Evaluation of peristalsis movement activity by the charcoal meal method

Firstly, we investigated whether mosapride and butyl scopolamine are appropriate as drugs for controlling gastrointestinal activity. As a result of evaluating peristalsis movement activity by the charcoal meal method, it was found the oral administration of mosapride caused the charcoal meal to proceed inside the small intestine and that movement of the charcoal meal was greatly suppressed by administration of butyl scopolamine via the tail vein (Fig. 3). The results showed the peristalsis movement activity can be changed by administration of these drugs.

3.1.2. Plasma concentrations of acetaminophen in combination with mosapride and butyl scopolamine

It was revealed that mosapride and butyl scopolamine change peristaltic activity. In the following experiments, acetaminophen, which is known to be affected by peristalsis movement activity [12], was used as a model drug. We investigated by measuring plasma concentrations whether the

administration of mosapride and that of butyl scopolamine affect the absorbability of other drugs. As a result, the C_{max} (from 23.32±13.1 to 35.53±5.7 μg/mL) and AUC (from 70.44±30.9 to 113.05±19.3 hr*μg/mL) of acetaminophen in plasma in the case of combined administration of mosapride increased. Although there was no significant difference in C_{max} and AUC when mosapride was administered, a tendency to increase was confirmed. On the other hand, when acetaminophen was used in combination with butyl scopolamine, there were decreases in both C_{max} (from 34.96±4.8 to 19.57±6.0 μg/mL) and AUC (from 76.43±9.5 to 51.85±8.4 hr*μg/mL) (Fig. 4, Table 1, 2). T_{max} was shortened when mosapride was administered (from 1.35±0.5 to 0.58±0.1 hr). T_{max} didn't change much when butyl scopolamine was administered (from 1.00±0.0 to 0.92±0.1 hr).

3.2. Plasma concentrations of nilotinib in combination with mosapride and butyl scopolamine

Since the absorption of acetaminophen used as a model drug changed when gastrointestinal activity was changed by mosapride or butyl scopolamine, the same study was conducted for the case of nilotinib. The results showed that there were increases in C_{max} (from 436.3±189.5 to 1284.5±287.9 ng/mL) and AUC (from 2550.7±982.2 to 6800.5±2423.3 hr*ng/mL) when mosapride was used in combination and decreases in C_{max} (from 564.2±42.1 to 163.8±80.4 ng/mL) and AUC (from 3528.8±383.2 to 943.1±496.4 hr*ng/mL) when butyl scopolamine was used in combination (Fig. 5, Table 3, 4). The results were the same as those for acetaminophen, but the magnitudes of changes were greater than those in the case of acetaminophen.

3.3. Plasma concentration of nilotinib during acceleration of gastrointestinal motility in rats treated with biliary duct cannulation

In sham rats, the combined use of mosapride increased C_{max} (from 715.6±80.8 to 1144.5±328.9 ng/mL, $p<0.08$ by Student's t-test) and AUC (from 1765.6±231.6 to 2273.4±494.9 ng/mL, Table 5). On the other hand, the experimental results obtained in rats that underwent biliary

duct cannulation treatment showed that the plasma concentration of nilotinib profile did not change even when mosapride was used in combination (C_{max}: from 86.3±34.0 to 188.5±101.8 ng/mL, AUC: from 232.1±98.8 to 557.8±224.7 hr*ng/mL, Fig. 6, Table 6).

3.4. Nilotinib solubility in bile

When the nilotinib suspension was mixed with bile for 15, 30, 45, 60 min, the percentage of nilotinib dissolved was 5.9%, 7.6%, 22.3%, 64.2%, respectively (Fig. 7). Also, when the percentage of bile was 0%, 25%, 50%, the percentage of nilotinib dissolved was 19.6%, 25.8%, 64.2% respectively (Fig. 8).

3.5. Bile secretion during administration of butyl scopolamine

The cumulative biliary secretion of rats administered of butyl scopolamine was 24.6 mL/kg B.W. (Fig. 9). There was no difference compared to the control (24.0 mL/kg B.W.).

Discussion

Nilotinib is a poorly absorbable drug having both low solubility and low membrane permeability. It was shown that the absorption of nilotinib is influenced by the secretion of stomach acid and bile. However, since the involvement of other factors has not yet been clarified, we decided to clarify whether digestive tract movement contributed to the fluctuation in absorption of nilotinib. Many orally administered drugs are absorbed mainly from the small intestine after being dissolved in the stomach, and that gastrointestinal motility can therefore be accelerated by the concomitant use of drugs that affect gastrointestinal activity. Generally, as the rate at which the drug is discharged from the stomach (GER) and the speed at which it passes through the digestive tract increase, C_{max} increases and t_{max} decreases. On the other hand, when gastrointestinal motility is suppressed, GER decreases and drugs stay in the gastrointestinal tract, and C_{max} therefore decreases and t_{max} is thought

to be prolonged. Therefore, we decided to control gastrointestinal motility using mosapride and butyl scopolamine and to measure the change in plasma concentration of nilotinib.

First, we confirmed by using the charcoal meal method that peristalsis movement activity could be changed by mosapride or butyl scopolamine (Fig.3). It was shown that peristalsis movement can be enhanced by mosapride and inhibited by butyl scopolamine. In addition, in the study in which acetaminophen, which is known to change absorption due to alteration of gastrointestinal activity, was used as a model drug, the absorption of acetaminophen increased (C_{max} and AUC increased) when mosapride was used in combination (Fig. 4A). However when acetaminophen was combined with butyl scopolamine, the absorption of acetaminophen decreased (C_{max} and AUC decreased, Fig. 4B). Therefore, it was shown that the use of a combination of mosapride and butyl scopolamine enables evaluation of the influence of orally administered drugs on the absorption process. The changes in absorption of nilotinib when using these drugs showed the same tendency as that in the case of acetaminophen, but the magnitudes of changes were larger (Fig. 5A, B). Also, when GER increases, AUC does not change, even if it influences C_{max} or T_{max}, but AUC increased in these experiments. As an explanation for this, administration of mosapride or butyl scopolamine might change the pH in the stomach. The pH in the stomach after administration of the drugs was investigated, but little change was observed (data not shown). Also, since nilotinib is known as dual substrate for CYP3A4 and P-gp, we investigated the effects of mosapride on these transporters. There is no report that mosapride inhibits P-gp. Also, there are reports that mosapride does not inhibit CYP3A4, but promotes CYP3A4 [13]. Therefore, there is no possibility that the absorbabilityof nilotinib was changed by the pH in the stomach and the expression level of the transporter.

It was thought that the gastrointestinal activity affected not only the absorption process of nilotinib but also the emulsifying action of bile. Therefore, the same study was carried out in rats treated with biliary duct cannulation. As a result, even when mosapride was used concomitantly,

absorption of nilotinib did not change (Fig. 6A, B). Also, the absorption of nilotinib in cannulated rats was reduced. The mixing time of nilotinib and bile depended on the solubility of nilotinib (Fig. 7). Furthermore, the amount of bile in total volume depended on the solubility of nilotinib (Fig. 8). Therefore, it is believed that this is because the amount of bile excreted in the intestinal tract decreased and the amount of nilotinib dissolved in bile decreased. In addition, bile secretion was measured because there was a possibility that nilotinib absorption was reduced due to the decrease in the amount of bile secretion when butyl scopolamine was administered. But there was no difference compared to the control (Fig. 9). These results suggested that the enhancement of gastrointestinal activity accelerated the emulsification action of bile and the absorption of nilotinib increased.

The results indicate that the combined use of drugs affecting gastrointestinal motility may greatly affect the absorbability of nilotinib and that administration of an activator may be effective for patients in whom the blood level of nilotinib does not rise due to malabsorption of nilotinib.

Conclusion

We considered peristaltic movement to be a factor that changes the absorption of nilotinib and we confirmed fluctuation in the absorption of nilotinib by altering digestive tract activity. As gastrointestinal activity increased, the absorption of nilotinib increased, but the AUC of nilotinib changed significantly compared with the AUC of acetaminophen used as a model drug. Since this factor was thought to contribute to the emulsification by bile, fluctuation in the absorption of nilotinib was confirmed by the presence or absence of bile in the gastrointestinal tract by bile duct cannulation. Since nilotinib was hardly absorbed in the absence of bile, bile in the gastrointestinal tract may contribute greatly to the absorption process of nilotinib.

References

[1] Peng B, Dutreix C, Mehring G, Hayes MJ, Ben-Am M, Seiberling M, Pokorny R, Capdeville R,

Lloyd P. Absolute bioavailability of imatinib (Glivec) orally versus intravenous infusion. J Clin Pharmacol. 2004;44(2):158-62. https://doi.org/10.1177/0091270003262101

[2] Xia B, Heimbach T, He H, Lin TH. Nilotinib preclinical pharmacokinetics and practical application toward clinical projections of oral absorption and systemic availability. Biopharm Drug Dispos. 2012;33(9):536-49. https://doi.org/10.1002/bdd.1821

[3] Budha NR, Frymoyer A, Smelick GS, Jin JY, Yago MR, Dresser MJ, Holden SN, Benet LZ, Ware JA. Drug absorption interactions between oral targeted anticancer agents and PPIs: is pH-dependent solubility the Achilles heel of targeted therapy? Clin Pharmacol Ther. 2012;92(2):203-13.https://doi.org/10.1038/clpt.2012.73

[4] Minematsu T, Giacomini KM. Interactions of tyrosine kinase inhibitors with organic cation transporters and multidrug and toxic compound extrusion proteins. Mol Cancer Ther. 2011;10(3):531-9. https://doi.org/10.1158/1535-7163.MCT-10-0731.

[5] Yin OQ, Gallagher N, Fischer D, Demirhan E, Zhou W, Golor G, Schran H. Effect of the proton pump inhibitor esomeprazole on the oral absorption and pharmacokinetics of nilotinib. J Clin Pharmacol. 2010;50(8):960-7. https://doi.org/10.1177/0091270009346061.

[6] Fujimi A, Takahashi N, Miura M, Kanisawa Y, Ono K, Sawada K. Bile acid is important for gastrointestinal absorption of nilotinib. Eur J Clin Pharmacol. 2012;68(11):1575-6. https://doi.org/10.1007/s00228-012-1282-x.

[7] Sato Y, Kobayashi M, Itagaki S, Hirano T, Noda T, Mizuno S, Sugawara M, Iseki K.Protective effect of lutein after ischemia-reperfusion in the small intestine. Food Chem. 2011;127(3):893-8. https://doi.org/10.1016/j.foodchem.2011.01.096.

[8] Yakubu MT, Nurudeen QO, Salimon SS, Yakubu MO, Jimoh RO, Nafiu MO, Akanji MA, Oladiji AT, Williams FE. Antidiarrhoeal Activity of Musa paradisiaca Sap in Wistar Rats. Evid Based Complement Alternat Med. 2015;2015:683726. https://doi.org/ 10.1155/2015/683726.

[9] Matsumoto M, Ishige A, Yazawa Y, Kondo M, Muramatsu K, Watanabe K. Promotion of

intestinal peristalsis by Bifidobacterium spp. capable of hydrolysing sennosides in mice. PloS One. 2012;7(2):e31700. https://doi.org/ 10.1371/journal.pone.0031700.

[10] Rouf AS, Islam MS, Rahman MT. Evaluation of antidiarrhoeal activity Rumex maritimus root. Journal of ethnopharmacology. 2003;84(2-3):307-10.

[11] Zhao Y, Harmatz JS, Epstein CR, Nakagawa Y, Kurosaki C, Nakamura T, Kadota T, Giesing D, Court MH, Greenblatt DJ. Favipiravir inhibits acetaminophen sulfate formation but minimally affects systemic pharmacokinetics of acetaminophen. Br J Clin Pharmacol. 2015;80(5):1076-85. https://doi.org/ 10.1111/bcp.12644.

[12] Nimmo J, Heading RC, Tothill P, Prescott LF. Pharmacological modification of gastric emptying: effects of propantheline and metoclopromide on paracetamol absorption. Br Med J. 1973;1(5853):587-9.

[13] Young-Hoon K, Young-Ji B, Hyung Soo K, Hey-Jin C, Jae-Suk Y, Ji-Soon S, Won-Keun S, Yong-Moon L, Kyoung-Moon H. Measurement of Human Cytochrome P450 Enzyme Induction Based on Mesalazine and Mosapride Citrate Treatments Using a Luminescent Assay. Biomol Ther. 2015:23(5):486-92. http://dx.doi.org/10.4062/biomolther.2015.041

Table 1 Plasma concentration profile of acetaminophen when combined with mosapride

	control	mosapride
C_{max} (μg/mL)	23.32±13.1	35.53±5.7
T_{max} (hr)	1.35±0.5	0.58±0.1
AUC (hr*μg/mL)	70.44±30.9	113.05±19.3

Dose: 50 mg/kg, n=3-5, mean±S.D.

Table 2 Plasma concentration profile of acetaminophen when combined with butyl scopolamine

	control	butyl scopolamine
C_{max} (μg/mL)	34.96±4.8	19.57±6.0*
T_{max} (hr)	1.00±0.0	0.92±0.1
AUC (hr*μg/mL)	76.43±9.5	51.85±8.4

Dose: 50 mg/kg, n=3, mean±S.D. *P<0.05 vs. control by Student's t-test

Table 3 Plasma concentration profile of nilotinib when combined with mosapride

	control	mosapride
C_{max} (ng/mL)	436.3±189.5	1284.5±287.9*
T_{max} (hr)	4.7±0.9	3.3±0.9
AUC (hr*ng/mL)	2550.7±982.2	6800.5±2423.3

Dose: 8.0 mg/kg, n=3, mean±S.D. *P<0.05 vs. control by Student's t-test

Table 4 Plasma concentration profile of nilotinib when combined with butyl scopolamine

	control	butyl scopolamine
C_{max} (ng/mL)	564.2±42.1	163.8±80.4**
T_{max} (hr)	4.0±1.7	3.3±0.9
AUC (hr*ng/mL)	3528.8±383.2	943.1±496.4**

Dose: 8.0 mg/kg, n=3, mean±S.D. **P<0.01 vs. control by Student's t-test

Table 5 Plasma concentration profile of nilotinib when combined with mosapride in sham rats

	control	mosapride
C_{max} (ng/mL)	715.6±80.8	1144.5±328.9
T_{max} (hr)	2.0±0.0	4.0±0.0
AUC (hr*ng/mL)	1765.6±231.6	2273.4±494.9

Dose: 8.0 mg/kg, n=3, mean±S.D.

Table 6 Plasma concentration profile of nilotinib when combined with mosapride in rats that underwent biliary duct cannulation treatment

	control	mosapride
C_{max} (ng/mL)	86.3±34.0	188.5±101.8
T_{max} (hr)	2.7±0.9	2.7±0.9
AUC (hr*ng/mL)	232.1±98.8	557.8±224.7

Dose: 8.0 mg/kg, n=3, mean±S.D.

Figure legends

Fig. 1 Structural formula of nilotinib monohydrochloride monohydrate

Fig. 2 Measurement by the charcoal meal method

Based on the position of the cecum, a positive value was obtained when reaching the anal side, and a negative value was obtained when reaching the small intestine side.

Fig. 3 Arrival position of the charcoal meal (cm)

The medicinal solution was administered to male Wistar rats (8 weeks of age) under each condition. One hour after oral administration of the charcoal meal, the rats were sacrificed and an abdominal incision as made. The distance from the tip of where the charcoal meal had reached in the small intestine to the cecum was measured.

Fig. 4 Plasma concentrations of acetaminophen in combination with (A) mosapride and (B) butyl scopolamine

We used male Wistar rats (9 weeks of age). (A) Mosapride (0.6 mg/kg weight, closed square) or a vehicle (closed circle) was administered orally 15 minutes before acetaminophen solution (50 mg/kg weight) was administered orally. (B) Butyl scopolamine (4.0 mg/kg weight/mL, open square) or a vehicle (open circle) was continuously administered intravenously (1.0 mL/hr) sustainably from 1 hr before acetaminophen solution (50 mg/kg weight) was administered orally. Then nilotinib suspension (8.0 mg/kg weight) was administered orally. Blood was collected over time after administration, and plasma concentrations were measured by HPLC-UV.

Fig.5 Plasma concentrations of nilotinib in combination with (A) mosapride and (B) butyl

scopolamine

We used male Wistar rats (9 weeks of age). (A) Mosapride (0.6 mg/kg weight, closed square) or a vehicle (closed circle) was administered orally 15 minutes before nilotinib suspension (8.0 mg/kg weight) was administered orally. (B) Butyl scopolamine (4.0 mg/kg weight/mL, open square) or a vehicle (open circle) was continuously administered intravenously (1.0 mL/hr) sustainably from 1 hr before nilotinib suspension (8.0 mg/kg weight) was administered orally. Then nilotinib suspension (8.0 mg/kg weight) was administered orally. Blood was collected over time after administration, and plasma concentrations were measured by HPLC-UV.

Fig.6 Plasma concentrations of nilotinib in combination with mosapride in (A) sham rats and (B) cannulated rats.

We used male Wistar rats (9 weeks of age). With the rat anesthetized, the abdomen was incised along the midline and a tube was inserted into the bile duct. The incision was sutured and used 16-20 hours later for oral administration experiments. Mosapride (0.6 mg/kg weight) was administered orally 15 minutes before administration of nilotinib. Then a nilotinib suspension (8.0 mg/kg weight) was administered orally. Blood was collected over time after administration, and plasma concentrations were measured by UPLC-UV.

Fig. 7 Change of nilotinib solubility by mixing time with bile

The nilotinib suspension (2.4 mg/mL) of 100 μL was added to 100 μL of bile. These were vortexed for 15-60 min at 37°C. After centrifugation (750×g for 10 min at 4°C), a 200-fold dilution of the supernatant was used as a sample. The control was a 6.0 μg/mL nilotinib solution in methanol. Nilotinib concentrations were measured by UPLC-UV.

Fig. 8 Change of nilotinib solubility by amount of bile

The nilotinib suspension (2.4 mg/mL) of 100 µL was added to 100 µL of bile, water or equal mixture of bile and water. These were vortexed for 60 min at 37°C. After centrifugation (750×g for 10 min at 4°C), a 200-fold dilution of the supernatant was used as a sample. The control was a 6.0 µg/mL nilotinib solution in methanol. Nilotinib concentrations were measured by UPLC-UV.

Fig. 9 Bile secretion during administration of butyl scopolamine

We used male Wistar rats (9 weeks of ages) with suppressed peristalsis movement. At the same time as administration of butyl scopolamine, a cannulation tube was inserted into the bile duct and bile drained was collected for 6 hours.

Fig. 1

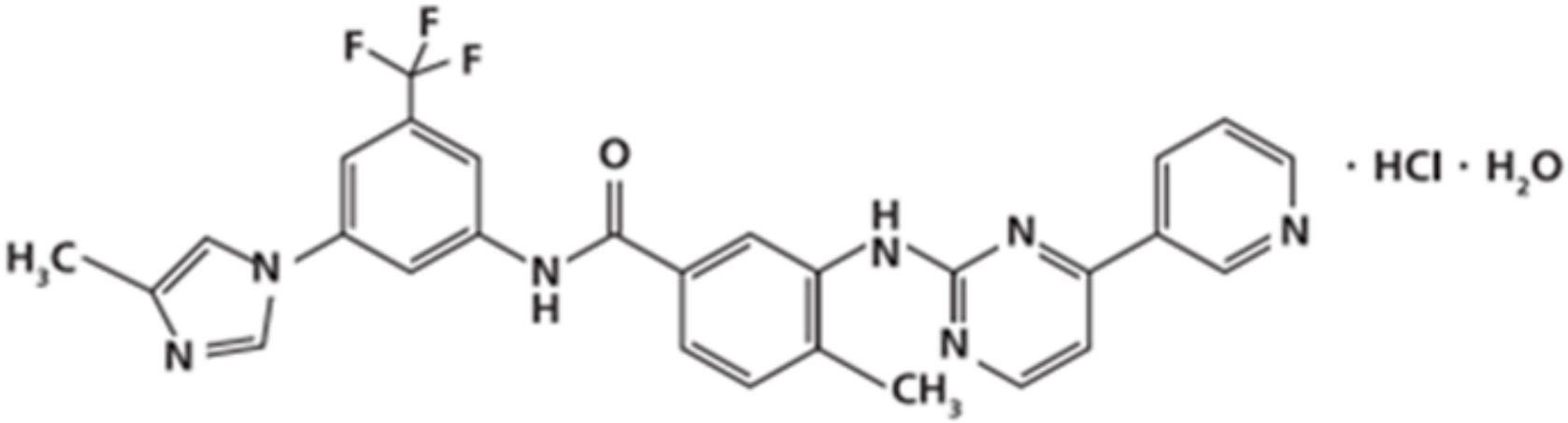


Fig. 2

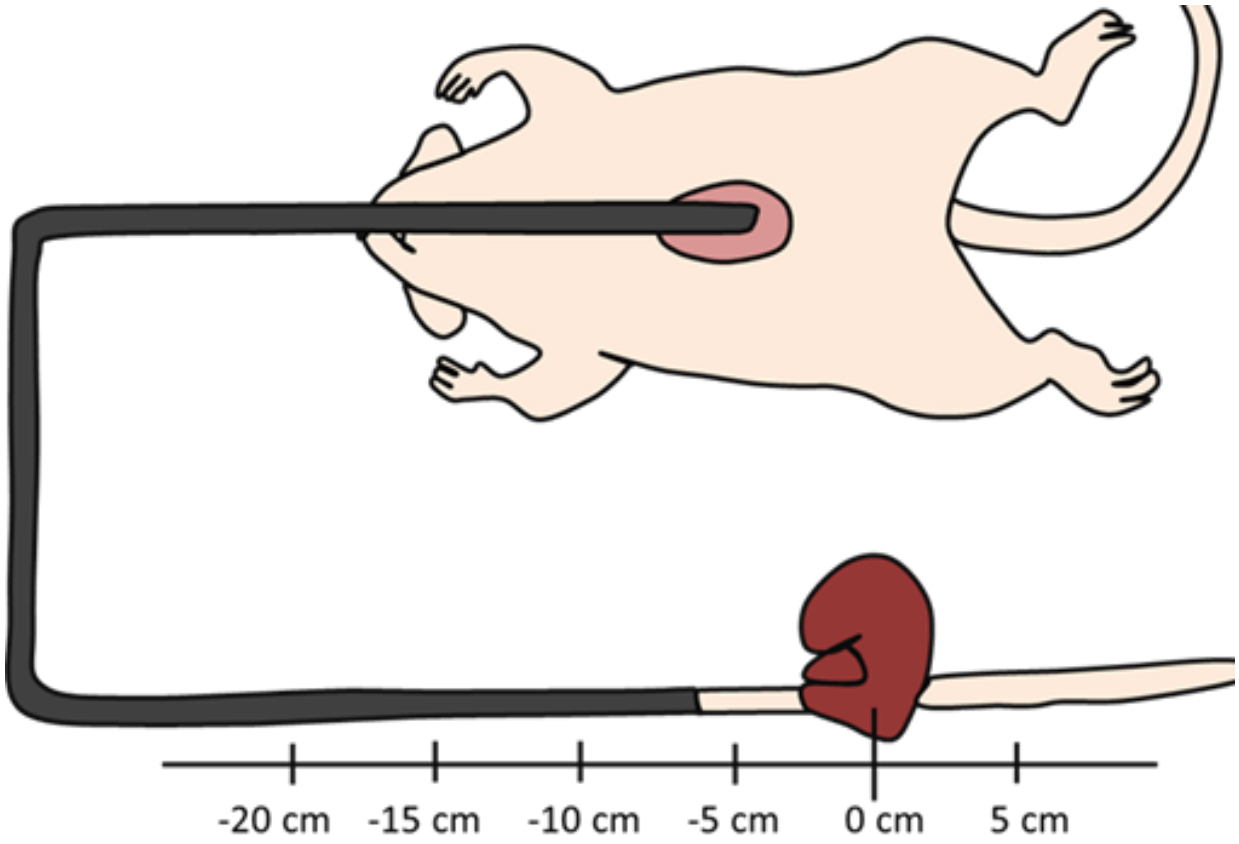


Fig. 3

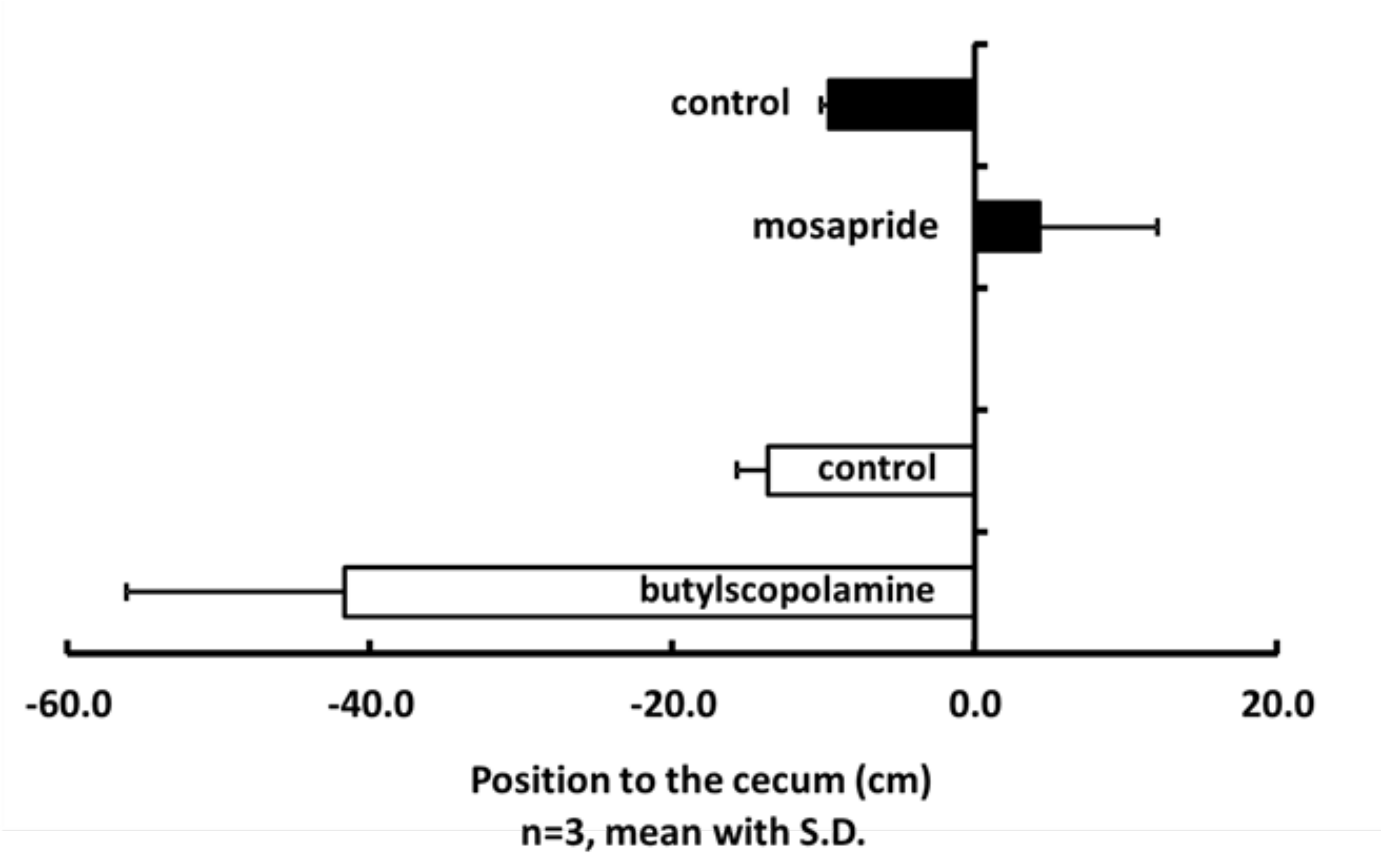

control
mosapride
control
butylscopolamine
-60.0
-40.0
-20.0
0.0
20.0
Position to the cecum (cm)
n=3, mean with S.D.

Fig. 4 (A)

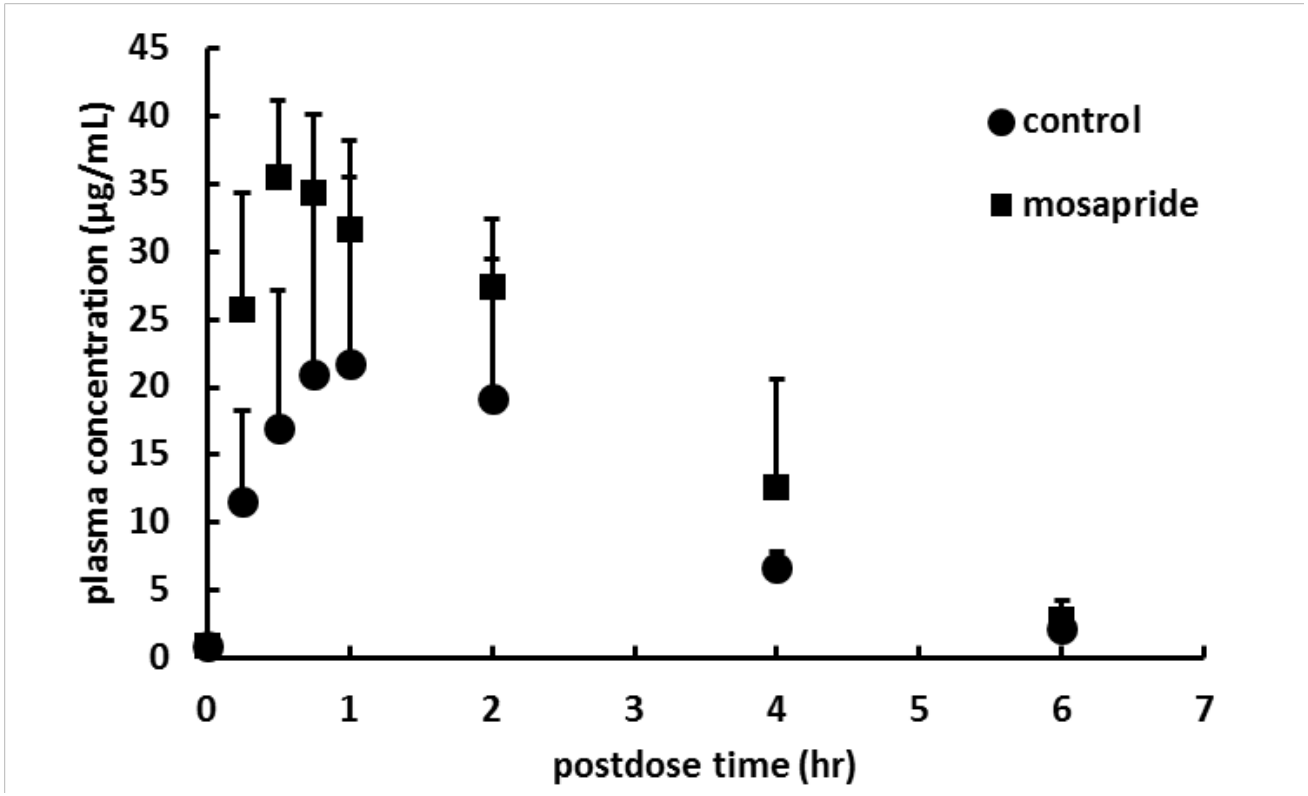

plasma concentration (μg/mL)
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
postdose time (hr)
control
mosapride

Fig. 4 (B)

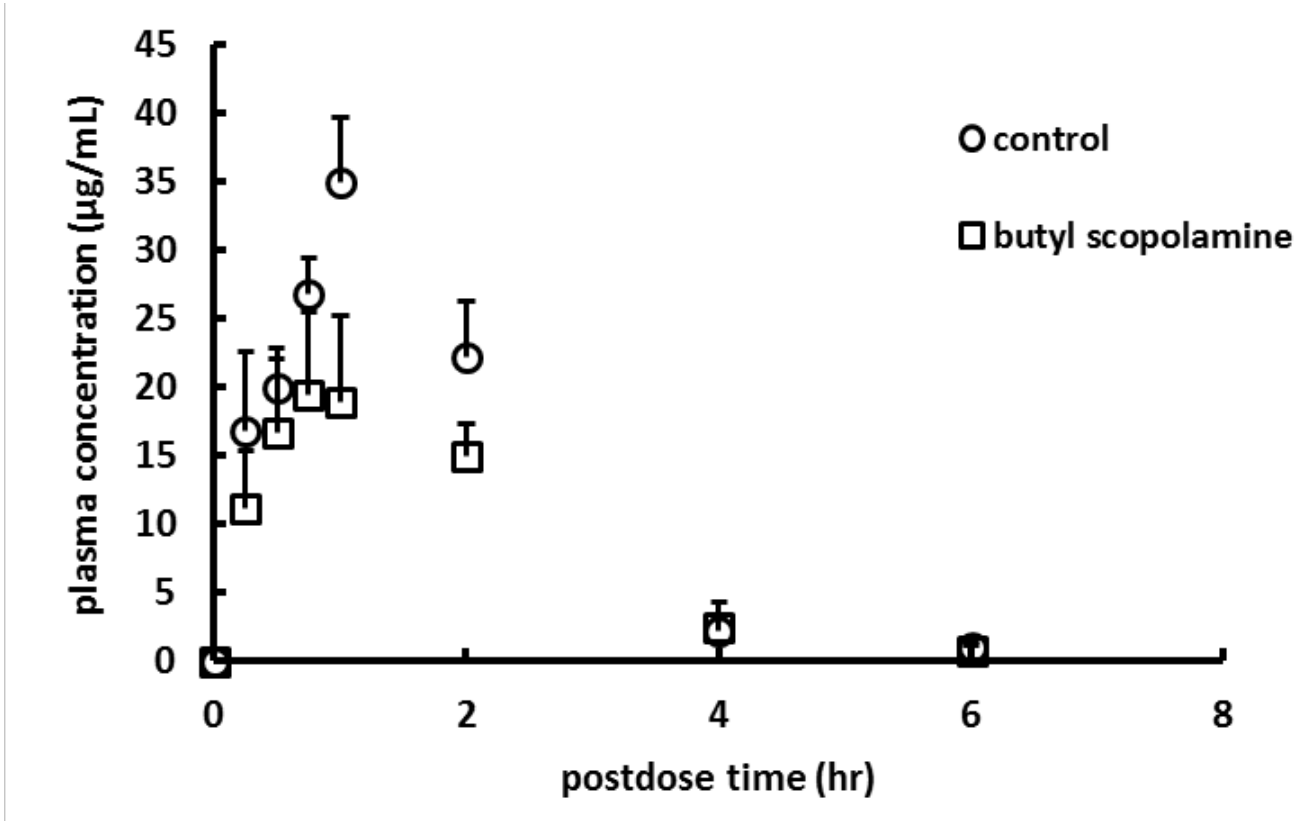

plasma concentration (µg/mL)
45
40
35
30
25
20
15
10
5
0
0
2
4
6
8
postdose time (hr)
control
butyl scopolamine

Fig. 5 (A)

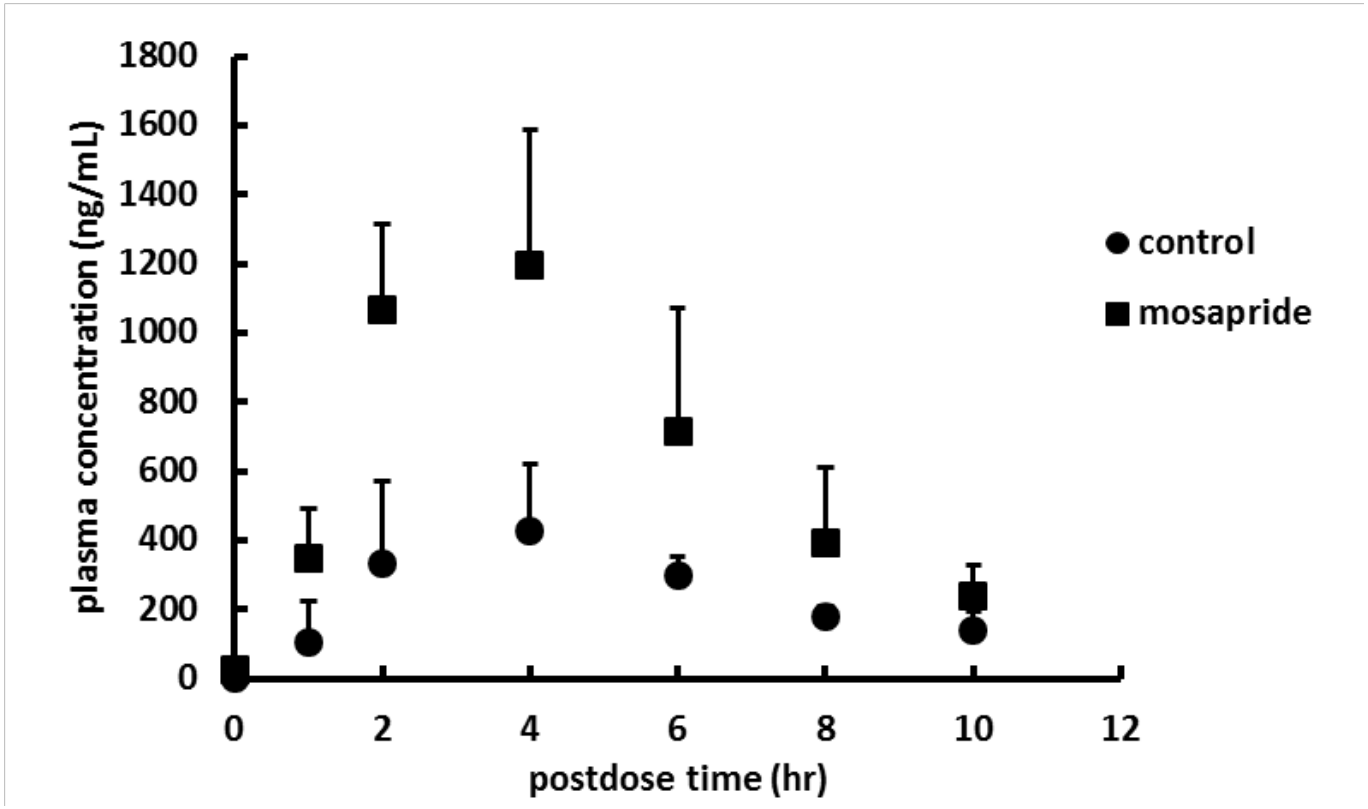

plasma concentration (ng/mL)
1800
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
postdose time (hr)
control
mosapride

Fig. 5 (B)

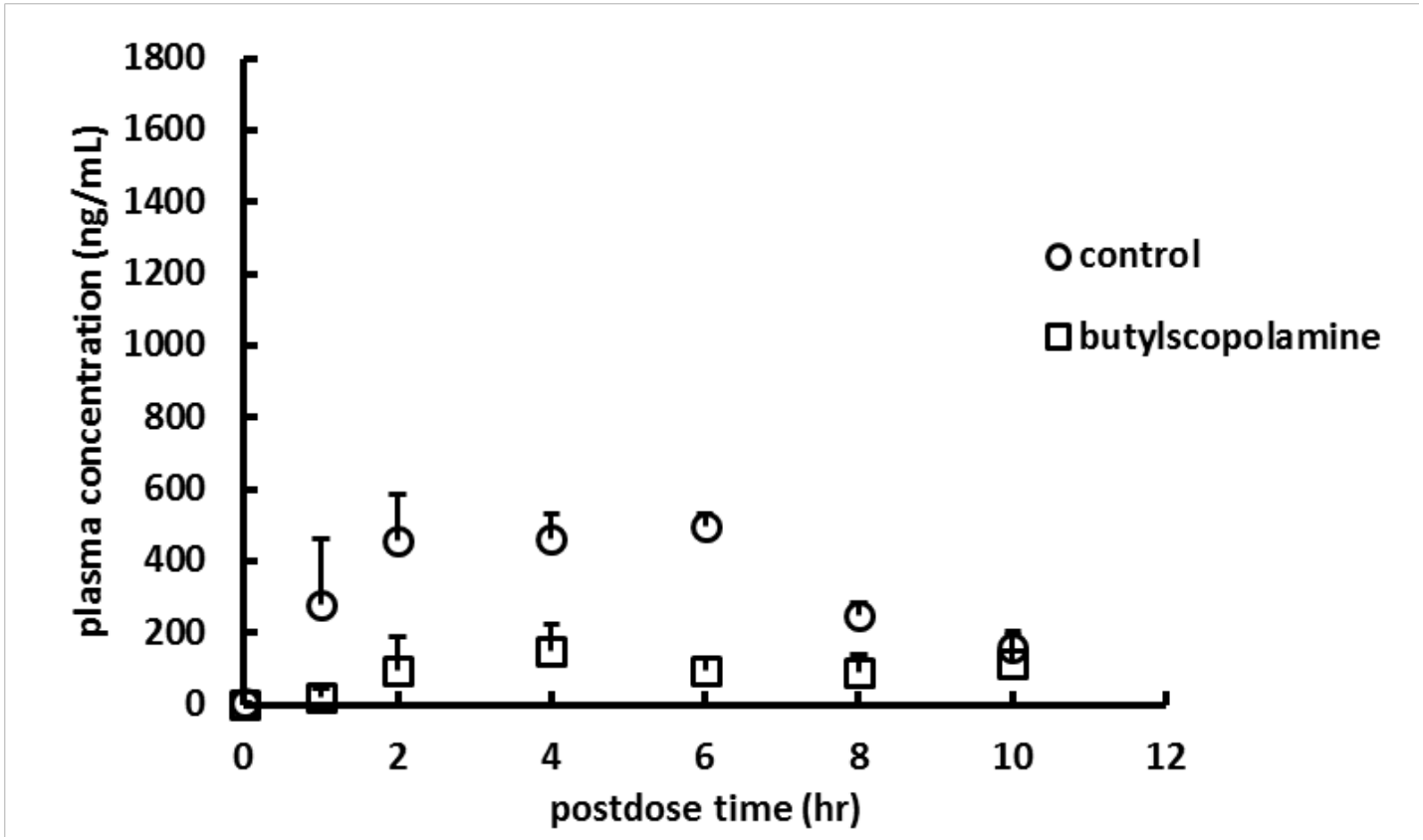

1800
1600
1400
1200
1000
800
600
400
200
0
plasma concentration (ng/mL)
control
butylscopolamine
0
2
4
6
8
10
12
postdose time (hr)

Fig. 6 (A)

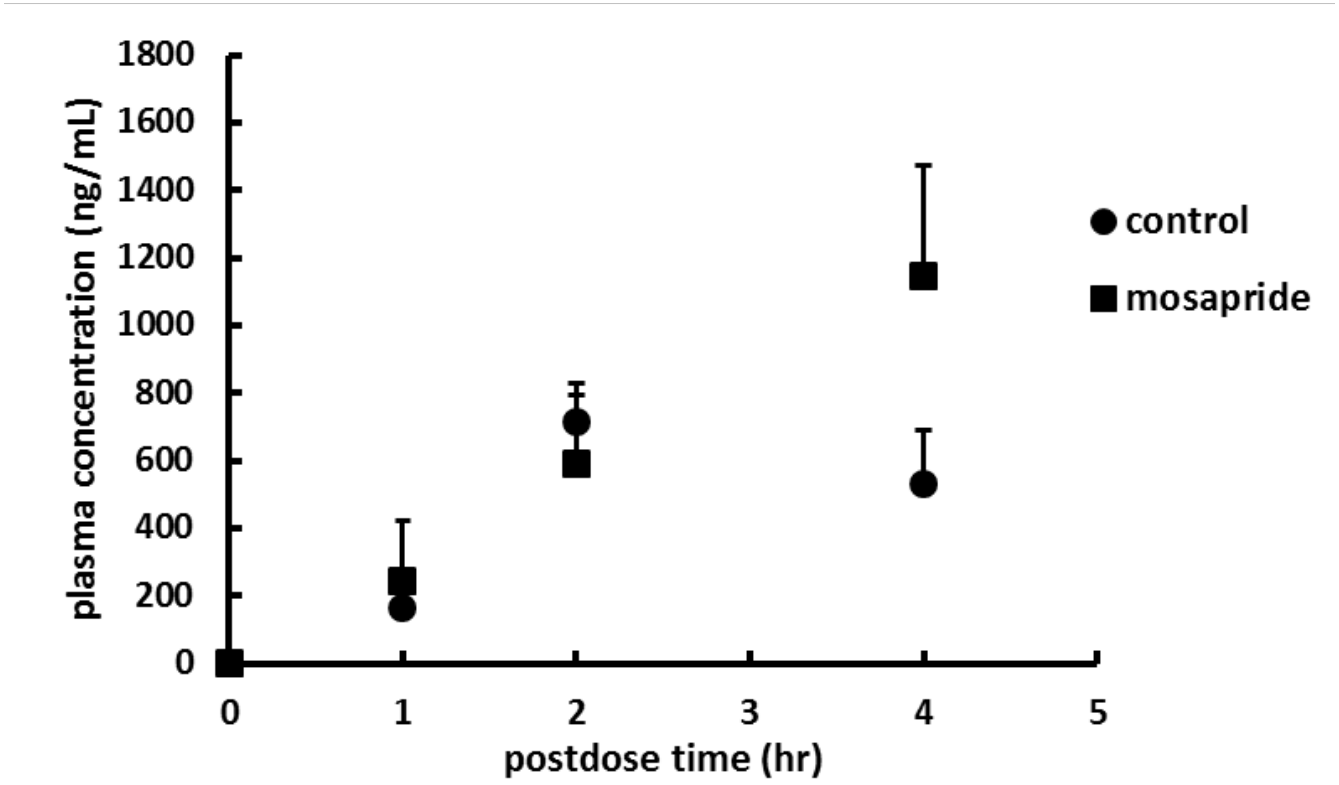

plasma concentration (ng/mL)
1800
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
postdose time (hr)
control
mosapride

Fig. 6 (B)

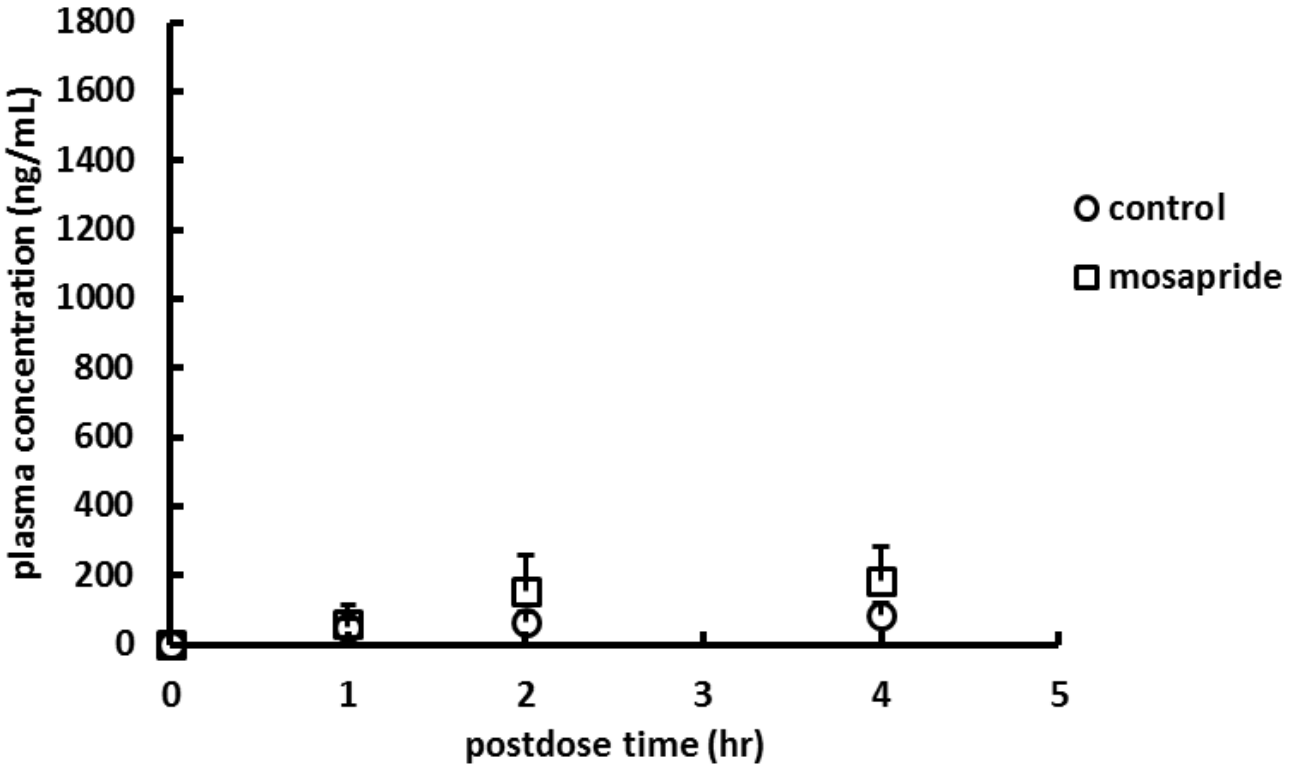

1800
1600
1400
1200
1000
800
600
400
200
0
plasma concentration (ng/mL)
0
1
2
3
4
5
postdose time (hr)
control
mosapride

Fig. 7

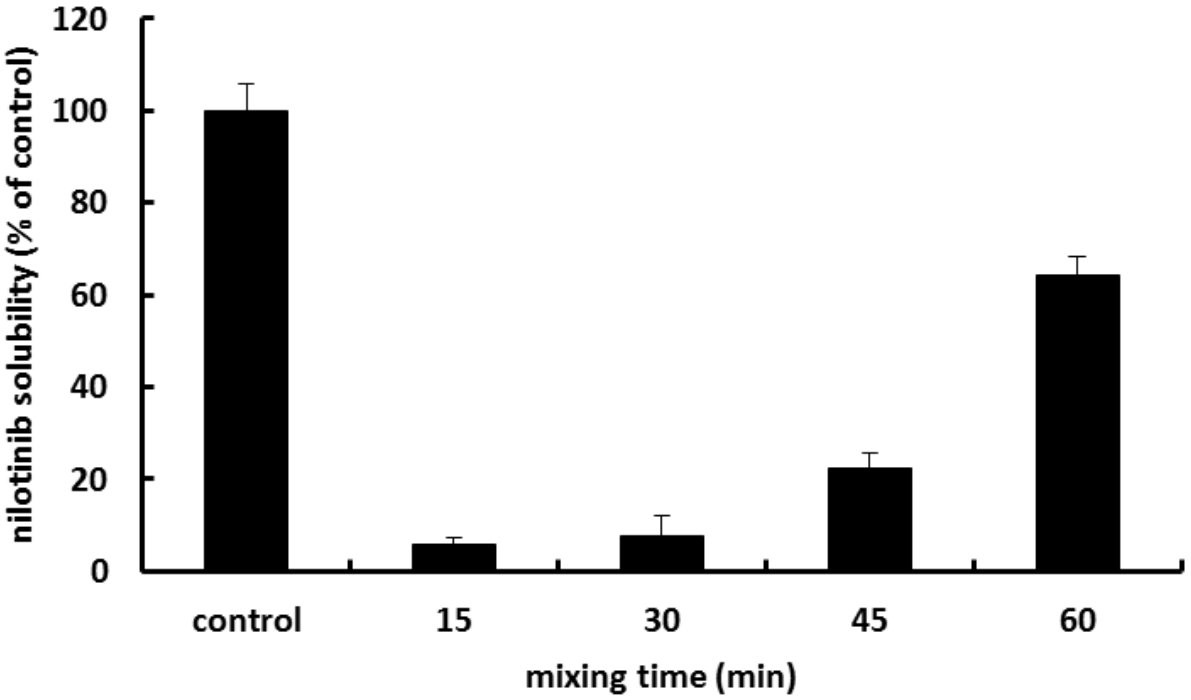

120
100
80
60
40
20
0
nilotinib solubility (% of control)
control
15
30
45
60
mixing time (min)

Fig. 8

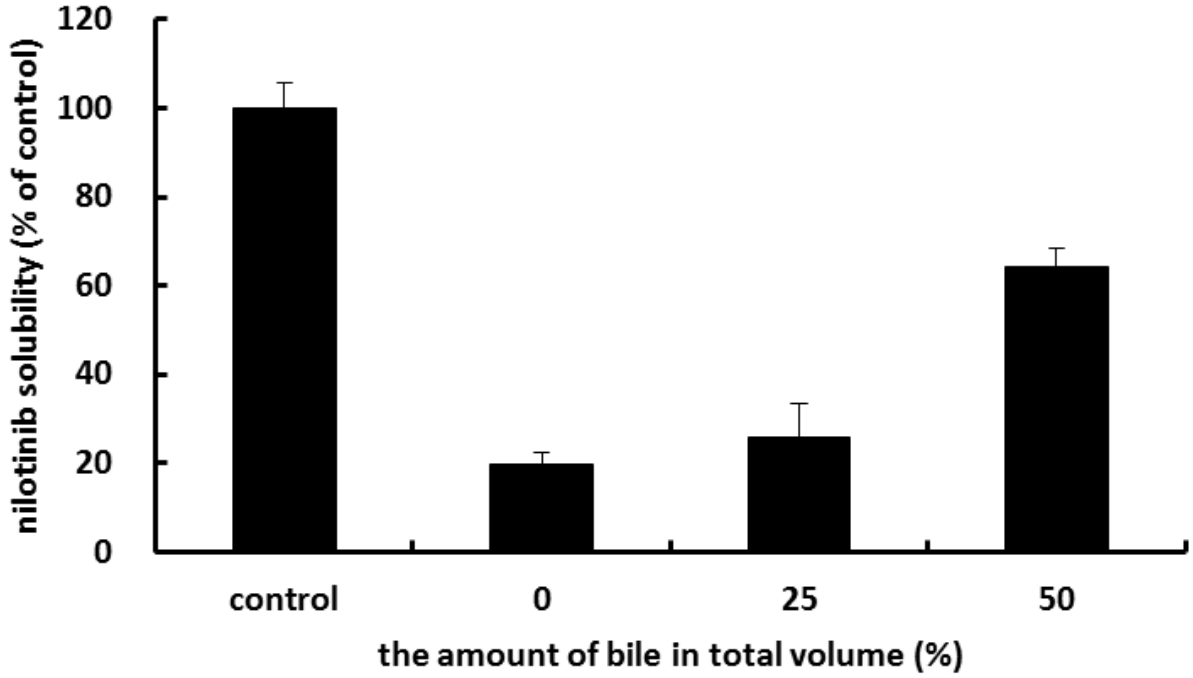
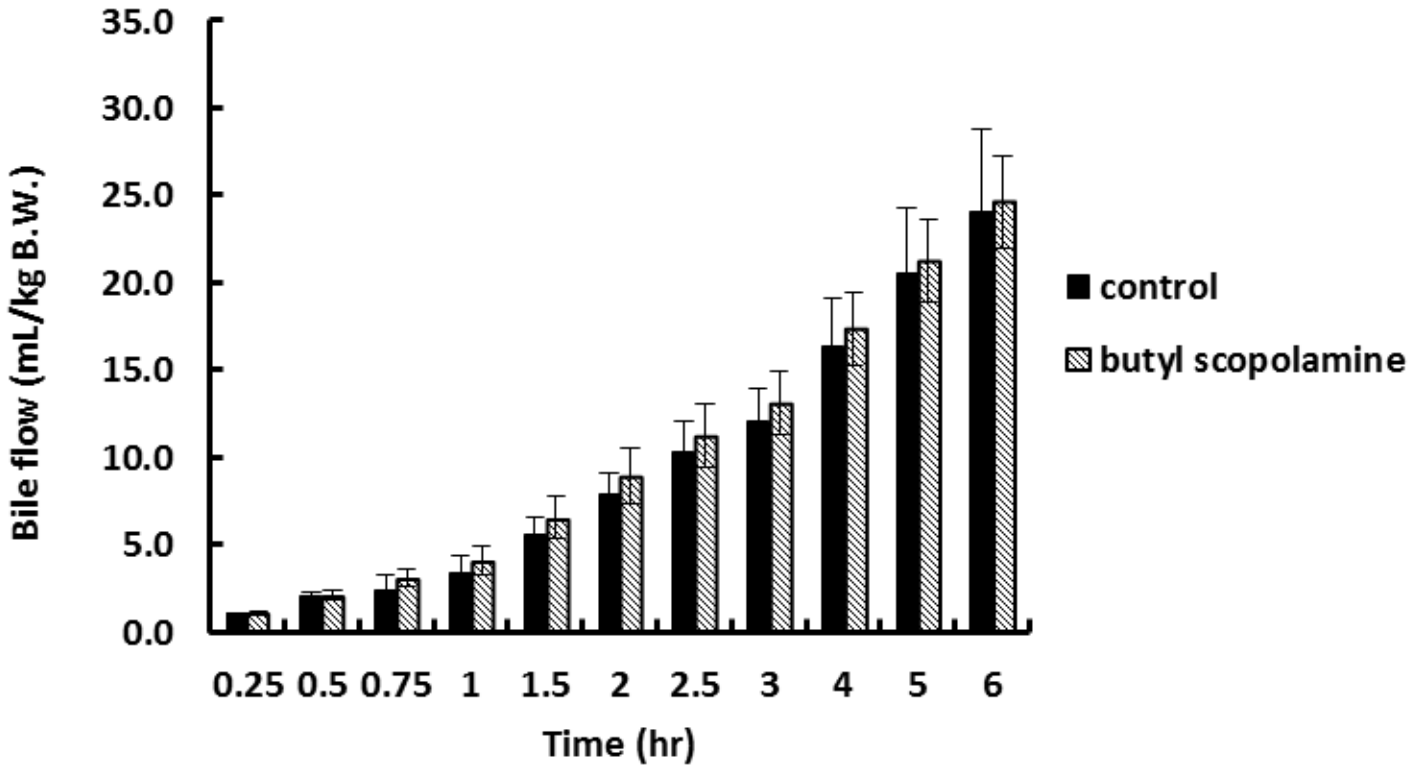

nilotinib solubility (% of control)
120
100
80
60
40
20
0
control
0
25
50
the amount of bile in total volume (%)

Fig. 9


35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Bile flow (mL/kg B.W.)
0.25 0.5 0.75 1 1.5 2 2.5 3 4 5 6
Time (hr)
control
butyl scopolamine