筑　波　大　学

博 士 （医 学） 学 位 論 文

# Effects of gastric acid secretion on pharmacokinetics of lapatinib and erlotinib

（胃酸分泌の変動がラパチニブ及びエルロチニブの薬物動態に及ぼす影響）

２０１７

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# Contents

## Abbreviations

| | |
|---|---|
| AST | asparatate aminotransferase |
| ALT | alanine aminotransferase |
| AUC | area under the blood concentration time curve |
| C/D | concentration-to-dose |
| CL/F | oral clearance |
| CTCAE | common terminology criteria for adverse events |
| CV | coefficient of variation |
| EGFR | epidermal growth factor receptor |
| $H_2$RB | histamine $H_2$ receptor blocker |
| HPLC | high-performance liquid chromatography |
| ILD | interstitial lung disease |
| IS | internal standard |
| Ka | absorption rate constant |
| LC-MS/MS | high-performance liquid chromatography with tandem mass-spectrometric detection |
| LDH | lactate dehydrogenase |

| | |
|---|---|
| LLOQ | lower limit of quantification |
| NSCLC | non-small cell lung cancer |
| ODS | octadecylsilyl silica-gel |
| OFV | objective function value |
| PK | pharmacokinetic |
| PPI | proton pump inhibitor |
| PPK | population pharmacokinetics |
| T-Bil | total bilirubin |
| TDM | therapeutic drug monitoring |
| TKI | tyrosine kinase inhibitor |
| UV | ultraviolet |
| Vd | volume of distribution |

# Introduction

Tyrosine kinases are involved in cancer cell proliferation, apoptosis, angiogenesis and metastasis. A number of tyrosine kinase proteins were identified and have been shown to play a role in the development of several types of cancer [Krause DS et al, 2005]. Tyrosine kinase inhibitors (TKIs) specifically target the ATP-binding site of tyrosine kinases, implicated in cell signaling and thus influencing both cell proliferation and survival [van Erp NP et al, 2009; Scheffler M et al, 2011]. The epidermal growth factor receptor (EGFR) is a part of a subfamily of four closely related receptors: EGFR, HER-2/*neu* (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4). The receptors exist as inactive monomers, which dimerize after ligand activation [Sedlacek HH, 2000]. Lapatinib is a potent inhibitor of the EGFR and ErbB-2 tyrosine kinase [Rusnak DW et al, 2001]. Erlotinib is selective inhibitors for EGFR tyrosine kinase [Moyer JD et al, 1997]. Lapatinib and erlotinib are used for the treatment of breast cancer and non-small cell lung cancer (NSCLC), respectively [Geyer CE et al, 2006; Shepherd FA et al, 2005; Rosell R et al, 2012; Zhou C et al, 2011].

Therapeutic drug monitoring (TDM) has been confirmed to be valuable for administration plan of various therapeutic drugs, especially drugs with narrow therapeutic windows and large individual pharmacokinetic (PK) variability [Begg EJ et al, 2001]. TDM helps to avoid therapeutic failures due to poor compliance or insufficient dose and adverse or toxic events including over dose or drug-drug interactions [Ried LD et al, 1990]. TDM is also effective in the field of anticancer chemotherapy when the relationship between systemic exposure and clinical response is found. For example, trough concentration monitoring of imatinib, which is used for patients with chronic myeloid leukemia, could be used to assess for clinical response [Picard S et al, 2007]. Similar strategies can be used for other TKIs.

Food and drug interactions need to be considered as factors of PK variability in TKIs [Singh BN et al, 2004], since they may affect the blood concentration affecting the clinical effects and adverse events. It has been reported that the intestinal absorption of TKIs was affected by the pH variation in the stomach under food intake and gastric acid suppressants administration [Ohgami M et al, 2013]. Among the gastric acid suppressants, histamine $H_2$ receptor blockers ($H_2$RB) and proton pump inhibitors (PPI),

potent inhibitor gastric acid secretion, provide higher gastric pH [Miner PB Jr et al, 2007], resulting in reducing solubility of TKIs in gastric juice. Therefore, concomitant use of gastric acid suppressants potentially decrease the systemic exposure of TKIs, though the magnitude is different between $H_2$RB and PPIs co-administration [Ohgami M et al, 2013]. The area under the blood concentration time curve (AUC) for lapatinib and erlotinib are increased by 325% and 109%, respectively, when administered after food intake [Koch KM et al, 2009; Ling J et al, 2008], and are decreased by 26% and 46%, respectively, when co-administered with PPI in healthy subjects [Koch KM et al, 2013; Kletzl H et al, 2015]. It is recommended that lapatinib and erlotinib should be administered under fasting state and avoided the co-administration of gastric acid suppressants in their indication of package insert [TYKERB (lapatinib) prescribing information; TARCEVA (erlotinib) prescribing information]. However, it has never been confirmed the change in pharmacokinetics of lapatinib and erlotinib under food intake or co-administration of gastric acid suppressants in clinical setting.

To assess the impacts of gastric acid status, food intake and gastric acid suppressants co-administration on the pharmacokinetics of lapatinib

and erlotinib, the author conducted the following investigations: (1) development of the method for determining of lapatinib and erlotinib in human plasma; and (2) TDM for lapatinib and erlotinib in patients with breast cancer and NSCLC, respectively, under the various gastric status.

This thesis comprises 2 chapters as follows: chapter 1 describes the high-performance liquid chromatography (HPLC) equipped with ultraviolet (UV) detector for the determining lapatinib and erlotinib in human plasma, chapter 2 describes that effect of dose timing on the blood concentration of lapatinib in patients with breast cancer and the effects of gastric acid suppressants co-administration on the plasma concentration of erlotinib in patients with NSCLC.

## Chapter 1

## A simple high-performance liquid chromatography UV method for the determination of lapatinib and erlotinib in human plasma

Lapatinib and erlotinib, anilinoquinazoline derivatives, are selective inhibitors for the EGFR tyrosine kinase [van Erp NP et al, 2009; Scheffler M et al, 2011]. Lapatinib is used to treat advanced or metastatic breast cancer in human EFGR type 2-overexpressing tumors in combination with capecitabine [Geyer CE et al, 2006]. Erlotinib is used to treat advanced or metastatic non-small cell lung cancer [Shepherd FA et al, 2005; Rosell R et al, 2012; Zhou C et al, 2011] and pancreatic cancer in combination with gemcitabine [Moore MJ et al, 2007]. Lapatinib and erlotinib are currently prescribed at a fixed dose of 1250 mg/day and 150 mg/day, respectively. It has been reported that large interindividual variability in systemic exposure was found under the fixed-dose regimen [Gao B et al, 2012; Widmer N et al, 2014]. Because pharmacokinetic–pharmacodynamic studies have demonstrated a correlation between plasma concentrations and clinical outcomes [Gao B et al, 2012; Widmer N et al, 2014], TDM may be useful for assessing the clinical

outcomes and adverse events. One of the reasons for the limitations of lapatinib and erlotinib TDM is the assay method, in which HPLC with tandem mass-spectrometric detection (LC-MS/MS) has been commonly used in previous studies [Haouala A et al, 2009; Hsieh S et al, 2004; Götze L et al, 2012; Andriamanana I et al, 2013; Honeywell R et al, 2010]. Since LC-MS/MS is not generally available in clinical institutions such as hospitals and outpatient clinics, an alternative way to determine both drugs is required.

In the present study, a simple HPLC UV method was developed for the determination of lapatinib and erlotinib in human plasma.

## Materials and Methods

### *Chemical and reagents*

Lapatinib and erlotinib were purchased from LC laboratories (Woburn, MA, USA). HPLC grade acetonitrile, methanol, and trifluoroacetic acid were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The chemical structure of lapatinib and erlotinib is indicated in Figure 1.

*Preparation of solutions*

A stock solution containing 1 mg/mL of lapatinib or 1 mg/mL of erlotinib was prepared in the mobile phase solvent. Each solution was stored at −20 °C until use. Working solutions and control samples for confirming assay precision were prepared using human plasma (COSMO BIO Co. Ltd., Tokyo, Japan).

Working solutions of lapatinib and erlotinib were prepared at concentrations of 0.125–8.00 μg/mL. Quality control samples were prepared using different stock solutions of lapatinib and erlotinib. Concentrations of quality control samples were 0.125, 1.00, and 8.00 μg/mL for lapatinib and erlotinib. Each drug can be used as an internal standard (IS) for measuring the other one. IS solutions were prepared as 10.0 μg/mL solution in distilled water.

*Chromatographic conditions*

The HPLC system (Agilent 1120 Compact LC) consisted of an isocratic pump, auto sampler, a UV detector, and EZChrom Elite

WorkStation® (Agilent Technologies, Santa Clara, CA, USA). Separation of the compounds of interest was achieved using an octadecylsilyl silica-gel (ODS) column (TSK-gel 5 μm; 4.6 × 150 mm, Tosoh, Tokyo, Japan). The column was maintained at room temperature. The detection wavelength was set at 316 nm. The mobile phase solvent consisting of acetonitrile, methanol, water, and trifluoroacetic acid (26:26:48:0.1) was pumped at a flow rate of 1.0 mL/min.

*Assay procedures*

An aliquot of plasma sample (200 μL) spiked with 50 μL of IS solution was treated with 700 μL of acetonitrile to precipitate the proteins. After vortex mixing for 20 s, the sample was centrifuged at 5000 g for 10 min and the supernatant was evaporated to dryness. The residue was reconstituted with 100 μL of mobile phase and 20 μL was injected into the HPLC. Calibration curves were obtained at concentrations of 0.125, 0.250, 0.500, 1.00, 2.00, 4.00, and 8.00 μg/mL for lapatinib and erlotinib.

Recovery was evaluated at concentrations of 0.125, 1.00, and 8.00 μg/mL for lapatinib and erlotinib. Extraction recovery was obtained by

comparing the peak height of the extracted control sample with that of the identical concentration of determinants in the mobile phase without extraction. Experiments were carried out on five replicates for each determinant.

Intra-day assay precision was evaluated at concentrations of 0.125, 1.00, and 8.00 μg/mL for lapatinib and erlotinib, measuring five replicates for each level. Inter-day assay precision was evaluated by repeated analysis performed on five different days. Coefficients of variation (CVs: %) were calculated within single runs (intra-day assay) and between different assays (inter-day assay), and the bias between nominal and measured concentrations was assessed.

The stability for both drugs in the plasma samples was assessed at the concentrations of 0.125, 1.00, and 8.00 μg/mL for freeze-thaw, short-term, long-term, and autosampler stability. Samples for assessing freeze-thaw stability underwent three cycles of freeze and thaw. Samples for assessing short-term stability were stored for 24 hr at room temperature or at 4 °C; for long-term stability, for 21 days at −20 °C; and for autosampler stability, for 24 hr at room temperature.

*Application*

The method was applied to determine lapatinib and erlotinib concentrations in patients with breast cancer (n = 4) and NSCLC (n = 4), respectively. Blood samples were collected into heparinized collection tubes between 8 and 24 hr after the drug administration for determining the trough levels of lapatinib and erlotinib. Steady-state concentrations were monitored in patients treated with lapatinib or erlotinib on a once-daily schedule in an outpatient setting.

This study was approved by the Ethics Committee of Ibaraki Prefectural Central Hospital (approval no.: 26-4 dated April 23, 2014) and University of Tsukuba Hospital (approval no.: H27-148 dated October 16, 2015). An informed consent was obtained from all patients.

## Results

Typical chromatograms for control and patients' samples are shown in Figure 2. Retention times of erlotinib and lapatinib were 4.8 and 8.5 min, respectively (Figure 2B). No interference was observed in the

chromatograms for both control and patients' samples (Figure 2C-a and 2D-a). Co-administered drug for a patient was capecitabine, which did not interfere in determining lapatinib (Figure 2C-a and 2C-b). Co-administered amlodipine and L-carbocisteine with erlotinib did not interfere for determining erlotinib (Figure 2D-a and 2D-b). Other concomitant drugs (famotidine, ranitidine, lansoprazole, dexamethasone, pitavastatin, mecobalamin, potassium chloride, brotizolam, duloxetine, metoclopramide, ursodeoxycholic acid, dimethicone, meloxicam, silodosin, mirabegron, teprenone, dried ferrous sulfate, minocycline, and loperamide) also did not appear in the chromatogram for determining lapatinib and erlotinib.

The calibration curves for lapatinib and erlotinib were linear in the range of 0.125–8.00 μg/mL (Y = 0.3650X−0.0016, $r$ = 0.9999) and (Y = 0.6817X−0.0554, $r$ = 0.9999), respectively. Each drug concentration was measured five times to make the calibration curves. The CVs for measured peak height ratios at each drug concentration were less than 4.7%.

Mean recovery of lapatinib and erlotinib at the concentrations of 0.125, 1.00, and 8.00 μg/mL was higher than 89.9% with the CVs less than 3.5% (Table 1).

The CVs for intra- and inter-day assays were, respectively, less than 2.1% and 5.1% for lapatinib and less than 2.8% and 6.1% for erlotinib. The biases for intra- and inter-day assays were, respectively, less than 1.1% and 8.1% for lapatinib and 6.4% and 6.7% for erlotinib (Table 2).

The stabilities under various storage conditions were examined at the concentration of 0.125–8.00 μg/mL for lapatinib and erlotinib. The biases for freeze-thaw, short-term, long-term, and autosampler stability were 0.9–14.0%, 5.3–14.6%, 0.6–6.0%, and 2.0–11.4% with the CVs less than 5.9% for lapatinib and 6.9–14.7%, 0.3–12.5%, 4.7–11.3%, and 3.4–8.2% for erlotinib with the CVs less than 4.9% for erlotinib, respectively (Table 3).

Patients' plasma samples of lapatinib (n = 24) and erlotinib (n = 15) were analyzed. Intrapatient variability (CVs: %) for plasma concentrations of lapatinib and erlotinib were 15.7–35.4% and 9.6–21.5%, respectively (Table 4). A typical case for monitoring plasma lapatinib is presented in Figure 3 (Case 2). The plasma concentrations of lapatinib were 1.87–2.24 μg/mL with the occurrence of a skin rash (grade 1–2). The plasma concentration of lapatinib was 2.77 μg/mL, when interstitial pneumonitis appeared, prompting cessation of the lapatinib administration.

## Discussion

The advantage of the present HPLC is to detect both lapatinib and erlotinib in a same chromatogram; therefore, each drug can be used as an IS for measuring the other one because both TKIs are not used concomitantly in a patient under cancer chemotherapy. The present HPLC method had sufficient selectivity and sensitivity for the determination of plasma lapatinib and erlotinib. HPLC conditions were optimized by convenient common ODS columns, UV detection, and simple mobile-phase solvents. The total run time for chromatographic analysis was less than 10 min (Figure 2). The recoveries of lapatinib and erlotinib were higher than 89.9%. Intra- and inter-day assay precision was sufficient with the CVs less than 6.1%, almost identical to those in the previous method [Escudero-Ortiz V et al, 2013; Faivre L et al, 2011]. The lower limit of quantification (LLOQ) for lapatinib and erlotinib was 0.125 μg/mL, which was 25-fold higher than that observed with previous LC-MS/MS methods [Haouala A et al, 2009; Honeywell R et al, 2010]. LLOQs of the present method, however, was enough to determine trough concentrations for lapatinib (0.3 μg/mL) and erlotinib (1.4 μg/mL) in

the Phase I study involving patients with breast cancer or NSCLC [Burris HA 3rd et al, 2005; Burris HA 3rd et al, 2009; Hidalgo M et al, 2001]. Similar plasma concentrations of erlotinib have been also reported in population pharmacokinetics (PPK) study for lung cancer patients [Fukudo M et al, 2013].

It has been reported that metabolites for lapatinib and erlotinib were associated with hepatotoxicity [Teo YL et al, 2012; Czejka M et al, 2013]. Determining the metabolites, therefore, may provide clinically useful information. Although OSI-420, a erlotinib metabolite, can be distinguished from erlotinib on the chromatogram with the present HPLC, the sensitivity for determining OSI-420 was not enough for the quantification. The therapeutic range for plasma concentration of lapatinib has not been established yet [Bouchalova K et al, 2010]. Interstitial lung disease (ILD) is one of the severe adverse events of lapatinib [Capri G et al, 2010], of whose association with the plasma concentration has been unclear. In this study, the plasma concentration of lapatinib in case 2 was elevated from 1.87 to 2.77 μg/mL when ILD appeared. This elevation may have been caused by chemotherapy-induced liver dysfunction as shown by the increased levels of

bilirubin (Figure 3). It has been reported that concomitant use of lapatinib and dexamethasone, a CYP3A4 inducer, increased the risk for hepatotoxicity due to the production of toxic lapatinib metabolites under CYP3A4 induction [Teo YL et al, 2012], though the metabolites were not determined in the present case. In this case, lapatinib was terminated because of ILD, of whose symptoms and CT scan imaging were improved after stopping lapatinib. Since capecitabine administration after stopping lapatinib did not induce ILD-related symptoms, it was considered that ILD in this case may have been caused by lapatinib administration.

The present method was successfully applied in clinical settings of lapatinib and erlotinib in cancer patients. This method is applicable to TDM of lapatinib and erlotinib in clinical institutes that do not have LC-MS/MS.

**Table 1** Recoveries of lapatinib and erlotinib in spiked plasma samples (n = 5)

| Lapatinib | | | | Erlotinib | | | |
|---|---|---|---|---|---|---|---|
| Concentration (μg/mL) | Mean ± SD (%) | CV (%) | Bias (%) | Concentration (μg/mL) | Mean ± SD (%) | CV (%) | Bias (%) |
| 0.125 | 90.4 ± 3.1 | 3.4 | −9.6 | 0.125 | 100.8 ± 3.0 | 3.0 | 0.8 |
| 1.00 | 89.9 ± 0.6 | 0.7 | −10.1 | 1.00 | 105.8 ± 3.2 | 3.0 | 5.8 |
| 8.00 | 91.7 ± 1.4 | 1.5 | −8.3 | 8.00 | 105.0 ± 3.1 | 3.0 | 5.0 |

**Table 2** Intraday and interday assay precision for the determination of lapatinib and erlotinib.

| Lapatinib | | | | Erlotinib | | | |
|---|---|---|---|---|---|---|---|
| Concentration (μg/mL) | Mean ± SD (μg/mL) | CV (%) | Bias (%) | Concentration (μg/mL) | Mean ± SD (μg/mL) | CV (%) | Bias (%) |
| Intra-day (n = 5) | | | | Intra-day (n = 5) | | | |
| 0.125 | 0.126 ± 0.003 | 2.0 | 1.0 | 0.125 | 0.133 ± 0.001 | 0.1 | 6.3 |
| 1.00 | 1.004 ± 0.009 | 0.9 | 0.4 | 1.00 | 0.978 ± 0.010 | 1.0 | −2.2 |
| 8.00 | 7.949 ± 0.125 | 1.6 | −0.6 | 8.00 | 8.028 ± 0.220 | 2.7 | 0.4 |
| Inter-day (n = 5) | | | | Inter-day (n = 5) | | | |
| 0.125 | 0.115 ± 0.006 | 5.0 | −8.0 | 0.125 | 0.133 ± 0.001 | 1.1 | 6.6 |
| 1.00 | 0.942 ± 0.044 | 4.6 | −5.8 | 1.00 | 0.956 ± 0.041 | 4.3 | −4.4 |
| 8.00 | 7.781 ± 0.303 | 3.9 | −2.7 | 8.00 | 7.549 ± 0.454 | 6.0 | −5.6 |

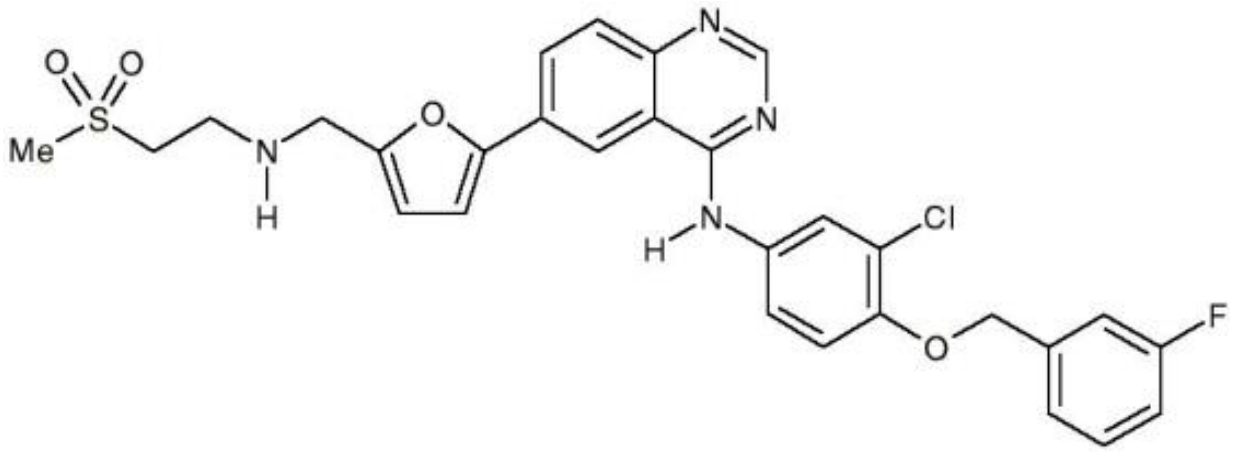
**Table 3** Stabilities of lapatinib and erlotinib under various storage conditions (n = 3).

| | | Lapatinib | | Erlotinib | |
|---|---|---|---|---|---|
| Condition | Concentration (μg/mL) | Bias (%) | CV (%) | Bias (%) | CV (%) |
| Freeze and thaw cycle 1 | 0.125 | −10.6 | 1.0 | 7.5 | 0.6 |
| | 1.00 | −5.5 | 1.4 | −6.9 | 1.4 |
| | 8.00 | −0.9 | 1.2 | −14.7 | 0.5 |
| Freeze and thaw cycle 3 | 0.125 | −14.0 | 4.0 | 7.9 | 1.3 |
| | 1.00 | −11.0 | 2.9 | −11.3 | 2.1 |
| | 8.00 | −6.8 | 3.6 | −7.4 | 2.9 |
| Short-term at RT (24hr) | 0.125 | −14.6 | 2.5 | 6.4 | 1.7 |
| | 1.00 | −10.7 | 1.4 | −0.3 | 1.9 |
| | 8.00 | −5.3 | 2.0 | −7.0 | 2.1 |
| Short-term at 4°C (24hr) | 0.125 | −10.3 | 1.3 | 12.5 | 1.3 |
| | 1.00 | −11.3 | 1.2 | −2.3 | 1.6 |
| | 8.00 | −10.4 | 1.4 | −10.4 | 4.9 |
| Long-term 21 days | 0.125 | −3.8 | 5.8 | 11.3 | 1.6 |
| | 1.00 | −6.0 | 0.8 | −4.7 | 2.7 |
| | 8.00 | −0.6 | 0.2 | −7.7 | 3.0 |
| Autosampler (24hr) | 0.125 | −11.4 | 0.3 | 8.2 | 0.7 |
| | 1.00 | −6.5 | 0.6 | −3.4 | 4.8 |
| | 8.00 | −2.0 | 0.9 | −7.7 | 2.0 |

**Table 4** Plasma concentration of lapatinib and erlotinib

| | Case | Age/Sex | BW (kg) | Dose (mg/kg) | Day | Time after Administration (hr) | Concentration (μg/mL) | | CV (%) | Adverse events (Grade) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | No. of sample | Mean ± SD | | |
| Lapatinib | 1 | 51/F | 47.0 | 26.6 | 659–708 | 24 | 4 | 1.96 ± 0.33 | 17.0 | |
| | 2 | 50/F | 51.5 | 24.3 | 36–71 | 24 | 6 | 2.20 ± 0.35 | 15.7 | Rash (2)<br>Interstitial pneumonitis (2) |
| | 3 | 72/F | 52.6 | 23.8 | 49–147 | 8 | 6 | 2.06 ± 0.73 | 35.4 | Diarrhea (1) |
| | 4 | 58/F | 58.0 | 21.6 | 22–85 | 24 | 8 | 2.00 ± 0.62 | 30.9 | |
| Erlotinib | 1 | 67/M | 62.2 | 2.41 | 126–203 | 24 | 4 | 0.80 ± 0.16 | 20.1 | Rash (1)<br>Diarrhea (1) |
| | 2 | 65/M | 55.0 | 1.82 | 351–405 | 24 | 3 | 1.39 ± 0.13 | 9.6 | Rash (2) |
| | 3 | 52/M | 63.7 | 1.18 | 56–120 | 24 | 5 | 1.40 ± 0.30 | 21.5 | Rash (3)<br>Diarrhea (1) |
| | 4 | 79/F | 48.0 | 0.52 | 690–774 | 24 | 3 | 0.26 ± 0.03 | 10.7 | Rash (1) |

(a)




(b)

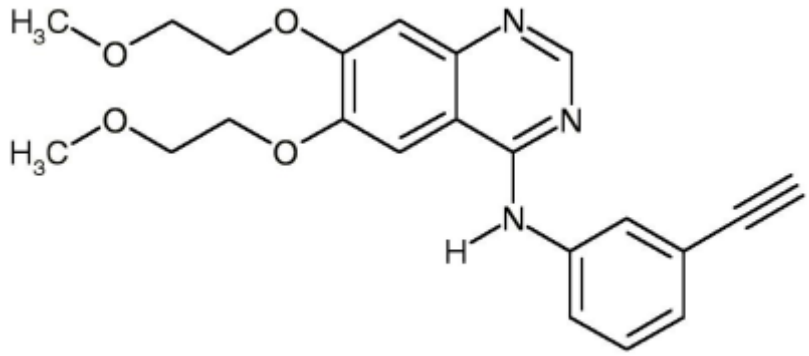



**Figure 1** Chemical structures of lapatinib (a) and erlotinib (b).

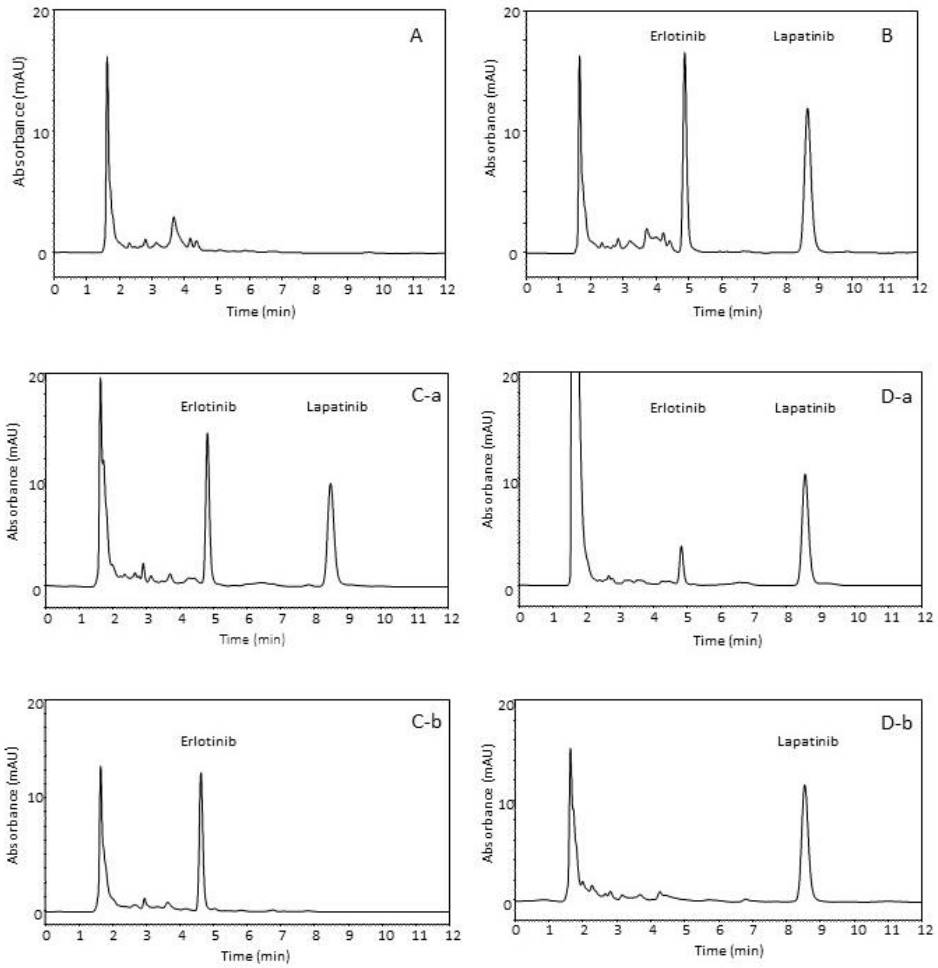



**Figure 2** Typical chromatograms: A, Blank human plasma; B, Blank human plasma after the addition of 2.5 μg/mL of lapatinib and erlotinib; C-a, Plasma sample from a patient treated with 1250 mg lapatinib once daily (the concentration of lapatinib was 2.34 μg/mL); C-b, Plasma sample from the same patient after stopping lapatinib administration; D-a, Plasma sample from a patient treated with 50 mg erlotinib once daily (the concentration of erlotinib was 0.67 μg/mL); D-b, Plasma sample from the same patient after stopping erlotinib administration.

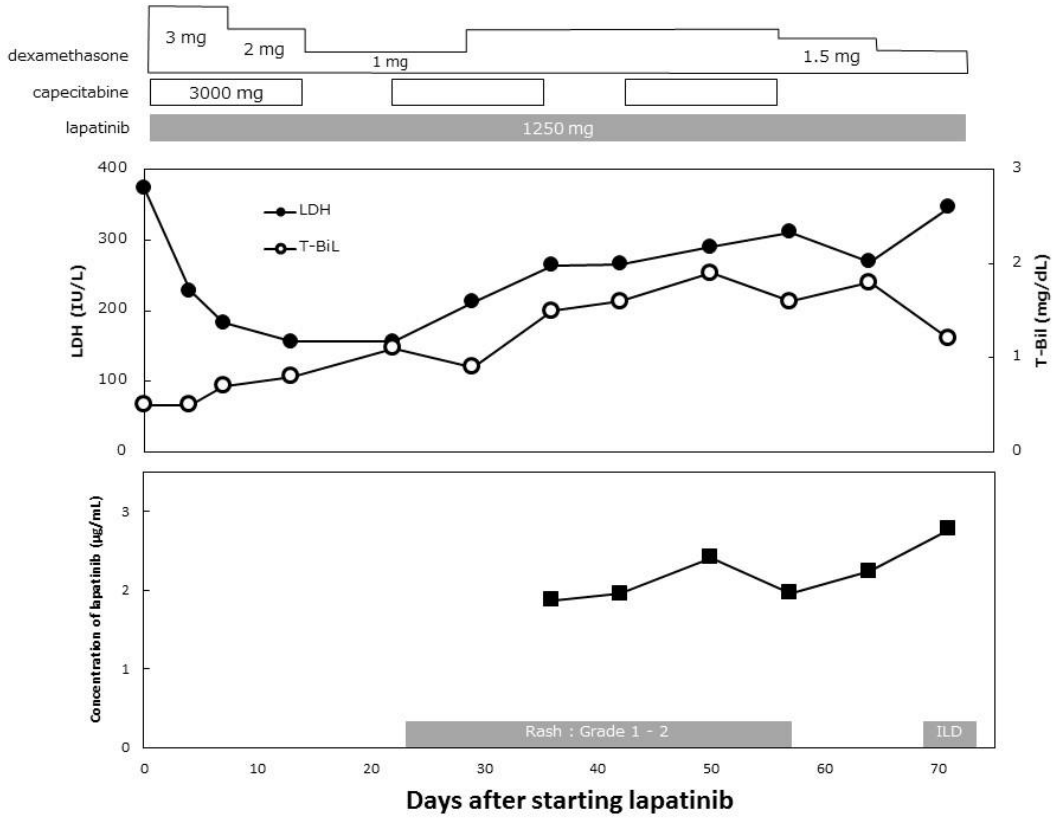



**Figure 3** Clinical course of case 2 treated with lapatinib. The plasma concentration of lapatinib was gradually increased due to total bilirubin (T-Bil) elevation from 0.5 to 1.9 mg/dL when interstitial lung disease (ILD) appeared on day 71. ILD was confirmed by computed tomography scan image and elevation of lactate dehydrogenase (LDH) (346 IU/L), which were recovered after stopping lapatinib administration.

## Chapter 2

## 2-1 Effect of dose timing on the blood concentration of lapatinib in patients with breast cancer.

Lapatinib, a TKI, is indicated for treatment of HER2-dominant breast cancer in combination with capecitabine [Geyer CE et al, 2006]. Since intestinal absorption and subsequent blood concentration of lapatinib is affected by food intake [Koch KM et al, 2009], the package insert recommends administration under fasting conditions, either 1 hr pre- or post-prandial. However, it is unclear as to whether or not both dose timings provide a similar blood lapatinib concentration. Here, we report the differences in blood lapatinib concentration under at least 1 hr pre- or post-prandial fasting states and the result that post-prandial fasting provides the higher levels.

### Patients and Methods

Twenty-one patients were recruited from Kyoto University Hospital, Shikoku Cancer Center, and Ibaraki Prefectural Central Hospital. As

chemotherapy for breast cancer, patients received 21 [13–33] mg/kg/day of lapatinib and blood samples were collected 8–26 hr after administration to measure trough levels at steady state. Patients were divided into two groups according to the dose timing: at least 1 hr before (before group) or 1 hr after food (after group). Blood lapatinib concentration was determined by HPLC as previously described [Ohgami M et al, 2016]. Adverse events, consisting of grade 2 or higher of rash and diarrhea (Common Terminology Criteria for Adverse Events (CTCAE) ver.4.0) were also examined in the study groups. All aspects of this study were approved by the Ethics Committee of each institution.

## Results and Discussion

Trough lapatinib concentration was significantly higher in the after group compared with the before group (1,405 [230–2,486] vs. 340 [167–1,288] ng/mL, $P$ = 0.003) (Table 5). The concentration-to-dose (C/D) ratio of lapatinib was also significantly higher in the after group (66 [13–115] vs. 17 [7–54] ng/mL/mg/kg, $P$ = 0.001) (Table 5). Adverse events (grade 2, 3) comprised of rash and diarrhea seemed to be more frequent in the after

group, though this difference was not significant (Table 5).

Lapatinib concentrations in the after group were approximately 4 times higher than the before group even though both groups were fasted according to the dosage instructions. This effectively means that higher blood concentration can be achieved with 1 hr post-prandial dosing. The intestinal absorption of lapatinib may be enhanced forming micelles with bile salts, which are secreted after food intake.

Although the therapeutic range for blood lapatinib concentration has not been established, it has been reported that keeping the trough level at 550 ng/mL was safe and effective [Fumoleau P et al, 2014]. The toxicity range for lapatinib-induced adverse events, such as rash and diarrhea, is also currently unclear but Burris and colleagues reported that lapatinib-induced rashes might be associated with systemic exposure [Burris HA 3rd et al, 2009]. In our present results, the frequency of lapatinib-induced rashes was higher in the after group, in which blood lapatinib concentrations were as high as 1,234 [539–1,899] ng/mL. No relationship between systemic exposure and clinical outcome of lapatinib has been found in current investigation of Japan Breast Cancer Research Group

including present data.

In conclusion, even if lapatinib is administered according to the recommendation of the package insert, blood lapatinib concentrations are different between the indicated 1 hr pre- or post-prandial dose timings. Because trough concentration is potentially associated with adverse events, clinical trials comparing both dose timings are required to assess the practical therapeutic range with respect to lapatinib. These data suggested that 1hr pre-prandial dose timing of lapatinib may be able to manage the lapatinib-induced rash without diminishing the therapeutic efficacy.

**Table 5** Patient characteristics and lapatinib concentration

| | Dose timing of lapatinib | |
|---|---|---|
| | Before food (n = 8) | After food (n = 13) |
| Age (years)* | 66 (31–72) | 50 (28–72) |
| Body weight (kg) | 50 (32–56) | 52 (38–76) |
| Dose of lapatinib (mg/kg) | 21 (18–32) | 21 (13–33) |
| Sampling time after taking lapatinib (hr) | 24 (8–26) | 23 (13–25) |
| Concentration of lapatinib (ng/mL)** | 340 (167–1288) | 1405 (230–2486) |
| C/D (ng/mL/mg/kg) ** | 17 (7–54) | 66 (13–115) |
| Adverse events (Grade 2 ≤) | | |
| Rash/ Diarrhea | 0/2 (0%/25%) | 3/6 (23%/46%) |

Data present as median (range) or number (percent). * $P < 0.05$, ** $P < 0.01$

## 2-2 Effects of proton pump inhibitor co-administration on the plasma concentration of erlotinib in patients with non-small cell lung cancer

Erlotinib, an EGFR TKI, is used for treating of advanced or metastatic NSCLC [Shepherd FA et al, 2005; Rosell R et al, 2012; Zhou C et al, 2011] and pancreatic cancer [Moore MJ et al, 2007]. Because erlotinib is weakly basic, its solubility is reduced in gastric juice under elevated pH condition, and therefore intestinal absorption of this agent is impaired [TARCEVA (erlotinib) prescribing information]. A pharmacokinetic study of healthy subjects reported that the concomitant administration of the PPI, omeprazole, or the $H_2$RB, ranitidine, with erlotinib reduced blood concentration of erlotinib [Kletzl H et al, 2015]. The current information for prescribing erlotinib recommends that concomitant use of PPI should be avoided if possible. If patients need to be treated with an $H_2$RB such as ranitidine, erlotinib must be administered 10 hr after the $H_2$RB dosing and at least 2 hr before the next $H_2$RB dose [TARCEVA (erlotinib) prescribing information].

However, clinical implications of the drug interaction between gastric acid suppressants and erlotinib, with regard to treatment outcome, have been controversial. There have been conflicting reports that concomitant use of gastric acid suppressants reduced the efficacy of erlotinib [Chu MP et al, 2015; Lam LH et al, 2016] and remained unchanged [Hilton JF et al, 2013; Kumarakulasinghe NB et al, 2016]. To date, there are no reports evaluating the use of plasma erlotinib for drug interaction in patients with NSCLC. In the present study, we measured plasma concentration of erlotinib to assess the effects of gastric acid suppressants, PPIs and $H_2$RB, co-administration on intestinal absorption of erlotinib in patients with NSCLC.

## Patients and methods

*Patients and sample collection*

Forty-two patients receiving erlotinib therapy for NSCLC were recruited from Ibaraki Prefectural Central Hospital and University of Tsukuba Hospital. Patients were classified into 3 groups: (1) without co-administration of gastric acid suppressants (control group: n = 22), (2) with co-administration of PPI (PPI group: n = 12), and (3) co-administration

of $H_2RB$ ($H_2RB$ group: n = 6). The blood samples (n = 343) were collected at 240 [7–1099] days after starting of erlotinib. Data including sex, age, body weight, EGFR status, histological subtype stage at diagnosis, smoking, prescription drugs, and adverse events were collected from paper and electronic medical records. Thirty-three patients (78.6%) had EGFR mutation. Of the 42 patients, 12 (28.6%) administered PPI, 6 (14.3%) administered $H_2RB$. Adverse events, skin rash and diarrhea, were evaluated using CTCAE Version 4.0. Association of adverse events (rash and diarrhea) with trough plasma concentration of erlotinib was examined in 250 blood samples from 33 patients.

The study was approved by the Ethics Committees of Ibaraki Prefectural Central Hospital and the University of Tsukuba Hospital. Informed consent was obtained from all participants.

*Determination of plasma erlotinib*

Steady state plasma concentrations of erlotinib were measured using HPLC, as previously described [Ohgami M et al, 2016]. An aliquot of plasma sample (200 μL) spiked with 50 μL of internal standard solution (lapatinib)

was treated with 700 μL of acetonitrile to precipitate the proteins. After vortex mixing, the sample was centrifuged at 5000 g for 10 minutes and the supernatant was evaporated to dryness. The residue was reconstituted with 100 μL of mobile phase and the 20 μL was injected into the HPLC. The detection wavelength was set at 316 nm. Mobile phase solvent consisting of acetonitrile, methanol, water, and trifluoroacetic acid (26:26:48:0.1) was pumped at a flow rate of 1.0 mL/min. The detection limit of HPLC is as low as 0.125 μg/mL. Intra- and inter-day validations at 0.125–8.00 μg/mL are 0.1–2.7% and 1.1–6.0%, respectively [Ohgami M et al, 2016].

*Population pharmacokinetics analysis*

The PPK of plasma erlotinib was performed with Phoenix NLME (Version 1.1; Pharsight Corp, St Louis, Missouri, USA). The analysis included 343 blood samples from 42 patients. The blood samples were collected at 22.3 ± 6.1 hr after dosing of erlotinib. Oral clearance (CL/F) of erlotinib was estimated using the one-compartment model with first-order absorption, according to a previous report [Emoto-Yamamoto Y et al, 2015]. Considering the limited data available for the absorption phase, the

absorption rate constant (Ka) and volume of distribution (Vd) were fixed at the reported value of 1.97/hr and 163 L, respectively [Emoto-Yamamoto Y et al, 2015].

The proportional model for the interpatient variability of PK parameter was as follows:

$$\theta i = \theta_{TV} e^{\eta i},$$

where $\theta i$ is the parameter value for the $i$th individual, $\theta_{TV}$ is the typical value of the parameter in the population, and $\eta$ is a random variable with a mean of 0 and variance of $\omega^2$. A multiplicative error model for residual error, which describes the difference between the observed and predicted concentration was as follows:

$$C_{obs} = C_{pred}(1+\varepsilon),$$

where $C_{obs}$ is the observed concentration, $C_{pred}$ is the predicted concentration, and $\varepsilon$ is a random variable with a mean of 0 and a variance of $\sigma^2$.

The influences of 4 continuous covariates, age, body weight, total-bilirubin, and serum creatinine, and 3 categorical covariates, sex, smoking status, and concomitant use of PPI, on CL/F were evaluated in the population analysis. A liner model and a power model with normalization on

the covariate to a median were used for a continuous covariate. The relationship between CL/F and a categorical covariate was evaluated as follows:

$$CL/F = \theta_1 \theta_2^{IND},$$

where IND was an indicator variable. Forward inclusion and backward elimination techniques were used for model building. Weighted residuals and a change in the objective function value (OFV, -2 log likelihood) were noted in the model building process. A decrease in the OFV of greater than 3.84 ($P < 0.05$, $d.f. = 1$) was considered statistically significant when each covariate was factored into the base model (univariate analysis). All significant covariates in the univariate analysis were added to the base model. The importance of each covariate was reevaluated using the backward elimination technique. During backward selection, an increase in OFV of greater than 3.84 ($P < 0.05$) was required to justify the retention in the final model. The predictive performance of the final population model was assessed using bootstrap analysis. Resampling was repeated 1000 times and the estimates (2.5–97.5th percentiles) from bootstrap analysis were compared with those from the original dataset.

*Statistical analysis*

The C/D ratios for erlotinib, which were calculated as plasma trough concentrations divided by dose per kilogram of body weight, and estimated CL/F were compared among the 3 groups using Kruskal-Wallis tests followed by Mann-Whitney tests. Statistical analysis was performed using SPSS 22 for Windows (SPSS IBM Japan Inc, Tokyo, Japan). A $P$ value less than 0.05 was considered to be significant.

## Results

Patient characteristics of the present study are shown in Table 6. The median [range] plasma concentration of erlotinib was 0.80 [0.10–2.60] μg/ml. Association of adverse events, rash and diarrhea, with trough plasma concentration of erlotinib was examined. Plasma concentrations of erlotinib correlated with severity of rash assessed by CTCAE (Figure 4). Significant difference in trough erlotinib concentration was observed between grade ≤ 1 and grade ≥ 2 (0.67 [0.10–1.85] vs. 1.02 [0.43–2.60] μg/mL, $P < 0.01$). No patient had grade 3 rash in PPI and $H_2$RB groups (Figure 4). No difference

was found in plasma concentration of erlotinib between the patients with and without diarrhea (data not shown).

Plasma concentrations of erlotinib were compared among 3 groups: control, PPI, and $H_2$RB groups (Table 7). Erlotinib dose and plasma concentration in the $H_2$RB group were higher than those in the control group (Table 7). On the other hand, plasma concentration of erlotinib in the PPI group was lower than those in the control group, even though the erlotinib dose was higher than the control group (Table 7).

The C/D ratio in the PPI group was significantly lower compared with the control group (0.39 [0.08–0.76] vs. 0.51 [0.28–1.28] μg/mL/mg/kg, $P < 0.05$) (Table 7). Lower C/D ratio was also observed in the $H_2$RB group (0.48 [0.33–0.81] μg/mL/mg/kg), though the difference was not statistically significant compared with the control group.

PPK analysis was performed to estimate the oral CL/F. The final model included concomitant use of PPI as a significant covariate for estimating CL/F (Table 8). The final model for erlotinib CL/F was:

$$\mathrm{CL/F} = \theta_1 \theta_2^{\mathrm{PPI}},$$

where PPI is 1 for concomitant use of PPI and 0 for without PPI.

The estimates of the parameter with 95% confidence intervals are shown in Table 8. The inter-patient variability in CL/F for base model (no covariates) and the final model were 51.9% and 48.9%, respectively. Goodness-of-fit plots for the final model indicated a satisfactory correlation between observed and predicted concentration of plasma erlotinib (Figure 5), suggesting that the final model was relevant. Model validation, performed on the basis of 1000 bootstrap replicates of which parameter estimates (2.5–97.5th percentiles) were in close agreement with those of the final model, also supported the adequacy of the final model (Table 8). Concomitant use of PPI was the only significant covariate for estimating CL/F in the final model. The estimated CL/F in the PPI group were significantly higher than the control group (5.55 [3.36–14.52] vs. 3.95 [2.01–10.44] L/hr, $P < 0.05$) (Figure 6). The estimated CL/F in the $H_2$RB group also showed a higher (4.82 [2.08–6.32] L/hr) value, though statistically significance was not observed (Figure 6).

## Discussion

The most common toxicity associated with anti-EGFR therapy is a

dermatologic reaction, particularly acneiform eruption [Agero AL et al, 2006]. It has been reported that the blood concentration of TKI was associated with the development of skin toxicity in chemotherapies including lapatinib and erlotinib [Burris HA 3rd et al, 2009; Steffens M et al, 2016]. Results of the present study also showed a correlation between plasma concentration of erlotinib and severity of skin rash (Figure 4), but not of diarrhea. This means that blood concentration monitoring for plasma erlotinib might be useful for assessing and managing the skin toxicity of this drug.

The results of the present study revealed that concomitant use of gastric acid suppressants, PPI and $H_2$RB, reduced the plasma concentration of erlotinib due to drug interaction at gastrointestinal tracts, where elevated pH produced lower solubility of erlotinib in the gastric juice [TARCEVA (erlotinib) prescribing information]. This drug interaction was observed to a remarkable degree in PPI co-administration compared with $H_2$RB. The C/D ratio and estimated CL/F of erlotinib in the PPI group were significantly altered; the C/D ratio and CL/F showed 24% reduction and 41% elevation, respectively, compared with the control group (Table 7). The $H_2$RB group also provided similar results; the C/D and CL/F showed 6% reduction and 22%

elevation, respectively (Table 7), though the difference was not statistically significant.

The PPK model for estimating the CL/F included concomitant use of PPI as a significant covariate. This means that PPI co-administration alters pharmacokinetics of erlotinib via elevation of the CL/F. It is considered that elevation of CL/F under PPI co-administration is associated with both increased metabolic clearance and decreased intestinal absorption of erlotinib [Kletzl H et al, 2015]. In the present case, later mainly contributed to alter CL/F of erlotinib rather than former, which is due to possible activity of PPI as the CYP3A4 inducer, a responsible enzyme for hepatic metabolism of erlotinib [Ling J et al, 2006]. Enzyme induction of PPI is very weak or negligible clinically, even though the PPI acts as an inducer for CYP enzymes in in-vitro cell culture [Curi-Pedrosa R et al, 1994]. On the contrary, effects of PPI on gastric acid suppression leading reduction in intestinal absorption of erlotinib is strong and obvious compared with enzyme induction.

Although the timing of erlotinib administration was largely fixed at 2 hr after food in the morning, PPI dose timing differed between the patients: morning, evening, and before sleeping. Because PPIs strongly suppress

gastric acid secretion and keep the gastric pH elevated over a 24 hr period, the effect of PPI co-administration on plasma erlotinib concentration might be similar at any dose timing. On the other hand, difference in the dose timing of $H_2$RB provided different results. Four patients whose dose timing of $H_2$RB was once a day in the evening did not show any reduction of plasma concentration of erlotinib because the suppression of gastric secretion was not sufficient to make any drug interaction the next morning when the patients took their erlotinib dose.

Effects of concomitant use of gastric acid suppressants on the therapeutic outcome of erlotinib are controversial. It has been reported that gastric acid suppressant co-administration related to shorter progression-free survival in the patients with NSCLC [Chu MP et al, 2015; Lam LH et al, 2016], though the blood concentration of erlotinib was not determined. Petrelli and colleagues reported that the patients with NSCLC who developed skin rash had an increased clinical response compared with patients without rash [Petrelli F et al, 2012]. Both reports seem to be very important when considering the impact of our present results in terms of the plasma concentration of erlotinib. Co-administration of PPI may be

associated with unfavorable clinical outcome of erlotinib therapy, decreasing plasma erlotinib concentration and risk of skin rash. PPI co-administration may therefore cause difficulties in maintaining steady-state plasma concentrations with fixed doses of erlotinib. When patients require administration of gastric acid suppressants, especially PPI, dose adjustment, using therapeutic drug monitoring, would be required to improve the therapeutic outcome of erlotinib.

**Table 6** Patient characteristics (n = 42)

| Characteristics | Data |
|---|---|
| Male/Female | 18/24 |
| Age (years) | 66.5 ± 7.1 |
| Body weight (kg) | 59.0 ± 11.9 |
| EGFR status (wild/mutation/unknown) | 4/33/5 |
| Histology (adeno/squamous) | 41/1 |
| Smoking (never/former/unknown) | 17/18/7 |
| Treatment line (1st/2nd/3rd ≤) | 12/19/11 |
| Dose (mg/body)* (25/50/75/100/150) | 7/9/9/24/18 |
| Sampling time after dose (hr) | 22.3 ± 6.1 |
| Concomitant gastric acid suppressants (PPI/ $H_2$RB) | 12/6 |
| PPI | |
| Lansoprazole | 9 |
| Omeprazole | 1 |
| Esomeprazole | 1 |
| Rabeprazole | 1 |
| $H_2$RB | |
| Famotidine | 4 |
| Ranitidine | 2 |

Data present as mean ± SD.

* There are overlapped data in the same patient.

**Table 7** Patient characteristics in control, PPI, and $H_2RB$ groups

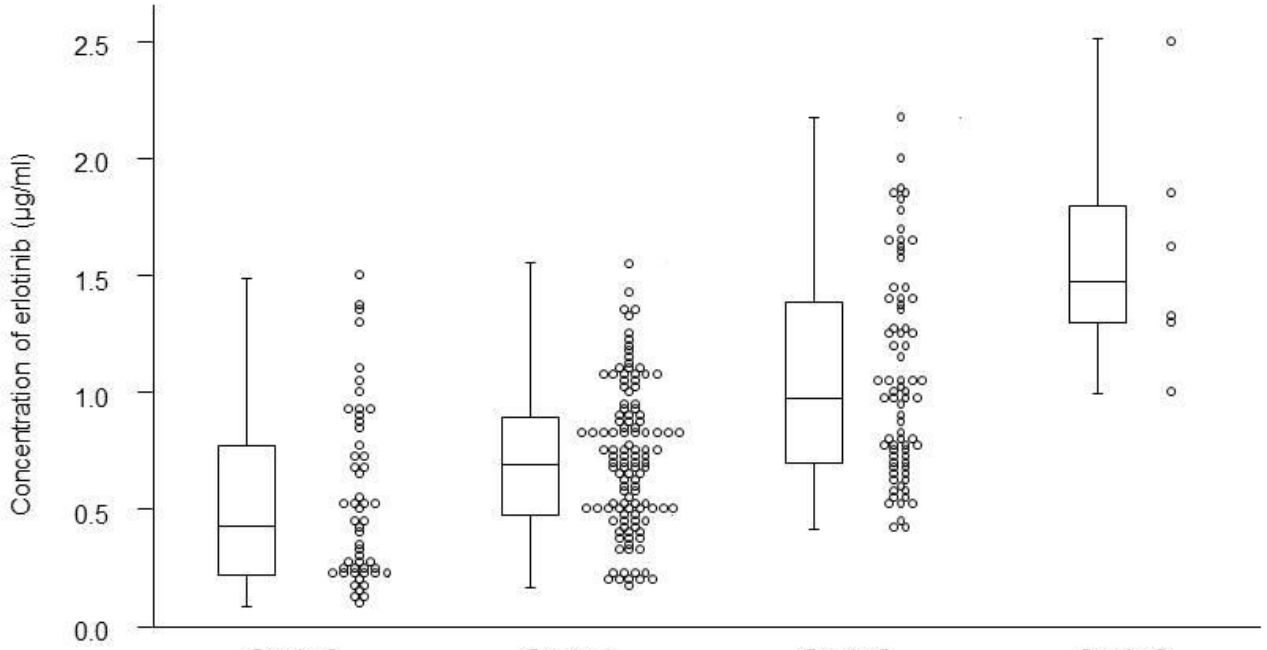
| | | Control (n = 24) | PPI (n = 12) | $H_2RB$ (n = 6) |
|---|---|---|---|---|
| No. of sample | | 205 | 65 | 73 |
| Male/Female | | 14/10 | 2/10 | 2/4 |
| Age (years) | | 66.6 ± 6.8 | 66.8 ± 7.9 | 65.5 ± 8.5 |
| Body weight (kg) | | 59.7 ± 9.2 | 56.9 ± 11.7 | 56.7 ± 14.8 |
| AST (IU/L) | | 23.8 ± 7.9 | 21.4 ± 6.1 | 29.4 ± 6.0 |
| ALT (IU/L) | | 23.0 ± 14.2 | 15.1 ± 4.7 | 19.9 ± 5.7 |
| Serum Creatinine (mg/dL) | | 0.81 ± 0.24 | 0.72 ± 0.60 | 0.89 ± 0.31 |
| Dose of erlotinib(mg/kg) | | 1.49 ± 0.70 | 1.87 ± 0.86 | 2.10 ± 0.61* |
| Concentration of erlotinib (μg/mL) | | 0.77 (0.16–2.60) | 0.66 (0.10–1.35) | 0.98 (0.53–1.84) † |
| C/D (μg/ml)/(mg/kg) | | 0.51 (0.28–1.28) | 0.39 (0.08–0.76)* | 0.48 (0.33–0.81) |
| CL/F (L/hr) | | 3.95 (2.01–10.44) | 5.55 (3.36–14.52)* | 4.82 (2.08–6.32) |
| Rash | Grade 0/1/2/3 | 2/7/9/6 | 3/5/4/0 | 1/1/4/0 |
| Diarrhea | Grade 0/1 | 16/8 | 9/3 | 5/1 |

Data present as mean ± SD or median (range). *$P$< 0.05: vs. control, †$P$< 0.05: vs. PPI

AST: Asparatate aminotransferase, ALT: Alanine aminotransferase

**Table 8** Population pharmacokinetic parameter estimates for erlotinib and its bootstrap validation

| | Final model | | Bootstrap (n = 1000) | |
|---|---|---|---|---|
| Parameter | Estimates | 95%CI | Estimates | 2.5th, 97.5th |
| $\theta_1$ | 4.36 | 3.49, 5.23 | 4.40 | 3.57, 5.48 |
| $\theta_2$ | 0.445 | 0.063, 0.827 | 0.462 | 0.029, 1.047 |
| Interpatient variability (%CV) | 48.9 | 38.0, 59.8 | 48.1 | 37.3, 58.9 |
| Residual variability (%CV) | 26.0 | 21.8, 30.1 | 25.8 | 22.1, 30.0 |




| | Grade 0 | Grade 1 | Grade 2 | Grade 3 |
|---|---|---|---|---|
| Control | 23 | 67 | 58 | 6 |
| PPI | 16 | 38 | 2 | 0 |
| $H_2RB$ | 13 | 12 | 15 | 0 |

**Figure 4** Correlation between rash grade and plasma concentration of erlotinib.

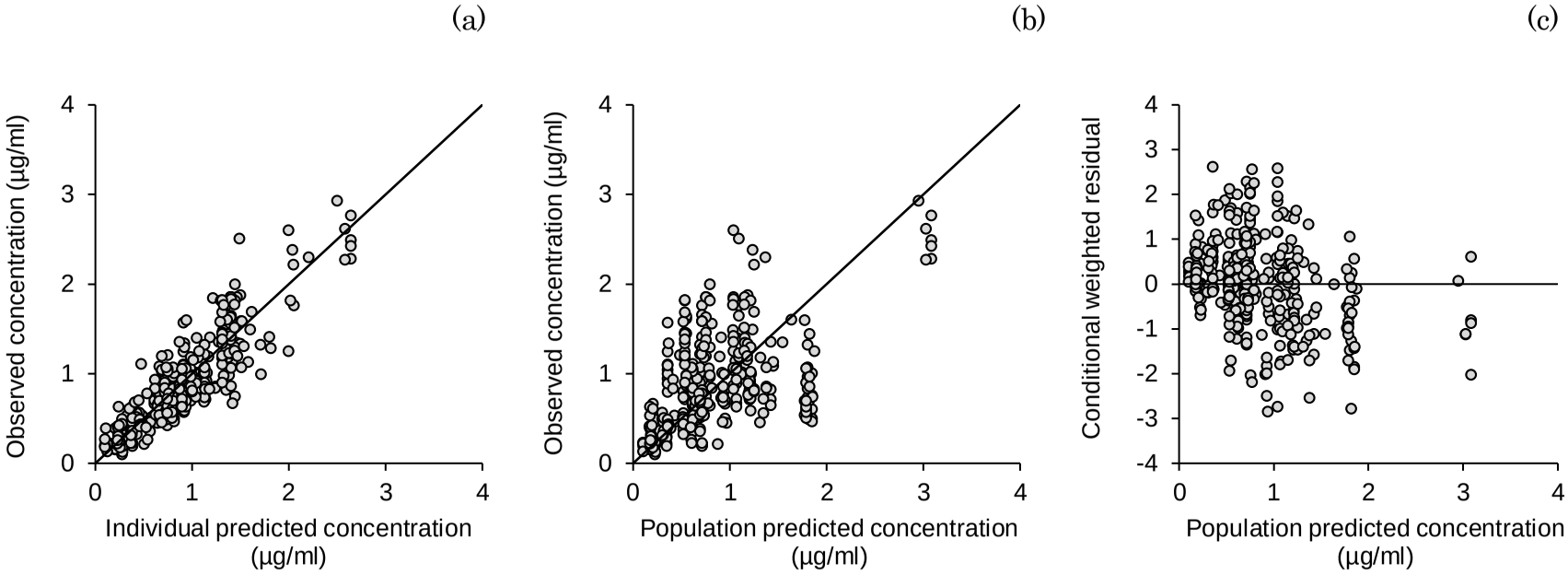



**Figure 5** Goodness- of-fit plots for the final model with covariate; observed versus individual predicted concentrations (a), observed versus population predicted concentrations (b), and conditional weighted residuals versus population predicted concentrations (c).

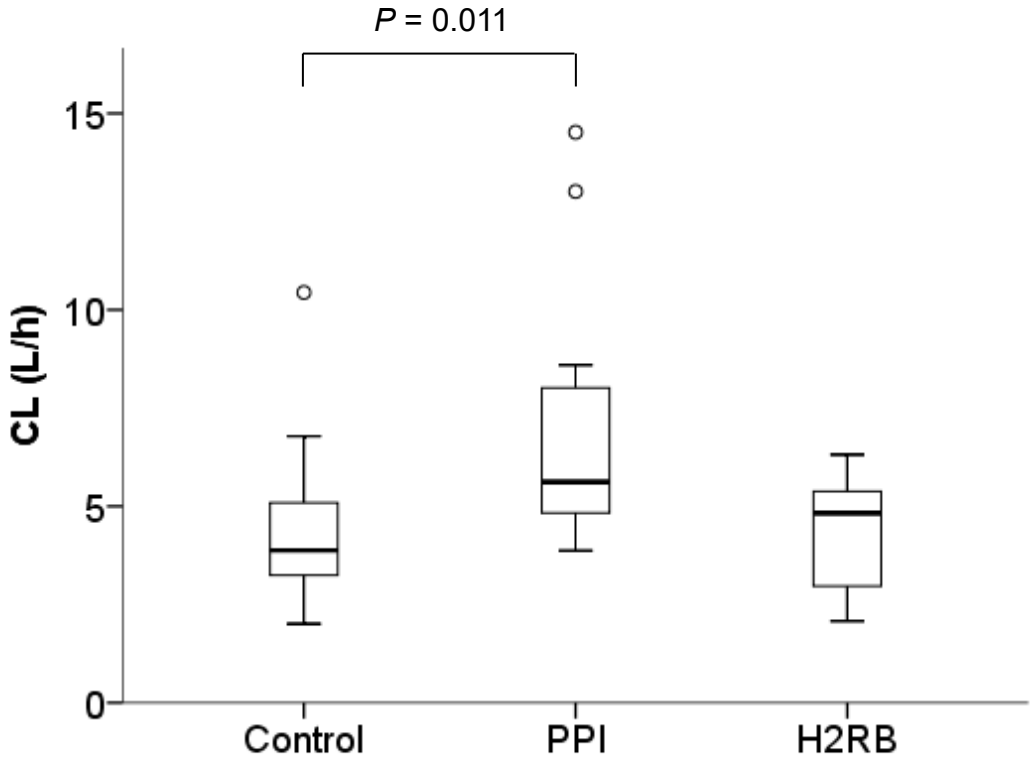



**Figure 6** Effects of co-administration of gastric acid suppressants on erlotinib CL/F using Bayesian estimation method in the 3 groups; control, PPI, and $H_2$RB.

# Conclusion

The author clarified three findings in this thesis as follows:

1) The author developed a simple HPLC UV method for determination of lapatinib and erlotinib in human plasma for conducting the TDM in cancer patients.

2) Blood lapatinib concentration was 4 times higher at least 1h post-prandial dose timings than at least 1h pre-prandial, even though the both timing was under fasting conditions according to the package insert of lapatinib.

3) Blood erlotinib concentrations under concomitant with gastric acid suppressants were lower than those without gastric acid suppressants via drug interaction suppressing the intestinal absorption of erlotinib. The magnitude of this drug interaction was more pronounced in co-administration of PPI compared with $H_2$RB.

The author concludes that TDM is useful for assessing the dose timing of lapatinib and drug interaction of erlotinib with PPI, because gastric acid secretion affected the absorption of both drugs.

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