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Short communication

Determination of CH330331, a novel 4-anilinoquinazoline inhibitor of epidermal growth factor receptor tyrosine kinase, in human Caco-2 monolayers by high performance liquid chromatography with ultraviolet detection: Application to a trans-epithelial transport study

Hai-Yan Sun [a,b], Su Guan [a], Hui-Chang Bi [a], Qi-Biao Su [a], Wen-Lin Huang [c], Balram Chowbay [d], Min Huang [a,**], Xiao Chen [e], Chun-Guang Li [f], Shu-Feng Zhou [g,*]

[a] Institute of Clinical Pharmacology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510080, China
[b] School of Applied Chemistry and Biological Technology, Shenzhen Polytechnic, Shenzhen, Guangdong 518055, China
[c] Sun Yat-sen University Cancer Center, Sun Yat-sen University, Guangzhou 510080, China
[d] Clinical Pharmacology Lab, Division of Medical Sciences, National Cancer Centre, Singapore 169610, Singapore
[e] Department of Pharmacy, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
[f] The Chinese Medicine Research Group, Division of Chinese Medicine, RMIT University, Melbourne, Australia
[g] School of Life Sciences, Queensland University of Technology, 2 George Street, GPO Box 2434, Brisbane, Queensland 4001, Australia

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Abstract

4-Anilinoquinazolines (e.g. Iressa and Glivec) are a class of epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitors widely used to treat non-small cell lung cancer and other tumors. However, low clinical response rate, resistance, and host toxicity of currently available EGFR-TK inhibitors prompt the development of second generation of TK inhibitors with improved efficacy, selectivity, and less resistance. CH330331 is a recently synthesized novel 4-anilinoquinazoline analog with confirmed anticancer activity in vitro and in vivo. To predict its oral pharmacokinetic behavior and transport nature in the intestine before entering clinical trials, we have developed and validated a high performance liquid chromatographic (HPLC) method for the determination of CH330331 in Caco-2 (a human colon cancer cell line) monolayers. The developed HPLC method was sensitive and reliable, with acceptable accuracy (90–110% of nominal values) and precision (intra- and inter-assay R.S.D. < 10%). The total running time was within 10 min, with acceptable separation of the target analytes. The lower limit of quantitation (LLOQ) value for CH330331 was 200 ng/ml when an aliquot of 100 μl sample was injected onto the HPLC. The validated HPLC method was applied to characterize the epithelial transport of CH330331 in Caco-2 monolayers. The transport of CH330331 across the Caco-2 monolayers from the apical to basolateral side was 8- to 10-fold higher than that from the basolateral to apical side. Co-incubation of sodium azide or MK-571, but not verapamil, significantly inhibited the apical to basolateral transport of CH330331. These findings provide initial evidence that the intestinal absorption of CH330331 is mediated by an active mechanism. Further studies are required to explore the interaction of CH330331 with ATP-binding cassette transporters and the possible influence on its pharmacokinetics and pharmacodynamics.
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Keywords: HPLC; Validation; CH330331; Caco-2 cells

Abbreviations: AP, apical; BCRP, breast cancer resistance protein; ANOVA, one-way analysis of variance; BL, basolateral; CV, coefficient of variation; EGFR, epidermal growth factor receptor; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulphoxide; HBSS, Hanks' balanced salt solution; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[4-butanesulfonic acid]; LLOQ, lower limit of quantitation; LOD, limit of detection; MK-571, 3-[[[3-[2-(7-chloro-2-quinolinyl)-(E)-ethenyl]phenyl][[3-(dimethylamino)-3-oxopropyl]thio]methyl]thio]propionic acid; MRP, multidrug resistance associated protein; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide; P_{app}, permeability coefficient; P-gP, P-glycoprotein; QC, quality control; TK, tyrosine kinase; TEER, transepithelial electrical resistance

* Corresponding author at: Division of Pharmacy, School of Life Sciences, Queensland University of Technology, 2 George Street, GPO Box 2434, Brisbane, Queensland 4001, Australia. Tel.: +61 7 31381340; fax: +61 7 31381534.
** Co-corresponding author at: Institute of Clinical Pharmacology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510080, China. Tel.: +86 20 87334521; fax: +86 20 873 34718.
E-mail addresses: huangmin@mail.sysu.edu.cn (M. Huang), s4.zhou@qut.edu.au (S.-F. Zhou).

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1. Introduction

Multiple components of mitogenic signaling pathways in normal and neoplastic cells have been identified, including the large family of protein kinases, which serve as critical components of signal transduction pathways. These protein kinases play a critical role in diverse biological processes, such as control of cell growth, metabolism, differentiation, and apoptosis [1]. The signal transduction of human epidermal growth factor receptor (EGFR) tyrosine kinases (TKs) that is frequently expressed in epithelial tumors is closely associated with tumor growth, angiogenesis, invasion, and metastasis [2–4]. The EGFR was the first receptor to be proposed as a target for cancer therapy [5], and recent insight into the role of receptor TK function in cancer cells culminated in the design of highly selective TK inhibitors. After two decades of intensive research, a series of antineoplastic agents have been developed and synthesized which preferentially inhibit EGFR tyrosine kinase [6,7]. To date, there are several anti-EGFR agents available for chemotherapy in the clinic [4]. These compounds have been widely used as oral agents to treat multifold tumors, including breast cancer, stomach cancer, ovary cancer, small cell lung cancer, and non-small cell lung cancer [4]. 4-Anilinoquinazolines, including gefitinib (Iressa), imatinib melysate (Glivec), and erlotinib (Tarceva) (Fig. 1), are a class of orally available synthetic small molecules designed to bind to the intracellular kinase domain of TKs [8–12]. These compounds are competitive inhibitors at the ATP binding site [13]. Treatment of appropriately selected patients with these drugs can alter the natural history of their disease and improve survival with a response rate of 5–10% in non-small cell lung cancers with activating mutations within the EGFR kinase domain [7]. However, despite the marked antitumor effect in animal studies, clinical response to these compounds is still poor for a high proportion of the cancer patients after failure of at least one prior chemotherapy regimen [7]. Furthermore, the cancer cell may develop resistance to these TK inhibitors due to acquired mutations in the *EGFR* gene [14,15]. All currently available TK inhibitors have some minor to moderate host toxicities [7]. Therefore, there is a need to develop second generation of novel anti-EGFR agents and TK inhibitors with advantages of lesser resistance and higher efficacy compared to first generation compounds. In a hope of identifying new TK inhibitors with improved specificity, selectivity and clinical efficacy, CH330331, an analog of 4-anilinoquinazoline, has been recently synthesized by the Sun Yat-sen University Cancer Center (Guangzhou, China). Its chemical structure is shown in Fig. 1.

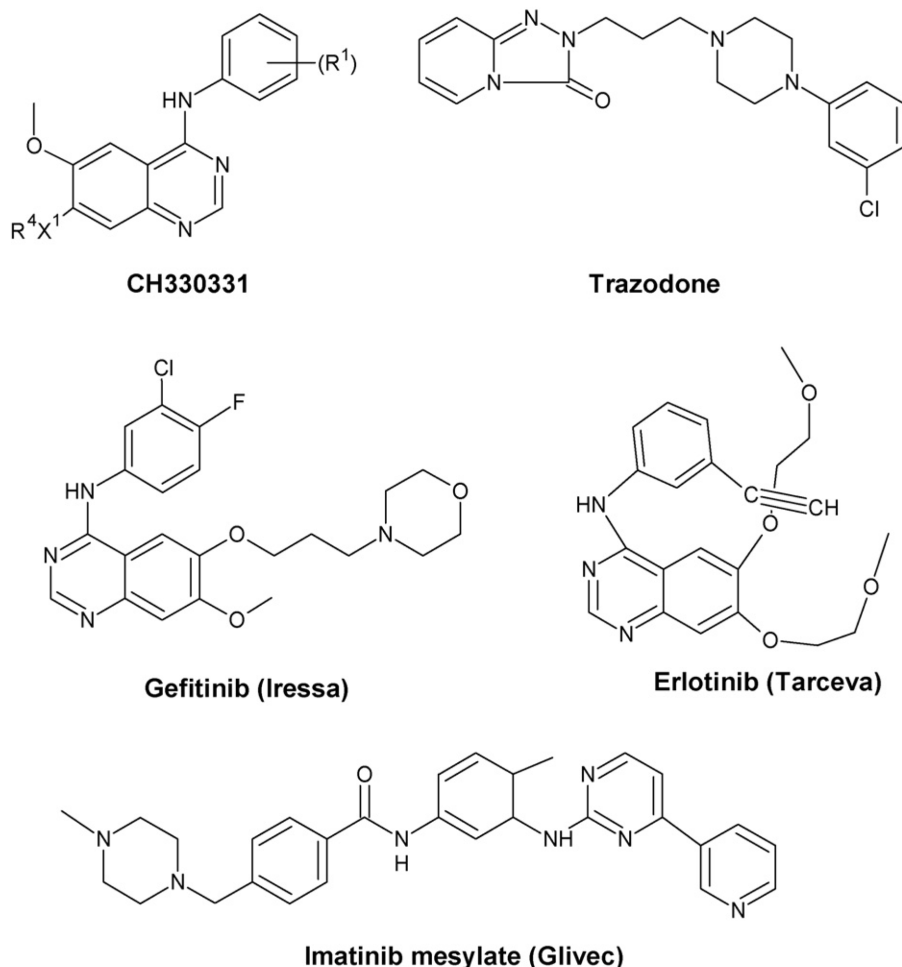

Fig. 1. Chemical structures of CH330331, trazodone (used as an internal standard), gefitinib (Iressa), Erlotinib (Tarceva) and imatinib mesylate (Glivec).

CH330331 is supposed to be given orally for chronic therapy of non-small cell lung cancer and other cancers. It appears stable in aqueous medium at physiological pH and biological matrices such as plasma, bile, and urine (unpublished data, Huang et al.). As a very novel anticancer agent, data is lacking on the oral absorption, disposition and transport, and pharmacokinetics of CH330331 in animals and humans. To date, there is no high performance liquid chromatography (HPLC) method reported for its determination in any biological matrices. In particular, imatinib mesylate, gefitinib, and erlotinib have been found to have a moderate bioavailability (45–55%) [16–18] and high affinity towards breast cancer resistance protein (BCRP/ABCG2) but a low affinity towards P-glycoprotein (P-gp/MDR1/ABCB1) [19,20]. These compounds can potentiate the anticancer activity of irinotecan in mice and reversed resistance mediated by BCRP [21]. They also significantly increased the oral bioavailability of BCRP substrate drugs [22]. Genetic mutations in *BCRP* gene have been found to considerably affect the elimination, efficacy, and toxicity of imatinib and gefitinib [23–25]. Because of the structural similarity, we hypothesize that CH330331 may interact with some drug transporters and its oral pharmacokinetic behaviors may resemble those of imatinib and gefitinib. To predict the oral absorption and oral pharmacokinetic properties of CH330331 before it enters Phase I trials, we used a human colon cancer cell line, Caco-2 cells, which have been widely used to investigate and predict intestinal permeability and transport of a number of drugs [26–28], to characterize its transport. Therefore, we developed a sensitive and reliable HPLC method with ultraviolet detection to quantitate CH330331 in Caco-2 monolayers. This fully validated HPLC method was successfully applied in a transport characterization of CH330331 in Caco-2 cell monolayers.

2. Materials and methods

2.1. Chemicals and reagents

CH330331 (purity > 99%, as determined by thin layer chromatography) was synthesized by the Sun Yat-sen University Cancer Center, Guangzhou, China. Trazodone (a selective serotonin reuptake inhibitor used as internal standard, IS), sodium azide, verapamil, and dimethyl sulphoxide (DMSO) were purchased from the Sigma–Aldrich (St. Louis, MO). The choice of trazodone as the internal standard is because its molecular weight (=371.86 Da) and Log *P* value (=2.553) are comparable to those of CH330331. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, 0.05% trypsin–ethylenediaminetetraacetic acid, penicillin–streptomycin, and non-essential amino acids were all obtained from Invitrogen (Carlsbad, CA). The leukotriene antagonist, 3-[[[3-[2-(7-chloro-2-quinolinyl)-(E)-ethenyl]phenyl][[3-(dimethylamino)-3-oxopropyl]thio]methyl] thio]propionic acid (MK-571), was a gift from Dr. Ford Hutchinson (Merck Frosst Canada Inc., Kirkland, Quebec, Canada). Hank's balanced salt solution (HBSS) was prepared by dissolving appropriate solutes in 1 l Milli-Q water [5.95 g *N*-[2-hydroxyethyl]piperazine-*N*′-[4-butanesulfonic acid] (HEPES), 0.14 g $CaCl_2$, 0.40 g KCl, 0.06 g KH_2PO_4, 0.047 g $MgCl_2$, 0.10 g $MgSO_4{\cdot}7H_2O$, 8.00 g NaCl, 0.35 g $NaHCO_3$, 0.048 g Na_2HPO_4, and 4.5 g D-Glucose, and pH was adjusted to 6.0 using 1 M NaOH or 1 M HCl. The solution was sterilized by filtering through a 0.22-μm filter.

2.2. Cell culture

The Caco-2 cell line was obtained from the American Type Culture Collection (Manassas, VA). The cells were cultured in DMEM supplemented with 10% fetal bovine serum, 1% nonessential amino acids, and 100 U/ml penicillin and gentamicin in an atmosphere of 5% CO_2 and 90% relative humidity at 37 °C and given fresh medium every 3–4 days.

2.3. Cytotoxicity assay

The 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was used to determine the cytotoxicity of CH330331 in Caco-2 cells as previously described [29]. The drug was dissolved in DMSO and then diluted in culture medium and added to the cultures 24 h after cell seeding. Cells were exposed to the drug at different concentrations (10–80 μg/ml) in culture medium for 24 h, after which 0.05 mg MTT was added to each well, and the plates were further incubated for 4 h. Thereafter, supernatants were removed and the purple precipitate was dissolved in 100 μl DMSO. The absorbance of formazan, a metabolite of MTT, was measured at a wavelength of 595 nm using a microplate reader (Tecan Instrument Inc., Research Triangle Park, NC).

2.4. Transport experiments

The transport of CH330331 in Caco-2 monolayers was investigated using the methods described previously [30,31]. The transepithelial electric resistance (TEER) of the monolayers was examined routinely before and after the experiment. The monolayers were used for the transport study when the effective TEER exceeded 250 Ω cm^2 and the permeability of Lucifer yellow was $\leq 0.1 \times 10^{-6}$ cm/s. CH330331 was dissolved in HBSS and added to the apical (AP) and the basolateral (BL) sides. Transport experiments of CH330331 in the monolayers were performed when the cells had reached integrated confluence 21 days after seeding. Cells were rinsed twice with HBSS buffered with 25 mM HEPES (pH 6.8) before transport study. A pH of 6.8 for the transport study was chosen as it was close to the intestinal pH value and this pH resulted in maximum AP to BL and BL to AP transport of CH330331. After two washes with warm HBSS, the plates were incubated at 37 °C for 15 min, and the TEER was monitored and inserts distributed evenly between treatments based on the measured TEER values. CH330331 (5, 10, and 20 μg/ml) was loaded to the AP or BL side, and an aliquot (50 μl) of sample was collected from the receiving side at predetermined times (30, 60, 90, and 120 min) over 120 min. After each sampling, 50 μl of HBSS with 25 mM HEPES were added to the receiving side to maintain a constant volume.

To identify which drug transporters were involved in the intestinal absorption of CH330331, the inhibitory effect of

sodium azide (an ATP synthesis inhibitor), verapamil (a P-gp inhibitor), and MK-571 (a multidrug resistance associated protein (MRP1/2) inhibitor) on CH330331 transport was investigated by adding 100 μM of each inhibitor to both AP and BL side. All inhibitors were freshly prepared using DMSO with a final concentration of DMSO of 1.0% (v/v). The apparent permeability coefficient (P_{app}) was determined as described [30,31].

2.5. Determination of CH330331 by HPLC

2.5.1. HPLC instrumentation

An Agilent 1100 HPLC system (Santa Clara, CA) consisted of an auto-sampler, a pump, and a double-wavelength ultraviolet detector at an operation wavelength of 248 nm. The analytical column was a BDS reversed-phase C18 column (150 mm × 4.6 mm I.D., particle size 5 μm, ELITE, Dalian, China). The mobile phase at a flow-rate of 1.0 ml/min consisting of acetonitrile: ammonium acetate (40:60, v/v, pH 7.4 adjusted by triethylamine with ammonium acetate solution before mixture with acetonitrile) was degassed before use.

2.5.2. Sample preparation

Transport studies were conducted by incubating CH330331 with HBSS at either apical or basolateral side of Caco-2 monolayers. An aliquot (200 μl) was collected from the transport buffer (HBSS) and filtered by 0.22-μm filter unit (Millipore Co., Billerica, MA) and 100 μl of the filtrate was injected onto the HPLC. The mixture with trazodone (2 μg/ml) was completed automatically by the HPLC system.

2.5.3. Calibration curves

Calibration curves for CH3303331 determination in the transport buffer (HBSS) were constructed over the concentration range of 0.2–50 μg/ml. Sample preparation of standards was the same as for unknown samples. The ratio of peak area of CH330331 to that of internal standard, and linear least-squares regression analysis weighted according to the reciprocal of peak area ratio squares was conducted to determine the slope, intercept, and coefficient of determination by Excel 2000.

2.5.4. Sensitivity and selectivity

The lower limit of quantitation (LLOQ) was defined as the minimum concentration which could be determined with acceptable accuracy (i.e. recovery between 80% and 120%) and precision (coefficient of variation (CV) < 20%) [32]. The limit of detection (LOD) was the amount which could be detected with a signal to noise ratio of 3. The selectivity of the method was examined by determining if interfering chromatographic peaks were present in blank HBSS or in the presence of various compounds, including irinotecan, sodium azide, verapamil, and MK-571.

2.5.5. Accuracy and precision

Quality control (QC) samples containing CH330331 were prepared from weighings independent of those used for preparing calibration curves. Final concentrations of low, medium, and high QC samples were 0.2, 5, and 20 μg/ml, respectively. These samples were prepared on the day of analysis in the same way as calibration standards. The performance of the HPLC method was assessed by analysis of 15 QC samples (5 each of low, medium, and high concentrations) on a single assay day to determine intraday accuracy and precision, and 15 QC samples (five each of low, medium, and high concentrations) on each of 5 consecutive assay days to determine inter-day accuracy and precision.

2.5.6. Stability

The stability study was carried out to choose an appropriate experimental pH for transport studies of CH330331. To examine the stability of CH330331, the compound at 1 or 10 μg/ml was incubated in HBSS at different pH values (6.0, 6.8, and 7.4) at 37 °C over 2 h. At indicated time points, an aliquot of 100 μl of the stock solution was collected and processed as standard sample.

2.6. Data analysis

Data are presented as mean ± S.D. The initial statistical analysis to evaluate the differences was carried out by a one-way analysis of variance (ANOVA) test with a significance level of $P < 0.05$.

The apparent permeability coefficient (P_{app}, cm/s) of CH330331 in Caco-2 monolayers is expressed in cm/sec and calculated as following equation:

$$P_{app} = \frac{\Delta Q}{\Delta t} \times \frac{1}{60} \times \frac{1}{A} \times \frac{1}{C_0} \quad (1)$$

where $\Delta Q/\Delta t$ is the permeability rate (μg/s); A is the surface area of the membrane (cm^2); and C_0 is the initial drug concentration in the donor chamber (μg/ml). Samples from all time points calculated.

3. Results and discussion

We report here on a validated HPLC method for the determination of CH330331 in HBSS. Representative chromatograms from HBSS with added CH330331 and IS are shown in Fig. 1. Under the chromatographic conditions used for the analysis of CH330331, the retention times for internal standard and CH330331 were 5.8 and 8.6 min, respectively. The total chromatography run time was 10 min. The peak of the analyte was slightly skewed to the right. We evaluated peak skew using the asymmetry coefficient $A_s = b/a$, where b is the distance after the peak maximum and a is the distance before the peak maximum, both a and b being measured at 10% of the total peak height. The asymmetry coefficients for CH330331 were between 1.08 and 1.27 (Fig. 2).

Matrix-specific interfering peaks that required modification of the mobile phase composition were not observed in any cases, including in the presence of drugs such as irinotecan, verapamil, and MK-571. In addition, no metabolites of CH330331 were detected in the culture medium of Caco-2 cells.

Calibration curves were linear over the concentration range of 0.2–50 μg/ml with the mean correlation coefficients >0.999 ($n = 8$). The mean y intercepts were 0.004–0.03 ($n = 5$) for

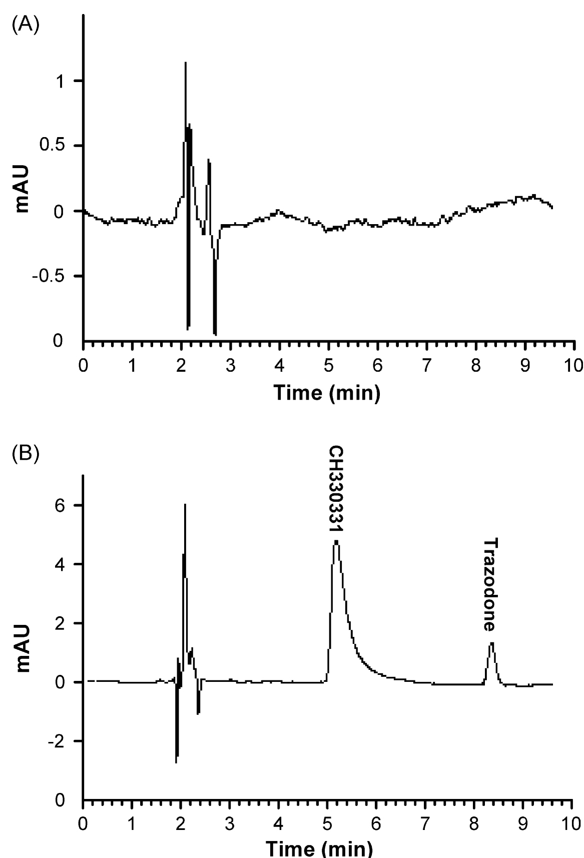

Fig. 2. Representative chromatographs of CH330331 and trazodone (IS) in Hanks balanced salt solution (HBSS). (A) Blank HBSS; and (B) HBSS containing CH330331 at 10 μg/ml and trazodone at 2 μg/ml.

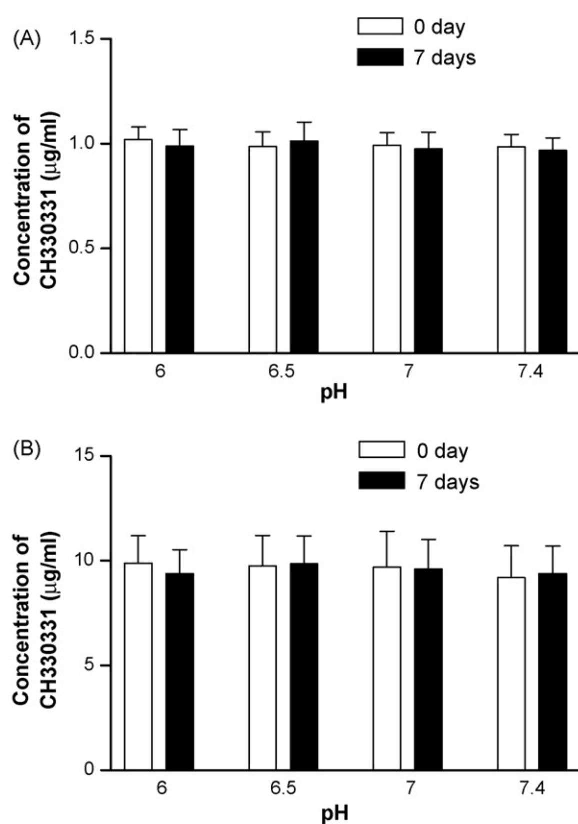

Fig. 3. Stability of CH330331 at 1 (A) and 10 μg/ml (B) in Hanks balanced salt solution at different pH conditions at 37 °C. Data are the mean ± S.D. of at least three independent determinations.

the analyte. For each point on the calibration curves for the analyte, the concentrations back-calculated from the equation of the regression analysis were within acceptable limits for accuracy and precision of ± 20%. A linear regression of the back-calculated concentrations versus the nominal values provided a unit slope and an intercept not significantly different from zero. The distribution of the residuals showed random variation, was normally distributed and centered on zero. The bias was not statistically different from zero, and the 95% confidence intervals included zero (data not shown).

The differences between the theoretical and actual concentration and the relative standard deviation were less than 10% at any QC concentrations. The results for precision and accuracy are shown in Table 1. The LLOQ of the assay was 200 ng/ml for a 100 μl aliquot of CH330331 in HBSS. The LOD of the assay was 50 ng/ml.

CH330331 at 1 and 10 μg/ml in HBSS at pH 6.0, 6.5, 7, and 7.4 was stable, whereas no significant degradation was observed over 7 days stored at 4 °C (Fig. 3).

As shown in Table 2, CH330331 at a concentration of 40 μg/ml or below did not show significant cytotoxic effect on Cacxo-2 cells, but an increase in its concentration to 60 and 80 μg exhibited marked cytotoxicity toward Caco-2 cells. The maximum concentration of CH330331 used for our transport studies was 20 μg/ml, which was non-toxic towards the Caco-2 cell monolayers, especially since the incubation time (up to 2 h) was shorter than the time employed for the MTT test (24 h).

Table 1
Accuracy and precision of the HPLC method for the analysis of CH330331 in Hanks balanced salt solution (HBSS, pH 7.4, $n = 5$ for both intar- and inter-day assays)

Theoretical conc. of CH330331 (μg/ml)	Measured conc. of CH330331 (μg/ml) (mean ± S.D.)	% Recovery of theoretical conc. of CH330331	R.S.D. (%)
Intra-day			
5	4.75 ± 0.08	95.00	1.68
10	10.10 ± 0.36	101.02	3.56
20	19.96 ± 1.24	99.80	6.21
Inter-day			
5	4.63 ± 0.05	92.60	1.08
10	9.91 ± 0.23	99.10	3.25
20	20.06 ± 1.02	100.30	5.08

Table 2
Cytotoxicity of CH330331 towards Caco-2 cells ($n = 3$)

Groups	CH330331 conc. (μg/ml)	Absorbance	P-value
1	HBSS (control)	0.641 ± 0.067	–
2	10	0.621 ± 0.064	0.60
3	20	0.618 ± 0.050	0.51
4	30	0.569 ± 0.042	0.02
5	40	0.555 ± 0.069	0.08
6	50	0.543 ± 0.052	0.04
7	60	0.518 ± 0.076	0.02
8	70	0.490 ± 0.105	0.01
9	80	0.466 ± 0.153	0.01

We applied this validated HPLC method to the transport study of CH330331 in Caco-2 monolayers. After incubation with CH330331 at 5–20 μg/ml loaded at either AP or BL side, the sample was collected from the other side for HPLC analysis. The transport rate of CH330331 from the AP to BL side was 8- to 10-fold higher than from BL to AP side, though the transport of CH330331 from A to B appeared to be steady within 2 h (Figs. 4 and 5). The P_{app} values of CH330331 at 5–20 μg/ml from the AP to BL side were 2.65–4.41 × 10^{-5} cm/s, with a marked increase in P_{app} values from AP to BL at increased CH330331 concentrations. Though the transport of CH330331 from AP to BL appeared to be steady within 2 h, the transport of the opposite direction appeared to be linear with the increase of the incubation time. In addition, the transport of CH330331 (10 μg/ml) from AP to BL side was significantly decreased in the presence of the ATP inhibitor sodium azide or MK571 (Table 3). However, verapamil (a P-glycoprotein inhibitor) had no significant effect on the transport of CH330331.

In the present study, we developed and validated a sensitive and reliable HPLC method for the determination of CH330331, a novel EGFR-TK inhibitor, in the transport buffer for Caco-2 cell monolayers. The validation data indicate that the method was sensitive and reliable, with acceptable accuracy (90–110% of true values) and precision (intra- and inter-assay CV < 10%). The total running time was within 10 min, with satisfactory separation of the target compounds. CH330331 was stable in HBSS at pH 6.0–7.4. Therefore, the risk of degradation of CH330331 can be reduced dramatically during sample analysis. The LLOQ value was 200 ng/ml. This sensitivity was high enough to quantitate the low concentrations of CH330331 in the receiving chamber of the monolayers when using low loading concentrations.

The results indicate that CH330331 had a relatively high apparent permeability coefficient in Caco-2 monolayers. The high P_{app} value of CH330331 from AP to BL side suggested

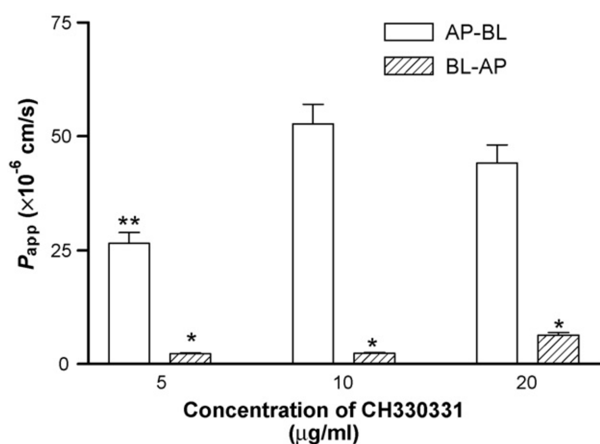

Fig. 4. Bi-directional transport of CH330331 in Caco-2 monolayers incubated over 60 min. Data are the mean ± S.D. of at least three independent determinations. (*) $P < 0.05$ (AP to BL vs. BL to AP); (**) $P < 0.01$ (5 vs. 10 or 20 μg/ml).

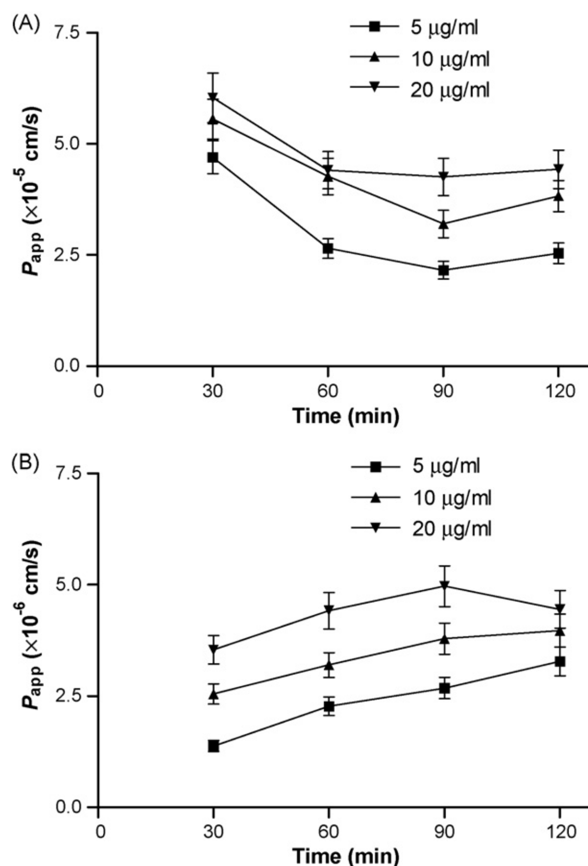

Fig. 5. The apical to basolateral (AP) and basolateral to apical (BL) transport of CH330331 in Caco-2 monolayers incubated over 120 min. Data are the mean ± S.D. of at least three independent determinations.

Table 3
Apical (AP) to basolateral (BL) and BL to AP transport data of CH330331 across Caco-2 cell monolayers ($n = 3$)

Treatment	Transport direction	P_{app} (cm/s)
CH330331 (10 μg/ml)	AP → BL	$(3.67 \pm 1.02) \times 10^{-5}$
+Verapamil (100 μM)	AP → BL	$(3.48 \pm 0.80) \times 10^{-5}$
+Sodium azide (10 mM)	AP → BL	$(9.29 \pm 2.11) \times 10^{-6}$**
+MK-571 (50 μM)	AP → BL	$(6.20 \pm 1.19) \times 10^{-6}$*
CH330331 (10 μg/ml)	BL → AP	$(3.41 \pm 0.86) \times 10^{-6}$
+Verapamil (100 μM)	BL → AP	$(4.92 \pm 1.63) \times 10^{-6}$
+Sodium azide(10 mM)	BL → AP	$(3.18 \pm 1.16) \times 10^{-6}$
+MK-571 (50 μM)	BL → AP	$(3.20 \pm 0.97) \times 10^{-6}$

* $P < 0.05$.
** $P < 0.01$.

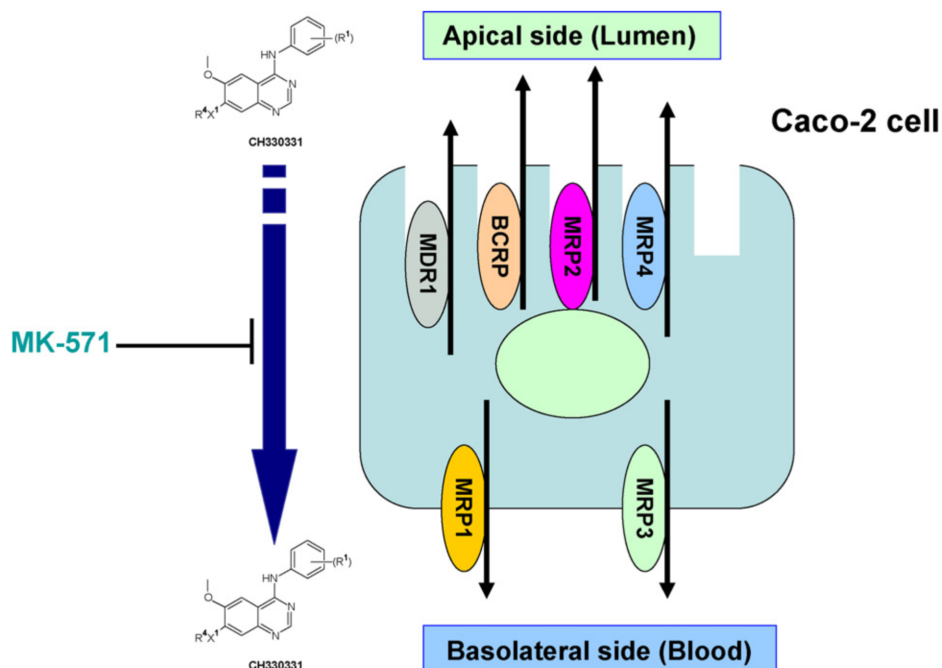

Fig. 6. A multitude of ATP-binding cassette transporters located on the apical and basolateral sides of Caco-2 cells. CH330331 had a polarized transport with facilitated apical to basolateral flux (absorption) which might be mediated by multidrug resistance associated protein 1 (MRP1) as MK-571 significantly reduced the polarized transport of CH330331. P-glycoprotein (MDR1/P-gp) appeared not to participate the intestinal transport of CH330331.

CH330331 might have moderate to high oral bioavailability. The permeability of CH330331 from AP to BL side was higher than that from BL to AP side, suggesting the involvement of an active mechanism facilitating intestinal absorption of this drug. The transport of CH330331 from AP to BL side remained unchanged with the increase of substrate concentration and the incubation time while the transport from BL to AP side was time and concentration-dependent.

Drugs that cross the apical membrane may be substrates for apical efflux transporters such as P-gp, BCRP and MRP2, and they can be selectively inhibited by verapamil and MK571, respectively (Fig. 6). In contrast, drugs within the colon epithelial cells are subject to efflux via MRP1 and MRP3 located at the BL side. The P_{app} values of CH330331 decreased dramatically with the presence of MK571 but not verapamil, suggesting the involvement of MRP1. However, the BL to AP transport of CH330331 was not significantly altered by these inhibitors. Therefore, MRP1, but not P-gp, may be involved in the absorptive transport of CH330331 in the gut. All these findings indicate a polarized transport of CH330331 with involvement of an active mechanism. However, further study is needed to confirm the role of P-gp, BCRP, MRP1/2, and other transporters in the intestinal absorption of CH330331.

In conclusion, we developed and validated a sensitive and reliable HPLC method for the determination of CH330331 in Caco-2 cell monolayers. The validated method was successfully used to characterize the transport of CH330331 in Caco-2 monolayers, and found that the transport of CH330331 was rapid, facile, and saturable, and might involve an active mechanism. Further research of the absorption properties of CH330331 should be undertaken.

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