



Pharmacokinetic/pharmacodynamic analyses of chymase inhibitor SUN13834 in NC/Nga mice and prediction of effective dosage for atopic dermatitis patients

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ARTICLE INFO

Article history:

Received 28 April 2011

Received in revised form 20 May 2011

Accepted 23 May 2011

Available online 12 June 2011

Keywords:

Atopic dermatitis

Chymase inhibitor

SUN13834

NC/Nga

PK/PD analysis

ABSTRACT

A chymase inhibitor SUN13834 has been shown to improve skin condition in animal models for atopic dermatitis. In the present study, effective dosages of SUN13834 for atopic dermatitis patients were predicted by pharmacokinetic/pharmacodynamic (PK/PD) analyses of SUN13834 in NC/Nga mice, which spontaneously develop atopic dermatitis-like skin lesions. For the PK/PD analyses, we utilized the minimum effective plasma concentration of unbound SUN13834 in late-phase reaction of trinitrochlorobenzene (TNCB)-induced biphasic dermatitis in mice, based on the assumption that the minimum effective plasma concentrations are the same among the two animal models. In late-phase reaction of biphasic dermatitis, SUN13834 was most effective when its plasma concentration was highest at the elicitation, and the minimum effective plasma concentration of unbound SUN13834 at the elicitation was calculated to be 0.13–0.2 ng/mL. Oral administration of SUN13834 improved dermatitis in NC/Nga mice at 15 mg/kg (twice a day; *bid*) and 30 mg/kg (once a day; *qd*), but not at 60 mg/kg (every other day; *eod*). At the three dosages, the duration times over the plasma level of 0.13–0.2 ng/mL were 16.1–20.3, 10.7–12.2 and 7.8–8.8 h, respectively, suggesting an importance of maintenance of the minimum effective plasma concentration for at least about 10–12 h. The clinical effective dosage predicted in this paper is also discussed in relation to a recently conducted Phase 2a study.

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1. Introduction

Chymase is a chymotrypsin-like serine protease stored in mast cell granules [1] and hydrolyses a variety of physiological substrates including angiotensin I [2]. Chymase is also known to play a role in pathogenesis of atopic dermatitis, e.g. i) an intradermal injection of chymase to mouse skin elicits edema as well as inflammatory cell accumulation [3,4], ii) there are several reports suggesting a correlation between a certain single nucleotide polymorphism (SNP) of chymase gene and incidence of atopic dermatitis [5–7].

SUN13834 is a potent, selective orally-administered chymase inhibitor that is currently under clinical development for the treatment of atopic dermatitis. Chymase inhibitors including SUN13834 (or SUN13350, the free form of SUN13834) and SUN C8257 have been shown to improve dermatitis in several animal models of atopic dermatitis [3,8,9]. The mechanisms of such chymase inhibitors for ameliorating dermatitis likely include inhibition of inflammatory cell recruitment and activation of stem cell factor [8].

In the present study, we performed pharmacokinetic/pharmacodynamic (PK/PD) analyses of SUN13834 in NC/Nga mice, which spontaneously develop atopic dermatitis-like skin lesions along with IgE hyperproduction only in a so-called conventional environment [10]. We first estimated the minimum effective concentration of unbound SUN13834 for late-phase skin reaction of TNCB (trinitrochlorobenzene)-induced acute biphasic dermatitis in mice [11] and used it for the analyses, assuming that the atopic dermatitis-like skin lesions of NC/Nga mice are caused by repeated late-phase reactions that are thought to be related to human atopic dermatitis, and are sensitive to existing atopic dermatitis treatments. We also predicted in this paper the effective plasma concentrations and durations of SUN13834 for the treatment of atopic dermatitis in humans, and compare these findings to the results obtained in a recent Phase 2a clinical study (Rikken et al., 40th annual meeting of the European Society for Dermatological Research 2010, and the manuscript in preparation).

2. Materials and methods

2.1. Mice

Conventional NC/Nga mice were bred and maintained in the Laboratory Animal Facilities of the Jikei University School of Medicine. BALB/c and NC/Nga mice, under specific pathogen free (SPF)

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conditions, were purchased from Charles River Japan (Kanagawa, Japan). The efficacy studies of SUN13834 with the conventional NC/Nga mice were performed according to the Guideline for Animal Experimentation of the Jikei University (Jikei University, 1988). All the other animal studies were approved by the Ethics Committee for Animal Experiments of Biomedical Research Laboratories, Asubio Pharma Co., Ltd. and performed in accordance with the Guideline for Animal Experiments of the laboratories.

2.2. Chymase inhibitor

SUN13834 (6-(5-chloro-2-methoxybenzyl)-4-alkylaminocarbonyl-1,4-diazepane-2,5-dione) was synthesized in Asubio Pharma Co., Ltd. as described [12,13]. The IC_{50} values of SUN13834 for mouse chymase (mMCP-4) and human chymase were 0.078 μ mol/L and 0.14 μ mol/L, respectively. The inhibition of chymase by SUN13834 is specific in that it has little effect on other enzymes such as tryptase, cyclooxygenase-1 (COX-1) and angiotensin converting enzyme (ACE).

2.3. Effect of SUN13834 on IgE-mediated biphasic skin reaction in mice

To sensitize mice, 1.0 mL of anti-2,4,6-trinitrophenol (anti-TNP) mouse IgE solution (culture supernatant of IGEL b4 cells; cells were obtained from American Type Culture Collection, VA, USA) was injected intravenously to female BALB/c mice. On the next day of sensitization, the biphasic skin reaction was elicited by an application of 20 μ L of 1% 2, 4, 6-trinitro-1-chlorobenzene (TNCB; Nacalai tesque Inc., Kyoto, Japan) dissolved in acetone/olive oil (1:9) to the both sides of mouse right ear (10 μ L/site). Late-phase (24 h after elicitation) reaction was examined by measuring ear thickness using a thickness gauge (Digimatic Indicator, Mitutoyo, Tokyo, Japan), and an increase in ear thickness was determined by subtracting the ear thickness before the elicitation. As a control, the acetone olive oil was applied to sensitized mice. SUN13834 dissolved in 0.5% hydroxypropyl cellulose (HPC; Nippon Soda Co., Ltd., Tokyo, Japan) in water was administered orally or intraperitoneally 1 h before the elicitation.

2.4. Effect of SUN13834 on dermatitis in NC/Nga mice

SUN13834 suspended in 0.5% HPC in water was orally administered to 8 week-old NC/Nga mice at the doses of 15 mg/kg (twice a day; *bid*), 30 mg/kg (once a day; *qd*) and 60 mg/kg (every other day; *eod*). As a control, 0.5% HPC in water was administered to mice instead of SUN13834. The severity of dermatitis was determined according to the criteria of Leung et al. [14] at 2 and 3 weeks after the first administration. Briefly, five skin symptoms, itch, erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness, were scored on a scale of 0–2, and the total skin score was expressed as the sum of the individual scores [15].

2.5. PK analyses in mice

SUN13834 was administered to BALB/c and NC/Nga mice at SPF condition, and the plasma samples were collected at 1 h (BALB/c mice) or 0.5, 1, 3, 6 and 24 h (NC/Nga mice) after the administration. The concentrations of SUN13834 in plasma samples were measured by using LC–MS/MS (UPLC–TQD, Waters Corporation, Milford, MA, USA or API4000, AB SCIEX, Foster, CA, USA). Then plasma unbound concentrations of SUN13834 in BALB/c mice and NC/Nga mice were calculated by using the ratio of the unbound fraction to total SUN13834, i.e. approximately 2%, which was determined *in vitro*. The duration times over the effective plasma concentration at steady state in NC/Nga mice were calculated by WinNonlin® (ver. 5.2.1, Pharsight Corporation, St. Louis, MO, USA).

2.6. Statistical analysis

The statistical analysis was performed by using EXSUS Version 7.6 (Arm Systex Co., Ltd., Osaka, Japan). The p-value less than 0.05 was considered significant.

3. Results

3.1. Estimation of minimum effective plasma concentration of SUN13834 in murine biphasic dermatitis model

In our preliminary experiments using TNCB-induced acute biphasic dermatitis model in mice, an oral (*p.o.*) administration of SUN13834 at 1 h before the TNCB elicitation was most effective in the treatment of late-phase skin reaction as compared with oral treatment at 3 h or 15 min before elicitation, or at 2 h after elicitation (data not shown). PK data suggested that the effects of SUN13834 were related to its plasma concentrations at the time of elicitation with TNCB, which may be consistent with the thought that SUN13834 needs to exist around chymase, as the enzyme is secreted from mast cells upon stimulation by IgE–antigen complex.

As shown in Fig. 1, the effects of SUN13834 administered 1 h before TNCB elicitation, on the late-phase reaction in the model were dose-dependent, e.g. the increase of ear thickness was significantly inhibited by an oral administration of SUN13834 at 2, 10 or 50 mg/kg, but not at 0.4 mg/kg (Fig. 1A) or by an intraperitoneal (*i.p.*) administration of SUN13834 at 0.4 or 2 mg/kg, but not at 0.08 mg/kg, (Fig. 1B). Measurements of plasma concentration of unbound SUN13834 revealed that 0.130 ng/mL and higher concentrations were effective, while 0.016 ng/mL was not (Table 1, $n=6$ for each dose). These data suggest that the minimum effective plasma concentration of unbound SUN13834 in the late-phase skin reaction of TNCB-induced biphasic dermatitis is approximately 0.13–0.20 ng/mL.

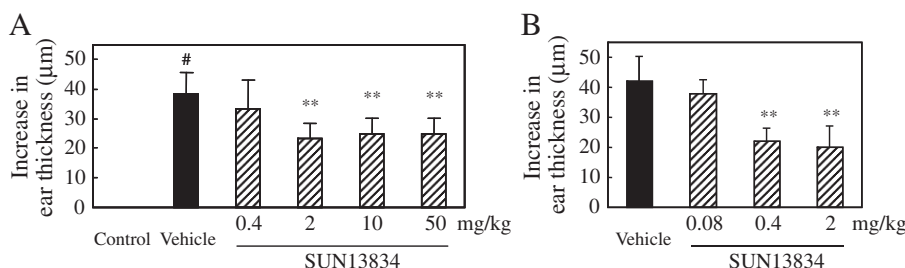


Fig. 1. Effect of SUN13834 on late-phase reaction of murine biphasic skin reaction. Biphasic skin reaction was induced by topical application of 1% TNCB solution to the sensitized BALB/c mice. Ear thickness was measured at 24 h after the application of TNCB solution, and an increase in ear thickness was determined by subtracting the ear thickness before the elicitation. As a control (Control group), the acetone/olive oil was applied to sensitized mice. SUN13834 was administered *p.o.* (A) or *i.p.* (B) 1 h before the TNCB challenge. Data are mean $\pm$ SD ($n=12$). #, $p<0.01$ Aspin–Welch's t-test vs. Control group, **, $p<0.01$ Dunnett's test vs. Vehicle group.

Table 1

Plasma concentrations of SUN13834 after administration of the compound to BALB/c mice.

Dose (route)	Plasma concentration of SUN13834 ^a	Efficacy
0.4 mg/kg (i.p.)	0.203 ± 0.042	Yes ^b
0.4 mg/kg (p.o.)	0.016 ± 0.008	No
2.0 mg/kg (p.o.)	0.130 ± 0.054	Yes ^b

^a Unbound SUN13834 (ng/mL) at 1 h after administration (mean ± SD, n = 6).

^b Significantly effective in late-phase reaction of biphasic dermatitis model in mice.

3.2. PK/PD analyses of SUN13834 in NC/Nga mice

Next, we performed PK/PD analyses of SUN13834 in NC/Nga mice, which show spontaneous chronic dermatitis and thus are thought to be a useful model for human atopic dermatitis. In the analyses, we used the following assumptions: i) the mechanisms underlying the development of dermatitis in NC/Nga mice are the same as those for the late-phase reaction in TNCB-induced biphasic dermatitis model, although the lesions of NC/Nga mice develop due to continuing exposure to natural antigens in the environment, in contrast to the acute biphasic model that is induced by a single challenge with a hapten; ii) long-lasting skin inflammation in NC/Nga mice is the result of repeated late-phase skin reactions caused by natural antigens. Based on these assumptions, it would be possible to estimate how long the plasma concentration of SUN13834 should be over the minimum effective plasma concentration of unbound SUN13834 by evaluating the PK of doses required to improve the atopic dermatitis-like skin lesion in NC/Nga mice.

As shown in Fig. 2, oral administration of SUN13834 improved skin clinical score in NC/Nga mice at the doses of 15 mg/kg (twice a day; *bid*, Fig. 2A) and 30 mg/kg (once a day; *qd*, Fig. 2B), but not at 60 mg/kg (every other day; *eod*, Fig. 2C). Measurement of the plasma levels of unbound SUN13834 following the administration of the compound to NC/Nga mice at the three dosages and the analysis of the PK data revealed that the duration time that 15 mg/kg (twice a day; *bid*), 30 mg/kg (once a day; *qd*) and 60 mg/kg (every other day; *eod*) were over the minimum effective plasma concentration, i.e. 0.13–0.2 ng/mL, was 16.1–20.3 h, 10.7–12.2 h and 7.8–8.8 h, respectively (Fig. 3 and Table 2, n = 9 for each dose). These results suggest that the duration of the minimum effective plasma concentration of unbound SUN13834 is about 10–12 h for improvement of the atopic dermatitis-like lesions in NC/Nga mice.

3.3. Prediction of effective dosage of SUN13834 for atopic dermatitis patients

Finally, we predicted the effective dosages of SUN13834 for the patients with atopic dermatitis, presuming that the pathogenesis and

mechanisms for NC/Nga mice are the same as those of human atopic dermatitis. Since the IC₅₀ value of SUN13834 for human chymase (0.14 μmol/L) is approximately 2-fold that of mouse chymase (0.078 μmol/L, the IC₅₀ for mast cell protease-4, mMCP-4), the effective plasma concentration of unbound SUN13834 in the late-phase skin reaction of biphasic dermatitis is considered to be 0.26–0.40 ng/mL. Thus, the maintenance of this plasma concentration for more than 10–12 h is thought to be effective for human atopic dermatitis.

4. Discussion

In the present study, we performed PK/PD analyses of the effective and ineffective doses of the chymase inhibitor SUN13834 on atopic dermatitis-like lesions of NC/Nga mice, utilizing the minimum effective plasma concentration determined from the late-phase reaction of a biphasic dermatitis model. The results indicated that maintaining a plasma concentration of unbound SUN13834 at more than the minimum effective plasma concentration (0.13–0.20 ng/mL) for 10–12 h is important for improvement of atopic dermatitis-like lesions in mice. Considering the difference of IC₅₀ values of SUN13834 between mouse and human, we estimated that the dosage to retain the plasma concentration of the compound at 0.26–0.40 ng/mL for more than 10–12 h could be effective in human atopic dermatitis.

Recently, we conducted Phase 2a clinical study, in which the protocol was made based on both the data of Phase 1 clinical study and the prediction carried out in the present study. When SUN13834 was orally administered to atopic dermatitis patients at 50 mg (three times a day; *tid* = 150 mg/day in total), the plasma concentration of unbound SUN13834 continued to be over 0.26–0.40 ng/mL for 22–23 h, and the Eczema Area and Severity Index (EASI) scores of the patients were improved compared with that of placebo-treated group (Rikken et al., 40th annual meeting of the European Society for Dermatological Research 2010, and the manuscript in preparation). However, as the time over minimum effective concentration in the clinic trial (22–23 h) was still much longer than half a day, the effect of the compound at lower doses should be examined so as to confirm if the assumptions and models used in this study are reasonable.

PK/PD analyses are generally performed so as to elucidate the relationship between the administered drug concentration in body fluids and pharmacological effect, and provide the effective means for translating *in vitro* and animal data to human clinical pharmacology [16]. However, most of such analyses have been done for the compounds with the same mechanism as those that have already been used in humans, and it is difficult to perform such analyses for compounds with novel mechanisms of action. This is because it is uncertain whether or not human disease and the corresponding animal models are completely the same and whether or not the targeting

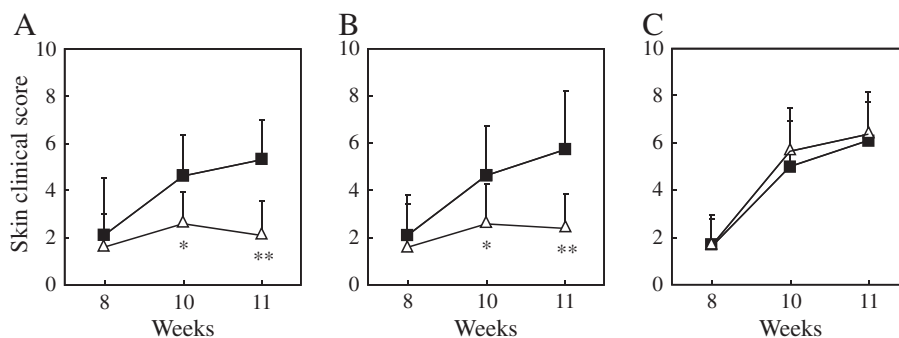


Fig. 2. Effect of SUN13834 on skin clinical score in NC/Nga mice. SUN13834 was orally administered to 8 week-old NC/Nga mice at the doses of 15 mg/kg (*bid*, A), 30 mg/kg (*qd*, B) and 60 mg/kg (*eod*, C) and the severity of dermatitis was determined on 2 and 3 weeks after the first administration of SUN13834 as described in the Materials and methods section. Closed square; Control (Vehicle-treated) mice, open triangle; SUN13834-treated mice. Data are mean ± SD (n = 10–11). *, p < 0.05, **, p < 0.01 compared with Control mice (Wilcoxon-test).

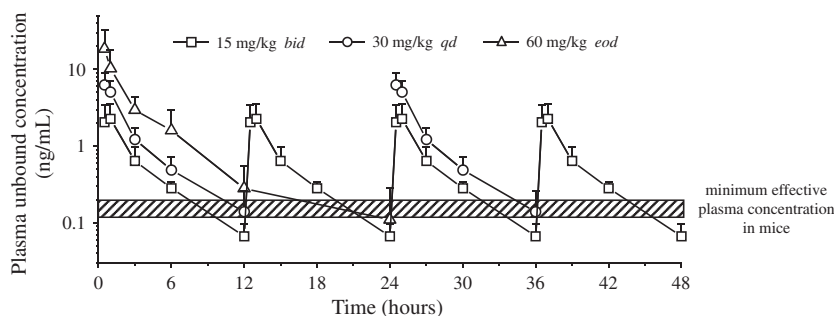


Fig. 3. Changes in the plasma level of unbound SUN13834 after oral administration of SUN13834 in SPF NC/Nga mice. SUN13834 was administered to NC/Nga mice at the doses of 15, 30 and 60 mg/kg (*p.o.*, *n* = 9 for each doses). The plasma samples were collected at 0.5, 1, 3, 6 and 24 h after the administration of SUN13834, and the concentration of unbound SUN13834 was determined as described in the **Materials and methods** section. The administration was only once, and the line graph for the repeated administration was prepared by prediction. The minimum effective plasma concentrations estimated in biphasic dermatitis model are indicated with the hatched box. Open square, 15 mg/kg, *bid*; open circle, 30 mg/kg, *qd*; open triangle, 60 mg/kg *eod*.

mechanism contributes equally to human and the models. In this respect, the analyses based on some assumptions, like those reported here, might be a useful procedure, while there is a limitation for the analyses, *e.g.* IgE-mediated reactions, like the TNCB-induced dermatitis used here, seems to be associated with extrinsic type of AD, but not in another type, intrinsic AD, which exhibits normal serum IgE levels.

One of the hypotheses generated here was that the skin lesions are developed by continuing exposure to environmental antigens, and we thought that the minimum effective plasma concentration of SUN13834 must be maintained for up to 24 h in the blood and the skin to be effective. Hence, it is quite interesting that the duration of the effective plasma concentration necessary for the pharmacological effect was approximately 10–12 h, half a day. This might be because mast cells cannot reconstitute their secretory granules containing chymase for a period of time once the cells release the granules.

There was a difference between the minimum effective plasma concentration of unbound SUN13834 for the late-phase in biphasic dermatitis (0.13–0.20 ng/mL) and the IC_{50} value of SUN13834 for mouse chymase mMCP-4 (0.078 μ M/L, *i.e.* 43.9 ng/mL). The discrepancy could be explained by the difference in the activity of SUN13834 between an artificial substrate (Suc-Ala-Ala-Pro-Phe-MCA) and physiological substrates *in vivo*, such as pro-IL-1 β , [17], pro-IL-18 [18], membrane-bound SCF [19], which could participate in skin inflammation. It is also possible that there is a synergistic effect on skin inflammation by the inhibition of more than two substrates.

For the PK/PD analyses of chymase inhibitors, it is valuable to measure chymase activity in the tissues. Although we attempted to assess chymase activity in the skin using these models, we could not obtain reasonable data. We think that the chymase released from mast cells immediately reacts with the substrates around the cells, and therefore it may be difficult to detect the activity using an artificial exogenous substrate. Recently, Belkowski et al. have reported cleaved secretory leucocyte protease inhibitor (cSLPI) found in the sputum of atopic asthmatic patients, which could be a biomarker for chymase inhibitors [20,21]. cSLPI might also be detected in the skin lesion or

serum of atopic dermatitis patients and used as a marker for PK/PD analyses in atopic dermatitis and its animal models.

Acknowledgments

We are grateful to Mr. Robert F. Reed for critical reading of the manuscript.

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Table 2

Duration time in NC/Nga mice over the minimum effective plasma concentration of unbound SUN13834 for late-phase reaction of biphasic dermatitis.

Dose (frequency)	Duration ^a over 0.13 ng/mL	Duration ^a over 0.20 ng/mL
15 mg/kg (<i>bid</i>)	20.3 ± 1.5 (18–22.8)	16.1 ± 2.1 (12.9–18.9)
30 mg/kg (<i>qd</i>)	12.2 ± 4.1 (5.8–21.8)	10.7 ± 3.8 (4.9–19.6)
60 mg/kg (<i>eod</i>)	8.8 ± 4.3 (5.5–17.2)	7.8 ± 3.7 (4.6–15.2)

^a Duration (hours, mean ± SD, *n* = 9) estimated using the data of Fig. 3. The values in the parenthesis indicate the range of the data, *i.e.* minimum to maximum.

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