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A sensitive enzyme immunoassay (EIA) for quantitation of the topical anti-inflammatory agent SCH 40120 in unextracted human plasma

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Abstract: SCH 40120 is a potent acute anti-inflammatory agent under development for topical treatment of dermal inflammatory and allergic disorders such as atopic dermatitis, contact dermatitis and psoriasis. In order to support percutaneous absorption studies, a competitive enzyme immunoassay (EIA) was developed to determine SCH 40120 in unextracted plasma samples. SCH 38280, a carboxylated analogue of SCH 40120, was used as the hapten and conjugated with bovine thyroglobulin (Thy). The hapten–Thy conjugate was used as the immunogen to immunize rabbits for antibody production. The hapten was also coupled to horseradish peroxidase (HRP) to form SCH 38280–HRP, which was used as the tracer. The EIA can detect SCH 40120 concentrations as low as 50 pg ml^{-1} of plasma, and can reliably quantitate SCH 40120 in plasma samples from 100 pg ml^{-1} to 10 ng ml^{-1} with good linearity, accuracy and precision. A variety of structurally related compounds and potential metabolites did not significantly cross-react with the antibodies, except for a few analogues. The availability of this sensitive assay makes it possible to evaluate the pharmacokinetics of SCH 40120 in man.

Keywords: *SCH 40120; enzyme immunoassay; 5-lipoxygenase inhibitor; topical anti-inflammatory agent; drug analysis; clinical analysis.*

Introduction

SCH 40120 {10-(3-chlorophenyl)-6,8,9,10-tetrahydrobenzo[b][1,8]naphthyridin-5(7H)-one} (Fig. 1) is a novel potent acute anti-inflammatory agent that has been shown to be an *in vitro* and *in vivo* inhibitor of the production of leukotrienes (LTs) [1, 2]. It is a new drug candidate under development for dermal treatment of inflammatory and allergic disorders such as atopic dermatitis, contact dermatitis and psoriasis. Initial studies with radiolabelled SCH 40120 indicated that the drug was well absorbed following both oral and subcutaneous administration and extensively metabolized both in rat and dog. However, only 5% of a topically applied 0.8% ^{14}C-SCH 40120 cream formulation was absorbed into the systemic circulation of the dog following a 6-h application [unpublished data, Schering Corp. Reports, 1989–1992]. Thus the concentration of the parent drug in plasma after topical administration is presumed to be in the low pg ml^{-1} range which would be very difficult to accurately quantitate by conventional chromatographic methods. Accordingly, development of a sensitive, specific and reproducible immunoassay for this drug was pursued in order to assess the pharmacokinetics of SCH 40120 in man. The following are reported in this paper: the conjugation of SCH 38280, a carboxylated analogue of SCH 40120, to thyroglobulin (Thy) and horseradish peroxidase (HRP); the production of SCH 40120 specific antibodies in rabbits; and the development and validation of a competitive enzyme immunoassay (EIA) for the determination of SCH 40120 in human plasma.

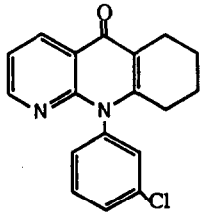

Figure 1
Chemical structure of SCH 40120.

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Experimental

Apparatus

The miocrotitre plate in which the EIA was run was read on a Bio-Tek EL 309 automatic plate reader (Bio-Tek Instruments, Winooski, VT). Centrifugation was performed at room temperature on an IEC Centra 8R Refrigerated Centrifuge (International Equipment, Needham Hts, MA). A microplate mixer was used during sample incubation (IKA Schuttler MTS4, Fisher Scientific, Springfield, NJ). Pipetting solutions of the antiserum, the enzyme conjugate and the enzyme substrate to the plate was done with a Brinkman eight-Channel Pipette (Fisher Scientific, Springfield, NJ). Washing of the plate was done with a Costar Octapette (Costar, Cambridge, MA). All the standard curves and their lot–logit linear regression curves were plotted on a Macintosh IIsi Computer with Cricket Graph software.

Materials

SCH 40120 (batch no. 19908-74-2), SCH 38280 (batch no. 18716-9), and other Schering compounds were from Compound Distribution Center, Schering–Plough Research Institute, Bloomfield, NJ. Bovine thyroglobulin (Thy), bovine serum albumin (BSA), Tween-20, complete and incomplete Freund's Adjuvant, normal rabbit serum and goat anti-rabbit IgG (Cat. no. R5756) were purchased from Sigma (St Louis, MO). The horseradish peroxidase (EIA grade) was from Boehringer Mannheim (Indianapolis, IN). The microtitre plate was purchased from Nunc (Roskilde, Denmark, distributed by Laboratory Disposable Products, N. Haledon, NJ). The horseradish peroxidase substrate, K-Blue™ (tetramethylbenzidine, TMB), was purchased from Elisa (Lexington, KY). The blank human plasma was obtained from Biological Specialities (Landsdale, PA). The blank plasma of rat, mouse and dog were from Pel-Freez Biologicals (Rogers, AR, USA).

Buffer solution composition

(1) EIA buffer(1): 0.1 M phosphate-buffered saline (PBS), 0.01% Tween-20, 0.2% BSA, pH 7.4; (2) buffer(2): 0.1 M PBS, 0.01% Tween-20, pH 7.4; (3) coating buffer: 0.1 M Na_2CO_3, pH 9.6; (4) washing buffer: 10 mM PBS, 0.05% Tween-20, pH 7.4; (5) stopping solution: 1 M phosphoric acid.

Assay solution preparation

(1) *Standard dilutions.* The SCH 40120 standard stock solution was prepared by dissolving 10 mg of accurately weighed SCH 40120 in 1.0 ml DMF to achieve a concentration of 10 mg ml^{-1}. Then, the stock solution was diluted in 50% DMF/buffer(2) to achieve a concentration of 100 $\mu g\ ml^{-1}$, which was then diluted in blank plasma to achieve a concentration range from 50 pg ml^{-1} to 10 ng ml^{-1}.

(2) *Antisera dilutions.* The rabbit antisera (no. L108) was diluted 100 times in buffer(2). It was then further diluted 100 times in the EIA buffer (final dilution: 1/10,000).

(3) *SCH 38280–HRP solution.* The enzyme conjugate (batch no. 29199-5) was first diluted 100 times in buffer(2), and then diluted 500 times in the EIA buffer (final dilution: 1/50,000).

Rabbit (immunization species)

New Zealand white rabbits were purchased by and housed in the Laboratory Animal Center, Schering–Plough Research Institute, Bloomfield, NJ. The rabbits were maintained on a standard diet of fresh vegetables and dry food.

Preparation of the immunogen

The SCH 38280 (10.0 mg), N-hydroxysuccinimide (NHS, 3.9 mg) and dicyclohexyl carbodiimide (DCC, 7.0 mg) were mixed and stirred in 0.8 ml dimethylformamide (DMF) for 4 h at room temperature. Then 30 mg of bovine thyroglobulin (Thy) in 4.5 ml of 0.2 M $NaHCO_3$, pH 8.5 was added to the reaction mixture slowly with vigorous stirring, followed by the addition of 11 mg of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDC). The reaction mixture was stirred at room temperature overnight and then dialysed extensively against three changes of water. After the dialysis, clear solution of the hapten–protein conjugate was obtained which was then aliquoted (~2 mg protein), lyophilized and stored at −20°C.

Preparation of SCH 38280–HRP

The SCH 38280 (0.1 mg), NHS (0.04 mg) and DCC (0.07 mg) were mixed and stirred in 0.1 ml DMF for 4 h at room temperature. Then the horseradish peroxidase (HRP, 1.0 mg) in 0.5 ml of 0.2 M $NaHCO_3$, pH 8.5

was added to the reaction mixture with stirring, followed by the addition of 0.1 mg of EDC. The reaction mixture was stirred at room temperature overnight and then applied onto a pre-washed Pierce GF-5 Desalting Column. The enzyme fraction was eluted by water in the void volume. The fraction was purified once more by the same procedure. About 2 ml of the purified enzyme conjugate solution was obtained. It was mixed with 2 ml glycerol, which was then aliquoted to 0.1 ml and stored at −20°C.

Immunization schedule

The SCH 38280–Thy conjugate (1 mg protein content) in 0.5 ml saline was emulsified with 0.5 ml of Completed Freund Adjuvant and injected intradermally into the backs of two female New Zealand white rabbits (10 weeks old). Booster injections of the same dose with an equal volume of Incomplete Freund Adjuvant were carried out monthly. The rabbits were bled from an ear vein 7–14 days following each booster injection. The serum was obtained after centrifugation of the blood at 2000 ***g*** for 10 min at room temperature and stored at −20°C.

The double antibodies EIA procedure

The anti-rabbit IgG was diluted 100 times in the coating buffer and coated to plate by incubation of 50 μl (2 μg IgG) per well at room temperature for 1.5 h. The plate was washed once with the washing buffer. The normal rabbit serum (NRS) and the antisera were diluted 10,000 times in the EIA buffer, respectively. The diluted NRS was added to the control wells and the diluted antisera was added to the rest of the wells (50 μl $well^{-1}$). The plate was incubated at room temperature for 2 h and washed once. The EIA buffer was added to each well (50 μl $well^{-1}$), followed by the addition of either the blank plasma, SCH 40120 standard dilutions in plasma, or the unknown plasma samples (50 μl $well^{-1}$) in duplicate. The plate was incubated at room temperature for 1 h. Following addition of 50 μl of the diluted SCH 38280–HRP (1/50,000 dilution in the EIA buffer) to each well, the plate was incubated at room temperature for 50 min and washed three times. The K-Blue substrate was added (0.1 ml $well^{-1}$) and the plate was incubated for 15 min at room temperature. The enzymatic reaction was terminated by the addition of 0.1 ml of 1 M phosphoric acid. The plate was then read on a Bio-Tek EL 309 plate reader at 450 nm.

Data analysis

The raw data (OD 450 nm) were analysed using regression of logit (B/B_0) vs log (C) where B is the mean OD value (at least in duplicate) after subtraction of the non-specific binding (NSB, see below) of the bound enzyme conjugate at drug concentration (C). B_0 is the mean OD value (at least in duplicate) after subtraction of NSB of the bound enzyme conjugate at zero drug concentration. NSB is the OD value of the bound enzyme conjugate in the presence of normal rabbit serum (in other words, in the absence of the antibodies). A logit transformation was performed on the B/B_0 ratio as follows:

$$\text{logit} = \ln\{(B/B_0)/[1 - (B/B_0)]\}$$

and logit (y) was regressed on log concentrations of SCH 40120 to generate an equation for a straight line in the form: $y = mx + b$, where y = logit and $x = \log(c)$. Therefore, the concentration of unknown sample was calculated from the regression equation as follows:

$$x = (y - b)/m, \text{ i.e. } C = \text{Anti-log}[(y - b)/m].$$

Results

Preparation of the immunogen and the enzyme conjugate

Since SCH 40120 is a small molecule and is therefore not immunogenic, it must be conjugated to an immunogenic protein to form a hapten–protein conjugate. Moreover, there is no readily usable functional groups on SCH 40120 for covalent conjugation with protein. Accordingly, SCH 38280, a carboxylated analogue of SCH 40120 was chosen to be the hapten. As shown in Fig. 2, it was conjugated to bovine thyroglobulin via a stable amide linkage [3]. Also, SCH 38280 was conjugated to horseradish peroxidase (HRP) via the same type of amide linkage. This conjugate was used as the 'tag' which binds to the antibodies and gives detectable signal after exposure to its substrate.

Immunization and antibody production

SCH 38280–Thy conjugate was used to

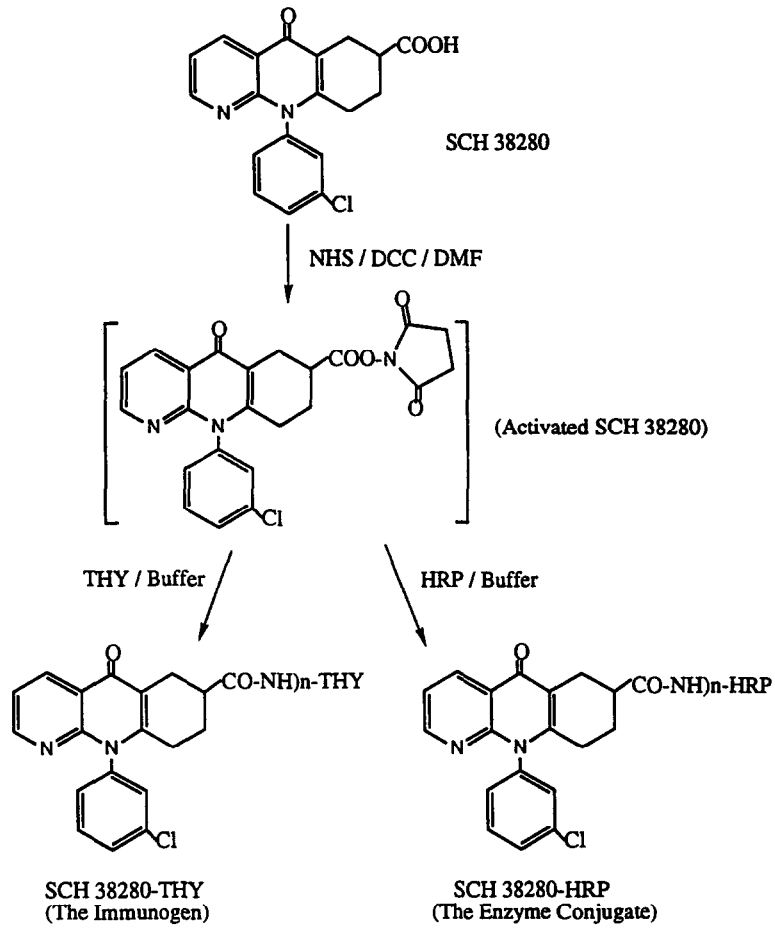

Figure 2
Synthetic scheme for the preparation of the immunogen and the enzyme conjugate.

immunize two rabbits, which then produced detectable antibodies to SCH 40120 after the first booster injection. One rabbit (no. L108) produced antibodies with higher titre than the other one, thus the antisera was further characterized. SCH 38280–HRP was used to titrate the rabbit antiserum. The titration curve clearly demonstrated that the rabbits produced high titre antibodies to SCH 40120 (Fig. 3). It was also demonstrated that the enzyme conjugate exhibited excellent tracer properties in terms of titre (1 to 100,000 dilution) and specific displacement by the free SCH 40120 in the assay media (see below).

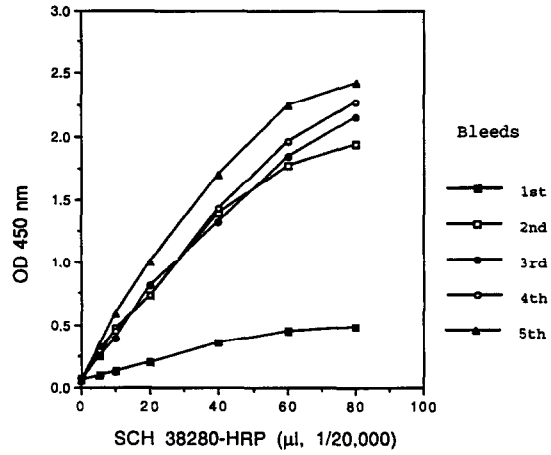

Figure 3
Titration curve of the rabbit antisera (no. L108). The antisera from different bleeds were diluted 10,000 times in the EIA buffer, and the enzyme conjugate (SCH 38280–HRP) was diluted 20,000 times in the same buffer. See Experimental section for experimental details.

Standard curve

A typical standard curve for SCH 40120 EIA in unextracted plasma obtained by plotting the B/B_0 values versus the log of the SCH 40120 standard concentrations exhibited the expected

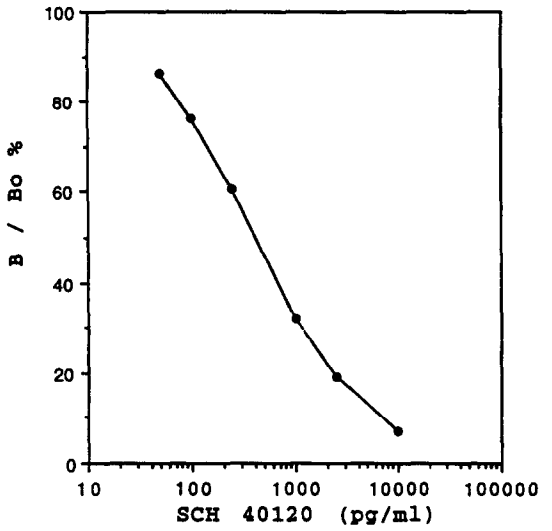

Figure 4
Standard curve of the SCH 40120 EIA. The SCH 40120 standards were prepared in blank plasma. The antisera (no. L108) was diluted 10,000 times and the enzyme conjugate (SCH 38280–HRP) was diluted 50,000 times, both in the EIA buffer. For experimental details see Experimental section.

sigmoidal shape (Fig. 4). Linearization of the curve was achieved by the logit transformation of the B/B_0 values and using linear regression. An excellent linear response was established

Table 1
Standard curve statistics

Standard curve no.	Slope	*y*-intercept	r^2
1	−1.9594	5.2043	0.999
2	−2.1054	5.9584	0.995
3	−1.7451	4.4178	0.997
4	−1.8928	4.9040	0.996
5	−1.6769	4.6767	0.996
6	−1.8791	4.9187	0.997
Mean	−1.8791	5.0133	0.997
SD	0.153	0.533	0.001
RSD (%)	8.15	10.62	0.14

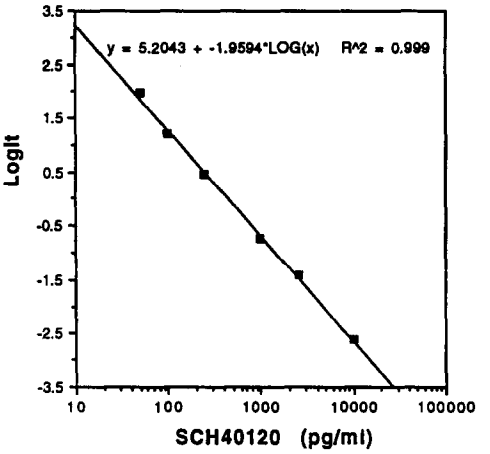

Figure 5
Standard curve in log–logit scale. The logit transformation of the original B/B_0 values is as follows: $\text{logit}(B/B_0) = \ln[(B/B_0)/(1 - B/B_0)]$.

between 50 pg ml^{-1} and 10 ng ml^{-1} in human plasma (Fig. 5). In order to assess the reproducibility of the standard curve, i.e. the consistency of the relationship between the response and concentration, standard curve parameters (slope, *y*-intercept and r^2) are summarized in Table 1. The variations (measured as % RSD) were about 8% for slope, 11% for *y*-intercept and only 0.1% for r^2. The back-calculated standard concentrations in six curves are summarized in Table 2; both the RSD (precision) and bias (accuracy) were below 15% for all concentrations. Therefore, the standard curve of the assay is linear between the concentrations 50 pg ml^{-1} and 10 ng ml^{-1}, and the relationship between the response and concentration is accurate and reproducible.

Specificity

The cross-reactivity of the anti-SCH 40120 antisera was examined by the competitive

Table 2
Standard concentration statistics

	SCH 40120 (pg ml^{-1})					
Standard curve no.	50	100	250	1000	2500	10000
1	44.5	108.0	259.8	1074.4	2414.5	9638.9
2	46.8	85.4	310.0	10727	2732.7	8606.7
3	42.3	116.4	255.4	1002.0	2706.2	9182.9
4	43.1	118.6	229.6	1147.2	2502.4	9279.9
5	48.3	118.1	231.0	842.9	2690.4	10457.1
6	44.7	98.3	303.0	1030.9	2317.0	9830.0
Mean	45.0	107.4	264.8	1028.4	2560.5	9499.2
RSD (%)	5.0	12.4	13.1	10.0	6.8	6.6
Bias (%)	10.1	7.4	5.9	2.8	2.4	5.0

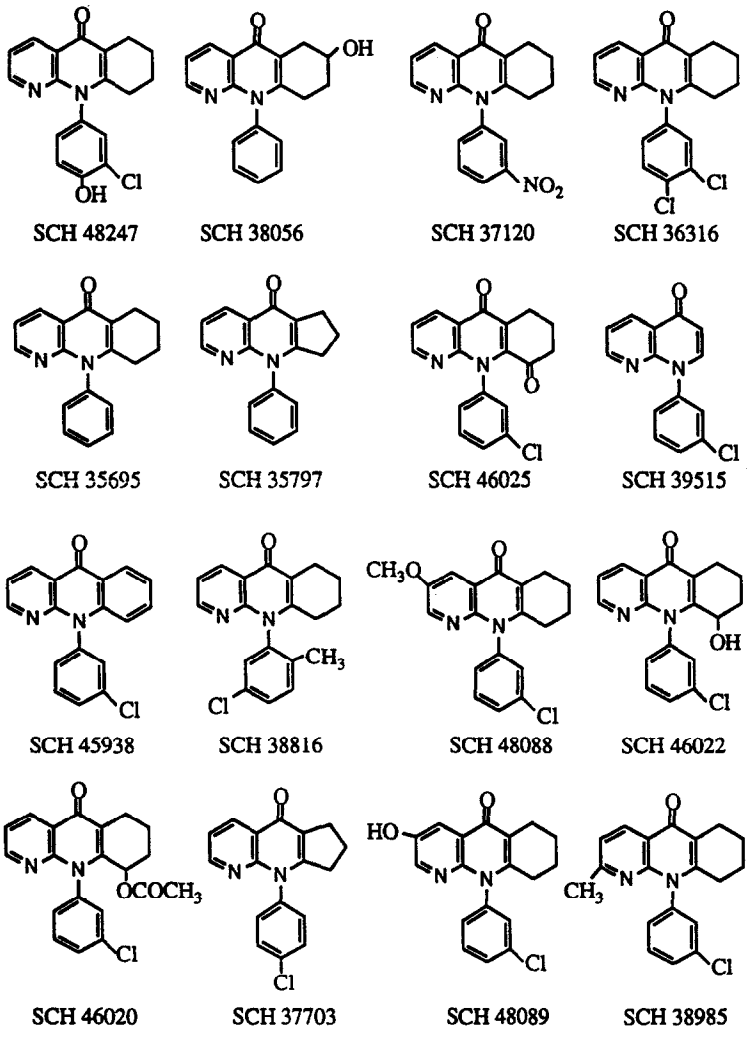

Figure 6
Chemical structures of the SCH 40120 analogues tested in the cross-reaction study.

binding of SCH 38280–HRP with a variety of structurally related compounds and potential metabolites of SCH 40120 (Fig. 6). The cross-reaction was observed as determined at 50% displacement of the enzyme conjugate (SCH 38280–HRP) and summarized in Table 3. Although no metabolite of SCH 40120 has yet been identified, the likely metabolites, such as SCH 48247, SCH 48089 and SCH 46022, did not cross-react with the antisera significantly. Among the few compounds that cross-reacted significantly, SCH 37120 (~13% cross-reaction) would not be a metabolite of SCH 40120 since the addition of a nitro group *in vivo* is highly unlikely. SCH 35695 (~10% cross-reaction), SCH 35797 (~13% cross-reaction) and SCH 38056 (~20% cross-reaction) have not been found *in vivo*, despite the considerable effort of metabolite identification studies

Table 3
SCH 40120 EIA cross-reaction study (*in vitro* competitive binding assay)

Compounds tested	IC_{50} (pg ml^{-1})	Cross-reactivity (%)
SCH 40120	454.9	100.0
SCH 38056	2152.8	21.1
SCH 35797	3389.2	13.4
SCH 37120	3589.9	12.7
SCH 35695	4430.3	10.3
SCH 38816	6780.1	6.7
SCH 46025	6896.9	6.6
SCH 45938	8033.5	5.7
SCH 48088	14319.0	3.2
SCH 36316	18406.6	2.5
SCH 39515	25894.8	1.8
SCH 46022	27195.5	1.7
SCH 38985	35634.9	1.3
SCH 46020	36093.7	1.3
SCH 37703	134306.4	0.3
SCH 48089	260000.0	0.2
SCH 48247	500000.0	0.1

Table 4
Estimation of the limit of quantitation (LOQ)

Spiked	SCH 40120 (pg ml^{-1}) Measured I	II	III	Mean	RSD (%)	Bias (%)
50.0	67.2	24.8	45.2	45.7	46.4	8.5
60.0	57.3	50.4	41.2	49.6	16.2	17.3
70.0	93.5	76.5	57.8	76.0	23.5	8.5
80.0	105.3	80.0	68.5	84.6	22.2	5.7
100.0	116.3	111.7	83.2	103.7	17.3	3.7
125.0	145.8	130.1	101.7	125.9	17.8	0.7

conducted in human, rat and dog [unpublished data, Schering–Plough Research Institute, Research Reports, 1989–1992]. Thus the antisera appears to be specific to SCH 40120.

Limit of quantitation (LOQ)

Although the 50 pg ml^{-1} was the lowest concentration in the standard curve (Table 2), a series of spiked SCH 40120 samples in human plasma were tested to further assess the LOQ of the assay. The LOQ was defined as the lowest concentration which can be measured with both RSD and bias less than 20%. As shown in Table 4, at 100 pg ml^{-1} the RSD was 17.3% and the bias was 3.7%. Therefore, the LOQ of the assay was estimated to be 100 pg ml^{-1} of plasma. Therefore, the EIA can precisely and accurately quantitate SCH 40120 in unextracted plasma samples ranging in concentration from 100 pg ml^{-1} to 10 ng ml^{-1}.

Precision and accuracy

To assess the precision and accuracy of the assay, spiked SCH 40120 in plasma at three concentrations representing the entire range of the standard curve were studied. The concentrations are 200 pg ml^{-1} (2 × LOQ), 1 ng ml^{-1} (near the centre) and 5 ng ml^{-1} (near the upper boundary of the standard curve). To assess the intra-assay precision and accuracy, these samples were assayed in six determinations per concentration in one run and each determination was run in duplicate. As shown in Table 5, the intra-assay precision (measured as % RSD) were 11.5% at 200 pg ml^{-1} and about 8% at both 1 and 5 ng ml^{-1} concentrations; and the intra-assay accuracy (measured as % bias) was less than 8% for all three concentrations. By assaying the same group of the spiked samples on different days, the inter-assay precision and accuracy were observed and shown in Table 6. The inter-assay precision (RSD) was about 16% or less, and accuracy (bias) was less than 5% at all three concentrations.

Table 5
Intra-assay precision and accuracy

Determination no.	Spiked SCH 40120 (pg ml^{-1}) 200	1000	5000
1	187.9	1064.9	5330.3
2	156.8	877.2	4243.6
3	220.6	892.1	4736.5
4	208.6	1038.2	4308.5
5	203.6	1000.2	4746.7
6	187.3	903.3	4405.3
Mean	194.0	962.7	4628.5
SD	22.3	81.7	404.6
RSD (%)	11.5	8.3	8.7
Bias (%)	3.0	3.7	7.4

Table 6
Inter-assay precision and accuracy

Determination no.	Spiked SCH 40120 (pg ml^{-1}) 200	1000	5000
1	210.7	1027.1	5354.4
2	187.3	1064.9	5330.3
3	177.9	957.4	4284.1
4	220.1	942.5	5479.8
5	219.1	1048.1	3555.6
6	195.0	1013.1	4936.9
Mean	201.7	1008.9	4823.5
SD	17.6	49.2	759.1
RSD (%)	8.7	4.9	15.7
Bias (%)	0.8	0.9	3.5

Validation with 'checking' samples

A group of randomly coded and spiked samples of SCH 40120 in human plasma at five concentrations (including zero) was assayed as 'unknown samples' to check the *in vitro* feasibility of the EIA. As shown in Table 7, the precision (% RSD) ranged from 6.9 to 18.2 and the accuracy (% bias) ranged from 3.4 to 9.5, clearly indicating that the assay is suitable

Table 7
Determination of the check samples

Random code	SCH 40120 (pg ml^{-1}) Spiked	Measured	Mean	RSD (%)	Bias (%)
D	100.0	104.0			
G	100.0	96.7	108.3	13.2	8.3
I	100.0	124.3			
A	200.0	153.3			
F	200.0	199.0	191.2	18.2	4.4
K	200.0	221.4			
B	500.0	439.8			
H	500.0	488.0	452.4	6.9	9.5
J	500.0	429.3			
C	5000.0	5268.7			
E	5000.0	5306.0	4830.2	16.4	3.4
L	5000.0	3916.0			

for quantitating SCH 40120 in unextracted plasma precisely and accurately.

Stability of SCH 40120 in human plasma

The effect of freeze/thaw cycles on the stability of SCH 40120 in plasma was studied. Spiked SCH 40120 in plasma at three concentrations (200, 1000 and 5000 pg ml^{-1}) were assayed after each freeze/thaw cycle for a total of three cycles (3 days). As summarized in Table 8, no significant changes were observed indicating that the drug is stable at this condition.

Application

The application of the EIA method for the determination of SCH 40120 in clinical samples (study no. C91-307-01, Schering–Plough) was studied. As shown in Fig. 7, the mean plasma concentration of SCH 40120 peaked at about 1.5 ng ml^{-1} (C_{max}) at about 6 h (T_{max}) after single topical administration of 1 g of 0.1% SCH 40120 cream for 12 h on psoriatic skin of psoriatic patients (n = 8). Also, the mean plasma drug concentration peaked at about 6.4 ng ml^{-1} (C_{max}) at about 4 h (T_{max}) when 1 g of 1% cream of SCH 40120 was administered, suggesting dose-related absorption. This result clearly demonstrated that the EIA method is suitable for the evaluation of the pharmacokinetics of SCH 40120 in man after topical administration.

Table 8
Effect of freeze/thaw on the stability of SCH 40120 in human plasma

Freeze/thaw cycle	Spiked SCH 40120 (pg ml^{-1}) 200	1000	5000
	Measured SCH 40120 (pg ml^{-1})		
1	210.7	1027.1	5354.4
Recovery (%)	105.4	102.7	107.1
2	177.9	957.4	4284.1
Recovery (%)	89.0	95.7	85.7
3	195.0	1013.7	4936.9
Recovery (%)	97.5	101.4	98.7

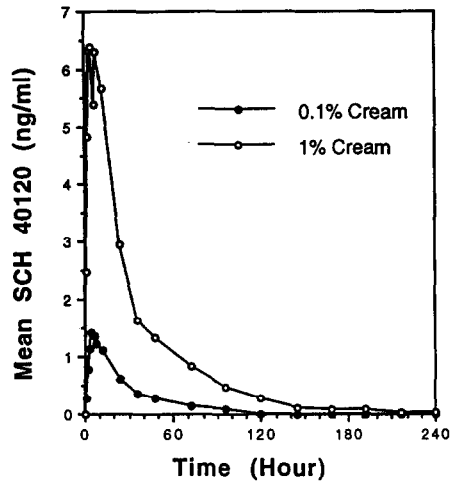

Figure 7
SCH 40120 concentrations in human plasma. A single dose of 1 g of 0.1% or 1% SCH 40120 cream was administered topically to psoriatic skin of each of the psoriatic patients (study no. C91-307-01, Schering–Plough). Blood samples were taken at times indicated and the plasma samples were assayed for SCH 40120 concentrations. The procedure is described in the Experimental section.

Discussion

Immunoassays are extremely useful methods to determine relatively small amounts of analytes in complex matrices. Enzyme

immunoassay (EIA) has emerged as a widely used technique to avoid the problems of radioactive waste disposal and limited shelf life of radiolabelled reagents [3–6]. The EIA described in this report is the first successful approach to quantitate SCH 40120 at pg ml^{-1} levels, which is essential for pharmacokinetic and toxicokinetic studies because of the low dose regimen and the topical use of this drug. In addition, this analysis is performed directly on plasma samples without prior extraction, which is important in the analysis of large number of samples generated in clinical studies. It should be noted that the sensitivity (limit of quantitation, LOQ), the term which is now widely used in literature with broad definitions or sometimes without proper definition, of the EIA was defined as the lowest concentration of plasma samples which can be precisely (RSD <20%) and accurately (bias <20%) quantitated. Therefore, it is more meaningful in real-life sample analysis. The cross-reaction study indicated that the majority of the SCH 40120 analogues did not significantly bind to the antibodies. The three compounds which showed significant cross-reactivity are neither found *in vivo* nor likely metabolites. Thus the assay appears to be specific to SCH 40120. The EIA described herein represents the simplest, yet robust and highly sensitive method available for the quantitation of SCH 40120 in human plasma.

Conclusions

A competitive enzyme immunoassay was developed and validated for the determination of SCH 40120 concentrations in human plasma samples. The method is specific, linear, sensitive, precise and accurate. The limit of quantitation is 100 pg SCH 40120 per ml plasma. The assay is suitable for evaluation of pharmacokinetics of SCH 40120 in man.

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