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Liquid chromatographic analysis in mouse, dog and human plasma; stability, absorption, metabolism and pharmacokinetics of the anti-HIV agent 2-chloro-5-(2-methyl-5,6-dihydro-1,4-oxathiin-3-yl carboxamido) isopropylbenzoate (NSC 615985, UC84)

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Abstract

NSC 615985 (UC 84) has demonstrated anti-HIV activity in the NCI–AIDS antiviral screen and was under consideration as an anti-AIDS drug. The compound was subsequently shown to be a non-nucleoside reverse transcriptase inhibitor (NNRTI). An HPLC method was developed for the analysis of NSC 615985 in mouse, dog and human plasma; and was used to study its stability in plasma and blood as well as its absorption and metabolism in mice. The method involved precipitation of plasma protein with three volumes of methanol followed by HPLC analysis of the supernatant. The HPLC analysis was carried out on a reversed-phase Nova-Pak C_{18} column with a mobile phase of KH_2PO_4 (0.01 M; pH 4.8)-acetonitrile (52:48, v/v) at a flow rate of 1 ml min^{-1} and quantification with a UV detector set at 259 nm. The lower limit of quantitation was 0.05 μg ml^{-1} in 1 ml of dog or human plasma or 0.1 μg ml^{-1} in 0.5 ml of mouse plasma. NSC 615985 was more stable in dog and human plasma than in mouse plasma, and was less stable in blood than in plasma of the three species investigated. Following bolus intravenous (IV) administration at 10 mg kg^{-1} to male CDF_1 mice, NSC 615985 elimination followed biexponential kinetics with half-lives of 1 and 7 min, and was extensively metabolized. NSC 615985 was very poorly absorbed following oral (PO) administration as a suspension in water or in 20% lipid emulsion (Liposyn II). Following bolus subcutaneous (SC) administration of [^{14}C]NSC 615985 at 10 mg kg^{-1}, relatively low concentrations of the parent compound were observed in three of 36 mice. One metabolite was tentatively identified in plasma of both the IV- and SC-treated animals as the sulfoxide of the parent compound. No parent compound was detected in the urine of NSC 615985 dosed mice. At least seven metabolites were present in urine; one metabolite (constituting 8–14% of urinary radioactivity) was tentatively identified as the carboxylic acid resulting from the hydrolysis of the isopropyl group

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from the parent compound. In summary, NSC 615985 was poorly absorbed following oral administration and extensively metabolized and eliminated following IV or SC administration. This unfavorable pharmacokinetic profile of NSC 615985 as well as its pattern of activity against NNRTI-resistant strains of HIV-1 precluded its progression to clinical trial; however, other members of the general chemical class are currently being evaluated by the NCI.
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Keywords: Reversed Phase LC; Absorption; Metabolism; Anti-HIV agent; NSC 615985; UC84; NNRTI

1. Introduction

NSC 615985 (UC84) [2-chloro-5-(2-methyl-5,6-dihydro-1,4-oxathiin-3-yl-carboxamido) isopropylbenzoate] is a derivative of 2-chloro-5-aminobenzoic acid in which both the amino and carboxyl groups are derivatized; its chemical structure is shown in Fig. 1. This chemical has demonstrated anti-HIV activity in the NCI AIDS antiviral screen and was under consideration as an anti-AIDS drug [1,2]. NSC 615985 has been shown to exhibit an exceptional ability to protect T_4 lymphocytes from HIV-1 induced cytopathic effects, and is also effective in the P24 protein and syncytium formation assays [3]. It has been reported that the projected therapeutic concentration of NSC 615985 is 1 μM [3]. NSC 615985 was subsequently shown to be an HIV-1-specific non-nucleoside reverse transcriptase inhibitor (NNRTI) [4]. The ability of NSC 615985 to select for a specific resistant mutant strain of HIV-1 and its cross-resistance profile against HIV-1 mutants selected by other classes of NNRTIs have been described [4,5].

The objectives of the present studies were to develop an analytical method for the analysis of NSC 615985 in human, dog and mouse plasma; to determine its stability in plasma and blood of the above mentioned species; and to study the absorption and metabolism of this compound in male CDF_1 mice in order to evaluate its potential as a drug development candidate for the treatment of HIV-1 disease.

2. Materials and methods

2.1. Materials

The following compounds were supplied by the NCI: unlabelled NSC 615985 (97.9–99.4% pure); [^{14}C]NSC 615985 (benzene ring-U-^{14}C), 32.1 mCi mmol^{-1}, 99.8% radiochemically pure (Research Triangle Institute, NC); 2-chloro-5-(2-methyl-5,6-dihydro-1,4-oxathiin-4-oxo-3-yl carboxamido) isopropylbenzoate, the sulfoxide of the parent compound; 2-chloro-5-(2-methyl-5,6-dihydro-1,4-oxathiin-4,4-dioxo-3-yl carboxamido) isopropybenzoate, the sulfone of the parent compound; 2-chloro-5-(2-methyl-5,6-dihydro-1,4-oxathiin-3-yl carboxamido) benzoic acid, the benzoic acid derivative remaining after the hydrolysis of the isopropyl group from NSC 615985. 2-Chloronitrobenzene (2-CNB) and 4-chloronitrobenzene (4-CNB), both 99% pure, were purchased from Aldrich (Milwaukee, WI). Mouse blood and plasma were obtained from male CDF_1 mice which were supplied by the NCI. Blood used as a source of plasma was collected using heparin as anticoagulant. Blood used for stability studies was collected using EDTA as anticoagulant. Plasma was separated from blood cells by centrifugation. Beagle dog plasma and blood were purchased from Marshall Farms (North Rose, NY). Heparin was used as the anticoagulant with the exception of using EDTA when blood was used for stability studies (when heparin was used blood coagulated after 24 h at 37°C). Human plasma and blood were obtained from the American Red Cross Blood Services, Northeast Region (Dedham, MA). The anticoagulant used was citrate-phos-

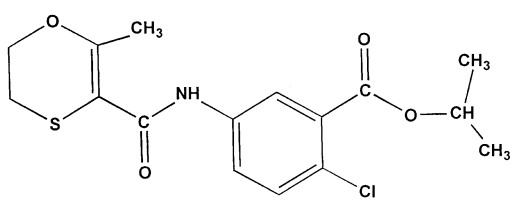

Fig. 1. Chemical structure of NSC 615985.

Table 1
Composition of the gradient HPLC solvent system

Time (min)	Composition of solvent system (%)[a]		Waters gradient controller number
	Pump A	Pump B	
Initial	95	5	
5	95	5	6 (linear)
12	46	54	
18	46	54	6
20	40	60	
24	40	60	
27	95	5	6
30	95	5	

[a] Pump A = 0.01 M KH_2PO_4, pH 4.8: acetonitrile, 9:1; pump B = acetonitrile.

phate-dextrose. All solvents used for chromatography were HPLC grade, purchased from Caledon (Georgetown, Ontario) and water was deionized and glass distilled. All HPLC solvents were filtered through 0.45 μm filters and deaerated by sonication and vacuum before use. All chemicals were analytical grade or better.

2.2. Liquid chromatography (HPLC)

The liquid chromatograph consisted of two model 6000A solvent delivery systems, a model 680 automated gradient controller, a model U6K universal injector, a WISP™ model 710B autosampler, a model 730 data module, a Nova-Pak™ C_{18} 4 μm 15 cm × 4 mm (i.d.) stainless steel column, a C_{18} Guard-Pak™ precolumn (all from Waters, Milford, MA), a Kratos 773 variable wavelength absorbance detector (Kratos, Ramsey, NJ) set at 259 nm, a Radiomatic Instruments Flo-One Beta model IC radioactive flow detector (Radiomatic, Tampa, FL) and a Hewlett-Packard 1040 M photodiode array detector (Hewlett-Packard, Palo Alto, CA). The following solvent systems were used:

2.2.1. Isocratic elution

The solvent system consisted of KH_2PO_4 (0.01 M; pH 4.8)-acetonitrile (52:48, v/v) at a flow rate of 1 ml min^{-1}. Most analyses were carried out with this system. It was used to determine the identity as well as chemical and radiochemical purities of the parent compound, to analyze dose formulations, to determine if the parent compound was present in urine, to obtain the initial metabolic profiles of NSC 615985 in plasma and urine, and to determine the concentrations of the parent compound in plasma.

2.2.2. Gradient elution

The gradient solvent system shown in Table 1, at a flow rate of 1 ml min^{-1}, was used to determine the metabolite profile of NSC 615985 in urine and plasma of treated mice.

2.3. Analysis of plasma for parent compound

Plasma protein was precipitated by the addition of three volumes of methanol to each volume of plasma, followed by mixing and centrifugation at $5000 \times g$ for 10 min. For compound concentrations of 1–100 μg ml^{-1}, the supernatant was analyzed directly after the addition of 2-CNB as an internal standard in a small volume of methanol (15 μl for 1.5 ml supernatant). The internal standard was added after protein precipitation in order to obtain consistent peak area for the internal standard. For compound concentra-

tions of 0.05–1 μg ml^{-1}, a volume of the supernatant (usually 1.5 ml) was evaporated to dryness and the residue was reconstituted in 0.25 ml of methanol containing the internal standard 4-CNB. 4-CNB was used instead of 2-CNB in the concentrated samples because of the presence of a small interference at the retention time of 2-CNB. The concentration of NSC 615985 was determined in the methanol supernatant of plasma with the isocratic HPLC method described earlier using a concomitant standard curve set up in plasma of the respective species.

2.4. Method evaluation

The method was evaluated prior to sample analysis. The precision (%CV) and accuracy (%bias) were calculated from the back calculated concentrations of three standard curves prepared in plasma. The results were satisfactory as indicated by CV values of $< 10\%$ and bias values of $< 9\%$. The lowest limit of quantitation was established as the lowest concentration in the standard curve where the %CV (three replicates) and bias from the back calculated concentrations were $\leq$ 20%. The specificity of the assay was established by the lack of interference peaks at the retention time of the internal standards and NSC 615985 in at least four different samples from each species evaluated. The recovery of NSC 615985 from plasma was determined using a standard curve set up in methanol. The methanol standard curve was prepared by diluting the methanol stock solution with methanol and the addition of the internal standard followed by HPLC analysis. The peak areas of the methanol standards were very similar to those obtained from identical concentrations prepared in the methanol supernatant of plasma as long as the injection volume of the methanol solutions did not exceed 25 μl. Also, standard curves prepared in different days showed similar parameters.

2.5. Stability of NSC 615985 in plasma and blood

The stability of NSC 615985 in mouse, dog and human plasma and blood was evaluated at concentrations of 0.1, 10 and 100 μg ml^{-1}. Plasma and blood samples containing NSC 615985 were incubated at 4 or 37°C, and plasma samples were also frozen and stored at -10°C. All samples were kept in the dark for periods ranging from 10 min to 4 weeks depending on the storage conditions. Plasma samples either spiked directly or obtained from spiked blood by centrifugation, were analyzed for the parent compound by HPLC. In addition, at each concentration duplicate zero-time controls were prepared in which methanol was added immediately after the addition of NSC 615985 to plasma or immediately after plasma was separated from blood spiked with NSC 615985. The fraction of the compound remaining in test samples was expressed relative to that found in the zero-time samples.

2.6. Animals and dosing

Male CDF_1 mice, weighing 25 ± 3 g (mean $\pm$ SD), were supplied by the NCI. The animals were housed in plastic cages. Food and water were allowed ad Libitum. Food consisted of Purina Rodent Chow (# 5008; Raltech, St. Louis, MO) and water was Cambridge, MA tap water.

NSC 615985 is practically insoluble in water (0.02–0.03 μg ml^{-1}). Consequently, dose formulations for the IV and SC administration were prepared as solutions in 20% lipid emulsion (Liposyn II; Abbott, Chicago, IL). For the IV and SC studies, [^{14}C]NSC 615985 (diluted to a specific activity of 16.05 mCi $mmol^{-1}$) was administered at 10 mg kg^{-1} of body weight. Dose formulation was prepared by stirring [^{14}C]NSC 615985 in Lipsoyn II at a nominal concentration of 2 mg ml^{-1} for 48 h in the dark at room temperature, then filtered through 0.45 μ filters. The concentration of NSC 615985 was determined radiochemically and chemically by HPLC prior to dosing in order to obtain the chemical and radiochemical purities and the specific activity of NSC 615985 in dose formulation. The analysis was carried out by diluting 20 μl sample of dose formulation in 5 ml of absolute ethanol (clear solution) followed by radiochemical (scintillation counting and HPLC-radioactive flow detector) and chemical analyses (HPLC–UV) of the clear solution. The concentra-

tion of NSC 615985 in dose formulations ranged from 0.6–0.8 mg ml^{-1}. Dose formulations for the IV and SC studies were used within 8 h of preparation. Dose formulations for the oral absorption studies were prepared as suspensions in water or Liposyn II at a nominal concentration of 75 mg ml^{-1}.

[^{14}C]NSC 615985 was administered at 10 mg kg^{-1} to unanesthetized male CDF_1 mice IV in a caudal vein or SC between the shoulder blades. For the PO study, NSC 615985 was administered at 750 mg kg^{-1} by gastric intubation to mice, that had been fasted for 18 h. Following dosing, four animals were sacrificed at specified time intervals for the collection of plasma. The mice were anesthetized with diethyl ether and blood was collected by heart puncture into Vacutainer® tubes containing heparin or EDTA (Becton–Dickinson, Rutherford, NJ), and was kept on ice. The animals were terminated by exsanguination. Plasma was obtained by centrifugation and was frozen at −10°C or lower pending analysis. Plasma from each animal was assayed separately.

Urine was collected from animals kept for 90 min or longer after IV or SC dosing with [^{14}C]NSC 615985. All animals of each time point that were used for urine collection were housed together in Nalgene metabolism cages (Nalge, Rochester, NY) which allowed separation of urine and feces. Therefore, urine from animals of each collection interval was pooled. Urine was kept on ice during collection and frozen until analysis.

2.7. Quantization of total compound equivalents

Radioactivity in duplicate urine or plasma aliquots was determined in Scint™-AXF scintillation fluid (Packard, Downers Grove, IL), using a Tracor Analytic Mark III liquid scintillation spectrophometer (Tracor, Austin, TX).

2.8. Analysis of parent compound in urine

Urine was analyzed by HPLC to determine if the parent compound was excreted in urine. Urine was injected directly after filtration through 0.45 μ filters and chromatographed using the isocratic solvent system. There was no significant non-specific binding to the filter as indicated by radiochemical analysis of urine samples before and after filtration.

2.9. Metabolite profile of NSC 615985 in plasma and urine

For characterization of plasma metabolite profile, the remaining plasma from the IV (2–30 min) and SC (20–240 min) dosed animals were combined by route of administration and treated with methanol as described above. Methanol extracts of plasma and unextracted urine were analyzed by the gradient HPLC using both the UV and the radioactive flow detector. After passing through the UV detector, column eluate was mixed with Flo-Scint™ II (Radiomatic, Tampa, FL) at a flow rate of 4 ml min^{-1} and then analyzed on line with the radioactive flow detector. The recovery from the HPLC was calculated by collecting the total eluate and determining the radioactivity using liquid scintillation counting.

2.10. Identification of plasma and urinary metabolites

NSC 615985 metabolites in plasma and urine were analyzed by HPLC using a diode array detector to obtain the retention time as well as the UV-visible spectrum of each metabolite present in appreciable amount. The authentic standards (the sulfoxide and sulfone of the parent compound, and the benzoic acid derivative remaining after the hydrolysis of the isopropyl group from NSC 615985; see chemical names under Materials) were analyzed similarly and their spectra were superimposed on those of metabolites which eluted at similar retention times.

2.11. Calculations and statistics

NSC 615985 concentrations in experimental plasma samples were determined by relating peak area ratios (NSC 615985/internal standard) to a concurrent standard curve for the compound set up in plasma. Samples which contain compound concentrations below the limit of quantitation (0.05 μg ml^{-1} for human and dog plasma and 0.1

Table 2
Parameters of standard curves[a]

Curve type	Species	Range (μg ml^{-1})	Slope	Intercept	Correlation coefficient
Plasma	Mouse	0.1–1	0.113	−0.0009	0.999241
		1–100	0.036	0.018	0.999841
	Dog	0.05–1	0.226	−0.003	0.999274
		1–100	0.046	−0.041	0.999766
	Human	0.05–1	0.152	0.002	0.999276
		1–100	0.037	0.021	0.998628
Methanol		0.1–5	0.078	−0.0047	0.999091
		2–50	0.169	−0.077	0.999737

[a] Each standard curve included at least five concentrations. Duplicate HPLC analyses of each of three replicates at each concentration were performed.

μg ml^{-1} for mouse plasma) were included as zero in the calculations. Therefore, the calculated mean values may be below the limit of quantitation.

Pharmacokinetic analysis was carried out using RSTRIP (Version 4.05) polyexponential computer program (Micromath, Salt Lake City, UT) to obtain initial estimates for the parameters and to select the pharmacokinetic model that best represented the data. The parameters were then refined by fitting the data using the least-squares method. The half-lives for the distribution ($t_{1/2}\alpha$) and elimination ($t_{1/2}\beta$) phases, the area under the plasma concentration-time curve (AUC∞), volume of distribution and total body clearance were calculated using classical methods [6].

3. Results

An analytical method for the assay of NSC 615985 in plasma was developed. The method involved precipitation of plasma protein with methanol and analysis of supernatant either directly or after concentration, depending on the concentration of NSC 615985 in plasma. As a result, two standard curves were needed to cover a concentration range of 0.05–100 μg ml^{-1}, one for the 0.05–1 μg ml^{-1} range and the other for the 1–100 μg ml^{-1} range. The samples were first analyzed without the concentration step. Samples containing concentrations outside the standard curve range were reanalyzed after concentration of the plasma-methanol supernatant. Table 2 shows the parameters of the two standard curves in plasma as well as the parameters of standard curves in methanol. The correlation coefficients were >0.9980. The bias values of the back calculated concentrations of the standard curves were $<9\%$ and the CV values of three replicates were $<10\%$. The recovery of NSC 615985 from plasma of the three species is shown in Table 3. The recovery values for the 1–100 μg ml^{-1} concentration range were satisfactory. For the lower concentration range, the recovery values were satisfactory for mouse and dog plasma, while for human plasma, relatively lower recovery values were obtained. The precision was satisfactory as indicated by CV values of $<10\%$ (Table 3). The lower limit of quantization (LOQ) was 0.05 μg ml^{-1} in 1 ml of dog or human plasma, or 0.1 μg ml^{-1} in 0.5 ml of mouse plasma. The LOQ was established as the lowest concentration in the standard curve where both the CV and bias were $\leq 20\%$. The method was selective as indicated by the absence of interfering peaks at the retention times of the internal standards and NSC 615985. Representative chromatograms of blank mouse plasma spiked with the internal standard alone and with the internal standard and NSC 615985 are shown in Fig. 2.

Tables 4 and 5 show the stability of NSC 615985 at various temperatures in plasma and blood of the three species investigated, respectively. NSC 615985 was stable in mouse, dog and human plasma for at least 4 weeks at −10°C (data not shown), and in dog and human plasma

for at least 24 h at 37°C (Table 4). Disappearance of the compound from mouse plasma was observed following incubation for 24 h at 37°C (Table 4). NSC 615985 was stable in dog blood for at least 24 h at 4°C and in mouse and human blood for at least 1 h at 4°C. Disappearance of the compound from blood of all three species was observed following incubation for 24 h at 37°C, most extensively from mouse blood (Table 5). Also, there was some indication of the disappearance of the compound from mouse and human blood incubated for 24 h at 4°C (Table 5). Overall, NSC 615985 appeared to have good stability under the handling and storage conditions likely to be encountered in pharmacokinetic studies.

Several studies were carried out to investigate the pharmacokinetics and metabolism of this drug candidate in male CDF_1 mice. Fig. 3 shows the plasma concentration-time profiles of NSC 615985 and NSC 615985 equivalents in mice following IV administration of [^{14}C]NSC 615985 at 10 mg kg^{-1}. NSC 615985-derived radioactivity was detected in all animals for up to 4 h following dosing, while NSC 615985 was detected in all animals for only up to 20 min following dosing, in addition to one animal from the 30 min time interval. The concentrations of NSC 615985 in plasma decreased more readily than those of NSC 615985 equivalents, indicating that metabolism plays a major role in the elimination of this drug candidate from plasma, particularly at the early time points. Based on AUC, approximately 84% of plasma radioactivity was due to metabolites.

Following PO administration of NSC 615985 at 750 mg kg^{-1} to male CDF_1 mice (as a suspension in Liposyn II or in water), the parent compound was present in plasma at relatively low concentrations (0.1–2 μg ml^{-1}) and in scattered animals (8 of 18 dosed mice, data not shown). The experiment was repeated and similar results were obtained. These studies conclusively showed that NSC 615985 was poorly and inconsistently absorbed from the gastrointestinal tract following PO administration as suspensions in water or Liposyn II.

Fig. 4 shows the concentrations of NSC 615985 and NSC 615985 equivalents in plasma of male CDF_1 mice at intervals following SC administration of [^{14}C]NSC 615985 at 10 mg kg^{-1}. The parent compound was quantifiable only in three of 36 animals and the concentrations were 0.21–0.33 μg ml^{-1}. The concentration of the compound equivalents appears to be increasing until 90 min after dosing, then remained essentially unchanged for at least additional 2.5 h.

Analysis of pooled plasma extracts with the gradient HPLC system indicated the presence of at least eight metabolites, four majors each constituting 5% or more and four minors, each constituting less than 5% of plasma radioactivity (Fig. 5). The peak eluted at 22 min was identified as the parent compound. The major metabolite (retention time 16 min), which constituted approximately 60% of the radioactivity present in pooled plasma extract (from 2–30 min for IV-treated mice), was tentatively identified as the sulfoxide of the parent compound. The identification was based on matching the retention time and the UV spectrum of the plasma metabolite with those of

Table 3
Recovery of NSC 615985 from mouse, dog and human plasma

Species	Concentration Added μg ml^{-1}	Recovery (%) mean ± CV[a]
Mouse	0.1[b]	115.7 ± 6.6
	1.0[b]	86.7 ± 1.5
	1.0[c]	83.2 ± 2.6
	10.0[c]	90.6 ± 8.7
	100[c]	75.74 ± 1.7
Dog	0.05[b]	94.9 ± 4.2
	0.1[b]	88.6 ± 4.2
	1.0[b]	83.6 ± 2.9
	1.0[c]	97.7 ± 1.3
	10.0[c]	88.3 ± 4.8
	100[c]	97.5 ± 7.9
Human	0.05[b]	89.4 ± 7.9
	0.1[b]	84.1 ± 7.9
	1.0[b]	61.5 ± 4.2
	1.0[c]	78.3 ± 4.7
	10.0[c]	86.8 ± 4.0
	100[c]	78.0 ± 2.2

[a] Each value is a mean ± RSD (%CV) of three replicates with duplicate HPLC analyses. Duplicate analyses were almost identical.
[b] Methanol extract of plasma was evaporated to dryness (1.6–3 ml) and the residue reconstituted in 0.25 ml of methanol.
[c] Methanol extract of plasma was analyzed directly.

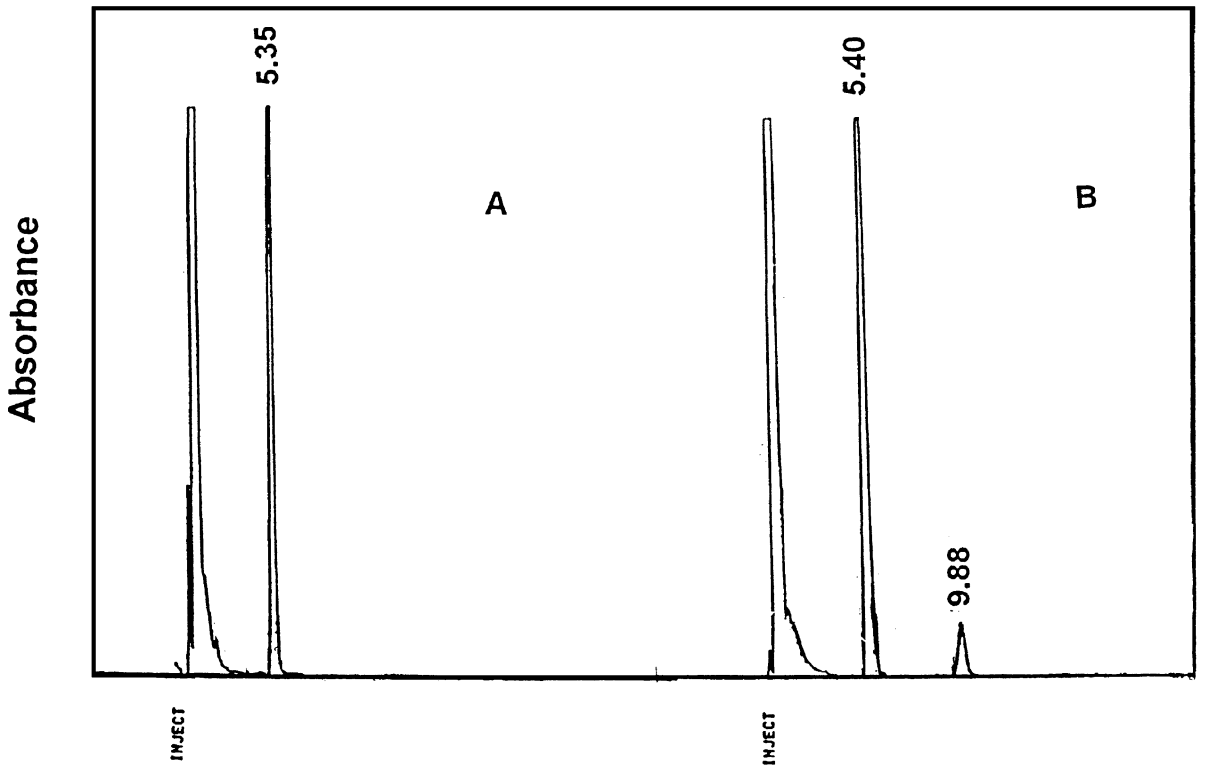

Fig. 2. Typical chromatograms of blank mouse plasma spiked with the internal standard alone (A) or with the internal standard and NSC 615985 (B).

the authentic standard (Fig. 6). The short wavelength end of the spectra was different probably due to impurities in the mouse plasma extract.

Pooled plasma extracts from the SC-dosed animals (20–240 min) were also analyzed by the gradient HPLC system. The metabolic profile was qualitatively similar to that of the IV sample (Fig. 5). However, the ratio of each metabolite was different. The metabolite eluted at 16 min constituted approximately 16% of the radioactivity in the extract versus 60% for the IV dosed animals. This metabolite was tentatively identified as the sulfoxide of the parent compound as described above.

Approximately 16 and 1.5% of the IV and SC doses, respectively, were excreted in urine within 4 h after dosing. Urine from the IV and SC studies was analyzed by HPLC. No parent NSC 615985, sulfoxide or sulfone of the parent compound were found in any of the urine samples analyzed. At least seven metabolites were present in urine (Fig. 7); four were majors each constituting 5% or more, and three were minors each constituting less than 5% of the urinary radioactivity. One metabolite (retention time 10–11 min), which constituted 8–14% of the urinary radioactivity from both the IV- and SC-dosed animals, was tentatively identified as the carboxylic acid, which is presumably formed by the hydrolysis of the isopropyl group from the parent compound. The identification was based on matching the retention time and the UV spectrum of the metabolite to those of the authentic standard (Fig. 8).

Table 4
Stability of NSC 615985 in mouse, dog and human plasma

Species	Concentration (μg ml^{-1})	NSC 615985 Remaining (%)[a]			
		Incubation period			
		4°C	37°C		
		24 h	10 min	1 h	24 h
Mouse	0.1	105	100	100	—[b]
	10	91	98	80	8
	100	96	98	97	28
Dog	0.1	98	105	107	88
	10	91	99	116	88
	100	90	99	100	93
Human	0.1	100	100	100	100
	10	100	94	93	98
	100	99	97	96	92

[a] Mean of duplicate determinations with duplicate HPLC injections each.
[b] Detectable but not quantifiable.

4. Discussion

Historically, NSC 615985 was the first in a large series of structurally-related synthetic compounds that was evaluated by the NCI as a possible candidate for the treatment of AIDS. Although the pharmacological and antiviral characteristics of NSC 615985 led to its exclusion as a developmental candidate, several related compounds remain under active consideration.

In order to obtain preclinical pharmacokinetic data in laboratory animals, an analytical method for NSC 615985 was developed. The method was subjected to extensive evaluation prior to use [7], and was found to be reliable for sample analysis. NSC 615985 was more stable in dog and human plasma than in mouse plasma, and was less stable in blood than plasma. A similar stability profile was observed with NSC 629243 which is a structurally similar compound [8].

NSC 615985 was rapidly eliminated from plasma of mice following IV administration at 10 mg kg^{-1}. Its disappearance followed biexponential kinetics with half-lives of approximately 1 and 7 min for the distribution and elimination phases, respectively. The systemic clearance was 138 ml min^{-1} kg^{-1} and the volume of distribution was 1349 ml kg^{-1}. Since no parent compound was detected in urine, the clearance was primarily attributable to extensive metabolism and/or biliary secretion. The metabolites were also rapidly cleared from plasma, as indicated by the rapid decline of the total compound equivalents. For example, at 60 min the concentration of NSC 615985 equivalents was approximately 11% of the value obtained at 2 min.

The projected therapeutic concentration of NSC 615985 is 1 μM, which is equivalent to 0.36 μg ml^{-1} [3]. The results of the present study indicate that therapeutic concentrations were observed in mouse plasma for only 20 min following IV dosing at 10 mg kg^{-1}. Following SC bolus administration at 10 mg kg^{-1}, NSC 615985-derived radioactivity was absorbed. However, concentrations below the therapeutic level were found in plasma of scattered mice (only three of 36 animals). These results are consistent with the finding that NSC 615985 rapidly disappeared from plasma following IV dosing.

No parent compound was detected in the urine of NSC 615985-treated animals. This is not unexpected, since the chemical is highly nonpolar. At least seven metabolites were present in urine of both the IV- and SC-dosed animals. One metabo-

Table 5
Stability of NSC 615985 in mouse, dog and human blood

Species	Concentration (μg ml^{-1})	NSC 615985 Remaining (%)[a]				
		Incubation Period				
		4°C		37°C		
		1 h	24 h	10 min	1 h	24 h
Mouse	0.1	98	50	<10	<10	<10
	10	107	77	94	71	8
	100	92	81	87	93	23
Dog	0.1	112	120	127	93	25
	10	95	98	88	88	70
	100	88	110	112	92	66
Human	0.1	98	86	86	86	79
	10	95	74	93	96	69
	100	113	76	93	103	67

[a] Mean of duplicate determinations with duplicate HPLC analyses each.

lite, which constituted 8–14% of the urinary radioactivity from both the IV- and SC-dosed mice, was identified as the carboxylic acid resulting from the hydrolysis of the isopropyl group from the parent compound. A metabolite which eluted at a similar retention time was also found in plasma extracts (Fig. 5). However, the level of this metabolite was too low for spectral identification. It is expected that the free carboxylic acid will not be active against HIV-1 as it was reported with a similar compound [5]. The hydrolysis of the carboxylic acid esters has been shown to occur with many other compounds and is catalyzed by carboxylesterases. These enzymes have been shown to be widely distributed in blood and tissues of mammalian species and are present in both soluble and membrane-bound forms [9].

Analysis of pooled plasma extracts indicated that at least eight metabolites were present. One metabolite, which constituted 60 and 16% of the radioactivity in the extracts for the IV- and SC-dosed animals, respectively, was tentatively iden-

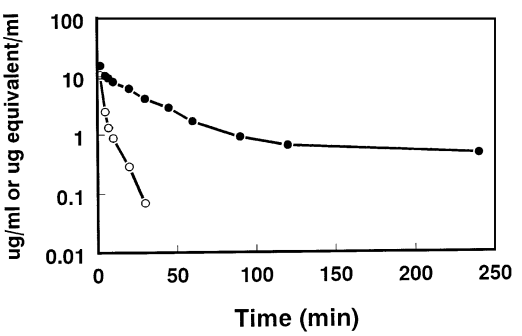

Fig. 3. Plasma concentration-time profiles of NSC 615985 (– ◦ –) and NSC 615985 equivalents (– • –) in male mice following intravenous administration of a 10 mg kg^{-1} dose. Each value is the mean of data from four mice.

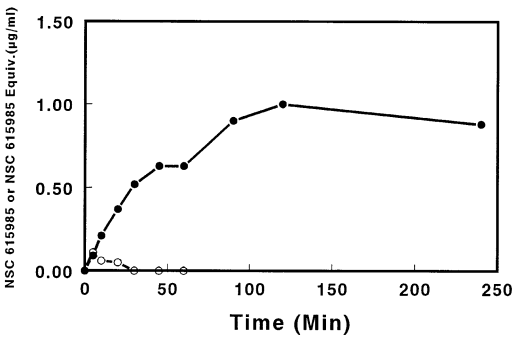

Fig. 4. Plasma concentration-time profiles of NSC 615985 (– ◦ –) and NSC 615985 equivalents (– • –) in male mice following subcutaneous administration of a 10 mg kg^{-1} dose. Each value is the mean of data from four mice.

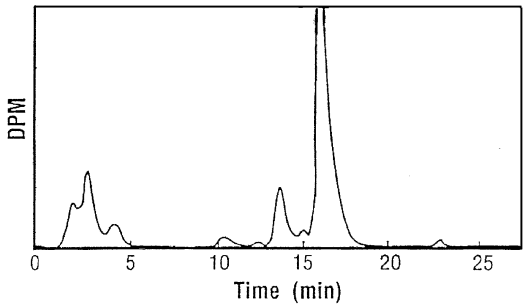

Fig. 5. Metabolite profile of NSC 615985 in mouse plasma (2–30 min IV) using the gradient HPLC elution system.

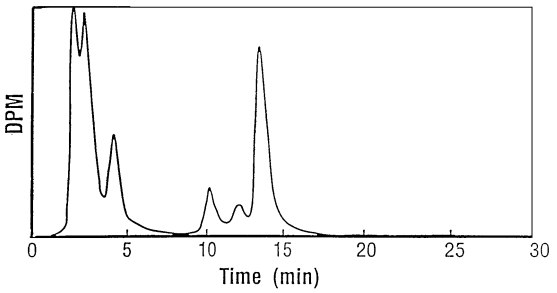

Fig. 7. Urinary metabolite profile of NSC 615985 in mice (0–90 min IV).

tified as the sulfoxide of the parent compound. The formation of sulfoxide is a common metabolic pathway of organic sulfide-containing xenobiotics [10]. This reaction has been shown to be mediated by both the cytochrome—P450 monooxygenase and the FAD—containing monooxygenase [10–12]. However, the tentative identification of the sulfoxide in plasma and its absence in urine suggest that the sulfoxide may be further metabolized to a more polar metabolite(s) prior to excretion in urine and/or secreted in bile and voided in feces. Studies with a similar compound, Vitavax (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin) have shown similar results indicating that vitavax sulfoxide was not excreted in urine of rats and rabbits treated with vitavax [13].

Although NNRTIs have been shown to be highly potent and specific against HIV-1 strains, the rapid development of mutant strains resistant for this class of compounds is becoming a major concern. For example, NSC 615985 is one of the most potent antiviral agents against wild-type HIV-1 (IIIβ) among the series of oxathiin carboxanilide derivatives evaluated. However, its potency against mutant viruses that contain the ILe-100, Lys-138, Ala-106 or Cys-181 mutation in their reverse transcriptase was at least three orders of magnitude lower than that observed for wild-type HIV-1 [4]. In addition, when an HIV-1 infected CEM cell culture was exposed to NSC 615985, a resistant strain of HIV-1 with a single amino acid change in reverse transcriptase (Glu →

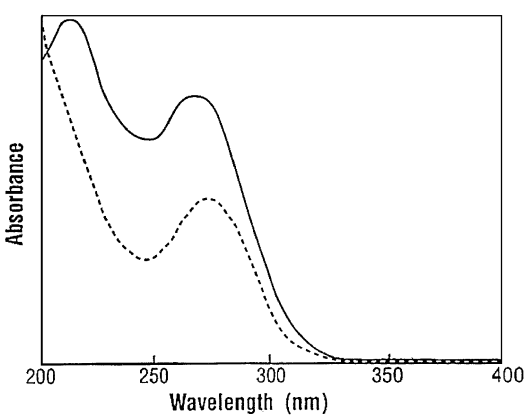

Fig. 6. UV spectrum of the plasma metabolite (2–30 min IV) eluted at 15.8 min (——) superimposed upon that of the authentic sulfoxide standard (- - -).

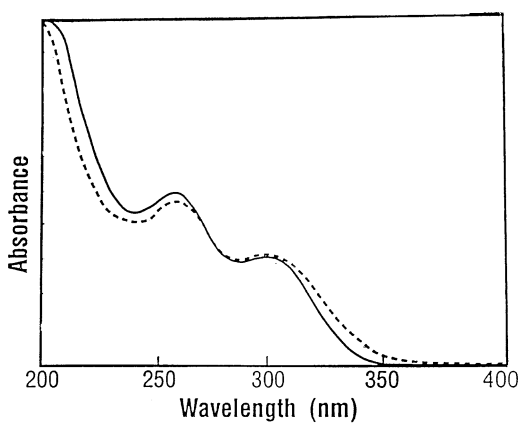

Fig. 8. UV spectrum of the urinary metabolite (0–90 min IV) eluted at 10.85 min (——) superimposed on that of authentic acid standard (- - -).

Lys in position 138) emerged [4]. Furthermore, results of the current study showed that NSC 615985 is rapidly cleared from blood following IV administration, and very poorly bioavailable following PO or SC administration. Such findings have resulted in the discontinuation of the development of NSC 615985 to focus the efforts on more promising candidates. Currently, a group of compounds belong to the general chemical class (thiocarboxanilide reverse transcriptase inhibitors) are being evaluated. Members of this class completely suppressed both HIV-1 replication and the emergence of resistant virus strains, have a better pharmacokinetic profile, and more active against mutant strains which are resistant to other HIV-1 specific reverse transcriptase inhibitors [5,14].

Abbreviations: NSC 615985, 2-chloro-5-(2-methyl-5,6-dihydr-1,4-oxathiin-3-yl carboxamido) isopropylbenzoate; 2-CNB, 2-chloronitrobenzene; 4-CNB, 4-chloronitrobenzene; NCI, National Cancer Institute.

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