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Development and validation of a sensitive solid-phase extraction and high-performance liquid chromatography assay for the novel antitumour agent CT2584 in human plasma

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Abstract

A HPLC assay and solid-phase extraction technique from human plasma has been developed and validated for the novel anticancer agent CT2584, 1-(11-dodecylamino-10-hydroxyundecyl)-3,7-dimethylxanthine, which has recently completed a phase I trial at the Christie Hospital, Manchester under the auspices of the CRC phase I/II committee. Following addition of CT2576, 1-(11-octylamino-10-hydroxylundecyl)-3,7-dimethylxanthine, as internal standard, a solid-phase extraction cartridge (100 mg cyanopropyl) was used to isolate the drug CT2584 from human plasma. Analysis was performed by reversed-phase chromatography. CT2576 was used as internal standard at a concentration of 4 $\mu g\ ml^{-1}$ for the quantification of CT2584 from plasma for the duration of this work. The lower limit of quantification for the drug CT2584 in buffer using this assay was found to be 0.0122 μM (0.008 $\mu g\ ml^{-1}$) and 0.048 μM (0.027 $\mu g\ ml^{-1}$) when extracted from human plasma.

Keywords: CT2584; 1-(11-Dodecylamino-10-hydroxyundecyl)-3; 7-Dimethylxanthine

1. Introduction

The development of anticancer agents has historically focused on inhibitors of DNA replication and cell division. However there are still many tumours that are either insensitive to or become resistant to these agents. Therefore discovery and development of new anticancer agents with unique pharmacological profiles is necessary to satisfy a major need in oncology.

CT2584, (Fig. 1A), is a novel cytotoxic agent developed for clinical use by Cell Therapeutics Inc (Seattle, WA, USA) and has recently completed clinical evaluation at the Christie Hospital, Manchester, UK, under the sponsorship of the Cancer Research Campaign Phase I/II Committee and in the USA. Although the mechanism of action of CT2584 is not fully understood, it is believed to exert it's antitumour activity by modulating the production of several phosphatidic acid species [1,2].

Phosphatidic acid is a signalling lipid which has been shown to be overexpressed in tumour cells. Cancerous and normal cells appear to differ in their lipid metabolism. Tumour cells take up unsaturated

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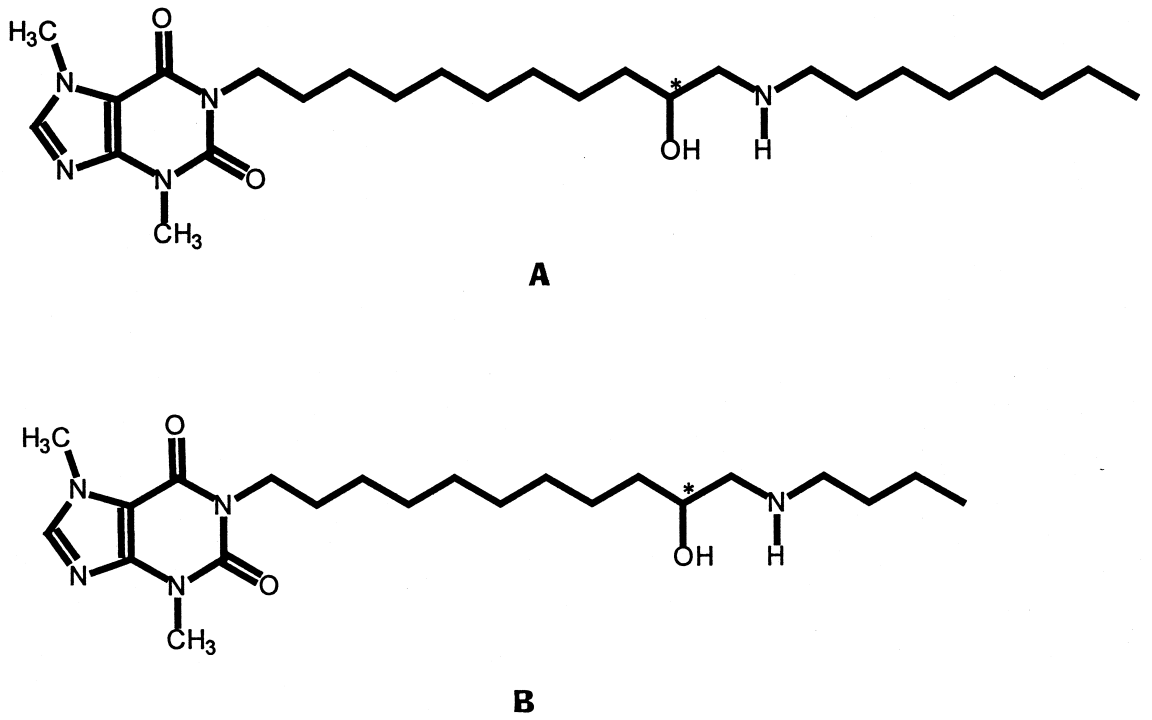

Fig. 1. (A) The structure of CT2584 1-(11-dodecylamino-10-hydroxyundecyl) 3,7-dimethylxanthine hydrogen methanesulphonate. (B) The structure of CT2576 1-(11-octylamino-10-hydroxyundecyl) 3,7-dimethylxanthine hydrogen methanesulphonate.

fatty acids and convert these to phosphatidic acid, which may give rise to the abnormally high membrane fluidity seen in some cancers [3–6]. Tumour cells also constitutively produce unsaturated phosphatidic acids, whereas normal cells do so only in response to certain receptor–ligand interactions.

Modulation of specific phosphatidic acids generated following receptor binding selectivity inhibits cellular responses to certain ligands.

Unsaturated phosphatidic acids are present at very low levels in normal resting cells. Therefore it may be proposed that modulators of phosphatidic acid generation by agents such as CT2584 would exert relatively few effects on quiescent cells, but could show selective effects against cycling tumour cells.

In addition to its direct cytotoxic activity, CT2584 exerts anti-angiogenic, anti-invasive and anti-adhesive effects at sub-cytotoxic concentrations. These properties may contribute to a broader spectrum of anti-tumour activity than that of a purely cytotoxic compound.

The objective of the phase I clinical trial in the UK was to determine the pharmacokinetic profile of CT2584. In order to do this a solid-phase extraction and high-performance liquid chromatography (HPLC) method has been developed and validated. Citrated blood samples were taken at various time points both during, and up to 72 h following the end of infusion.

Several analytical methods are available in the literature relating to the analysis of methyl xanthines and related compounds. The majority of methods use solvent [7,8] or solid-phase [9–11] extractions and occasionally acid precipitation [12] for sample preparation prior to chromatographic separation.

2. Experimental

2.1. Chemicals and reagents

CT2584 and CT2576 (Fig. 1B) standard material came from CTI (Seattle, WA, USA) and a 20 mg ml^{-1} solution of CT2584-HMS (formulation

inactive ingredients Polyoxyl 35 castor oil, Cremophore-EL, methanesulphonic acid, sodium hydroxide, sterile water), was manufactured by (Sanofi Winthrop, Kansas, USA) was used to develop the HPLC assay. HPLC grade acetonitrile and methanol were obtained from Sigma, (Poole, UK). Human plasma was obtained from the blood bank at Christie Hospital. Double distilled water was used throughout the study and was prepared *in house* in all glass distillation apparatus from alkaline permanganate.

Chloroacetic acid was obtained from Aldrich Ltd, (Poole, UK), and 10 *M* sodium hydroxide was obtained from BDH (Poole, UK). Cyanopropyl solid-phase extraction cartridges (100 mg) were obtained from Anachem (Luton, UK). The internal standard (CT2576, dissolved in 100% chloroacetate buffer pH3, 0.05*M*) was obtained from CTI.

2.2. Chromatographic system

HPLC analysis of CT2584 was carried out using a Gilson 306 solvent delivery system, a Gilson 117 UV Vis detector and an ASPEC XL autoinjector. The system was controlled using Gilson 715 controller software. This included data capture and processing. A wavelength of 273 nm was used for the duration of the work.

A 5 μm spherisorb cyanopropyl column (150 mm×4.6 mm I.D., Anachem, Luton, UK) was used, under isocratic conditions of 50% acetonitrile and 50% chloroacetate buffer (0.05 *M*, pH 3.0), to allow separation between the CT2584, the internal standard CT2576 and any remaining plasma components. The ratio of the two peak areas were used to quantitate the CT2584 extracted from human plasma. This was compared with quantitation using standard calibration curves constructed following extraction of CT2584 from human plasma over the concentration range 6.25–0.0122 μ*M*.

2.3. Blood collection

Several 4.5 ml samples of blood were collected from a peripheral indwelling venous canula (Becton Dickinson) following informed consent. Each sample was centrifuged at 2000 *g* for 20 min and the plasma stored at −30°C prior to analysis. Patients all had advanced refractory tumours and received drug as part of a phase I trial of CT2584 administered as a 6 h *i.v.* infusion *via* a central venous catheter.

This trial was carried out under the auspices of the Cancer Research Campaign Phase I/II Committee, and had approval from the South Manchester Ethics Committee.

2.4. Sample preparation

A solid-phase extraction technique for CT2584 from human plasma was developed. Several solid-phase extraction cartridges were evaluated including phenyl, cyanopropyl C18, C8 and C2. Reproducible extraction was best obtained using a 100 mg cyanopropyl cartridge under the following conditions at room temperature: (1) Condition the cartridge with 1 ml of acetonitrile. (2) Condition the cartridge with 1 ml of water. (3) Load 0.5 ml of plasma spiked with 100 μl of the internal standard CT2576 (4 μg ml^{-1}) onto the extraction cartridge. (4) Wash with 1 ml of water. (5) Elute the retained drug and internal standard with 1 ml of (10% 0.1 *M* chloroacetate buffer [pH3], and 90% acetonitrile). (6) Centrifuge samples in a microcentrifuge (13 000 *g*) for 10 min.

The samples were injected into a 200 μl sample injection loop and chromatographed at a flow of 1 ml min^{-1}, as described above.

3. Results

Standard curves prepared from formulated CT 2584 were found to be linear over the concentration range 0.0122 μ*M*–25 μ*M* (0.008–16 μg ml^{-1} +/− 0.016–12%). A correlation coefficient of 0.9998 and slope and intercept values of 1×10^{6} and −6880.8 were calculated. The lower limit of quantification for the drug CT2584 in buffer using this assay was found to be 0.0122μ*M* (0.008 μg ml^{-1} +/− 0.016%) (Fig. 2a).

Following extraction of CT2584 from human plasma over the concentration range 6.25 μ*M*–0.0122 μ*M* (3.94–0.008 μg ml^{-1} +/−0.02–12%), the lower limit of quantification for CT2584 was found to be 0.048 μ*M* (0.027 μg ml^{-1})(+/−5.04–12.0%) (Fig. 2c, peak 2) a value confirmed in a four day validation study. The limit of detection of CT2584 in plasma was found to be 0.024 μ*M* (Fig.

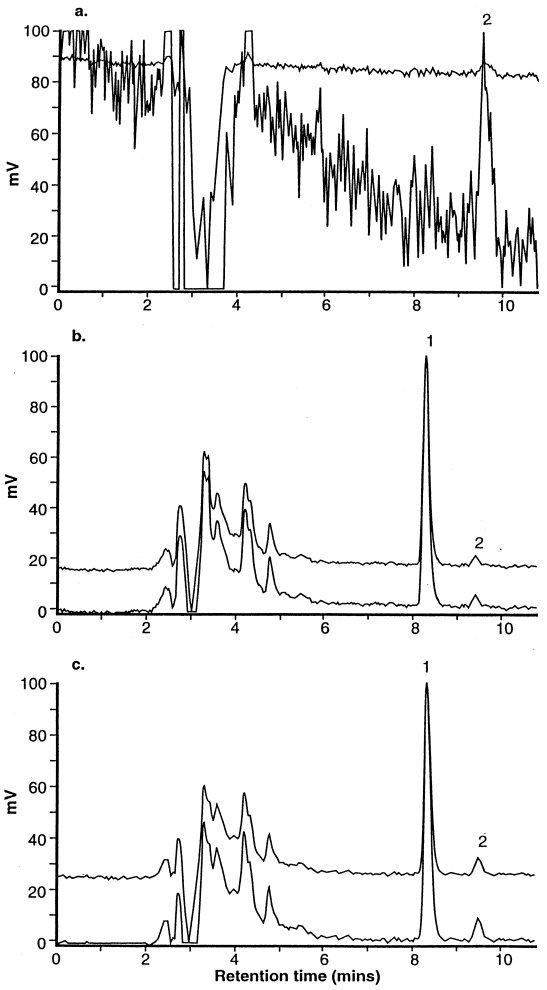

Fig. 2. (a) Chromatogram to show the lower limit of quantification for CT2584 in buffer 0.0122 μ*M*. (b) Chromatogram to show the limit of detection for CT2584 from plasma 0.022 μ*M*. (c) Chromatogram to show the lower limit of quantification for CT2584 from plasma 0.044 μ*M*.

2b, peak 2). Linear regression analysis was performed on the peak area ratio *vs* concentration data obtained from each day of the validation study and is shown in Table 1.

The inter- and intra-day precision and accuracy data for low, medium and high quality controls of CT2584 and also the five day validation study can be seen in Tables 2a and 2b.

Percentage extraction recoveries obtained over the concentration range 6.25–0.048 μ*M* and their respective inter- and intra- day precisions can be seen in Table 3.

Fig. 3 shows a typical chromatogram obtained following solid-phase extraction of patient plasma samples. The concentration of CT2584 in this instance was calculated to be 0.42 μg ml^{-1}. It can be seen that CT2584 elutes with a retention time of 9.7 min and is well separated from the internal standard CT2576, which elutes with a retention time of 8.4 min.

4. Discussion

CT2584 possesses a long substituted hydrocarbon side chain coupled to a methyl xanthine ring. The hydrocarbon side chain would confer lipophilic characteristics making this drug insoluble in water. This is consistent with the data shown in this study.

In order to evaluate the pharmacokinetic profile of this agent we have developed a sensitive, accurate and reproducible solid-phase extraction (SPE) method for the extraction and quantitation of CT2584 from human plasma followed by analysis using high-performance liquid chromatography. The method has been further improved through the use of an auto-

Table 1
Calibration data for CT2584 extracted from human plasma

Validation day	Slope	Intercept	Correlation coeff.
1	1×10^6	−103494	0.9909
2	1×10^6	−103964	0.9998
3	953454	−108339	0.9919
4	966754	−44420	0.9998

Table 2

	Concentration (μ*M*)							
	6.25	3.12	1.56	0.78	0.39	0.195	0.098	0.048
Inter-day precision and accuracy data for CT2584 isolated from human plasma								
Day 1	6.56	2.69	1.26	0.62	0.33	0.198	0.086	0.0578
Day 2	6.56	2.64	1.25	0.603	0.33	0.22	0.0933	0.047
Day 3	6.15	2.68	1.19	0.553	0.242	0.158	0..084	0.049
Day 4	6012	3.05	1.46	0.68	0.268	0.154	0.076	0.052
mean	6.35	2.77	1.29	0.61	0.293	0.183	0.085	0.052
S.D.	0.25	0.19	0.12	0.052	0.045	0.032	0.007	0.0047
%R.S.D.	3.87	6.92	9.11	8.50	15.24	17.97	8.39	9.15
%Accuracy	101.6	88.78	82.69	78.21	74.36	92.31	86.48	104

Intra-day precision and accuracy data for low, medium and high CT2584 quality controls isolated from human plasma

Std	Mean cone (μ*M*)	S.D.	%R.S.D.	% Accuracy
6.5	6.56	0.033	0.51	100.92
0.39	0.33	0.014	4.30	84.62
0.048	0.048	0.005	8.70	97.92

mated extraction system (ASPEC). Quantitation of extraction efficiencies of CT2584 from plasma was achieved using the addition of a 100 μl of 4 μg/ml of CT2576 (internal standard) prior to extraction of plasma.

The SPE technique developed was used to isolate and quantitate CT2584 from the plasma of patients taking part in a phase I clinical trial of CT2584 as a 6 h *i.v.* infusion.

A full pharmacokinetic study of this Phase I trial using the methods described in this study will be presented and discussed in a manuscript to be published at a later date.

Each plasma sample was extracted with a 100 mg SPE cartridge using 0.5 ml of plasma, although system is robust enough to allow lesser amounts of sample to be used. An elution system comprising of 1ml of 90% acetonitrile/10% chloroacetate buffer (0.1 *M*, pH 3) was used, but for additional certainty each cartridge was eluted with a further 1ml of acetonitrile, followed by 1 ml of a 70% acetonitrile/30%chloroacetate 0.1 *M* pH 3 buffer. Each of these eluent's were chromatographed, and yielded no additional elution of CT2584 or CT2576.

From the preliminary pharmacokinetic parameters a fairly rapid α decay of 0.24 h followed by a slow β decay of 6.71 h and a large volume of distribution of 1414 l would be consistent with the very lipophilic nature of this drug. CT2584 is a basic drug and its ionisation is supressed in a basic environment such

Table 3
Percentage recovery data for CT2584 extracted from human plasma over a four day validation study

Day	CT2584	Concentrations (μ*M*)						
	0.048	0.098	0.195	0.39	0.78	1.56	3.12	6.25
1	103.00	92.75	104.95	89.94	84.39	82.27	85.85	97.05
2	83.19	100.08	117.19	89.81	81.91	82.27	84.51	97.05
3	86.69	90.62	83.84	65.66	75.31	76.85	85.65	90.90
4	93.07	81.17	81.69	72.91	93.19	94.85	97.50	90.47
Mean	91.49	91.16	96.92	76.22	83.7	84.06	88.38	93.87
S.D.	8.3696867	6.748817	14.81885	10.71529	6.405708	6.610908	5.291623	3.186129
%R.S.D.	9.505811	7.403266	15.28977	14.05837	7.653176	7.864511	5.987353	3.394193
N	4	4	4	4	4	4	4	4

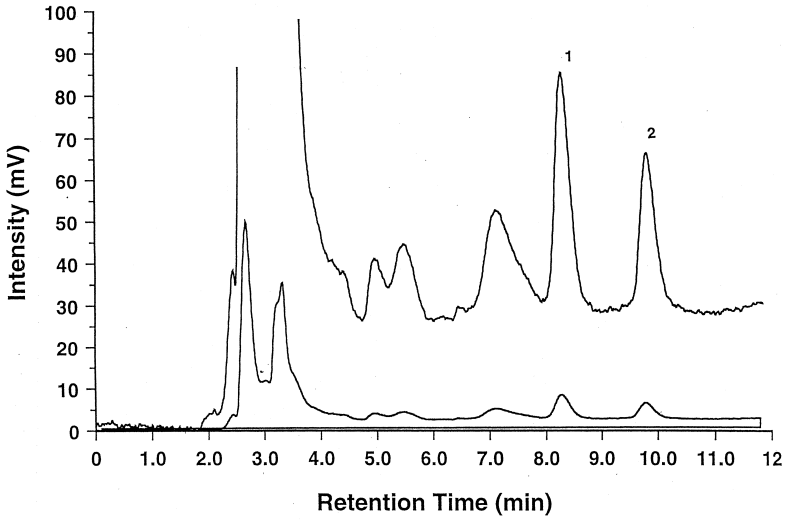

Fig. 3. Chromatogram of CT2584 (peak 2) and CT2576 (peak 1) (internal standard 100 μl of 4 μg ml^{-1}) extracted from human plasma using a solid-phase extraction technique and chromatographed on a reversed-phase chromatography system.

as plasma pH 7.4, this will enable its diffusion into tissues, where at lower pH, the extent of ionisation is increased, this would result in accumulation in the tissues.

5. Conclusion

A sensitive, accurate and reproducible solid-phase extraction and HPLC assay has been developed for the novel anti tumour agent CT2584 which allows the isolation from plasma and measurement of CT2584 down to 27 ng ml^{-1}.

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References

[1] E. Eiseman, S.M. Fujihara, D.C. Herrera, T. Le, G.E. Underiner, J.W. Singer, R E. Finney, J. Am. Soc. of Haem., 36th Annual Meeting, Dec. 2–6 1994, Nashville, Tennessee. (Abstract)
[2] G.C. Rice, C. Stringer, T. Le, M.J. Johnson, C. Brown, N. Jenkins, J.W. Singer. 86th meeting of the American Association for Cancer Research Toronto, Ontario, 18–22 March 1995 (Abstract)
[3] G. Deliconstantionos, Anticancer Res. 7 (1987) 1011.
[4] A. Raz, A.A. Ben-Ze'ev, Science 221 (1983) 1307.
[5] A. Raz, A.A. Ben-Ze'ev, Cancer Metastasis Rev. 6 (1987) 3.
[6] W.H. Moolenar, W. Kruijer, B.C. Tilly, I. Verlaan, A.J. Bierman, S.W. de Laat, Nature 323 (1986) 171.
[7] P.J. McNamara, D. Burgio, S.D. Yoo, Drug Metab. Dispos. 20 (1992) 302.
[8] W.E. lambert, M.A. Yousaf, B.M. Van liedekerke, J.E. Deroose, A.P. Deleenheer, Clin.Chem. 351 (1989) 298.
[9] Y. Hieda, S. Kashimura, K. Hara, M. Kageura, J. Chromatogr. B 667 (1995) 241.
[10] I.N. Papadoyannis, V.F. Samanidou, K.A. Georga, J.Liq.Chromatogr. Rel. Technol. 19 (1996) 2559.
[11] A. Mancinelli, S. Pace, A. marzo, E. Arrigoni Martelli, J. Chromatogr. 575 (1992) 101.
[12] D.T. Holland, K.A. Godfredsen, T. Page, J.D. Connor, J. Chromatogr. B 707 (1998) 105.