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Short communication

Application of a liquid chromatography–tandem mass spectrometry (LC/MS/MS) method to the pharmacokinetics of ON01910 in brain tumor-bearing mice

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ABSTRACT

ON01910 is a small molecular weight benzyl styryl sulfone currently under investigation as a novel anticancer agent. The purpose of the investigation was to develop a sensitive and reproducible liquid chromatography–tandem mass spectrometry (LC/MS/MS) method to quantitate levels of ON01910 in small amounts of five biological matrices; mouse plasma, feces, urine, normal brain and brain tumor. For all matrices, protein precipitation sample preparation was used that led to linear calibration curves with coefficients of determination greater than 0.99. The lower limit of quantitation (LLOQ) for all matrices was 5 ng/ml except that for mouse urine which was 10 ng/ml. The calibration standard curves were reproducible for all matrices with inter- and intra-day variability in precision and accuracy being less than 15% at all quality control concentrations except for the LLOQ in mouse plasma for which the accuracy was within 17%. The assay was successfully applied to characterize the systemic pharmacokinetics of ON01910 as well as its disposition in brain and brain tumor in mice. ON01910 exhibited a clearance of 3.61 ± 0.85 l/h/kg and a half life of 8.66 ± 3.30 h at 50 mg/kg dose given I.V.

1. Introduction

ON01910 (also referred to as ON 01910.Na and Estybon™) is a synthetic low molecular-weight anticancer agent (473.47 Da) that belongs to the class of benzyl styryl sulfones (Fig. 1). ON01910 causes a G2/M phase cell cycle arrest leading to mitotic inhibition [1]. ON01910 has exhibited both antitumor activity [1] and antiangiogenic activity [6] with a low toxicity profile in various preclinical tumor xenograft models, and is currently in Phase II clinical trials as a single agent and in combination with conventional chemotherapy in advanced and metastatic tumors.

Analysis of ON01910 in animal and human plasma using LC/MS/MS has been reported [2,3] based on sample sizes of 0.1 ml although for our studies, a sample size of 10 μl was the limit and a key consideration in the development of the assay. In addition, the LC/MS/MS method had to be applicable to normal brain and brain tumor, the latter being the intended site of action, which had not been previously investigated.

2. Materials and methods

2.1. Chemicals and standards

ON01910 and ON012380, the internal standard (Fig. 2), were synthesized in-house. Details on other chemicals, solvents and preparation of stock solutions, standards and quality control (QC) samples are presented in Supplementary information.

2.2. Chromatographic and mass-spectroscopic conditions

Method development and validation were performed with an LC/MS/MS (Agilent 1100 series HPLC, AB SCIEX API 4000 tandem mass spectrometer, Foster City, CA) using an ESI interface and operated in positive ion mode. Instrument control, data acquisition and processing for both chromatography and mass spectrometry were performed using the Analyst Software v 1.4.2 (AB SCIEX). Separation of the analyte was achieved at 40 °C using Luna 3 μm C18 column (50 mm × 2.0 mm i.d., Phenomenex, Inc., Torrance, CA, USA) protected by a C18 guard column. The mobile phase

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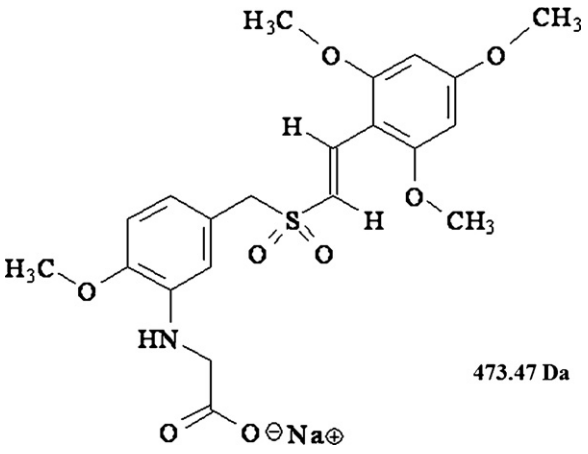

Fig. 1. Structure of ON01910.

used for the chromatographic separation was composed of acetonitrile:ammonium formate (10 mM) (30:70, v/v) that was delivered isocratically at a flow rate of 0.3 ml/min, and enabled a run time of 3 min.

Drug quantitation was performed by ESI-SRM using ion transitions of m/z 469.00 → 284.00 for ON01910 and 466.00 → 208.00 for the internal standard. The optimal declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) for ON01910 were 41 V, 5 V, 18 V and 9 V, respectively. The final DP, EP, CE and CXP of the internal standard used were 79 V, 5 V, 27 V and 6 V, respectively. Nitrogen was used as the nebulizer, curtain gas and collision gas.

2.3. Sample preparation

A protein precipitation sample preparation method was used for the analysis of ON01910 in mouse plasma, urine, feces, normal brain and brain tumor matrices. To aliquots of plasma (10 μl), urine (50 μl), feces (30 μl of a 5% homogenate), normal brain (20 μl of a 10% homogenate) and brain tumor (50 μl of a 10% homogenate), three times the volume of IS (internal standard) working stock solution (500 ng/ml of IS in methanol) was added and vortexed for 1 min followed by centrifugation for 5 min (plasma) or 15 min (rest of the matrices) at 15,000 rpm with 5 μl of the resultant supernatant injected into the LC/MS/MS.

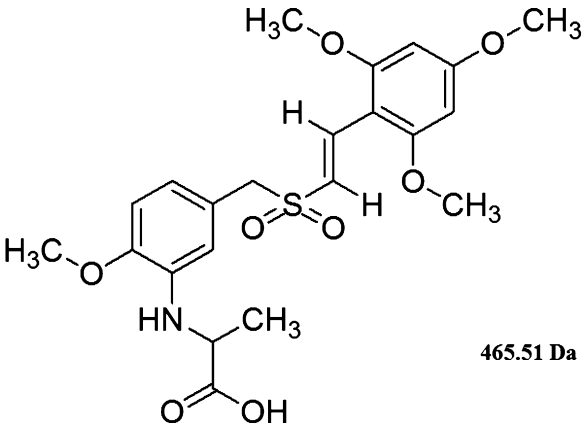

Fig. 2. Structure of internal standard, ON012380.

2.4. Method validation

Validation of the method in different matrices involved determining intra-day and inter-day variability as measured by linearity of the standard curve and accuracy and precision. Linearity of the method in each biological matrix was determined in five sets of calibration standards whereby a correlation coefficient (R^2) of ≥0.99 was considered satisfactory. Intra-day and inter-day variability in accuracy and precision was determined by analyzing replicates of QC samples prepared over a high to low concentration range either on the same day or separate days, respectively. Precision was expressed as the relative standard deviation of the determined concentrations while accuracy was expressed as percent bias [% Bias = ((mean of the measured concentration − added concentration)/added concentration) × 100]. Extraction efficiency or percentage recovery [(peak $\text{area}_{\text{sample}}$/peak $\text{area}_{\text{recovery control}}$) × 100] of the protein precipitation method was determined at multiple concentrations in triplicates by comparing the peak area of extracted QC samples to that of control samples prepared from methanolic supernatants spiked with analyte and IS.

2.5. ON01910 treatment and sampling

All animal studies were approved by the Institutional Animal Care and Use Committee. For systemic pharmacokinetic (PK) evaluation of ON01910, a single dose of 50 mg/kg ON01910 was given to healthy nude mice as a 10 min tail vein infusion followed by the collection of serial blood samples (20 μl) from a carotid artery cannula over a period of 24 h. A second steady-state pharmacokinetic study was conducted in mice bearing intracerebral tumors for the determination of brain and brain tumor disposition of ON01910. Detailed methodology on the systemic and brain and brain tumor pharmacokinetic studies is provided in Supplementary information.

3. Results and discussion

3.1. Chromatographic and mass spectrometric conditions

The isocratic mobile phase condition produced peaks for ON01910 and the internal standard with a total run time well within 3 min. Representative chromatograms of extracted blanks and ON01910 pharmacokinetic study samples in mouse plasma, urine, feces, brain and brain tumor are shown in Supplementary Fig. A.

The MS was operated in positive ion scan mode with ESI-SRM ion transitions of the protonated molecular ions (M+) at m/z of 469.00 and 466.00 for ON01910 and the internal standard, respectively. The molecular ion at 469 represents an ammonium adduct of the free base form of ON01910 and showed greater intensity compared to the molecular ion of the free base form (m/z = 452). The ammonium adduct disappeared in the absence of the ammonium formate mobile phase thus confirming the source of the ammonium ion. Supplementary Fig. B shows the MS/MS spectra of ON01910 and the internal standard, ON012380. The daughter ions, 284 and 208 from the precursor ions 469 (ON01910) and 466 (IS), respectively had the highest intensity among the possible daughter ions (see Supplementary Fig. B(a) and (b)).

3.2. Method validation

3.2.1. Calibration curve and lower limit of quantitation (LLOQ)

For all matrices, linear calibration curves were determined from the best-fit of the peak-area ratios (peak $\text{area}_{\text{analyte}}$/peak area_{IS}) vs. concentration using a weighing factor ($1/X^2$). The correlation coefficients were greater than 0.995 in all the matrices and the LLOQ

Table 1
Inter-day ($n = 5$) and intra-day ($n = 6$) assay accuracy and precision in determination of ON01910 in mouse plasma, urine, feces, normal brain and tumor by LC/MS/MS.

Biological matrix	Nominal concentration (ng/ml)	Inter-day validation			Intra-day validation		
		Mean observed concentration (ng/ml)	Precision (RSD)	Accuracy (% Bias)	Mean observed concentration (ng/ml)	Precision (RSD)	Accuracy (% Bias)
Plasma	10,000	10428.67	6.60	4.29	10333.33	2.00	3.33
	200	206.90	1.69	3.45	204.50	2.67	2.25
	10	10.15	4.79	1.47	9.86	8.24	−1.40
	5	4.71	9.44	−5.77	4.18	4.07	−16.50
Urine	10,000	10660.00	1.54	6.60	10633.33	1.65	6.33
	500	470.93	2.87	−5.81	475.33	1.50	−4.93
	10	10.85	5.40	8.46	11.40	1.36	14.00
Feces	10,000	9743.00	4.35	−2.57	9578.33	3.22	−4.22
	500	496.23	3.73	−0.75	508.50	6.25	1.70
	10	9.65	4.54	−3.47	10.21	6.35	2.10
	5	4.93	7.17	−1.46	4.91	4.32	−1.77
Normal brain	1000	1007.00	4.86	0.70	1021.33	1.84	2.13
	200	197.23	3.04	−1.38	193.50	8.60	−3.25
	10	9.94	3.25	−0.65	10.17	14.35	1.70
	5	5.06	7.10	1.19	5.37	4.40	7.47
Tumor	10,000	10336.67	2.41	3.37	10600.00	4.74	6.00
	200	183.10	5.15	−8.45	179.50	4.33	−10.25
	10	9.51	4.74	−4.87	9.31	4.19	−6.93
	5	4.82	6.65	−3.57	4.54	5.91	−9.17

was 5 ng/ml in all matrices except in urine in which the LLOQ was 10 ng/ml. Linearity was observed in the concentration ranges of 5 ng/ml–10 μg/ml in plasma, feces and tumor, 10 ng/ml–10 μg/ml in urine and 5 ng/ml–1 μg/ml in normal brain. Carry-over effects were not observed for the present method and an injector wash step was included after each sample injection in the method. The absence of carry-over effects was further confirmed by the lack of any differences in responses in standards and QCs when the injection order was from low to high or from high to low concentrations.

3.2.2. Accuracy and precision

The results of inter-day and intra-day accuracy and precision in various biological matrices are summarized in Table 1. The intra-day and inter-day accuracy and precision for plasma, urine, feces, normal brain and tumor matrices were well within 15% at all QC concentrations except for intra-day accuracy of plasma LLOQ which was within 17% and these values were considered acceptable [4].

3.2.3. Extraction efficiency

The relative recovery of ON01910 from various biological matrices is summarized in Supplementary Table A. The extraction efficiency at different QC concentrations from plasma, urine, feces, normal brain and brain tumor ranged from 23 to 27%, 96 to 106%, 105 to 115%, 85 to 90% and 90 to 95%, respectively. Although the extraction efficiency from mouse plasma was low and attributed to high plasma protein binding (93–97%, data not shown) and a pronounced matrix effect, the method was very sensitive with a LLOQ of 5 ng/ml and was sufficient for PK studies of ON01910 in mouse that facilitated serial blood sampling with a minimal plasma sample volume of 10 μl. In addition, the method validation results demonstrated that the method was robust and consistent with good accuracy and reproducibility which indicated no compromise caused by the relatively low recovery. The higher recoveries of ON01910 from brain and tumor tissues may be due to the dilution of proteins in the 10% homogenate.

3.3. Application to pharmacokinetic study

The LC/MS/MS method was successfully applied to study the pharmacokinetics of ON01910 in healthy nude mice receiving 50 mg/kg intravenous dose of ON01910 (see Supplementary Fig. C and Table B). A serial blood sampling study design was employed to determine the systemic PK properties of ON01910 that allowed for repetitive collection of small blood volumes (~20 μl/sample) and enables a reduction in inter-animal variability and the number of animals required to complete the PK assessment. The percentage of unchanged ON01910 excreted in urine and feces was found to be 9.8 ± 2.7 and 70.4 ± 14.7, respectively indicating biliary clearance to be the major route of elimination of ON01910 with a half-life of 8.66 h.

In brain tumor-bearing mice under steady-state conditions, the steady-state brain and brain tumor concentrations in a representative animal were found to be 0.22 μg/ml and 0.92 μg/ml, respectively, which yielded steady-state brain to plasma and brain tumor to plasma ratios of 0.029 and 0.121, respectively. Although ON01910 distribution into brain tumor is greater than in normal brain due to the compromised BBB [5], its distribution is low and suggests it may be a poor candidate for brain tumor chemotherapy.

4. Conclusions

In conclusion, we have developed a sensitive, specific and robust method for the quantitation of ON01910 in mouse plasma, urine, feces, brain and brain tumor that allowed the use of small plasma volumes of 10 μl and a serial sampling design that minimizes the number of animals and inter-animal variability. In addition, the method was successfully applied to brain and brain tumor samples that were used to assess the ability of ON01910 to penetrate the BBB, which under the conditions of this investigation were low and potentially a limiting factor in its development for brain tumor chemotherapy.

Conflict of interest

Dr. E.P. Reddy is the scientific founder, stock holder, board member and paid consultant of Onconova Therapeutics Inc. He is also one of the inventors of the patents that describe the compounds described here.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.08.003.

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