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Overcoming extractability hurdles of a ^{14}C labeled taxane analogue milataxel and its metabolite from xenograft mouse tumor and brain tissues

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ABSTRACT

Taxane analogue milataxel have been shown to bound to proteins/tissues irreversibly. Extraction of the aforementioned bound drug and metabolite was proven to be difficult task. Nonetheless, an extraction method had to be developed to accurately determine drug concentration in tissues over time. This method would enable Taxolog, Inc. (Fairfield, NJ, USA) to accurately map the fate of drug in mice and it would also enable to better design drug dosing scheme for its maximum efficiency. A productive extraction technique for milataxel (MAC-321, TL-139) in nude mice with various xenograft human tumors was developed by extracting analytes from tumors using a novel extraction procedure and analyzing samples by LC–MS. This extraction technique entails disrupting tissue cells with hexane followed by acidic methanol (MeOH), with the aid of a tissuemizer and sonic cell disrupter. An average extractability of 75% was achieved as confirmed by the recovery of ^{14}C labeled milataxel, as compared to 4–48.5% extraction efficiency using solvents and/or combination of solvents such as acetonitrile (ACN), ethanol, ethyl acetate, MeOH/acetic acid in water, and chloroform/MeOH. This extraction technique allowed for quantitation of milataxel and its major metabolite s-lactate (M-10) from tumors and brain tissue samples using HPLC coupled with electro-spray ionization mass spectrometry (HPLC-ESI-MS). Ratios of M-10 metabolite to milataxel were determined to be approximately 3:1 and 2:1 in SKMES human lung carcinoma tumors and A-375 melanoma tumors, respectively, and declined in concentration over 20 days. However, levels of milataxel and M-10 were determined to be equal at 8 h in HCT-15 human colon carcinoma tumors with M-10 levels dropping sharply over a 10-day period.

1. Introduction

Taxol®, which occurs naturally in the pacific yew tree and was first isolated by Monroe Wall and his colleague Mansukh Wani, was found to have efficacy against cancerous cells [1]. Taxol is a member of the taxane family of diterpenes, which are found in nature, but only in a limited supply. In late 1989, Dr. Robert Holton of Florida State University developed an efficient synthesis technique using metal alkoxide catalysis to that provided suitable yields for manufacturing [1]. Since the advent of Taxol, analogues of this compound have also been synthesized such as Taxotere®, therefore an effective means of extracting and analyzing taxanes in biological samples is desirable.

The analysis of paclitaxel analogues in biological tissue samples utilizing mass spectrometry was demonstrated by the work by Bradley et al. who studied paclitaxel with a fatty acid chain attached (docosahexanoic acid). The fatty acid chain led to an increased uptake by cancerous cells and reduced toxicity [2]. These authors used LC–MS–MS to determine levels of paclitaxel and dihydroxy aceto (DHA)-paclitaxel in plasma and tumors; however, the method of extraction of analytes from tumors was not disclosed in this article. Poon et al. examined paclitaxel and paclitaxel metabolites obtained by incubation with human liver microsomes utilizing HPLC and a VG Quattro triple quadropole MS (ESI) [3].

Non-radiolabeled extractions of taxanes from tumor tissues have been reported. A method to isolate and quantitate docetaxel in the 10–20 μg range from human plasma was developed by Loos et al. using a liquid/liquid extraction technique employing ACN/butyl chloride in a ratio of 1:4 [4]. Ciccolini et al. also developed a simple extraction technique to isolate docetaxel using diethyl ether with an extraction efficiency of 95% [5]. A solid phase extraction technique was also developed for docetaxel and its metabolites from human plasma using a cyano end-capped column and analyzing by HPLC by Rosing et al. [6]. An SPE technique was also developed by Sottanil to isolate and quantitate paclitaxel from human plasma by mass spectrometry [7].

In contrast there are limited literature references to ^{14}C labeled taxane extractability studies. Research by de Valeriola et al. demon-

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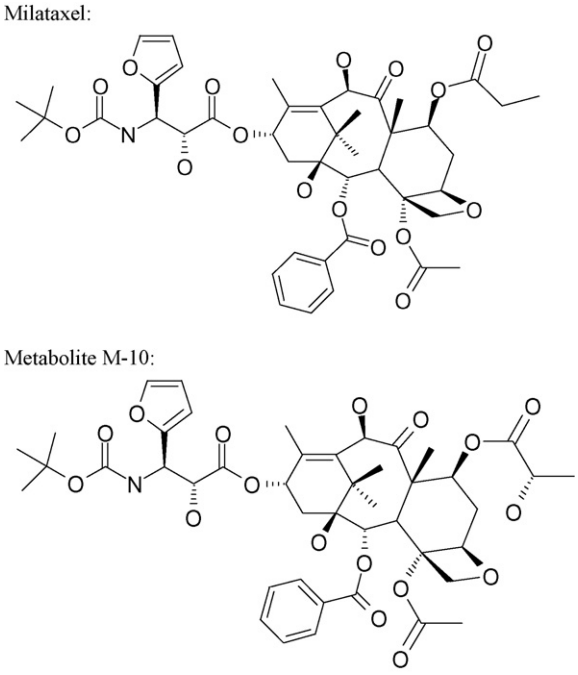

Fig. 1. Structures of milataxel (TL-139) and metabolite M-10.

strated excretion balance pharmacokinetics and metabolism of docetaxel and related compounds in 3 advanced refractory cancer patients using ^{14}C labeled docetaxel [8]. A single 100 mg/m^2 dose of ^{14}C labeled docetaxel (30 μci/m^2) was infused intravenously over a 1 h period. These authors characterized docetaxel plasma protein binding and determined levels of the parent and related compounds in plasma, plasma ultrafiltrate (PULF), blood, feces, urine, saliva, and breath. Docetaxel concentrations were determined in plasma and PULF using HPLC coupled to β-detector. Docetaxel levels were extracted using C2 bond elute SPE cartridges and concentrations were determined by HPLC. They determined docetaxel metabolites appear in plasma 8 h after infusion. Docetaxel was shown to rapidly bind to plasma protein after infusion. The HPLC results from plasma and PULF showed more than 90% of the drug to be protein bound in the first 8 h of post infusion.

Milataxel is a new taxane in the diterpene family of compounds, which is being developed by Taxolog, Inc. (Fairfield, NJ, USA). Currently, milataxel is in phase-II clinical trials in the U.S. The molecular structures of milataxel and its major metabolite M-10 are represented in Fig. 1. The objective of the experiments conducted at Taxolog, Inc. was to develop a suitable extraction technique that would enable detection, identification, and quantification of free or protein bound milataxel and metabolites present in xenograft mouse tumors. The information from these extraction experiments would provide a technique to study the pharmacokinetic and pharmacodynamic (PK/PD) characteristics of the parent and metabolites in tumor tissues. Considering the low extraction yields (4–48.5%) using known extraction techniques from tumor tissue containing milataxel compounds, a desired procedure would extract a minimum of 50% of milataxel and related compounds. As the conventional extraction approach using common solvents or their mixtures did not yield acceptable recoveries, the need for an unconventional procedure was required. All evidence pointed at protein binding and it was hypothesized that the fatty tumor tissues should be completely solubilized in a unique solution without compromising the integrity of the compounds. This was sought to free "protein-bound" parent and metabolite and expose them to a secondary extraction solvent such as acidic MeOH. The combination of acidic MeOH had experimentally shown to extract out approximately an order of magnitude higher ^{14}C milataxel and related residues when compared to other solvents or their combinations (Table 1). HPLC-ESI-MS was used to maximize detection for the quantitation of these components.

2. Experimental

2.1. Chemicals and reagents

Milataxel analytical reference standard with 99% purity was provided by Taxolog, Inc., Fairfield, NJ, USA. The solvents used were acetonitrile, methanol, hexane, ethanol, chloroform, and ethyl acetate of HPLC grade purity and purchased from Sigma, St. Loius MO, USA. Acetic acid with 99.9% purity was purchased from J.T. Baker, Phillipsburg, NJ, USA.

2.2. Sample preparation

Radiolabeled milataxel was administered either orally or intravenously to Harlan female nude mice bearing A-375 tumors (Piedmont Research Center, Morrisville, NC, USA) [9]. The tissues were shipped to MPI Research, Exygen Research Division (State College, PA, USA) for extraction experiments. Extraction experiments included those performed on single tumors, as well as multiple tumor blends. Prior to each extraction, a representative portion of each tissue sample was weighed and combusted using a biological material oxidizer (BMO). A liquid scintillation counter (LSC) was used to determine the total amount of radioactivity present in each sample prior to extraction.

All glassware was silanized prior to use to prevent the analytes from binding to the glass surface. Glassware was initially rinsed with a 30% dimethyldichlorosilane solution in toluene followed by a toluene and methanol rinse before allowing to air dry. The combustion efficiency of the BMO was calculated daily by fortifying duplicate ^{14}C cocktail aliquots with radioactive glucose, followed by combustion of duplicate aliquots of mannitol, dosed with a like amount of radioactive glucose and counted by LSC to determine daily efficiency (Table 1).

Tumor samples were small (100–400 mg), fibrous, wet, and sticky, making homogenizing difficult. Attempts to freeze the tumor

Table 1
Typical %recovery for extraction of milataxel from xenograft mice tumors using variety of solvents and mixtures of solvents.

Extraction solvents	Tumor weight (g)	Amount tumor combusted (g)	% Combustion efficiency	Total or corrected DPM for combusted tumor	Total or corrected DPM for extracted tumor	% Recovery C-14 milataxel and metabolite
Ethanol	0.35	0.022	87.6	3530	1,120	**1.8**
Chloroform: MeOH 50:50	0.385	0.022	87.6	3530	1,960	**3.1**
Ethyl Acetate	0.385	0.022	87.6	3530	4,180	**6.6**
Acidic MeOH 50:50	0.385	0.022	87.6	3530	30,690	**48.5**
Acetonitrile	0.102	0.032	84.8	5527	583	**3.1**
Hexane	0.131	0.01	94.7	959	340	**3.0**
Hexane/Acidic MeOH[a]	**0.131**	**0.01**	**94.7**	959	12,400	**99.0**

[a] Tumor tissues tissuemized in hexane and then acidic:MeOH added to extract out free drug and related residues.

with liquid nitrogen and shatter it with a mortar and pestle proved unsuccessful. Additional attempts to blend a tumor using a grinder and dry ice with a control tumor were unsuccessful. Dicing/mixing tumors with a razor blade were found to be the most practical technique for homogenization and extraction experiments. The moisture content of the tumors was high and maintaining tumor weight during processing was difficult. Tumors were stored in capped vials to prevent dehydration.

2.3. Separation and detection techniques

The chromatographic separation consisted of a gradient separation and was performed on a Jones Chromatography Genesis C18 column (50 mm × 2.1 mm, 4 μm) with mobile phase A (MP-A) = 45% ACN:55% (0.1% acetic acid in water), mobile phase B (MP-B) = 100% ACN, flow rate 0.5 mL/min, column temperature 35 °C. The gradient condition included 100% MP-A for 12 min then switched to 100% MP-B at 12.1 min and kept it until 20 min to allow elution of all bound matrix co-extractives from the column.

A PerkinElmer SCIEX API 365 turbo ion spray MS with a liquid introduction interface (positive ion mode, source temperature 350 °C) was utilized to detect and quantitate parent milataxel and the major metabolite M-10. Milataxel with molecular weight of 853.9 (mono isotopic mass 853.3) and its major in vitro metabolite M-10 with a molecular weight of 869.9 (mono isotopic mass 869.3) both demonstrated affinity to sodium adducts. Therefore, the molecular masses for parent compound and metabolite to observe were 876.3 and 892.3 (M + Na) amu, respectively. The main transition observed for the M-10 metabolite was 892–621.5 with minor transition at 892–294 and 238. The main transition observed for milataxel was 876–605 with minor transitions at 876–294 and 238. The product ions for both compounds were scanned from 0 to 5 min.

2.4. Preliminary extraction experiments

Evaluation of a typical extraction scheme involved placing a tumor sample into a 5 mL glass vial, adding 1 mL of solvent, and homogenizing for 1 min at 30,000 rpm. The extract was filtered using a 0.2 μm PVDF syringe filter into an LC vial for analysis (*note*: filter membrane compatibility test with cold milataxel in various solvents had been conducted prior to this extractability study and was shown to be suitable). The sample was assayed against a 0.1 μg/mL working standard solution of milataxel (due to lack of external standard for M-10 metabolite the levels of this metabolite were measured against milataxel standard assuming similar mass spectral response). Extraction of milataxel and metabolite M-10 from tumor samples was evaluated using the following solvent systems: MeOH, ACN, ethanol, ethyl acetate, or combination of solvents such as 95% MeOH/5–0.1% acetic acid in water (acidic MeOH), and chloroform/MeOH (Table 1). Analysis of the milataxel and M-10 metabolite using HPLC-UV/β-ram detection (Shimadzu LC-10, IN/US Systems Model 3) did not show sufficient ^{14}C response or UV absorbance to detect the target analytes. The samples were then analyzed and detected by LC–MS.

Each extraction technique using particular solvent and/or solvent mixtures was evaluated using tissues from mice dosed with radiolabeled milataxel and mass balance was determined for all milataxel related compounds in the starting tissue, intermediate steps, and final extracts by BMO and LSC. This allowed for a direct determination of the extraction efficiency of each given solvent system.

2.5. Hexane/acidic methanol extraction

A modified extraction technique was evaluated for milataxel and metabolite in oral (PO) and intravenous (IV) treated nude Harlan mice bearing A-375 and SKMES xenograft tumors. The following is a general description of the extraction and analysis.

Tumor samples were weighed into a scintillation vial; tumor samples were diced and hexane was added then homogenized with a tissuemizer. This homogenization step using hexane appeared to be different from previous extractions in that the tumor tissues appeared to be nearly completely solubilized. Following hexane tissue disruption, a solvent mixture of 95% MeOH/5–0.1% acetic acid in water (acidic MeOH) was added and samples were again tissuemized. This was followed by sonic cell disruption to facilitate extraction. The resulting solution was centrifuged to precipitate the matrix solids. The supernatant was transferred to a pear-shaped flask and the solvents removed by rotary evaporation. The resulting extracts were reconstituted in MeOH for analysis. The extracts were also evaluated using HPLC equipped with an UV/β ram detector. In contrast to results obtained using the previous extraction systems, it was now possible to detect both milataxel and M-10 using the β ram detector. Using the new hexane/acidic MeOH water technique samples were subsequently analyzed by LC–MS to quantitatively determine the levels of the parent and metabolite.

With extraction efficiency established, recovery of ^{14}C labeled milataxel samples from intravenous and orally dosed mice bearing A-375 tumors were determined. Samples were analyzed using LC–MS.

Mice with SKMES xenograft tumors were treated with a single intravenous dose of milataxel using a 5% ethanol/95% Liposyn® vehicle. Tumor samples taken at different time points were analyzed by LC–MS to determine concentrations of milataxel and M-10.

Using the productive extraction and quantitation technique demonstrated with SKMES and A-375 tissues, parent and metabolite were evaluated in an additional tumor type. Mice implanted with HCT-15 (colon cancer) tumors were dosed orally and intravenously and the tumors were dissected over time [11].

3. Results and discussion

A typical system suitability for concentration ranges 0.01–0.5 mg/mL ($n = 5$) resulted %RSD = 12.4 with correlation coefficient (R^2) = 0.999 for parent, and %RSD = 8.6 with R^2 = 0.997 for metabolite M-10 indicated good system linearity. Recovery experiments were conducted using direct spiking of control (milataxel untreated) tumor samples. Typical recoveries for parent and metabolite ranged from 92% to 98%.

Simple extractions techniques with 100% ACN to extract milataxel from SKMES tumor cell lines harvested at different time points ranging from 8 h to 20 days are shown in Fig. 2. Subsequent

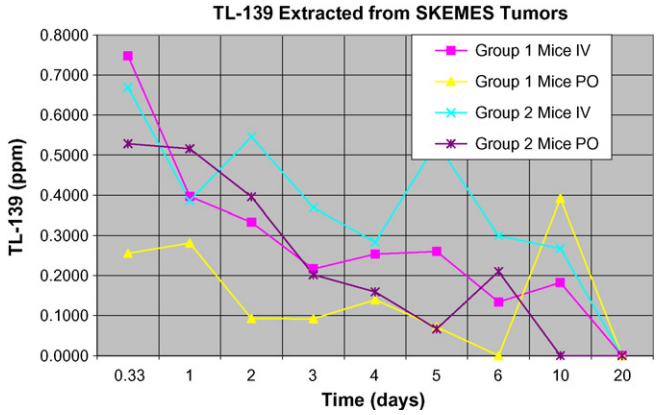

Fig. 2. Tumor concentration of milataxel (TL-139) in oral (PO) and intravenously (IV) dosed SKMES tumor bearing mice using an ACN extraction technique and LC–MS analysis.

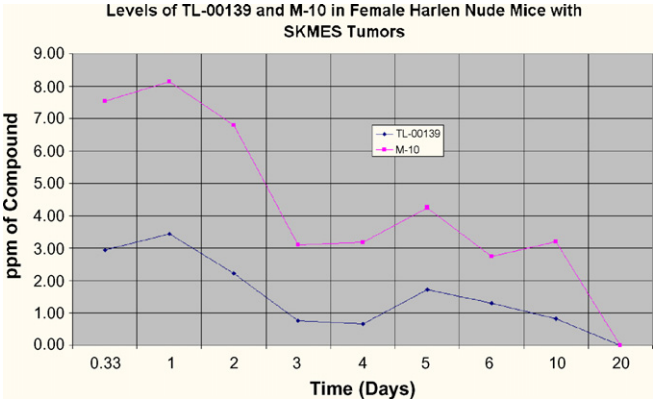

Fig. 3. Levels of milataxel (TL00139) and M-10 in female Harlan nude mice with SKMES mouse tumor using hexane/acidic MeOH extraction technique. A single intravenous injection of milataxel (73 mg/kg) in a 5% ethanol/95% Liposyn® vehicle was administered to mice with SKMES tumors. The hexane/acidic MeOH extraction technique was used to extract related residues from tumor tissues.

experiments demonstrated superior extraction recovery using hexane/acidic MeOH in the same SKMES cell lines (Fig. 3 and Table 1) as well as in limited A-375 cell lines. Observed concentrations of milataxel and M-10 in HCT-15 tumor cell lines from oral and intravenously dosed mice are shown in Figs. 4a and b, and 5a and b.

Using the common solvents as described earlier for extraction, only 2–48.5% of ^{14}C labeled milataxel and related compounds were extracted from tumor tissues when analyzed by BMO and LSC (Table 1) [10]. In results obtained using acetonitrile as the extraction solvent, the highest concentration of parent found in tumors from IV and PO dosed animals (Fig. 2) was approximately 0.53 and 0.75 ppm, respectively. M-10 was detected, but the levels observed were not quantifiable, it was concluded that the evaluated extraction techniques were inadequate. One possible cause for the low extraction values was presumed protein binding and a more elaborate extraction technique was explored.

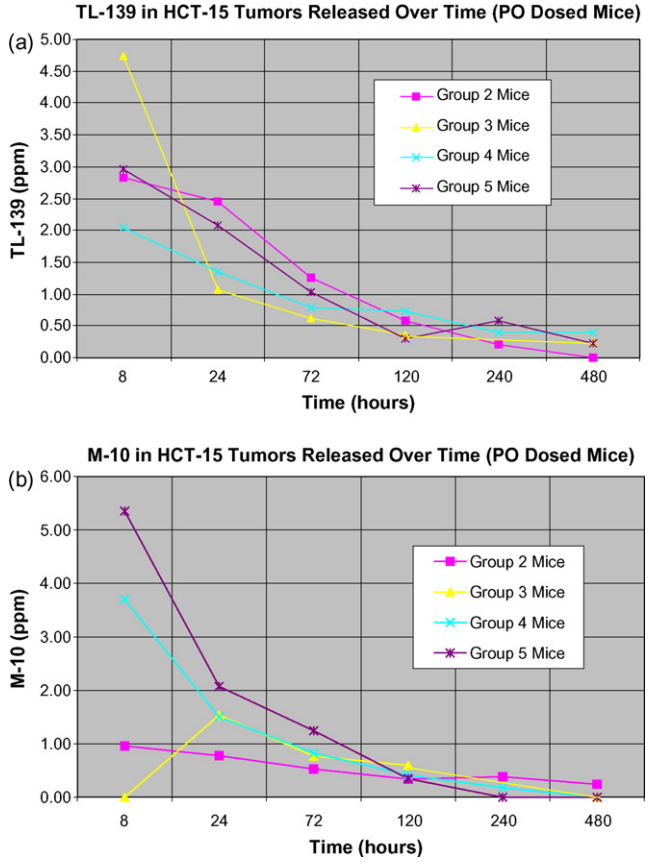

Fig. 4. (a) Quantification of milataxel (TL-139) in orally dosed mice with HCT-15 tumors by LC–MS using hexane/acidic MeOH extraction technique. (b) Quantification of M-10 in orally dosed mice with HCT-15 tumors by LC–MS using hexane/acidic MeOH extraction technique.

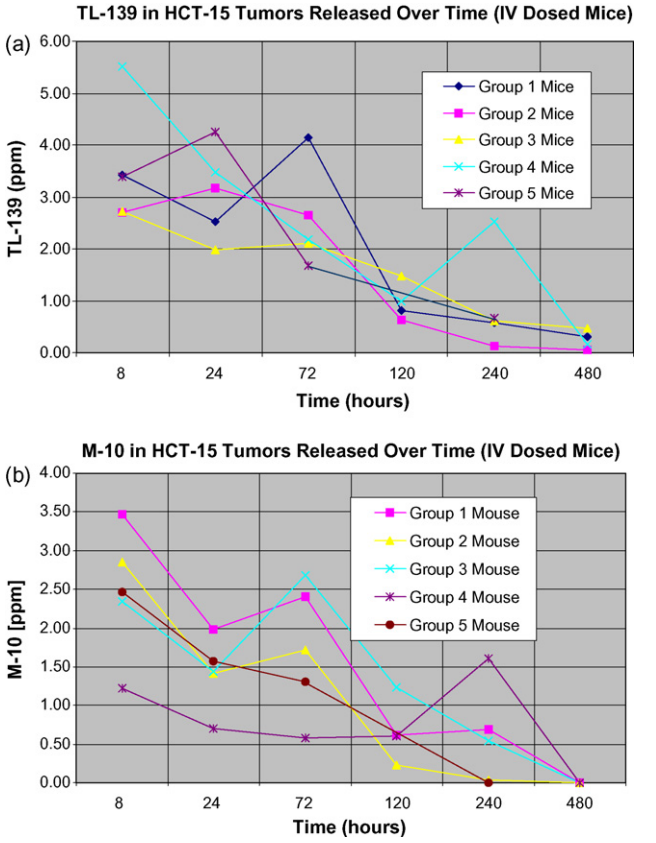

Fig. 5. (a) Quantification of milataxel in intravenously dosed mice with HCT-15 tumors by LC–MS via hexane/acidic MeOH extraction method. (b) Quantification of M-10 in intravenously dosed mice with HCT-15 tumors by LC–MS via hexane/acidic MeOH extraction method.

Hexane with the aid of sonic cell disruption, tissue penetration with a tissuemizer followed by acidic MeOH exposure, improved extraction efficiencies for radiolabeled milataxel from tumor tissue from less than 49–99% (Table 1). In general, utilizing BMO and LSC and mass balance equations, an average of 75% extraction efficiency was observed for parent and related radiolabeled compounds. Similar IV dosed SKMES tumors processed using the hexane/acidic MeOH extraction technique and subsequent analysis by LC–MS yielded approximately 8 ppm M-10 and 3.5 ppm parent (Fig. 3). Unlike most hydroxylated metabolites that are more soluble in aqueous and organic solvents, M-10 metabolite proved to be more difficult to retrieve from tumor tissue. It is possible that the M-10 binds to tissues differently than the parent. The PO dosed mice bearing SKMES tumors showed slightly lower concentration of milataxel, but the drug residence time was similar.

IV dosed SKMES group #2 mouse and PO dosed day-10 group #1 mouse tumors (Fig. 2) show relatively higher concentration of drug levels on day 5 and day 10, respectively. Both of these tumors shared one similar physical characteristic, they both were darker in color and appeared dehydrated. The observed higher drug concentrations may be due to differences in tumor necrosis and or excretion rates related to the tumor condition.

Table 2
Summary table of ^{14}C-TL00139 (milataxel) and M-10 metabolite in female nude mice bearing A-375 tumor determined by PE SCIEX API 300: PO vs. IV dosage.

Sample ID	Dosing route	Tumor weight (g)	TL00139 (ng/tumor)	TL00139 (ppm)	M-10 (ng/tumor)	M-10 (ppm)
Mouse 6, Group 1, 24 h	IV (73 mg/kg)	0.4595	200.3	0.436	721.9	1.571
Mouse 6, Group 3, 24 h	PO (104 mg/kg)	0.3759	206.4	0.549	682.0	1.814

Fig. 3 shows LC–MS results for the levels of parent and metabolite M-10 in the remaining female Harlen Nude Mice SKMES tumors. As with other SKMES bearing mice, these sets of mice were also dosed with a single IV injection of 73 mg/kg milataxel in a 5% ethanol/95% Liposyn® vehicle. Fig. 3 presents the data obtained using the improved extraction technique using hexane/acidic MeOH, demonstrating the improved recovery of ^{14}C to approximately 75%. The highest level of parent found was 3.44 ppm in the 24 h sample with 8.14 ppm levels of M-10 metabolite found. The concentration of parent detected was 5 times larger than what was obtained when using ACN as the extraction solvent (*note*: the animals in both ^{14}C and cold milataxel treated groups were dosed with similar levels of drug). The ratio of metabolite to parent was approximately 2.5–1 for most time points.

Due to limitation in number of A-375 ^{14}C tumors, only 2 × 24 h PO and IV dosed mice were compared using the hexane/acidic MeOH extraction technique (Table 2). The purpose for this comparative study was to determine concentration differences and the relative amount of the parent in comparison to metabolite M-10 in A-375 tumors. A second objective was to compare these concentrations to what was observed in SKMES tumors. Considerable differences in the drug and metabolite concentrations may explain pharmacological activity differences for milataxel in A-375 vs. SKMES. The PO and IV dosed mice at 24 h contained relatively the same levels of both parent and metabolite as shown in Table 2. It appears that distribution characteristics for parent and metabolite are similar, regardless of the route of administration. Both PO (104 mg/kg) and IV (73 mg/kg) QDx1 dosages are representative of the maximum tolerated doses and absolute oral bioavailability in mice was not determined.

Figs. 4 and 5 display levels of milataxel and M-10 in ppm for PO and IV dosed nude mice bearing HCT-15 tumors. As shown in Fig. 4a and b, milataxel concentrations in PO dosed mice tumors harvested at 8 h ranged from 2 to 5 ppm while 0–5 ppm levels were determined for M-10. M-10 levels dropped rather quickly by 24 h and almost leveled off with milataxel (except for one group "group 2 mice PO"). Traces of parent milataxel were detected on day 20-post drug administration, while M-10 was not detected past day 10 in either set of PO dosed mice. Fig. 5a and b demonstrates the levels of M-10 to be, in general, equal to or less than parent in IV dosed HCT-15 bearing nude mice. This is a rather different pattern than what has been seen before for milataxel in IV and/or PO dosed SKMES and A-375 tumor lines in that although initial distribution appears to follow previous tumor data, M-10 elimination rate exceeds that of parent. This may be related to the high level of MDR1 in the HCT-15 cell line and the predicted efflux of M-10 [11]. M-10 in vitro cytotoxicity (IC50) values are larger than Milataxel in cell lines with pgp expression [12].

In general, ratios of M-10 to milataxel were larger in SKMES and A-375 tumors, while in HCT cell lines the ratio was almost equal.

The physical appearance of HCT-15 tumor greatly differed from A-375 and SKMES. In general, 8 h–5 day tumors in SKMES and A-375 tumors were somewhat oval/round in shape, more fatty-like and light gray-pinkish in color while HCT-15 tumors were irregular in size, elongated, hard, and appeared dehydrated and grayish in color. The inconsistency in shape and size posed analytical challenges with sample processing. For example, in PO dosed mice group 3, the 8 h tumor only weighed 26.4 mg while tumor weights had increased to ~128 mg by day 5. In contrast, generally, early time point harvests for SKMES tumors were larger while decreasing in size as time had progressed and were easily cut and homogenized. SKMES tumor weights were more consistent than HCT-15 tumors. The level of milataxel was detectable in two cases in 20-day samples while no detectable residues were observed for M-10 in HCT-15 tumors.

Using the hexane/acidic MeOH extraction technique, 8 mice brains from mice treated with radiolabeled milataxel were evaluated for extraction efficiency. The brain tissues were collected across different genders and groups of milataxel treated xenograft mice (i.e. Group 2 Mouse 1 (Male, 6 day, IV), Group 2 Mouse 6 (Male, 6 day, IV), Group 4 Mouse 1 (Male, 6 day, PO), Group 4 Mouse 6 (Male, 6 day, PO), Group 2 Mouse 1 (Female, 6 day, IV), Group 2 Mouse 6 (Female, 6 day, IV), Group 4 Mouse 1 (Female, 6 day, PO), and Group 4 Mouse 6 (Female, 6 day, PO)). Based on the mass balance equation, 62.4% ^{14}C labeled milataxel and related compounds were extracted. The final extracts were analyzed semi-quantitatively using HPLC-MS. There were no detectable levels of metabolite M-10 in the brain tissue.

4. Conclusion

The novel extraction technique evaluated and discussed in this study was unique and allowed for the recovery of at least 75% of the bound compounds from tumor and brain tissues. The extraction efficiency of the hexane/acidic MeOH extraction technique may be related to the nature of the tissues. Tumor and brain tissues, in comparison to liver, digestive tracts, plasma, sciatic nerve, etc., are fattier in nature. Its feasible that hexane disrupts fatty tissues better than non-lipophilic, protic, and aprotic solvents. This unique extraction system allowed for adequate detection of M-10 metabolite that would not have been possible with conventional extraction techniques. Although the data suggest that drug was bound to the brain sample, it was not clear if the drug resided in the vascular or internal brain tissue. Future studies should include departmental examination of milataxel in the brain. A 3-dimensional tissue-imaging using MALDI-TOF-MS is a current technology that may provide more explicit tissue compartment information.

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References

[1] F. Stephenson, A Tale of Taxol, FSU Research in Review, vol. XII, No. 3, Tallahassee, FL, 2002, p. 12.
[2] M.O. Bradley, C.S. Swindell, F.H. Anthony, P.A. Witman, P. Devanesan, N.L. Webb, S.D. Baker, A.C. Wolf, R.C. Donehower, Elsevier J. Control. Release 74 (2001) 233.
[3] G.K. Poon, J. Wade, J. Bloomer, S.E. Clarke, J. Maltas, Rapid Commun. Mass Spectrom. 10 (1996) 1165.
[4] W.J. Loos, J. Verweij, K. Nooter, G. Stoter, A. Sparreboom, J. Chromatogr. B Biomed. Sci. Appl. 693 (1997) 437.
[5] J. Ciccolini, J. Catalin, M.F. Blachon, A. Durand, J. Chromatogr. B Biomed. Sci. Appl. 759 (2001) 299.
[6] H. Rosing, V. Lustig, F.P. Koopman, W.W. Ten Bokkel Huinink, H.J. Beijnen, J. Chromatogr. B Biomed. Sci. Appl. 696 (1997) 89.
[7] C. Sottanil, M. Rapid Commun. Mass Spectrom. 12 (1998) 251.
[8] D.L. de Valeriola, C. Brassinne, C. Gaillard, Proceedings of the American Association of Cancer Research (AACR), vol. 34, 1993, p. 343.

[9] D. Dexter, A. Meshaw, G. Collins, S.F. Chen, B. Hollister, Oral and IV Dosing Studies of Taxolog Agent TL-139 on Three Schedules in Nude Mice Bearing the A375 Human Melanoma Xenograft, NC (Report #18), Piedmont Research Center, Morrisville.
[10] P.D. Connolly, Extractability and Profiling of milataxel and Related Residues From Mouse Tissues Treated with Radiolabeled Drug, Exygen Research, Analytical Report 064-006.
[11] D. Sampath, C. Discafani, C. Beyer, M. Nunez, M. Hari, H. Liu, T. Annable, S. Musto, P. Gallagher, C. Rios, F. Loganzo, L.M. Greenberger, Proceedings of the 16th. Symposium on "Molecular Targets and Cancer Therapeutics" EORTC-NCI-AACR, Geneva, Switzerland, September 28–October 01, 2004.
[12] H. Safarpour, X. Tong, M. Bielawski, R. Holton, H. Wilcox, Proceedings of the American Association of Cancer Research (AACR), 2006 (Poster #512).