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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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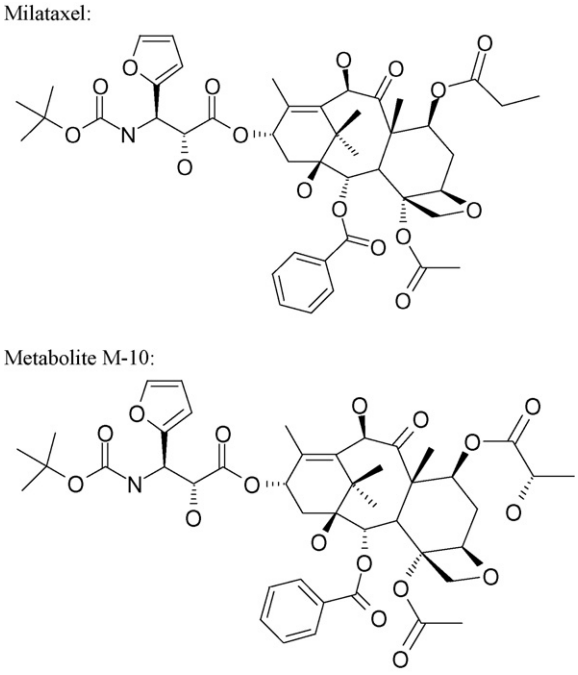

Fig. 1. Structures of milataxel (TL-139) and metabolite M-10.

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.