Evaluation of Drug Disposition Using Transgenic Animal Models and Quantitative Whole Body Autoradiography

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ABSTRACT

The important role of membrane transporters in drug absorption and disposition, therapeutic efficacy and adverse drug reactions as well as for drug-drug interactions is nowadays well recognized. The identification of the membrane transporters that influence the disposition and safety of drugs in development is a major challenge in preclinical drug evaluation. *In vivo* animal models are an important tool to investigate the interplay between uptake/efflux transporters and metabolizing enzymes and the relative importance of each transporter or enzyme considering overlapping substrate specificity. Current study demonstrates the utility of using transgenic mice lacking efflux transporter gene(s) and quantitative whole body autoradiography (QWBA) to study the role of the individual transporters in the disposition and elimination of a radiolabeled drug. Wild type (*wt*) FVB mice and ABC transporter family knock-out (KO) mice on FVB background (Mdr1a/b$^{(-/-)}$, Bcrp$^{(-/-)}$, and Mrp2$^{(-/-)}$) were provided by Taconic (Hudson, NY). ^{14}C-Taxol was selected as a probe and the brain as a primary target organ. The time course of plasma and tissue distribution of ^{14}C-taxol equivalents was determined first, and based on the results two time points (6 and 24 hours post-dosing) were selected for comparison of ^{14}C-taxol distribution in the *wt* and KO mice. At both evaluation time points, concentrations of ^{14}C-taxol equivalents in the brain higher than the *wt* were observed for the Mdr1a/b$^{(-/-)}$ mice (10- to 20-fold difference at 6 hours post-dosing and 60- to 200-fold difference at 24 hours post-dosing), but not for the Bcrp$^{(-/-)}$ and Mrp2$^{(-/-)}$ mice. This proof of concept study demonstrated the utility of using transgenic mice models and QWBA for elucidation of the role of individual transporters in drug disposition.

INTRODUCTION

The important role of membrane transporters in drug absorption and disposition, therapeutic efficacy and adverse drug reactions as well as for drug-drug interactions is well recognized. The identification of the membrane transporters that influence the disposition and safety of drugs in development is a major challenge in preclinical drug evaluation. More than 400 membrane transporters belonging to two major superfamilies, ATP-binding cassette (ABC) and solute carrier, have been annotated in the human genome [1]. Among these, P-glycoprotein (P-gp, *MDR1, ABCB1*), breast cancer resistance protein (BCRP, *ABCG2*), and multidrug resistance protein (MRP2, *ABCC2*) are known to have a significant impact on bioavailability of many drugs [2]. The techniques and methodology for studying drug-transporter interactions continually evolve. *In vitro* methods (membrane- and cell-based assays) are currently used to identify substrates and inhibitors for the individual transporters and for developing QSAR models. Decision trees for P-gp and BCRP substrate and inhibitor interactions based on results from bidirectional *in vitro* transporter assays have been described in the FDA guidance for drug interaction studies [3] and in the white paper membrane transporters in drug development [1]; probable P-gp and BCRP substrates/inhibitors are then recommended to be further assessed *in vivo*. *In vivo* animal models, particularly transgenic animals lacking one or more transporters, are important tools to investigate the interplay between uptake/efflux transporters and metabolizing enzymes and the relative importance of each transporter or enzyme considering overlapping substrate specificity.

In the current study we evaluated the utility of using transgenic mice lacking efflux transporter gene(s) and quantitative whole body autoradiography (QWBA) to study the role of the individual transporters in the disposition and elimination of a radiolabeled drug. Wild type (*wt*) FVB and ABC transporter family knock-out (KO) mice on FVB background (Mdr1a/b$^{(-/-)}$, Bcrp$^{(-/-)}$, and Mrp2$^{(-/-)}$) were used. ^{14}C-Taxol was selected as a probe and the brain as a primary target organ. P-gp and BCRP are both present in brain endothelium and therefore are likely to play a role in a drug disposition in the brain [4, 5]. First, the time course of plasma and tissue distribution of ^{14}C-taxol equivalents was determined in the FVB *wt,* and based on the results two time points (6 and 24 hours post-dosing) were selected for comparison of the ^{14}C taxol distribution in the *wt* and KO mice.

METHODS AND MATERIALS

FVB *wt* mice and efflux transporter KO mice on FVB background (Mdr1a/b$^{(-/-)}$, Bcrp$^{(-/-)}$, and Mrp2$^{(-/-)}$) were provided by Taconic (Hudson, NY). Animals were housed and handled following WIL research Laboratories, LLC standard operating procedures. The protocol for the study was approved by the Institutional Animal care Use committee.

Nonlabeled and ^{14}C-labeled taxol were purchased from Moravek Biochemicals, Inc., Brea, CA. On the days of dosing, taxol was formulated in Polysorbate 80:ethanol:sterile saline in 1:1:6 ratio (v/v/v).

Phase I: Six (3 per sex) FVB *wt* mice were administered a single intraperiotoneal (IP) injection of ^{14}C-taxol at 0.25 mg/ (5 µci)/animal. Blood, bladder urine and a select set of tissues were collected from one animal per sex at 2, 4, 6, 8, 12, and 24 hours postdose. The collected samples were analyzed for total radioactivity by liquid scintillation counting (LSC).

Phase II: Twelve FVB wt, Mdr1a/b$^{(-/-)}$, Bcrp$^{(-/-)}$, and Mrp2$^{(-/-)}$ KO mice (6 per sex from each strain) were administered a single IP injection of ^{14}C-taxol at 0.25 mg (5µCi)/animal. Blood was collected from 3 animals/sex/strain at 6 and 24 hours post-dosing. Whole blood and plasma were analyzed for total radioactivity by LSC. carcasses were processed for WBA (see next section).

Quantitative Whole Body Autoradiography (QWBA) Analysis

After euthanasia, the carcasses were submerged in a dry ice/hexane bath until frozen. The frozen carcasses blocked in 5% (w/v) carboxymethylcellulose (CMC). Standards and quality control (QC) samples were prepared by fortification of mouse whole blood with known amounts of ^{14}C-glucose. Sagittal, 30-µm thick sections of the CMC-embedded mouse carcasses, including QC standards, and CMC-embedded calibration standards, were collected and using transfer tape. The sections were dehydrated and placed on phosphor imaging plates. The exposure time was approximately 4 days. The imaging plates were scanned using a Fujifilm BAS-5000 phosphor imager. The images (autoradioluminograms) were overlaid, if necessary, with digital images of the sections to aid in the identification of tissue localization of the radioactivity. Approximately 30 tissues/animal were analyzed.

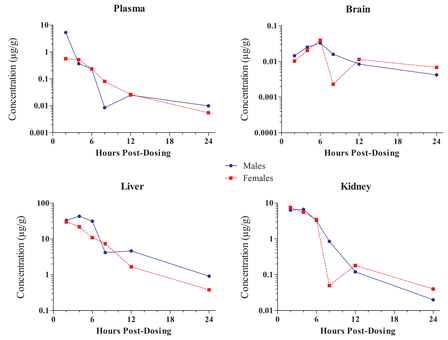

Figure 1 Phase I: Time course of ^{14}C-Taxol Equivalents in Plasma, Brain, Liver, and Kidney of FVB *wt* Mice after a Single IP Injection of ^{14}C-Taxol at 0.25 mg/animal.

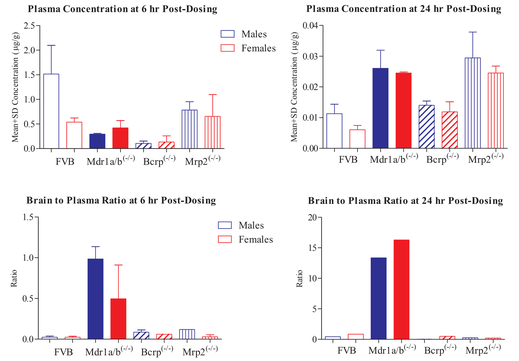

Figure 2 Phase II: Time course of ^{14}C-Taxol Equivalents in Plasma and Brain to Plasma Ratio in FVB *wt*, Mdr1a/b$^{(-/-)}$, Bcrp$^{(-/-)}$, and Mrp2$^{(-/-)}$ Mice after a Single IP Injection of ^{14}C-Taxol at 0.25 mg/animal.

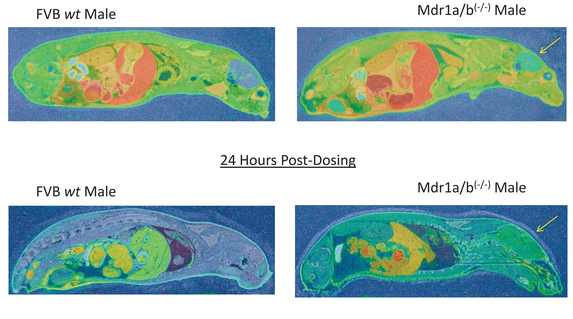

Figure 3 Whole Body Autoradioluminograms Overlaid with the Section Images for Representative FVB wt and Mdr1a/b$^{(-/-)}$ Male Mice at 6 and 24 Hours after a Single IP Injection of ^{14}C-Taxol at 0.25 mg/animal.

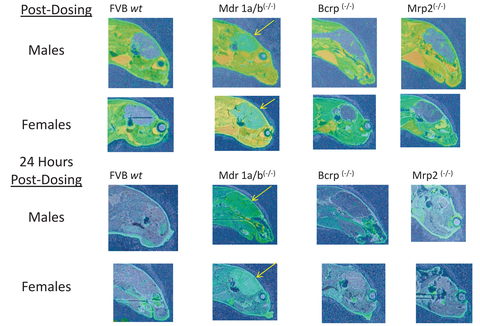

Figure 4 Autoradioluminograms Overlaid with the Section Images for Representative FVB *wt*, Mdr1a/b$^{(-/-)}$, Bcrp$^{(-/-)}$, and Mrp2$^{(-/-)}$ Mice at 6 and 24 Hours after a Single IP Injection of ^{14}C-Taxol at 0.25 mg/animal.

Table 1 Concentration of ^{14}C-Taxol Equivalents (ng/g) in Representative Tissues of FVB *wt*, Mdr1a/b$^{(-/-)}$, Bcrp$^{(-/-)}$, and Mrp2$^{(-/-)}$ Mice at 6 and 24 Hours after a Single IP Injection of ^{14}C-Taxol at 0.25 mg/animal

Strain	FVB *wt*		Mdr1a/b$^{(-/-)}$		Bcrp$^{(-/-)}$		Mrp2$^{(-/-)}$	
Tissue	Males	Females	Males	Females	Males	Females	Males	Females
6 Hours Post-dosing								
Adrenal gland	3050	3416	7542	11462	10742	3075	5432	1913
Brain	28.4	13.5	288	261	12.3	15.7	67.1	16.2
Heart	1811	1418	3088	3473	1071	881	2830	1516
Kidney	5071	6054	6146	6933	3481	5104	9844	10921
Liver	32275	15912	42275	30828	18765	13589	50084	23177
Lung	2228	2449	4426	5555	1038	1739	5386	1890
Pancreas	4926	5007	5005	5905	3345	4986	6734	4085
Spleen	3338	3077	6078	7449	2382	3566	5078	1578
Thymus	1510	878	1763	2510	666	1243	1286	1178
24 Hours Post-dosing								
Adrenal gland	9.13	12.5	NSA	715	63.3	281	NSA	NSA
Brain	2.83	6.41	498	405	0.197	9.06	9.34	5.03
Heart	16.7	8.00	147	123	48.0	52.1	61.0	14.1
Kidney	26.0	49.0	194	374	392	436	1041	639
Liver	575	545	5242	3800	1415	1512	6846	3473
Lung	26.8	49.1	654	213	142	939	105	40.7
Pancreas	46.1	58.3	264	351	115	203	158	106
Spleen	7.72	37.9	234	252	236	127	57.2	34.6
Thymus	218	785	2167	1402	626	534	1458	477

NSA = No sample analyzed.

KO/*wt* ratio: <0.1 | >0.1<0.5 | >0.5<2 | >2<10 | >10

RESULTS

Phase I. The time course of ^{14}C-taxol equivalents in plasma, brain, liver, and kidney in the FVB *wt* mice is presented in Figure 1. Peak concentration of ^{14}C-taxol equivalents in brain was observed at 6 hours post-dosing in both male and female mice, and this time point was selected as a primary evaluation period for Phase II of the study.

Phase II. Plasma concentrations of ^{14}C-taxol equivalents at 6 and 24 hours post-dosing in the FVB *wt* and KO mice are presented in Figure 2.

For the 6-hour post-dosing time point, 3 animals/sex per strain from the FVB *wt* and Mdr1a/b$^{(-/-)}$ were processed for and analyzed by QWBA. Representative images of whole body autoradioluminograms (pseudo-colored in rainbow) overlaid with the digital image of the sections are presented in Figure 3. The interanimal variability in the concentration of the ^{14}C-taxol derived radioactivity among the animals from the same sex and strain was low and, therefore, single animals/sex/time point were analyzed by QBWA for the Bcrp$^{(-/-)}$ and Mrp2$^{(-/-)}$ mice at 6 hours post-dosing and for all strains at 24 hours post-dosing. Representative images of autoradioluminograms (pseudo-colored in rainbow) of the heads overlaid with the digital image of the sections are presented in Figure 4. The estimated brain-to-plasma ratio is illustrated in Figure 2. The concentrations of ^{14}C-taxol equivalents in selected tissues at 6 and 24 hours post-dosing are presented in Table 1; the ratio of the tissue concentration in KOs vs. *wt* was color coded as indicated in the table legend. At both evaluation time points, concentrations of ^{14}C-taxol equivalents in the brain higher than the *wt* were observed for the Mdr1a/b$^{(-/-)}$ mice (10- to 20-fold difference at 6 hours post-dosing and 60- to 200-fold difference at 24 hours post dosing), but not for the Bcrp$^{(-/-)}$ and Mrp2$^{(-/-)}$ mice. Higher concentrations of ^{14}C-taxol equivalents in many tissues were observed at 24 hours post-dosing in all 3 KOs compared to the *wt* which was likely due to impaired elimination of the taxol metabolites from liver and kidney.

CONCLUSIONS

This proof of concept study demonstrated the utility of using transgenic mice models and QWBA for elucidation of the role of individual transporters in drug disposition. The research strategy will be to determine the time course of plasma and tissue distribution of the radiolabeled drug in FVB *wt* mice for optimal selection of time point(s) to investigate the dispositionof the drug in the KO mice. The feasibility of using ^{14}C-taxol as a probe for evaluation of potential/ proposed P-gp inhibitors *in vivo* is under current investigation.

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