Important Considerations for the Use of ^{125}I-Labeled Proteins to Examine ADME Characteristics of Iodinated Compounds

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INTRODUCTION

Radiolabeled pharmaceutical compounds have been used to examine the absorption, distribution, metabolism and excretion (ADME) patterns of new drugs for decades. These studies rely on accurate in vivo drug concentration data (parent molecule and metabolites). Small molecule drugs are often radiolabeled with ^{14}C because they can be synthesized to reside in specific stable molecular positions, which provides reliable quantitation and imaging. Stable radiolabeling of large molecules with ^{14}C is often difficult and expensive. Most often large molecules are labeled with ^{125}I, which is relatively quick, easy and much less expensive, but ^{125}I-labeled proteins have issues that investigators need to be aware of. The stability of ^{125}I on large molecules is highly variable in vivo due to biodehalogenation. Anywhere from 5% to 80% of the ^{125}I can detach from a protein in vivo and result in confounding data due to free ^{125}I in tissues. Therefore ADME studies using ^{125}I-labeled large molecules must account for biodehalogenation and investigators and regulators must understand the possible affects on the interpretation of ADME data. The objectives of this study were to evaluate the use of ^{125}I in ADME studies, which include the determination of mass balance (MB) of total radioactivity from an ^{125}I-labeled protein (^{125}I-IgG$_{rat}$), and tissue distribution (TD) of the drug-derived radioactivity using quantitative whole-body autoradiography (QWBA).

MATERIALS AND METHODS

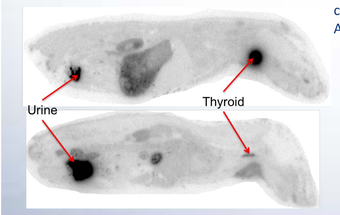
1) A Rat ADME study (MB and QWBA TD studies) of Sodium-^{125}Iodide (^{125}I-Na) (American Radiolabeled Chemicals, St. Louis, MO) and Rat ^{125}I-IgG$_{rat}$ (Vitrax, Placentia, CA) were conducted to examine the effect of biodehalogenation on determining ADME properties of iodinated large molecules.
2) A set of ^{125}I Blood calibration standards were constructed and examined to determine the upper and lower limits of quantitation for phosphor imaging technology (used for QWBA TD).
3) The effect of high concentrations of ^{125}I in closely associated tissues during QWBA image analysis (a.k.a. radioactive "flaring") was examined.
4) The effect of administering NaI via drinking water to saturate iodine-organifying tissues (e.g. thyroid, skin, salivary gland), and iodine transporters (kidney, stomach).

In Vivo Study Design

Group Number/ Sample	Study	Target Dose Level (mg/kg)	Target Radioactivity Level (µCi/kg)	Samples
1 / ^{125}I-rIgG	QWBA w/NaI in H_2O	2	20	Terminal plasma and carcass
2 / ^{125}I-rIgG	QWBA w/out NaI in H_2O	2	20	
3 / ^{125}I-rIgG	MB w/NaI in H_2O	2	20	Urine, feces, cage residue at intervals to 72 h; 1 carcass for QWBA, 2 carcasses for MB
4 / ^{125}I-rIgG	MB w/out NaI in H_2O	2	20	
5 / Na^{125}I	MB w/ NaI in H_2O	10	20	Urine, feces, cage residue at intervals to 72 h; 1 carcass for QWBA, 2 carcasses for MB
6 / Na^{125}I	QWBA w/NaI in H_2O	10	20	Terminal plasma and carcass
7 / Na^{125}I	QWBA w/out NaI in H_2O	10	20	

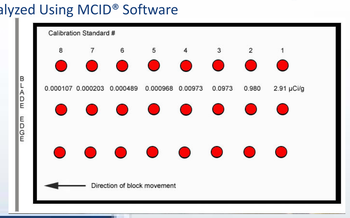
Mouse given IV dose of ^{125}I-Protein alone (top)

Mouse given IV dose of ^{125}I-Protein and NaI in drinking water. (bottom)



Evaluation of Calibration Standards for QWBA Image Analysis

Block of frozen CMC with holes filled with ^{125}I-blood at serial dilutions Sectioned at 40 µm thick w/ Leica CM3600 cryomicrotome at -20˚C Sections of Standards Imaged and Analyzed Using MCID® Software



Mass Balance & QWBA Analyses were performed using standard methods

Mass Balance – Amount of ^{125}I in urine (@ 0-8h, 8-24h, 24-48h, 48-72h), homogenized feces (@ 24 h intervals), cages residues (@ 24 h intervals), and homogenized carcasses (@72h) samples by gamma counting and % of administered ^{125}I was determined.

QWBA - carcass freezing, embedding, sectioning @ 40 µm, phosphor imaging, and Image analysis to determine tissue concentrations of ^{125}I radioactivity

Plasma Precipitation to assess biodehalogenation – Plasma was treated with/without Trichloroacetic acid (TCA) precipitation followed by gamma counting) to determine concentration and free and bound ^{125}I.

RESULTS

Mass Balance of ^{125}I-IgG alone

Specimen	Time	Mean % Recovery	SD	Cumulative
Feces	Pre-dose	0.00	0.00	0.00
	0-24 h	0.45	0.16	0.45
	24-48 h	0.50	0.12	0.95
	48-72 h	0.42	0.01	1.36
	Sub-total	1.36	0.03	
Urine	Pre-dose	0.00	0.00	0.00
	0-8 h	0.69	0.45	0.69
	8-24 h	4.84	0.53	5.53
	24-48 h	2.80	0.11	8.32
	48-72 h	2.09	0.03	10.41
	Sub-total	10.41	0.85	
Cage Rinse	24 h	0.68	0.18	0.68
	48 h	0.39	0.03	1.07
Cage Wash	72 h	1.14	0.25	2.20
Cage Residue	Sub-total	2.20	0.04	
Carcass	72 h	65.52	NC (n=2)	
Total		79.49	NC	

Mass Balance of ^{125}I-IgG w/NaI

Specimen	Time	Mean % Recovery	SD	Cumulative
Feces	Pre-dose	0.00	0.00	0.00
	0-24 h	0.35	0.20	0.35
	24-48 h	0.27	0.13	0.62
	48-72 h	0.29	0.19	0.92
	Sub-total	0.92	0.27	
Urine	Pre-dose	0.00	0.00	0.00
	0-8 h	4.88	0.99	4.88
	8-24 h	13.65	1.06	18.53
	24-48 h	6.11	0.71	24.64
	48-72 h	4.50	0.23	29.14
	Sub-total	29.14	0.56	
Cage Rinse	24 h	0.83	0.24	0.83
	48 h	0.42	0.09	1.26
Cage Wash	72 h	0.90	0.02	2.16
Cage Residue	Sub-total	2.16	0.26	
Carcass	72 h	61.23	NC (n=2)	
Total		93.04	1.00	

Mass Balance of ^{125}I-Na w/NaI

Specimen	Time	Mean v% Recovery	SD	Cumulative
Feces	Pre-dose	0.00	0.00	0.00
	0-24 h	0.44	0.12	0.44
	24-48 h	0.07	0.06	0.52
	48-72 h	0.01	0.01	0.53
	Sub-total	0.51	0.16	
Urine	Pre-dose	0.00	0.00	0.00
	0-8 h	35.16	13.32	35.16
	8-24 h	50.05	6.98	85.32
	24-48 h	1.35	0.48	86.56
	48-72 h	2.10	2.50	88.65
	Sub-total	88.76	5.76	
Cage Rinse	24 h	2.05	0.97	2.05
	48 h	0.45	0.21	2.51
Cage Wash	72 h	2.10	1.65	4.61
Cage Residue	72 h	1.54	1.96	6.15
Total		95.32	2.90	

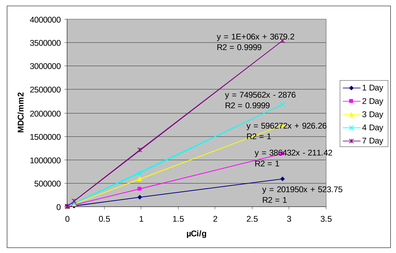
Mass Balance Observations:

1) Recovery of Radioactivity after dosing ^{125}I-IgG$_{rat}$ + NaI was higher than after dosing with ^{125}I-IgG$_{rat}$ alone.

2) Mass Balance was improved with co-administration of NaI in drinking water.

3) Loss of radioactivity in group treated with ^{125}I-IgG alone may have been due to carcass digestion in strong KOH where some ^{125}I was volatilized.

Image Calibration, and Limits of Quantitation LOQ



	µCi/g		
	24 h exp.	72 h exp.	168 h exp.
Av. Bckgrnd 5x5 mm	0.000077	0.000040	0.000004
Av. Bckgrnd 25x25 mm	0.000033	0.000048	0.000000
Av. Bckgrnd 5x5 mm, 5 mm from hi standard	0.002096	0.002205	0.002060
Av. Bckgrnd 5x5 mm, 10 mm from hi standard	0.000555	0.000493	0.000833
Av. Bckgrnd 5x5 mm, 15 mm from hi standard	0.000204	0.000204	0.000176
Low Stndrd used for Calibration Curve	0.000968	0.000107	0.000107

	µCi/g
	72 h exposure
Average Background 5x5mm	0.000040
Average Background 25x25mm	0.000048
5x5 mm; X + 3xSD	0.000136
5x5 mm; X + 5xSD	0.000200
25x25 mm; X + 3xSD	0.000151
25x25 mm; X + 5xSD	0.000219
Lowest Standard used for Calibration Curve	0.000107

The lowest standards (0.0001 – 0.0005 µCi/g) were unacceptable after a 1- or 2-day exposure time.
All standards (0.0001 – 2.91 µCi/g) were acceptable after a 3-, 4-, and 7-day exposures time.

"Flare' Effect for QWBA Image Analysis

Appearance of "flare from varying concentrations of radioactivity and at varying image contrast settings

1) After a 24, 72, and 168 h expo. the "Flare" distance was ~1-1.5 mm from the high standards.

2) Background readings were ≤10% higher than actual background readings at up to 1 mm away from the highest standard (10% at 1 mm, 7% at 1.5 mm, <1 % at 3 mm).

Effect of "Flare" from high source on background (or surrounding tissues)

Biodehalogenation

Treatment	Time Point	% ^{125}I-Protein
^{125}I-IgG w/NaI	1h	96.5
	48h	96.2
	72h	95.9
^{125}I-IgG alone	1h	95.5
	48h	97.1
	72h	96.2
^{125}I-Na w/NaI	1h	13.5
	48h	BQL
	72h	BQL
^{125}I-Na alone	1h	26.1
	48h	BQL

Stability of label of ^{125}I-Protein in Plasma

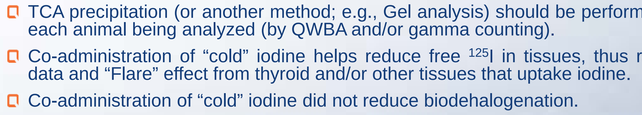
Rat ^{125}I-IgG appeared to be stably labeled in this experiment

^{125}I-Na appeared to associate with plasma proteins *in vivo*

# of Protiens Examined	Time point	% of ^{125}I-Protein in Plasma								
		10-20%	20-30%	30-40%	40-50%	~50-60%	60-70%	70-80%	80-90%	90-100%
n=11	5 min			1	2	1	2	2	2	1
n=5	30 min		3	2						
n=11	1h	1	3	2	2	3				
n=11	6h	3	2	1	1	2	1	1		
n=5	24h						1	1	1	2

Survey of other ^{125}I-Proteins in Plasma (w/ NaI): TCA precipitation results vary widely and may depend on the method of iodination and/or protein structure.; Appears most are relatively stable at early time points; Biodehalogenation appears to peak at ~0.5 – 6 h; Later time points show primarily labeled proteins.; Co-administration of NaI doesn't seem to effect Biodehalogenation.

Tissue Distribution by QWBA



Tissue	uCi/g					
	125I-Na w/NaI			125I-Na		
	1 h	48 h	72 h	1 h	48 h	
Blood	0.013	BQL	BQL	0.013	BQL	
Spleen	0.008	BQL	BQL	0.006	BQL	
Thymus	0.007	BQL	BQL	0.004	BQL	
Renal Cortex	0.010	BQL	BQL	0.007	BQL	
Renal Medulla	0.012	BQL	BQL	0.007	BQL	
Liver	0.009	BQL	BQL	0.005	BQL	
Urinary Bladder	0.204	BQL	BQL	0.064	BQL	
Urinary Bladder (contents)	0.220	0.001	0.001	0.078	0.001	
Adrenal Gland	0.012	BQL	BQL	0.005	BQL	
Thyroid Gland	0.026	0.003	0.013	0.136	0.317	
Salivary Gland	0.014	BQL	BQL	0.007	BQL	
Adipose (brown)	0.010	BQL	BQL	0.005	BQL	
Skin	0.013	0.001	0.001	0.014	0.004	
Prostate Gland	0.014	BQL	BQL	0.042	BQL	
Heart	0.009	BQL	BQL	0.006	BQL	
Skeletal Muscle	0.004	BQL	BQL	0.002	BQL	
Lung	0.014	BQL	BQL	0.009	BQL	
Large Intestine	0.011	BQL	BQL	0.000	BQL	
Small Intestine	0.011	BQL	BQL	0.019	BQL	
Stomach (gastric mucosa)	0.197	BQL	BQL	0.155	BQL	
Stomach (contents)	0.081	BQL	BQL	0.202	0.001	
TCA Results (% Bound)	13.50	NC	NC	26.10	NC	

Tissue	uCi/g					
	125I-IgG w/NaI			125I-IgG		
	1 h	48 h	72 h	1 h	48 h	72 h
Blood	0.098	0.037	0.032	0.091	0.034	0.031
Spleen	0.031	0.010	0.007	0.036	0.009	0.008
Thymus	0.009	0.005	0.004	0.003	0.005	0.004
Renal Cortex	0.014	0.008	0.007	0.020	0.008	0.006
Renal Medulla	0.016	0.009	0.008	0.025	0.009	0.007
Liver	0.027	0.008	0.006	0.030	0.008	0.005
Urinary Bladder	0.015	0.011	0.015	0.022	0.012	0.014
Urinary Bladder (contents)	0.048	0.007	0.004	0.018	0.001	0.002
Adrenal Gland	0.015	0.007	0.009	0.031	0.010	0.005
Thyroid Gland	0.011	0.006	0.010	0.077	1.747	1.808
Salivary Gland	0.034	0.015	0.012	0.005	0.009	0.006
Adipose (brown)	0.020	0.018	0.015	0.011	0.009	0.009
Skin	0.004	0.008	0.012	0.004	0.010	0.011
Prostate Gland	0.011	0.007	0.008	0.014	0.006	0.008
Heart	0.025	0.016	0.014	0.026	0.015	0.012
Skeletal Muscle	0.003	0.002	0.002	0.002	0.003	0.002
Lung	0.051	0.026	0.021	0.067	0.017	0.015
Large Intestine	0.004	0.006	0.018	0.006	0.005	0.006
Small Intestine	0.009	0.005	0.004	0.007	0.005	0.005
Stomach (gastric mucosa)	0.011	0.004	0.004	0.009	0.005	0.005
Stomach (contents)	0.005	0.001	0.001	0.008	0.003	0.003
TCA Results (% Bound)	96.50	96.20	95.50	95.50	97.10	96.20

1) Free ^{125}I was eliminated from tissues much faster than the ^{125}I-IgG (expected if stable radiolabel).

2) Co-administration of NaI increased elimination rate of free ^{125}I and blocked uptake in symporter tissues, thus enabling improved image analysis by decreasing areas of "flare" effect.

3) Co-administration did not seem to effect the concentration of free ^{125}I in skin or stomach.

4) Co-administration of NaI with ^{125}I-proteins that are resistant to biodehalogenation will still be helpful to reduce the "Flare" effect near tissue such as thyroid.

CONCLUSIONS

- Acceptable calibration curves were obtained after 3-, 4-, and 7-day exposure periods.
- LLOQ based on the lowest standard is efficient and effective without relying on background.
- High concentration calibration standards need to be placed away from other standards to avoid influence of "Flare" effect (at least 15 mm).
- The "Flare" distance from source did not change appreciably with longer exposure times. Most flare within 1 to 1.5 mm from source (10% at 1 mm, 7% at 1.5 mm, < 1 % at 3 mm)
- Co-administration of NaI in drinking water reduces localization of free ^{125}I in tissues thus reducing "hot" spots like thyroid and improves image sampling of surrounding tissues.
- Relatively larger animal models should be considered when performing tissue distribution by QWBA to facilitate tissue sampling of small tissues and to reduce effect of "Flare" on quantitation. (i.e., rat instead of mouse).
- Most biodehalogenation was observed at 0-6 h post-dose and can vary widely among different proteins.
- TCA precipitation (or another method; e.g., Gel analysis) should be performed on plasma and/or tissues of interest for each animal being analyzed (by QWBA and/or gamma counting).
- Co-administration of "cold" iodine helps reduce free ^{125}I in tissues, thus reducing influence on tissue concentration data and "Flare" effect from thyroid and/or other tissues that uptake iodine.
- Co-administration of "cold" iodine did not reduce biodehalogenation.
- Bio-Halogenation of endogenous proteins can occur after administration of ^{125}I.
- Co-administration of "cold" iodine decreases the amount of free ^{125}I and improved recovery at 72 h post-dose. (Did not achieve mass balance from rats given ^{125}I-IgG alone at 72 h post-dose).
- Proteins typically have much longer tissue half-lives than free ^{125}I, except for thyroid, which uptakes most free ^{125}I.
- Co-administration of "cold" iodine appears to shunt free ^{125}I to urine relatively quickly (0-6 h), thereby reducing confounding effect of flare and from "background" free ^{125}I.
- Tissue image measurements should be obtained at least 3 mm away from tissue with high concentrations to avoid "Flare" effect. Or tissues with suspected high levels (i.e. thyroid) can be removed from the section before imaging to avoid the "flare" effect altogether.

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