A Novel Method for the Early, Rapid Assessment of Tissue Distribution for New Drug Candidates

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Introduction

Considering the high rate of attrition for new drug candidates, it is increasingly important to understand the tissue distribution of novel compounds as early in the drug discovery process as possible. Elucidation of targeting, retention and accumulation properties can play a vital part in the decision to take a candidate further. The assessment of tissue distribution however is often a time-consuming process, involving expensive labelling and/or complicated analyses using mass spectrometry imaging.

Presented here are the results of a pilot study where five commercial drugs were analysed using a fast and cost-effective method to assess and compare their tissue distribution.

Methods

Five commonly available commercial drugs were selected for investigation:

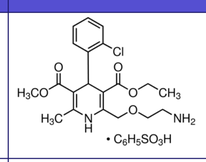
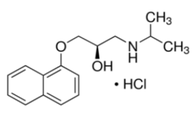
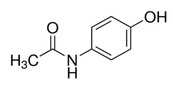
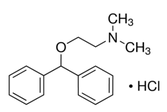
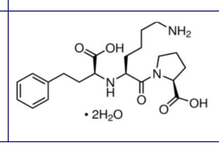
Compound (analysis times)	Structure	Dose route	Dose rate	Dose volume
Amlodipine (6 and 24 hours)		Oral	5 mg/kg	5 mL/kg
Propranolol (2 and 6 hours)		Oral	5 mg/kg	5 mL/kg
Paracetamol (1 and 6 hours)		Oral	5 mg/kg	5 mL/kg
Diphenhydramine (0.25 and 6 hours)		IV	1 mg/kg	10 mL/kg
Lisinopril (0.5 and 6 hours)		IV	1 mg/kg	10 mL/kg

Table 1: Study Design

To provide timely screening of test items, it was necessary to develop a rapid method of radiolabelling compounds of interest. This precludes synthesis, which is the usual way to make carbon-14 labelled compounds but can be a lengthy process. Tritium, however, can be introduced more quickly into molecules by exchange. There are many well-established methods using exchange with $^3H_2$ gas[1] or ^3H_2O and a catalyst. This method often gives multi-site labelling and, in the case of enolisable sites, the tritium label may be lost *in-vivo*. Recently a number of new methods have been published which allow the selective labelling of a wide range of molecules. These methods include photo-redox with ^3H_2O[2], Crabtree's catalyst/$^3H_2$[3], Kerr's catalysts/$^3H_2$[3], Fe catalyst/$^3H_2$[4] Ni catalyst $^3H_2$[5] and Ru nanoparticles/$^3H_2$[6].

A recent review [7] has shown that loss of ^3H label *in-vivo* is not often a problem. For this study, test materials were labelled using a simple tritium exchange method. Compounds were mixed with palladium black then exposed to tritium gas and heated. Resulting labelled material was adjusted to 1 mCi/mL using unlabeled material and stored in ethanol until required for analysis. Compounds were subjected to simple formulation in either ultra-pure water (oral dose) or sterile isotonic saline (intravenous dose).

Each dose formulation was administered to two male mice (CD1). One mouse was killed at T_{max} plus one later time point for each test material. Animals were frozen rapidly in a mix of hexane and solid CO_2 then processed for whole body autoradiography using procedures based on those outlined by Ullberg [8].

Frozen carcasses were sectioned using a Leica CM3600 Cryomicrotome and sections (30 µm thick) collected at two levels of the mouse body, to include as many tissues as possible. Sections were freeze-dried and analysed using a real-time digital autoradiographic imaging system.

Analysis

Based on gas detection, the real-time imager allows acquisition of autoradiography images by detecting the signal of each disintegration independently, thereby offering precise quantification of radio-distribution.

Quantification is independent of activity, with linearity over 5 orders of magnitude which is consistent over the entire field of view - in this case 20 x 20 cm. The result, a direct representation of the number of disintegrations detected, is obtained in real-time, with workable images acquired in just a few hours. Images from these sections were acquired overnight in runs of around 18 hours duration. At completion of acquisition, the number of counts within manually assigned regions of the resulting image can be determined, enabling simple quantification and calculation of tissue: blood ratios. (Figure 2).

Results

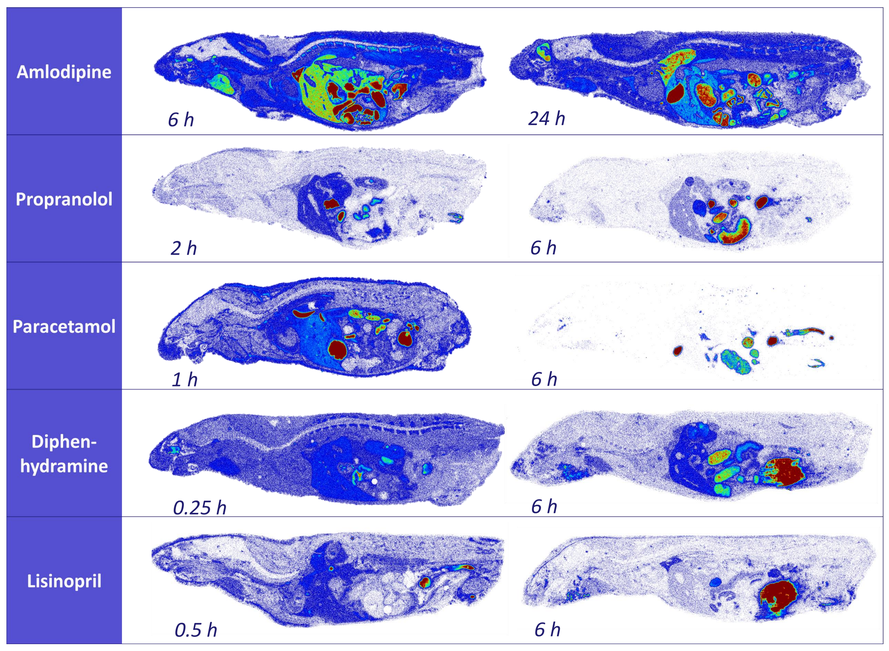


Figure 2: Autoradiography images obtained following an 18-hour acquisition

Images comparable with those obtained using the traditional approach of phosphor-storage plates and a 2-week exposure time were obtained after just 18 hours (Figure 1). Results were consistent with known distribution parameters for all compounds analysed. Using the plasma T_{max} plus one later time for analysis gave an indication of the rate of elimination from tissues, and enabled identification of potential accumulation/retention in target tissues.

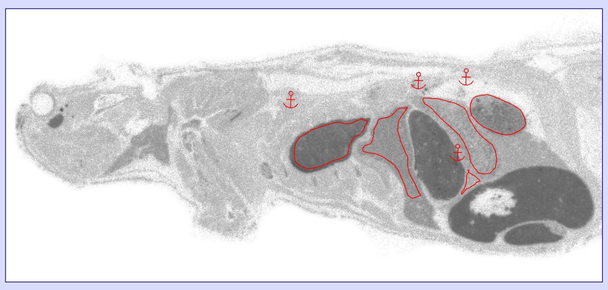


Figure 3: Quantification using imager software

Tissue	Tissue: blood ratios at 6 hours post-dose				
	Amlodipine	Propranolol	Paracetamol	Diphen-hydramine	Lisinopril
Brain	0.12	0.87	0.41	0.63	0.78
Kidney	9.78	3.69	16.5	5.81	48.9
Liver	9.76	5.88	6.97	7.61	7.33
Lung	22.1	1.87	2.44	2.43	58.1
Spleen	6.71	1.01	0.89	1.89	1.04

Table 2: Quantification results expressed as Tissue: blood ratios

Tissue: blood ratios were calculated for 5 tissues (Figure 3), allowing easy comparison of the distribution to these key tissues between test items (Table 2).

Conclusions

- **Rapid, cost-effective method for use in the support of drug discovery.**
- **From tritium labelling to results in as little as 2 weeks.**
- **Provides a clear indication of the distribution of the radiolabelled test material to as many as 40 different tissues within the rodent.**
- **Allows comparison of distribution to key tissues with basic quantification in the form of tissue: blood ratios to support observations.**
- **Can provide key target-engagement information for a panel of test materials to aid in the candidate selection process.**

References

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