A HIGH THROUGHPUT SCREENING ASSAY FOR CYP2D6 INHIBITION USING SPA

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Introduction

The P450 cytochromes (CYP) are a superfamily of enzymes found in the liver which catalyse the oxidative conversion of drugs and other lipophilic compounds to hydrophilic metabolites. CYP2D6 and CYP3A4, catalyse the metabolism of the major proportion of drugs available on the market. Inhibition of CYP-mediated metabolism accounts for a number of adverse drug-drug interactions [1], which often leads to withdrawal from the market [2,3].

A high throughput Scintillation Proximity Assay (SPA) was designed in collaboration with Merck to identify compounds that inhibit P450 CYP2D6[4]. It has subsequently been developed into a high throughput screening kit (product code: CFA772).

SPA is an innovative approach to high throughput screening which allows the rapid and sensitive assay of a wide variety of molecular interactions in a homogeneous system. As a result it is now routinely used in a broad range of applications including radioimmunoassays, receptor-ligand and enzyme assays.

SPA utilises microscopic beads containing scintillant that can be stimulated to emit light. This stimulation event only occurs when radiolabelled molecules of interest are bound to the surface of the bead. (fig. 1)

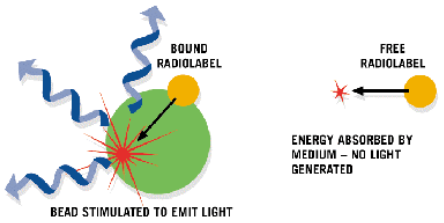

Figure 1. SPA concept diagram

The assay measures the conversion of a CYP2D6 substrate, [^{14}C]dextromethorphan to [^{14}C]formaldehyde, by selective capture of the product using yttrium silicate polyethyleneimine SPA beads (YSi-PEI SPA beads)[4].

Methods

CYP2D6 baculosomes (6.25µg) and [^{14}C]dextromethorphan (0.015µCi/~32,000cpm) were incubated for 15 minutes at 37°C in 75µl PBS buffer. 37.5µg NADPH in 25µl PBS buffer was added and incubated at 37°C for 30 minutes. 1mg YSi-PEI SPA beads in 50µl 0.05M NaOH was added to terminate the reaction. The plate was incubated overnight at room temperature and counted. Assay background was determined in the absence of NADPH. Test compounds were dissolved in DMSO and diluted in PBS to give a range of concentrations. They were added at the start of the assay in 25µl aliquots.

Results

Experimental data showed the kinetics of the assay to be linear with respect to enzyme concentration (fig. 2)

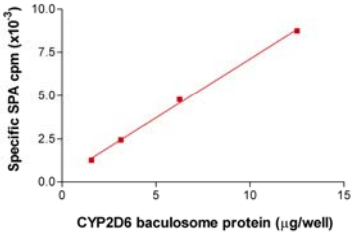

Figure 2. CYP2D6 titration. Values are means ±SEM (n=3).

The enzyme kinetics were also shown to be linear with respect to time for the 30 minute incubation period of the reaction (fig. 3). At 30 minutes signal:background was 9.

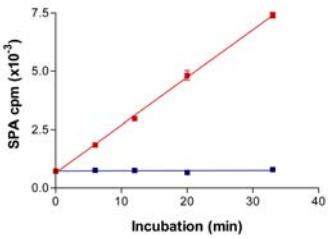

Figure 3. Time course over 30 minutes at 37°C. Totals (•), assay background (•). Values are means ±SD (n=3).

Non-linear regression analysis of 3 substrate curves obtained with [^{14}C] dextromethorphan gave a mean K_m of 2.9µM (fig. 4).

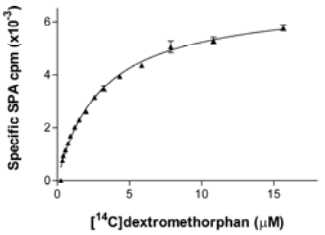

Figure 4. Representative substrate titration. Values are means ±SEM (n=3).

The IC_{50} of 9 inhibitors and 2 non-inhibitors of CYP2D6 were determined using the assay (fig. 5).

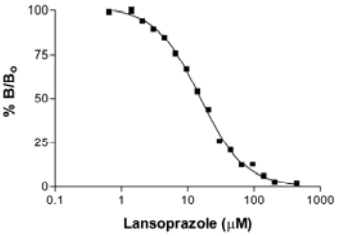

Figure 5. Lansoprazole inhibition of CYP2D6. Values are means ±SEM (n=3).

The K_m and IC_{50} values were used to determine k_i values for the inhibitors tested[5]. These results were compared with published k_i values[6-16]. (Table 1)

	k_i values µM		
Compound	SPA	95% confidence intervals	Literature
Omeprazole	295	256-342	240.7
Tranylcypromine	35.1	33.2-48.6	30
Tropisetron	16.5	12.6-21.6	14
Desipramine	4.1	3.8 - 4.4	12.5
Loratadine	1.99	1.75 - 2.27	2.7
Sulconazole	0.35	0.25 - 0.49	0.4
Quinidine	*0.0054*	*0.0058-0.010*	*0.018 – 0.043*
Lansoprazole	*7.0*	*6.3-7.7*	*44.7*
Metoclopromide	*23.1*	*17.5 - 30.5*	*4.7*
Diazepam	Determined not to inhibit CYP2D6 in the SPA assay or literature.		
Verapamil			

Table 1. Results obtained with a panel of test compounds. Compounds in italics are thought to be inactivators.

An excellent correlation was seen between the published and SPA values (fig. 6). Discrepancies occurred where the inhibitors were thought to be inactivators. This may be due to differences between the incubation periods for the literature and SPA assays, as the K_i of an inactivator is dependent on length of incubation time. However, the SPA assay has a 45 minute incubation period with test compounds and enzyme, hence, inactivators are detected by the assay.

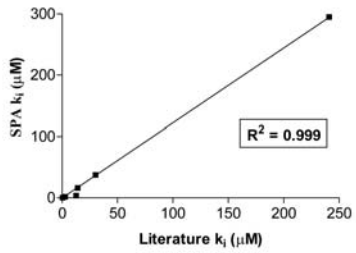

Figure 6. Correlation between literature and SPA k_i values. The k_i values determined for inactivators are not included.

The CYP2D6 assay was shown to have a Z' factor [17] of 0.89 (totals n=24, assay background n=24), showing that the assay is suitable for high throughput screening.

CONCLUSIONS

- We have developed a robust high throughput screening kit to measure CYP2D6 inhibition.
- The assay has a Z' factor of 0.89.
- There is an excellent correlation between K_i values generated in SPA and published values, for compounds which are not inactivators of CYP2D6.

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