A HIGH THROUGHPUT SCREENING ASSAY FOR HUMAN SERUM ALBUMIN BINDING USING SPA

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Introduction

Human serum albumin (HSA) is the most important carrier for acidic drugs in human plasma and has been shown to bind a large number of different compounds in a reversible manner. Several different ligand binding sites have been identified for HSA, two of which are major drug binding sites(1). Such drug-protein interactions are important in determining drug availability to, and elimination from, the body.

Amersham Biosciences has developed a high throughput Scintillation Proximity Assay (SPA) to identify compounds that bind to HSA. This assay utilises HSA captured with YSi beads, and two ligands, [^{3}H]ethynyloestradiol and [^{3}H]diazepam, which may be used in conjunction, or individually, to address two important HSA drug binding sites. The assay has been validated using a panel of nine compounds with known HSA binding profiles (35-99%) determined by equilibrium dialysis and ultra filtration.

Methods

HSA (10µg), underivatised yttrium silicate (YSi) bead (1mg), [^{3}H]ethynyloestradiol (0.02µCi/~20 000cpm) and/or [^{3}H]diazepam (0.08µCi/ ~65 000cpm) in a total volume of 40µl, were incubated for 20 hours at room temperature before counting. Non-specific binding was determined in the absence of HSA. Test compounds were dissolved in DMSO at a concentration of 2mM and added to the assay in 2µl aliquots to give a final concentration of 100µM in the assay.

Results

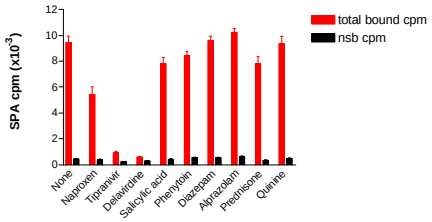

Figure 1. Binding of [^{3}H]ethynyloestradiol to HSA captured with YSi SPA beads. Competition against a panel of compounds with previously determined HSA binding profiles in order of decreasing binding to HSA (99-35%). Assay conditions were as previously described. Values are means ±SEM (n=3).

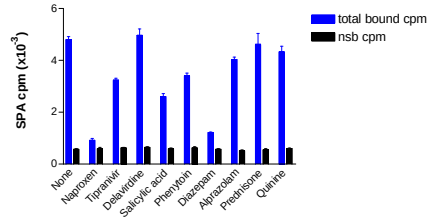

Figure 2. Binding of [^{3}H]diazepam to HSA captured with YSi SPA beads. Competition against a panel of compounds with previously determined HSA binding profiles. Assay conditions were as previously described. Values are means ±SEM (n=3).

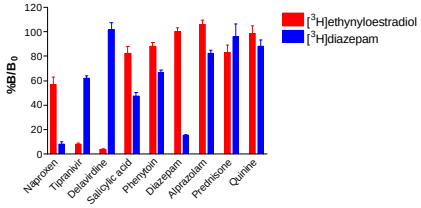

Figure 3. Binding of [^{3}H]ethynyloestradiol and [^{3}H]diazepam to HSA captured with YSi SPA beads as previously described. Binding data is normalised as %B/B$_0$. Values are means ±SEM (n=3).

The results show (figure 3) that there is very little interaction between [^{3}H]diazepam and [^{3}H]ethynyloestradiol binding to HSA, indicating that these two ligands bind to distinct HSA binding sites. Compounds such as naproxen, delavirdine and tipranivir that have been previously shown to bind 90-99% to HSA, using conventional methodologies, demonstrate selective binding to the two sites in the SPA assay; naproxen appears to bind preferentially to the diazepam site whilst delavirdine and tipranivir bind predominately to the ethynyloestradiol site (figure 3).

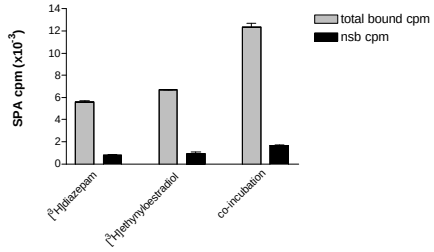

Figure 4. Binding of [^{3}H]diazepam and [^{3}H]ethynyloestradiol to HSA captured with YSi SPA beads. Assays were performed with the two ligands either incubated individually, or together, in the same well. Values are means ±SEM (n=3).

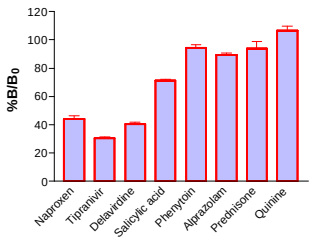

Figure 5. Binding of [^{3}H]diazepam and [^{3}H]ethynyloestradiol to HSA captured with YSi beads. Assays were performed with both ligands co-incubated in the same well. Values are means ±SEM (n=3).

As can be seen from figure 4, the total binding of the co-incubated [^{3}H]ligands is the sum of the individual ligand binding; thus providing further evidence that these ligands bind to two separate HSA binding sites. Performing the assay with the two ligands [^{3}H]diazepam and [^{3}H]ethynyloestradiol incubated together in the same well, allows the binding at both sites to be determined simultaneously (figure 5). When the assay is carried out in this format, there is a good correlation between the binding in the SPA assay and literature values of the % HSA binding, determined by ultra filtration and equilibrium dialysis, of the panel of compounds used in this study (figure 6).

This assay may therefore be used in two modes; as a high throughput screen to determine total binding to HSA at both sites, or as a high throughput site specific assay.

The assay is performed in a total volume of 40µl and is therefore amenable to use in 384-well format.

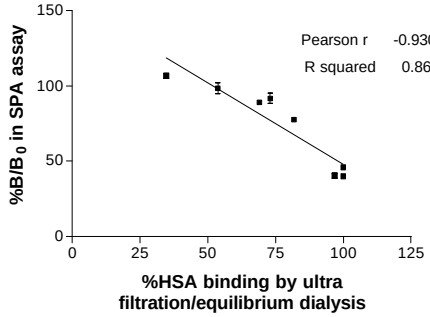

Figure 6. Correlation between %B/B$_0$ values obtained in the SPA and % binding to HSA determined by equilibrium dialysis or ultra filtration (literature values).

CONCLUSIONS

- We have developed a high throughput screening assay to measure HSA binding.
- The SPA assay utilises two radiolabelled ligands to determine binding at two important HSA drug binding sites.
- There is a good correlation between binding in the SPA assay and %HSA binding determined by equilibrium dialysis and ultra filtration (literature values).

References

1. ULRICH, K-H., *Pharmacological reviews,* **33**(1), 17-53, (1981).

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