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, [3H]ethynyloestradiol and [3H]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 (10g), underivatised yttrium silicate (YSi) bead (1mg), [3H]ethynyloestradiol (0.02Ci/20 000cpm) and/or [3H]diazepam (0.08Ci/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 [3H]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 [3H]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).

As can be seen from figure 4, the total binding of the co-incubated [3H]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, [3H]diazepam and [3H]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.

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


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