A HIGH THROUGHPUT SCREENING ASSAY FOR HUMAN α1-ACID GLYCOPROTEIN BINDING USING SPA AND LEADSEEKER TECHNOLOGIES

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Introduction

α 1-acid glycoprotein (AGP) and human serum albumin (HSA) are the most important drug binding proteins present in human plasma. HSA is considered to be largely responsible for the binding of acidic drugs whilst AGP is involved in the binding of basic drugs. Plasma AGP levels have been shown to vary considerably in certain disease states and therefore the determination of drug binding to AGP is important to the understanding of the pharmacokinetic and toxological properties of these drugs.

Amersham Biosciences has developed a high throughput Scintillation Proximity Assay (SPA) to identify compounds that bind to AGP. This assay utilises human AGP captured with wheat germ agglutinin yttrium silicate (WGA-YSi) SPA or yttrium oxide (WGA-YOx) LEADseeker™ beads, and a tritiated ligand, [^{3}H]propranolol. The assay was used to screen a panel of ten compounds, nine of which have previously been shown to bind to human AGP using conventional methodologies [(1)].

Method and Results

AGP (2.5μg), WGA-YSi or WGA-YOx bead (0.5mg) and [^{3}H]propranolol (0.03μCi/~35,000cpm) in a total volume of 40μl 50mM phosphate buffer, pH 7.5, were incubated for 25 hours at room temperature (20°C) SPA assays were counted using Packard TopCount™. LEADseeker assays were imaged for 5min, 3x3 binning with coincident averaging. Non-specific binding was determined in the absence of AGP. Test compounds were dissolved in DMSO at a concentration of 1mM and added to the assay in 1μl aliquots to give a final concentration of 25μM in the assay.

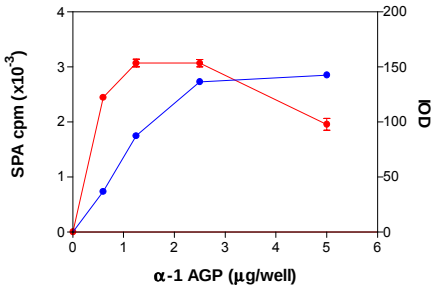

Figure 1. Binding of [^{3}H]propranolol to AGP captured with 0.5 mg YSi-WGA (●) SPA or YOx-WGA (●) imaging beads. Effect of increasing concentrations of AGP. Assay conditions were as previously described. Values are means ±SEM (n=3).

As can be seen from Figure 1, AGP was captured by both YSi-WGA and YOx-WGA beads. Maximum signal: background was achieved with 2.5μg/well AGP captured with 0.5mg SPA/LEADseeker bead. Therefore, 2.5μg/well AGP was used in subsequent assays. Signal: background was 7:1 for SPA assays, and 6:1 for LEADseeker assays.

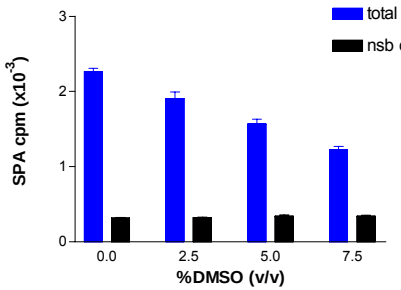

Figure 2. Binding of [^{3}H]propranolol to AGP captured with YSi-WGA SPA beads as previously described. Effect of DMSO addition. Values are means ±SEM (n=3).

As can be seen from Figure 2, the binding of [^{3}H]propranolol to AGP was slightly reduced by the addition of DMSO. In order to minimise this effect, compounds were added in 1μl DMSO to give a final concentration of 2.5% DMSO in the assay.

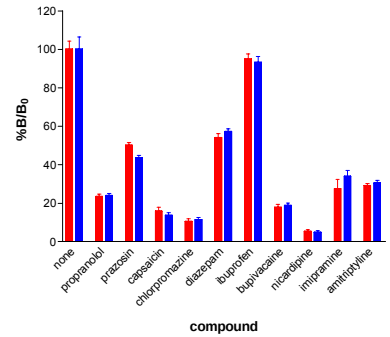

Figure 3. Binding of [^{3}H]propranolol to AGP captured with with SPA (●) or LEADseeker (●) beads. Competition against a panel of compounds. Binding was normalised as %B/B$_0$ to enable direct comparison of drug binding in both assays. Values are means ±SEM (n=3).

The results indicate (Figure 3), that the compounds imipramine, chlorpromazine, prazosin, nicardipine, bupivacaine, diazepam and amitriptyline, previously shown to bind to human AGP[(1)], displace [^{3}H]propranolol from AGP in both the SPA and LEADseeker assays. Capsaicin, not to our knowledge, previously shown to bind to human AGP, gave a high level of [^{3}H]propranolol displacement from AGP. Ibuprofen, previously shown to bind to human serum albumin [(2)], did not appear to displace [^{3}H]propranolol from AGP at the concentration tested.

Competition binding assays (Figure 4) were performed with propranolol, capsaicin and chlorpromazine; compounds shown in the assay to displace [^{3}H]propranolol from AGP (Figure 3).
EC_{50} values generated for capsaicin and chlorpromazine, 2.7 and 2.8μM respectively, were similar (Figure 4) whilst propranolol appeared to have a slightly higher EC_{50} (5.4μM). This indicates that capsaicin and chlorpromazine bind to AGP with slightly higher affinity than propranolol.

Therefore, the assay may be used to both identify, and determine the binding affinity of, compounds that bind to AGP.

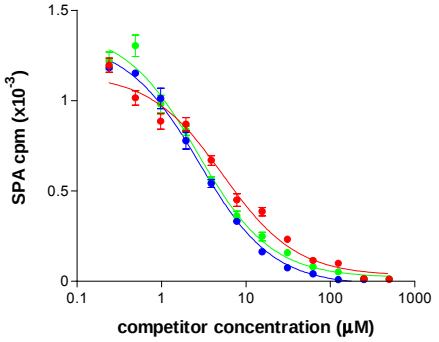

Figure 4. Binding of [^{3}H propranolol to AGP captured with YSi-WGA beads. Competition with propranolol (●) capsaicin (●) and chlorpromazine (●). A range of concentrations were prepared in DMSO and added in 1μl volumes. Values are means ±SEM (n=3).

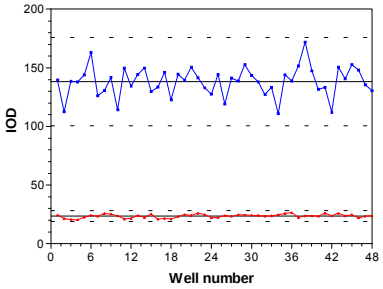

Figure 5. Z' factor analysis of LEADseeker assay, total binding (●) NSB (●). Solid lines indicate the mean of 48 observations; dashed lines indicate mean ± 3x standard deviation.

Z' [(3)] was determined for both SPA (data not shown) and LEADseeker (Figure 5) assays, and found to be 0.6 and 0.63 respectively, indicating that these assays are suitable for high throughput screening purposes.

Conclusions

- We have developed a high throughput screening assay to identify compounds that bind to human α 1-acid glycoprotein.
- The assay was shown to successfully identify compounds known to bind to α 1-acid glycoprotein.
- EC_{50} values were generated for capsaicin, chlorpromazine and propranolol.
- Z' values of >0.5 indicate that both the SPA and LEADseeker assays are suitable for high throughput screening purposes.

References

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