An Ultrafiltration Assay for Assessing the Extent of Whole Plasma Protein Binding of Antisense Oligonucleotides in Human and Various Species Plasma

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INTRODUCTION

ASOs are hydrophilic, poly-anionic, ~6-8 kDa molecules that are highly plasma protein bound (typically ~90%). ASOs bind to hydrophobic sites on plasma proteins, but do not displace small molecules, or vice versa. They display low affinity, non-specific, hydrophilic ASOs are hydrophilic, poly-anionic, ~6-8 kDa molecules that are highly plasma protein binding samples (pre-incubated at 37º C for at least 30 minutes to allow the ASO to bind to whole plasma protein) to the pre-treated filter, in three replicates. Incubate at rt for 10-15 min

EXPERIMENTAL CONDITIONS TESTED

1. Filter pre-treatment 1: 0.05%, 0.1%, 0.2%, 0.5%, 1%, 2% and 5% Tween 80

2. Filter pre-treatment 2: both ISIS-ASO-1 pre-treatment concentrations tested provided similar results

3. Spinning at: 3500 xg, 5000 xg, 7500 xg, 9000 xg, 12000 xg, 13000 xg and 14000 xg

4. Wash step (with or without) after pre-treatment with Tween 80

5. Wash step (with or without) after pre-treatment with ISIS ASO-1

EXPERIMENTAL CONDITIONS

Filters were pre-conditioned with Tween 80, and then with a non-specific ISIS ASO-1

Several ultrafiltration filters were assessed; the Nanosep© 30K Omega filter from Pall Life Sciences

RESULTS AND DISCUSSION

OBSERVATIONS

1. Filter pre-treatment 1 with Tween 80

2. Pre-treatment 2 with ISIS-ASO-1

3. Plasma sample ultrafiltration

4. Nuclease-dependent Hybridization ELISA

SAMPLE ANALYSIS

Typical hybridization ELISA data from back-calculated calibrations standards prepared in human, mouse and monkey plasma.

CONCLUSION

A robust and reliable ultrafiltration method was developed to determine the extent of the protein-binding of antisense oligonucleotides in human, cynomolgus monkey and mouse plasma. Details of the experimental approach used were presented along with key indicators that were found to be critical to the successful development of this ultrafiltration methodology.

Comparison of the performance of Millipore and Pall Life Sciences Nanosep® UF devices is summarized in Figure 2. Data shows that both UF devices provided very similar protein binding results and differences in % protein binding are within the intrinsic variability of the ELISA assay.

Filter pre-treatment 1: 0.05% to 2.0% Tween 80 had the same effect on blocking non-specific binding sites.

Filter pre-treatment 2: both ISIS ASO-1 pre-treatment concentrations tested provided similar results.

Centrifuge at 12,000 rpm, 10 min, rt

Incubate at room temperature (rt) for 10-15 min

Collect about 40 – 60 µL of the protein binding sample filtrate.

Wash membrane with water (300 µL x 2)

Centrifuge at 12,000 rpm, 10 min, rt

2. Pre-treatment 2 with ISIS-ASO-1

• Add 200 µL of ISIS-ASO-1

• Centrifuge at 12,000 rpm, 10 min, rt

3. Plasma sample ultrafiltration

• Add 200 µL of sample (250 ng/mL of specific ASO in water) or plasma protein binding samples (pre-incubated at 37º C for at least 30 minutes to allow the ASO to bind to whole plasma protein) to the pre-treated filter, in three replicates. Incubate at rt for 10-15 min

• Centrifuge at 12,000 rpm, 10 min, rt

• Collect 50 µL of the recovery filtrate

• Collect about 40 – 60 µL of the protein binding sample filtrate.

4. Nuclease-dependent Hybridization ELISA

Additional information about the protein binding assay is included in Table 2.

The lowest evaluated plasma concentration tested is expected to be near the anticipated clinical therapeutic dose peak plasma exposure levels. In addition, the highest evaluated plasma concentration tested exceeded peak plasma exposure levels observed in monkey toxicology studies.

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