Oligonucleotides represent a growing class of biotherapeutics currently being developed for a variety of indications. They are DNA or RNA molecules of approximately 20 nucleotides in length designed to hybridize to a target mRNA molecule, preventing its translation to a protein. In many cases, these large polyanionic molecules are developed with phosphorothioate backbones to improve their biological stability. Phosphorothioate oligonucleotides are biopharmaceuticals classified in the same class as antisense oligonucleotides, such that they have high aqueous solubility but low intestinal permeability, and are highly and quickly bound to plasma proteins (Deary, 2009; Su, et al., 2013). Despite the increased pharmaceutical development of phosphorothioate oligonucleotides, there are limited drug-drug interaction (DDI) data available on this class of biotherapeutics.

We previously examined the cytopathic P450 (CYP) inhibition potential of oligonucleotides and reported that the phosphorothioate, but not phosphodiester, backbone molecules caused potent inhibition of CYP1A2 and CYP2C9 in human liver microsomes (HLM) but not in cryopreserved human hepatocytes (CHH) (Dudley, 2006). In this study we expanded upon our previous work to include direct, time- (TDI) and metabolism-dependent inhibition (MDI) of CYP and UGT enzymes in pooled HLM and pooled CHH. Additionally, inhibition of the transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, Pgp and BCRP was also examined.

Materials and Methods

Oligonucleotides

Two ~20 base oligonucleotides with partial sequence homologies to glutathione peroxidase (5′-GCTGGTGGTCATCTGGGTGTAGT-3′) and beta actin (5′-CCTCGTTGGCGGATGTCTTTG-3′) were each synthesized with either a phosphorothioate or phosphodiester backbone (genetic structures shown in Figure 1) and were purchased from Integrated DNA Technologies (Coralville, IA).

Chemicals

Cholesterol (CDCA), digoxin, estradiol 17β-glucuronide, estrone sulfate, morphine, 1-naphthyl, saccharic acid 1,4-lactone, oxapax, oxapax-N-glucuronide and oxapax-N-proctoligand were purchased from Sigma (St. Louis, MO). Levoemodendrinone was a gift from Orion Corporation (Espoo, Finland).

Transfection of transporters

HEK293 cells transfected with the vector containing transporter cDNA by ADME & Tox. Research Institute, Sekisui Medical Co., Japan (kidney 293) cells expressing transporter (HEK293 cells transfected with vectors containing human transporter cDNA) were purchased from Integrated DNA Technologies (Coralville, IA). Oligonucleotides were transfected into HEK293 cells using FuGene HD Transfection Reagent (Promega, Madison, WI) at a concentration of 20 μM.

In Vitro CYP and UGT inhibition

The effects of oligonucleotides on CYP enzymes in HLM were evaluated in IC50 experiments with and without a preincubation step (in the presence and absence of NADPH) as described previously (Parkinson, et al., 2011). For CHH, incubations were conducted in 100 μl mixtures at 0.5 million cells/mL in KHB. Reactions were initiated by the addition of UDPGA and terminated by the addition of 100 μl of acetonitrile containing the appropriate internal standard. Precipitated protein was removed by centrifugation (6500 G for 10 min at 4°C) followed by LC/MS/MS analysis.

In Vivo uptake and efflux transporter inhibition

To evaluate inhibition of uptake transporters, MDCK cells over-expressing various uptake transporters were plated in 24-well plates and grown in an incubator (37°C, 95% relative humidity, 5% CO2) for two days. On the day of the assay, cells were pre-incubated with oligos or solvent control for 15 min followed by 2 min incubation with a 1.0 μM cold substrate. After the incubation, samples were stopped with 50:50 v/v ethanol:water. The biotransformation of specific substrate (10 μM for Caso-2 cells and 1 μM for MDCK-II cells) was measured in the presence of oligonucleotides. Oligos were added to both apical and basolateral sides for 120 min. After the incubation, samples were stopped with 50:50 v/v ethanol:water and analyzed by LC/MS/MS. IC50 values were determined with Graph (version 7.0; Eirthieric Software Ltd., Surrey, UK).

Results

As shown in Table 3, Figure 2 and Table 3, the results indicated that oligos #1 and #3 with the phosphorothioate backbones had little to no inhibitory effect on all CYP and UGT enzymes in HLM and CHH with the exception of oligo #3 in HLM for CYP2C19. Conversely, the oligos with the phosphorothioate backbone caused direct inhibition of CYP and UGT enzymes. The rank order of direct inhibition in HLM for oligo #2 was CYP1A2 > CYP2C8 > UGT1A2 > CYP3A4 while in CHH, the order was CYP1A2 > CYP2C8 > UGT1A2 > CYP2C9. The phosphorothioate oligonucleotides were found to inhibit all uptake transporters. Oligo #1 was found to inhibit OAT1 and OCT2; oligo #2 was found to inhibit OATP1B1, OATP1B3, OAT1, OAT3 and OCT2; oligo #3 was found to inhibit OAT1, OAT3 and OCT2; and oligo #4 was found to inhibit OATP1B1, OAT1, OAT3 and OCT2. While phosphorothioate oligos overall inhibited more uptake transporters, phosphorothioate oligos were inhibitory towards OAT1, OAT3 and OCT2. None of the oligos significantly inhibited the efflux transporters Pgp and BCRP.

Conclusions

In HLM, phosphorothioate but not phosphodiester oligonucleotides were found to be inhibitors of CYP and UGT enzymes.

In CHH, inhibition of CYP and UGT enzymes was dramatically less potent than in HLM, suggesting test system-dependent inhibition effects.

Both phosphorothioate and phosphodiester oligonucleotides were found to be inhibitors of uptake but not efflux transporters.

Overall these findings have implications on the design of in vitro DDI studies for oligonucleotide based therapeutics based upon test system-dependent differences.

References


