An assessment of the in vitro inhibition of cytochrome P450 enzymes (CYP), UDP-glucuronsyltransferases (UGT) and transporters by phosphodiester- or phosphorothioate-linked oligonucleotides

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Abstract

What is already known about this subject?

- Antisense oligonucleotides (ASOs) are increasingly being developed for many indications and are often modified with phosphorothioate linkages in lieu of phosphodiester linkages.
- Few in vitro DDI studies with ASOs have been performed, including the investigational imetelstat, volanesorsen, and ISIS 681257.
- No clinical DDIs have been reported.

What this poster adds:

- This study evaluated the inhibitory effects of two non therapeutic oligonucleotides with either phosphodiester (PD-GP and PD-Ac) or phosphorothioate (PT-GP and PT-Ac) linkages on the major drug-metabolizing CYPs and UGTs in both human liver microsomes (HLM) and cryopreserved human hepatocytes (CHH), and toward select transporters in expression systems.

Abstract:

Regulatory guidance documents do not have specific recommendations for ASO DDI evaluation, but these molecules do fall under the scope of overall DDI testing.

Aims: 1) Evaluate different ASOs as inhibitors of CYPs, UGTs and transporters in vitro. 2) Determine if in vitro inhibition of CYPs and UGTs in HLM by phosphorothioate ASOs is an artifact not observed in CHH.

Methods: Pooled HLM or CHH for CYP and UGT inhibition as well as HEK293 or MDCKII cell lines expressing the transporters were used. A range of concentrations of each ASO were incubated and /or pre-incubated (as applicable) with HLM, CHH or cells and then the probe substrate was added. Samples were analyzed using liquid scintillation counting or LC MS/MS.

Results: ASOs containing phosphodiester linkages had little-to-no inhibitory effect on any of the CYP and UGT enzymes in HLM with IC50 values generally greater than 100 µM. In contrast, phosphorothioate ASOs had inhibitory effects on most CYP and UGT enzymes evaluated. CYP1A2, 2C8 and UGT1A1 and 2B17 were most potently inhibited with IC50 values of 0.8 - 18 µM. There was little-to-no inhibitory effect in CHH. OATP1B1, OCT1, P-gp or BCRP were not inhibited by ASOs, while OATP1B3, OAT1 and 3, and OCT2 were inhibited with IC50 values ranging from 12 – 69 µM.

Conclusions: Inhibition of select uptake transporters was observed irrespective of oligonucleotide linkage. Phosphorothioate-based ASO inhibition of CYP and UGT enzymes in HLMs is an in vitro artefact without clinical relevance, and CHHs provide a more clinically relevant inhibitory evaluation of potential DDIs.

Introduction

Publicly available data with oligonucleotide therapeutics, such as eteplirsen, mipomersen, nusinersen, volenesorsen, and defibrotide, have shown no significant pharmacokinetic drug-drug interactions (DDIs) with co-administered medicines (University of Washington DDI database), suggesting that, as a class, ASOs do not affect the metabolic pathways of clearance of small molecule drugs. Upon performing pilot in vitro studies with proprietary phosphorothioate ASO molecules, we unexpectedly observed P450 inhibition in human liver microsomes (HLM). We hypothesized that this was not oligonucleotide sequence specific inhibition, but rather an artifact due to the presence of the phosphorothioate linkage. This observation prompted us to perform the present study with two generic non-therapeutic oligonucleotide sequences (partial sequences to glutathione peroxidase and beta-actin) with and without phosphorothioate linkages as surrogates for therapeutic ASOs. In the present study, phosphorothioate- and phosphodiester-linked oligonucleotides were evaluated as P450 and UGT inhibitors in both HLM and CHH. Furthermore, the potential for select transporter inhibition was also evaluated, specifically the uptake transporters such as organic anion transporting polypeptides (OATP), organic anion transporters (OAT), organic cation transporters (OCT) and the efflux transporters P glycoprotein (P-gp) and breast cancer resistance protein (BCRP). Overall this study reconciles in vitro DDI findings with the lack of interaction in clinical DDI reports for the oligonucleotide biomolecule class.

Materials & Methods

Note that this poster is based on the following publication which has complete details of the materials and methods: Kazmi F, Yerino P, McCoy C, Parkinson A, Buckley DB, and Ogilvie BW (2018) An assessment of the *in vitro* inhibition of cytochrome P450 enzymes, UDP-glucuronosyltransferases, and transporters by phosphodiester- or phosphorothioate-linked oligonucleotides. *Drug Metab Dispos* **46**:1066–1074.

A summary of the methods follows:

Oligonucleotides. Two ~20 base oligonucleotides with partial sequences towards glutathione peroxidase (5′ GCTCGTTCATCTGGGTGTAGT-3′) and beta actin (5′-CCTCCTCTTTGTTCCCTTCT-3′), each with either a phosphodiester or phosphorothioate linkage (generic structures shown in Figure 1) were purchased from Integrated DNA Technologies (Coralville, IA).

Test systems. Pooled human liver microsomes (HLM, n = 200, mixed gender) and pooled cryopreserved human hepatocytes (CHH, n = 100, mixed gender) were prepared from non-transplantable livers and characterized at XenoTech, LLC (Kansas City, KS). HEK293 cells expressing OATP1B1, OATP1B3, OAT1, OAT3 and OCT2 and control cells were obtained from the ADME & Tox. Research Institute, Sekisui Medical Co., Ltd. (Tokai, Japan); MDCKII cells transfected with BCRP were obtained from Netherlands Cancer Institute (Amsterdam, Netherlands); and Caco-2 cells were obtained from ATCC (Manassas, VA).

P450 and UGT inhibition. The effects of oligonucleotides on P450 enzymes in HLM were evaluated (n=2) in IC50 experiments with and without a 30 min preincubation step (in the presence and absence of NADPH) as described previously. For CHH, incubations were conducted in duplicate 100 µL mixtures containing 0.5 million cells/mL in Krebs-Henseleit buffer (KHB). Reactions were initiated by the addition of CHH to the oligonucleotides and incubations were conducted at 37°C with 95% humidity and 5/95% CO2/air for 0, 30 and 90 min, followed by addition of a P450 marker substrate (or the broad specificity UGT substrate 4-methylumbelliferone; 4-MU) at a concentration approximately equal to its Km. For UGT inhibition, assays were conducted as described previously. Briefly, oligonucleotides were incubated in duplicate at 37°C in 150 µL incubation mixtures containing pooled HLMs (≤ 0.1 mg/mL), Tris buffer (100 mM, pH 7.7), MgCl2 (10 mM), EDTA (1 mM, pH 7.4), saccharic acid 1,4-lactone (0.1 mM), UDP-GlcUA (10 mM), and a UGT marker substrate at a concentration approximately equal to its Km as described previously.

Materials & Methods (continued)

In vitro uptake and efflux transporter inhibition. To evaluate inhibition of uptake transporters, HEK293 cells expressing various human uptake transporters were plated in 24-well plates and grown in an incubator (37°C, 95% relative humidity, 5% CO_2) for two days. On the day of the assay, cells were pre-incubated with oligonucleotides or solvent control (n=2) for 15 min followed by a 2-min incubation with a combination of probe substrate (a mixture of cold and radiolabeled compound) and oligonucleotide. 50 nM estradiol 17-β-glucuronide substrate was used for OATP1B1 and OATP1B3 assays (with approximate equimolar radiolabeled and cold substrate); 1 µM p-aminohippuric acid for OAT1 (1:4 ratio of radiolabeled to cold substrate); 50 nM estrone 3-sulfate for OAT3 (approximately equimolar radiolabeled and cold substrate); and 5 µM tetraethylammonium bromide and 10 µM metformin for OCT1 and OCT2 respectively (all radiolabeled). Uptake assays were terminated by aspiration of the incubation media followed by washing the cells three times, first a rinse with ice cold PBS containing 0.2% BSA then two rinses with ice cold PBS. For analysis, cells were extracted with 0.1N NaOH for scintillation counting on a Microbeta2 instrument (Perkin Elmer, Waltham, MA). Evaluation of efflux transporter inhibition was conducted in Caco-2 (MDR1/P-gp) or MDCK-II cells (transfected with BCRP) plated on 24-well trans-well plates. The bidirectional permeability of specific probe substrates (10 µM digoxin for Caco-2 cells and 1 µM prazosin for MDCK-II cells) was measured (n=2) in the presence of oligonucleotides. Oligonucleotides were added to both apical and basolateral sides for 120 min. After the incubation, samples were stopped with 50:50 v/v methanol:water and analyzed by LC-MS/MS (as described previously).

Results

Table 1. IC_{50} values for P450 and UGT inhibition with phosphodiester (PD) or phosphorothioate (PT) oligonucleotides in human liver microsomes (HLM)

Enzyme	Substrate	IC_{50} (µM)											
		PD-GP			PT-GP			PD-Ac			PT-Ac		
		Direct	TDI	MDI	Direct	TDI	MDI	Direct	TDI	MDI	Direct	TDI	MDI
CYP1A2	Phenacetin	>100	>100	>100	0.8	0.8	1.8	>100	>100	>100	4.2	3.4	7.3
CYP2B6	Bupropion	>100	>100	>100	15	15	13	>100	>100	>100	39	20	22
CYP2C8	Amodiaquine	>100	>100	>100	1.1	0.6	0.6	>100	>100	>100	12	8.3	3.7
CYP2C9	Diclofenac	>100	>100	>100	97	36	47	>100	>100	>100	>100	53	57
CYP2C19	S-Mephenytoin	>100	>100	>100	21	8.7	19	29	36	>100	80	39	98
CYP2D6	Dextromethorphan	>100	>100	>100	81	41	>100	>100	>100	>100	>100	52	>100
CYP3A4/5	Midazolam	>100	>100	>100	>100	38	28	>100	>100	>100	>100	66	63
UGT1A1	Estradiol	>100	ND[1]	ND[1]	4.5	ND[1]	ND[1]	>100	ND[1]	ND[1]	5.4	ND[1]	ND[1]
UGT1A3	CDA	>100	ND[1]	ND[1]	51	ND[1]	ND[1]	>100	ND[1]	ND[1]	48	ND[1]	ND[1]
UGT1A4	Trifluoperazine	>100	ND[1]	ND[1]	>100	ND[1]	ND[1]	>100	ND[1]	ND[1]	>100	ND[1]	ND[1]
UGT1A6	1-Naphthol	>100	ND[1]	ND[1]	52	ND[1]	ND[1]	>100	ND[1]	ND[1]	55	ND[1]	ND[1]
UGT1A9	Propofol	>100	ND[1]	ND[1]	19	ND[1]	ND[1]	>100	ND[1]	ND[1]	42	ND[1]	ND[1]
UGT2B7	Morphine	>100	ND[1]	ND[1]	>100	ND[1]	ND[1]	>100	ND[1]	ND[1]	>100	ND[1]	ND[1]
UGT2B10	Levomedetomidine	>100	ND[1]	ND[1]	26	ND[1]	ND[1]	>100	ND[1]	ND[1]	41	ND[1]	ND[1]
UGT2B15	S-Oxazepam	>100	ND[1]	ND[1]	38	ND[1]	ND[1]	>100	ND[1]	ND[1]	45	ND[1]	ND[1]
UGT2B17	Testosterone	>100	ND[1]	ND[1]	7.2	ND[1]	ND[1]	>100	ND[1]	ND[1]	18	ND[1]	ND[1]

[1] Performed at 30 µM oligonucleotide

Table 2. IC_{50} values for P450 and UGT inhibition with phosphodiester (PD) or phosphorothioate (PT) oligonucleotides in human liver microsomes (HLM)

Transporter	Substrate	IC_{50} (µM)			
		PD-GP	PT-GP	PD-Ac	PT-Ac
OATP1B1	Estradiol 17-β-glucuronide	>100	90	>100	>100
OATP1B3	Estradiol 17-β-glucuronide	>100	14	>100	92
OAT1	*p*-Aminohippuric acid	46	12	52	16
OAT3	Estrone 3-sulfate	>100	26	69	>100
OCT1	Tetraethylammonium bromide	>100	>100	>100	>100
OCT2	Metformin	17	14	27	29
P-gp	Digoxin	>100	>100	>100	>100
BCRP	Prazosin	>100	>100	>100	>100

Figure 1. General structures of oligonucleotides with phosphodiester or phosphorothioate linkages

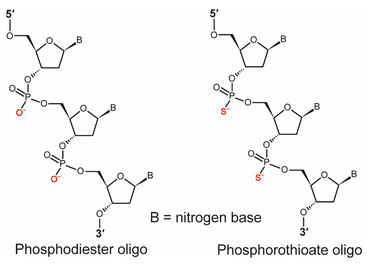


Figure 2. IC_{50} plots of CYP1A2, CYP2C8, and UGT1A1 inhibition by phosphodiester- or phosphorothioate-linked oligonucleotides in HLMs. The inhibition of CYP1A2 (phenacetin), CYP2C8 (amodiaquine), and UGT1A1 (estradiol) activity was assessed at 0.1–100 µM oligonucleotide in HLMs at 0.1 mg/ml, with or without a preincubation in the absence of NADPH cofactor (30 minutes) for the evaluation of direct inhibition and TDI, respectively, or in the presence of NADPH cofactor for the evaluation of MDI.

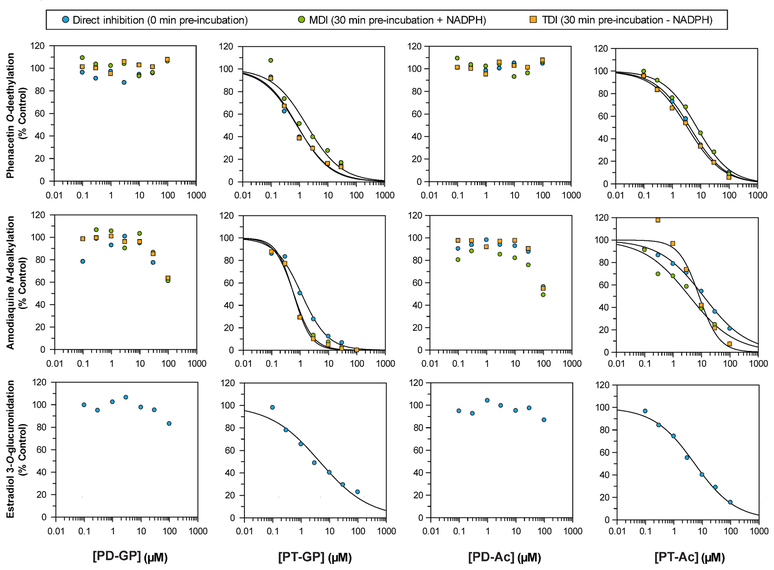


Figure 3. Assessment of the reversibility of CYP2C8 TDI by phosphorothioate-linked oligonucleotides in HLMs. Phosphorothioate oligonucleotides were incubated at 10 µM for 30 minutes in HLMs (0.0125 mg/ml), followed by ultracentrifugation (100,000*g*) and reisolation of the microsomal fraction. Residual CYP2C8 (amodiaquine) activity was assessed after protein normalization.

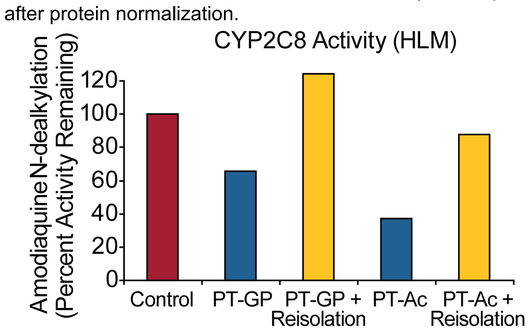


Figure 4. IC_{50} plots of CYP1A2, CYP2C8, and UGT inhibition by phosphodiester- or phosphorothioate-linked oligonucleotides in CHHs. Inhibition of CYP1A2 (phenacetin), CYP2C8 (amodiaquine), and overall UGT (4-methylumbelliferone) activity was assessed at 0.1–100 µM oligonucleotide in CHHs at 0.5 million cells/ml, with or without a preincubation (30 and 90 minutes) for the evaluation of direct inhibition and MDI.

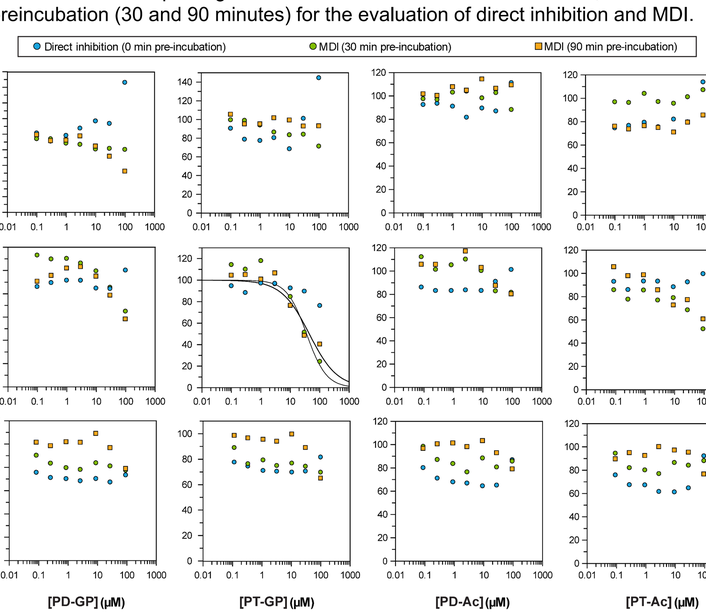
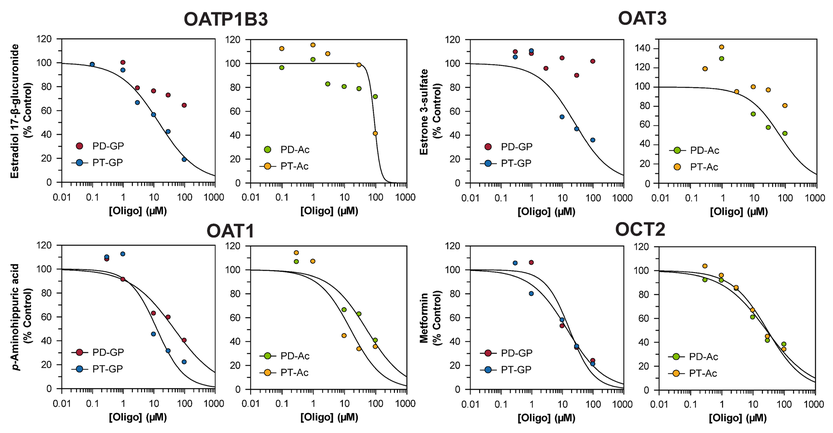


Figure 5. plots of OATP1B3, OAT1, OAT3, and OCT2 inhibition by phosphodiester- or phosphorothioate-linked oligonucleotides. Inhibition of OATP1B3 (estradiol 17-β-glucuronide), OAT1 (*p*-aminohippuric acid), OAT3 (estrone 3-sulfate), and OCT2 (metformin) activity was assessed at 0.1–100 µM oligonucleotide in transporter-overexpressing HEK293 cells.



Conclusions

- The data suggest that phosphorothioate-based oligonucleotide inhibition of P450 and UGT enzymes in HLMs is an in vitro effect without clinical relevance, and that CHHs provide a more clinically relevant inhibitory profile for use in in vitro to in vivo extrapolation (IVIVE) of DDIs.
- In vitro P450 inhibition studies with therapeutic phosphorothioate-based ASOs, and the thio-phosphoramidate, Imetelstat, and CHH has shown a lack of in vitro inhibition and consequently no predicted in vivo DDI (Shemesh et al., 2017; Kazmi et al., 2018), consistent with the results of this study.
- There is a lack of clinical interaction reports for ASOs in the literature, which would reconcile with the lack of in vitro CHH findings by ASOs in this study.
- **It is recommend that, for phosphorothioate-based ASOs as a molecule class, P450 and UGT inhibition studies be performed in CHH and not in HLM**

References

Poster based on: Kazmi F, Yerino P, McCoy C, Parkinson A, Buckley DB, and Ogilvie BW (2018) An assessment of the in vitro inhibition of cytochrome P450 enzymes, UDP-glucuronosyltransferases, and transporters by phosphodiester- or phosphorothioate-linked oligonucleotides. *Drug Metab Dispos* **46**:1066–1074.

Other references: Kazmi F, Sensenhauser C, ahd Greway T (2019) P450-Based DDI Potential of the Oligonucleotide Imetelstat. *Drug Metab Dispos* **47**:9–14.

Shemesh CS, Yu RZ, Warren MS, Liu M, Jahic M, Nichols B, Post N, Lin S, Norris DA, Hurh E, et al. (2017) Assessment of the drug interaction potential of unconjugated and GalNAc3-conjugated 29-MOE-ASOs. *Mol Ther Nucleic Acids* **9**:34–47.

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