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Review Article

Assessment of Drug-Drug Interaction Potential for Antisense Oligonucleotide Therapeutics

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Abstract

Antisense oligonucleotide (ASO) therapeutics have unique physicochemical properties, which differentiate them from small molecule drugs and large therapeutic proteins, in that ASOs are hydrophilic, highly water soluble, and poly-anionic, with molecular weight in the range of 6000-8000 Da. Although drug-drug interaction (DDI) assessment for ASOs largely follows the FDA/EMA guidelines established for small molecules, the unique characteristics of ASOs and low potential of DDIs are more like therapeutic proteins. In this paper, the literature was reviewed regarding *in vitro* and *in vivo* DDI assessments for ASOs related to major CYP450 enzymes and transporters. The clinical relevance and implications of ASO-associated DDIs are also discussed.

Keywords: *Antisense Oligonucleotide; ASO; Drug-Drug Interaction; DDI; CYP450; Transporters*

Introduction

Antisense oligonucleotides (ASOs) are short, chemically modified, synthetic, single-strand DNA/RNA like oligonucleotides (typically 16-20 nucleotides in length) that have the ability to hybridize with the target complementary RNA via sequence-specific Watson-Crick base paring, therefore, modulating the target RNA level and protein biosynthesis [1,2]. As a therapeutic platform, ASOs have been in drug development for over two decades, numerous chemical classes have been developed, and several compounds are approved for commercial use [3,4]. ASOs have unique physicochemical properties, which differentiate them from small molecule drugs and large protein drugs, in that ASOs are hydrophilic, highly water soluble, and poly-anionic, with molecular weight in the range of 6000-8000 Da. The PK properties of ASOs are remarkably similar across sequence, chemistry, and species, which makes their PK in humans highly predictable [5-10]. The clinical pharmacology characteristics of ASOs as a platform are generally considered to be well understood [5-8].

More recently one of the major breakthroughs was the GalNAc-conjugation of ASOs to target mRNA expressed in hepatocytes, which demonstrated prodrug-like properties, improving the potency up to 30-fold over unconjugated ASOs [6]. This led to a significant reduction in the clinical dose, thereby reducing the systemic exposure as well as exposure in extra-hepatic organs or tissues, thus improving the overall safety profiles of the ASOs. All GalNAc-conjugated ASOs in clinical development showed no impact on platelet level and no kidney related toxicities [6]. The clinical pharmacology profiles of GalNAc-ASOs have been reviewed recently [7]. Similar to unconjugated ASOs, GalNAc-conjugated ASOs demonstrated 1) lack of drug-drug interactions with small molecule drugs; 2) lack of QT prolongation at clinically relevant doses; 3) no or minimal effects on PK exposure for patients with mild or moderate renal or mild hepatic impairment (Ionis internal data); and 4) while being immunogenic following chronic treatment, the observed anti-drug antibodies behave like binding proteins, not neutralizing antibodies [7,8]. In this review, the potential of drug-drug interactions *in vitro* and *in vivo* are summarized and clinical implications are discussed for both unconjugated and conjugated ASOs.

In vitro CYP450-related Interactions

Both GalNAc-conjugated and unconjugated ASOs are metabolized by endo- or exonucleases which are ubiquitously expressed in all tissues or organs. GalNAc-conjugated ASOs behave like prodrugs, which deliver the parent ASOs preferentially to hepatocytes over non-parenchymal cells, where the GalNAc cluster is rapidly metabolized and excreted rapidly via biliary and renal routes, with the parent ASOs being metabolized slowly as unconjugated ASOs dosed directly. Neither unconjugated nor conjugated ASOs are substrates of any major CYP enzymes or transporters [9,11, 12]. Examples include *in vitro* studies in cryopreserved human hepatocytes with three unconjugated 2'-MOE-modified ASOs (ISIS 304801, ISIS 396443, and ISIS 420915) and one GalNAc-conjugated 2'-MOE ASO (ISIS 681257), which showed that none of these ASOs were inhibitors (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) nor inducers (CYP1A2, CYP2B6, and CYP3A4) of major cytochrome P450 isoforms [11] (Figure 1). Similar results were obtained for multiple ASOs of the same class (Ionis Internal Database). Thus, pharmacokinetic DDIs with small molecule drugs at CYP450 enzyme levels are not expected.

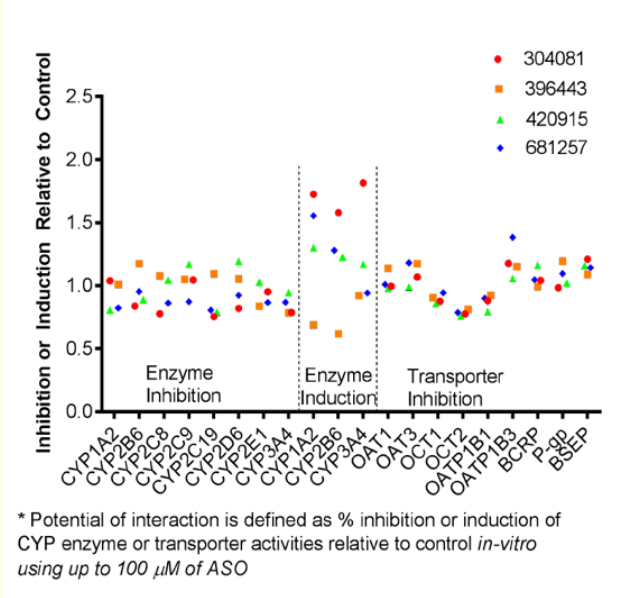

Figure 1: *Evaluation of In Vitro DDI Potential of 2'-MOE ASOs, without (ISIS 304801, ISIS 396443, and ISIS 420915) and with (ISIS 681257) GalNac-Conjugation [Shemesh., et al 2017].*

In vitro transporter-related interactions

ASOs with and without GalNAc3-conjugation are not substrates or inhibitors of hepatic uptake transporters such as organic cation transporter 1 (OCT1), organic anion transporting polypeptide 1B1 (OATP1B1), and OATP1B3, renal uptake transporters such as organic anion transporter 1 (OAT1), OAT3, and OCT2; and efflux transporters such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and bile salt export pump (BSEP) (Figure 1) [9, 11]. Thus, pharmacokinetic DDIs with small molecule drugs at transporter levels are not expected.

Clinical DDI studies

A clinical study in Type II diabetic patients administered ISIS 113715, a 2′-MOE-modified unconjugated ASO, did not show any changes in the C_{max} or AUC (geometric mean ratio was approximately 1.0 with and without ISIS 113715) of metformin (a substrate of OCT1 and OCT2, as well as MATE1 and MATE2), glipizide, or rosiglitazone indicating the lack of pharmacokinetic drug-drug interactions with these small molecules (Table 1). Similarly, no changes in the C_{max} and AUC of ISIS 113715 was observed with and without these small molecules (Table 2) [12,13].

Compound/ Molecular Target	Disease Indication	ASO Route/Dose	Co-administered Drug (Route/Dose)	Geometric Mean Ratio for Co-administered Drug (Test/Ref) [90% CI]	Published Reference
ISIS 113715/ PTP1B	Type II diabetes	SC/200 mg	Glipizide PO/5mg	Cmax: 1.02 [0.81 – 1.28] AUC: 1.02 [0.76 – 1.37]	Geary et al 2006
ISIS 113715/ PTP1B	Type II diabetes	SC/200 mg	Metformin PO/500 mg	Cmax: 0.91 [0.73 – 1.14] AUC: 0.96 [0.79 – 1.16]	Geary et al 2006
ISIS 113715/ PTP1B	Type II diabetes	SC/200 mg	Rosiglitazone PO/2 mg	Cmax: 1.00 [0.82 – 1.22] AUC: 0.91 [0.73 – 1.14]	Geary et al 2006
Mipomersen/ ApoB-100	Hyper-Cholestrolemia	IV/200 mg	Simvastatin PO/40 mg	Simvastatin: Cmax: 0.512 [0.341 – 0.770] AUC: 1.28 [0.899 – 1.82] Simvastatin acid: Cmax: 0.804 [0.659 – 0.982] AUC: 1.11 [0.888 – 1.38]	Yu et al 2009
Mipomersen/ ApoB-100	Hyper-Cholestrolemia	IV/200 mg	Ezetimibe PO/10 mg	Free ezetimibe: Cmax: 0.578 [0.405 – 0.824] AUC: 1.00 [0.777 – 1.27] Total ezetimibe: Cmax: 0.740 [0.568 – 0.963] AUC: 0.893 [0.699 – 1.14]	Yu et al 2009
Mipomersen/ ApoB-100	Hyper-Cholestrolemia	SC/200 mg	Warfarin PO/25 mg	R-Warfarin: Cmax: 1.06 [1.01 – 1.11] AUC: 1.11 [1.08 – 1.14] S-Warfarin: Cmax: 1.05 [0.997 – 1.11] AUC: 1.10 [1.06 – 1.13]	Li et al 2014
GalNAc-2'-MOE ASO (pelacarsen; ISIS 681257; TQJ230)	HVs	SC/40 mg	Warfarin PO/25 mg	R-Warfarin: Cmax: 1.04 [0.99 – 1.10] AUCinf: 1.06 [1.03 – 1.09] S-Warfarin: Cmax: 1.04 [0.98 – 1.10] AUCinf: 1.05 [1.03 – 1.08]	Ionis Internal Data (CS10)
GalNAc-2'-MOE ASO (pelacarsen; ISIS 681257; TQJ230)	HVs	SC/40 mg	Clopidogrel PO/75 mg	Clopidogrel Cmax,ss: 1.12 [0.99 – 1.27] AUC0-24h, ss: 1.07 [0.95 – 1.20]SR26334 (major metabolite) Cmax,ss: 1.02 [0.89 – 1.16] AUC0-24h, ss: 1.05 [1.01 – 1.10]	Ionis Internal Data (CS11)

Table 1: *Summary of Potentials Effects of 2′-MOE ASOs on the Pharmacokinetics of Co-Administered Small Molecule Drugs in Clinical Studies.*

Compound/ Molecular Target	Disease Indication	ASO Route/ Dose	Co-administered Drug (Route/Dose)	Geometric mean ratio for ASO (Test/Ref) [90% CI]	Published Reference
ISIS 113715/ PTP1B	Type II diabetes	SC/200 mg	Glipizide PO/5mg	Cmax: 1.06 [0.75 – 1.49] AUC: 0.95 [0.73 – 1.23]	Geary et al 2006
ISIS 113715/ PTP1B	Type II diabetes	SC/200 mg	Metformin PO/500 mg	Cmax: 0.93 [0.65 – 1.31] AUC: 0.98 [0.72 – 1.34]	Geary et al 2006
ISIS 113715/ PTP1B	Type II diabetes	SC/200 mg	Rosiglitazone PO/2 mg	Cmax: 1.46 [1.04 – 2.05] AUC: 1.15 [0.80 – 1.65]	Geary et al 2006
Mipomersen/ ApoB-100	Hyper-Cholestrolemia	IV/200 mg	Simvastatin PO/40 mg	Cmax: 0.978 [0.928 – 1.03] AUC: 1.00 [0.936 – 1.07]	Yu et al 2009
Mipomersen/ ApoB-100	Hyper-Cholestrolemia	IV/200 mg	Ezetimibe PO/10 mg	Cmax: 1.05 [0.864 – 1.28] AUC: 1.01 [0.924 – 1.11]	Yu et al 2009
Mipomersen/ ApoB-100	Hyper-Cholestrolemia	SC/200 mg	Warfarin PO/25 mg	Cmax: 1.17 [1.02 – 1.33] AUC: 1.17 [1.09 – 1.24]	Li et al 2014
GalNAc-2′-MOE ASO (pelacarsen; ISIS 681257; TQJ230)	Cardiovascular disease	SC/40 mg	Warfarin PO/25 mg	Cmax: 1.05 [0.94 – 1.17] AUC0-48h: 1.07 [1.02 – 1.12]	Ionis Internal data

Table 2: *Summary of Potentials Effects of Co-Administered Drugs on the Pharmacokinetics of 2′-MOE ASOs in Clinical Studies.*

Rosiglitazone has been shown to inhibit transporter activity of OCT1 and OCT2, as well as OATP1B1 and OATP1B3, but had no impact on ISIS 113715 pharmacokinetics, further suggesting that the ASOs are neither substrates for nor have interactions with these transporters of small molecule drugs [12]. Moreover, there was no evidence of DDI in clinic with mipomersen, another 2′-MOE-modified unconjugated ASO either as a perpetrator (Table 1) or victim (Table 2), after co-administration of simvastatin, ezetimibe, or warfarin [14,15]. More recently, two clinical DDI studies were carried out on a GalNAc-conjugated 2′-MOE-ASO with warfarin and clopidogrel. Results showed no changes in PK or PD of warfarin and clopidogrel (Table 1) or the PK of the ASO (Table 2) when co-administered together.

Discussion

ASOs as a chemical class with or without GalNAc-conjugation have demonstrated unique pharmacokinetic profiles. Both GalNAc-conjugated and unconjugated ASOs are rapidly absorbed into the systemic circulation following SC dosing. After reaching the maximum plasma concentration (C_{max} within 1 to 4 hours post dose), the plasma concentration declines rapidly, governed by rapid distribution to tissues, primarily liver and kidneys. The major difference between GalNAc-conjugated and unconjugated ASOs is that unconjugated ASOs distribute mostly to non-parenchymal cells such as Kupffer cells and endothelial cells, only a small fraction, less than 15% to hepatocytes [6], whereas GalNAc-conjugated ASOs distribute primarily to hepatocytes since the asialoglycoprotein receptor (ASGPR) is expressed primarily on hepatocytes [6,7]. Unconjugated ASOs are metabolized by ubiquitous endo- or exonucleases, and the chain-shortened metabolites are rapidly eliminated to urine due to low protein binding in plasma or tissues. The GalNAc-conjugated ASOs are metabolized after being internalized to hepatocytes, involving lysosomal hydrolase (N-acetyl-β-glucosaminidase) to cleave off GalNAc sugars from the linker, the lysosomal DNase II (deoxyribonuclease II) to cleave the linker off from the ASO, followed by rapid oxidative metabolism prior to biliary excretion or renal excretion [7,16]. All these metabolism and excretion pathways are considered as high-capacity processes considering the rapid degradation and eliminations of GalNAc-linker-associated metabolites (within 24 hours post dose) as observed in the ADME studies [16, and Ionis internal data]. Cumulative data as summarized in this review suggest that ASOs including GalNAc-ASOs are not substrates, inhibitors, or inducers of major cytochrome P450 enzymes *in vitro*, nor showing DDIs *in vivo* with small molecules that are predominately cleared through oxidative metabolic pathways [11-15]. Additionally, 2′-MOE-modified ASOs are not substrates or inhibitors of uptake or efflux membrane transporters (e.g., OATP, OAT, MDR1, etc.) [9,11]. No metabolic or transporter-based drug–drug interactions have been observed to date when ASOs are treated as either a perpetrator or victim in the study as shown in tables 1 and 2 and reported previously [12-15].

Pharmacological DDIs could take place at the down-stream level related to proinflammatory cytokine release caused by protein drug administration (FDA DDI Guidance 2020) which may affect CYP expression, thus affecting the enzyme activity and exposure for CYP substrates. Similar concerns could be raised for ASOs or any RNA related therapies because of the potential of cytokine release following SC administration. The clinical relevance of cytokine mediated DDIs is unlikely but may warrant further evaluation.

From the uptake perspectives, ASOs distributes to tissues mostly via receptor-mediated endocytosis and specifically ASGPR-mediated uptake into hepatocytes for GalNAc-conjugated ASOs. Thus, factors affecting the capacity or binding affinity of the ASGPR could potentially affect both the plasma AUC and liver tissue exposure as discussed elsewhere [7]. However, since the ASGPR-mediated uptake is a rapid process with a high capacity and short recycling time, approximately 10 - 15 min [17,18], and well conserved across species, minor effect on the initial rate of uptake may not have a major effect on the total amount of drug distributed to tissues over time considering the low uptake to or elimination from extra-hepatic organs including renal excretion. A PK/PD study in mice showed that greater than 50% reduction in ASGPR level did not affect the pharmacological activity of a GalNAc3-siRNA conjugate in animal models [19]. Nonetheless, the clinical relevance of DDIs at the ASGPR level remain to be determined.

Implications

Cumulative *in vitro* and *in vivo* DDI data, as discussed above, suggest that no clinical DDI interactions would be expected between 2′-MOE or cEt ASOs and small molecule drugs or therapeutic proteins. Conventional PK clinical DDI assessment is not suggested for similar 2′-MOE or cEt ASOs in development under general circumstances. Evaluation of ASOs with different chemistries, if needed, can be done first in vitro and results be compared to existing data from current chemistries to rule in/rule out DDI potential before embarking on a clinical DDI study. Pharmacodynamic interactions or proinflammatory cytokine-related down-stream interactions may be considered for routinely used co-medications, if justified on a case-by-case basis from the clinical perspectives. Similar findings and recommendations have been reported for siRNAs [20].

From an *in vitro* study design consideration standpoint, primary human hepatocytes are a more clinically relevant model than human liver microsomes. A pilot experiment may be needed to characterize the distribution kinetics and select an appropriate ASO dose/concentration in the incubate to ensure adequate and clinically relevant concentrations are achieved intracellularly prior to the *in vitro* DDI studies. ASOs have a high protein binding including CYP enzyme proteins in human liver microsomes, and hence the DDI potential could be over predicted in this system [21]. ASOs distribute to tissues via carrier-mediated endocytosis, and stay mostly in endosomes and lysosomes, where it is degraded; only a small fraction of ASO molecules would escape and distribute to nucleus or other microenvironment to exert its pharmacology. Thus, using an *in vitro* system that mimics the endocytosis process *in vivo*, such as hepatocytes, would be recommended.

Conclusions

Antisense oligonucleotide (ASO) therapeutics as a platform have unique physicochemical properties and pharmacokinetic profiles, which differentiate them from small molecule drugs and large therapeutic proteins. Although not as large as therapeutic antibodies, ASOs are much bigger than small molecule drugs. Thus, ASOs behave more like large molecules, they don't seem to share the same transporters for small molecule drugs. Similarly, ASOs are metabolized by endo- or exonucleases, and not by CYP450 enzymes. Although the metabolism of the GalNAc-linker may involve hydrolysis and oxidative enzymes, these pathways are considered as high-capacity kinetic processes. No major CYP450-related interactions are expected nor observed as supported by current literature data in hepatocytes or in humans. No further *in vitro* DDI studies nor clinical DDI studies would be recommended for this class of 2'-MOE- or cEt-ASOs with or without GalNAc conjugation unless new chemical modifications are made, in such case selected *in vitro* DDI studies may be conducted to determine if the platform knowledge and recommendation can be further generalized. For pharmacological interactions e.g., concomitant medications, a population PK/PD approach may be adopted and the effect of co-medication on ASO and vice versa, can be evaluated in a covariate analysis to assess any meaningful impact on exposure, efficacy, or safety.

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