Development of a Plasma Stability Assay for Oligonucleotides

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Abstract

The plasma stability assay plays an important role in drug discovery and development phases. Unstable compounds tend to have rapid clearances and short half-lives, resulting in poor *in vivo* performances. Oligonucleotides (oligos), including small interference RNAs (siRNAs) and antisense oligonucleotides (ASOs), present unique challenges in plasma stability study design using traditional protocols considering their properties such as relatively high molecular weight, high nonspecific binding, and metabolizing via specific metabolic enzymes compared to small molecule compounds. In order to investigate the effects of anticoagulants, status/sources of plasma, and serum on the metabolism of oligos, we compared the differences between: 1) EDTA-K2 and heparin as an anticoagulant, 2) fresh plasma and frozen plasma, 3) frozen plasma and frozen serum, and established the plasma stability assay for siRNAs and ASOs in this presentation.

Objectives

The objective of the study was to establish an *in vitro* accurate and reliable method combined with LC-MS/MS analysis for determining the plasma stability of siRNA and ASOs in relevant preclinical species.

Methods

Oligonucleotides were purchased from MedChem Express. The 21-mer unmodified ASO, ISIS 1049 (WuXi AppTec Control 01) (5'-GCCGAGGTCCATGTCGTACGC-3') was purchased from Sangon Biotech.

Matrix
- **Plasma and serum**
  - Frozen plasma was purchased from BioIVT.
  - Fresh plasma and serum was prepared in house.
  - Anticoagulant: EDTA-K_2 and heparin.

Incubation
- **Incubation procedure**
  - Anti-adsorption plate was used in the assay.
  - The siRNAs or ASOs were spiked to blank matrix.
  - Samples of 100 µL were incubated in a CO_2 incubator for 0, 1, 2, 4 and 24 hour.

Sample processing
- **Liquid-liquid extraction**
  - Phenol/chloroform/isoamyl alcohol (25:24:1) and dichloromethane were used as extraction solvents
  - After centrifugation, the aqueous layer was removed for LC-MS/MS analysis

Calculations
- The remaining was calculated by the following equation:
- $\%Remaining = \frac{\text{PAR of analyte to internal standard at each time point}}{\text{PAR of analyte to internal standard at T=0}} \times 100$

Table 1. Comparison of half-life of 5 ASOs between the in house and literature data in mouse, monkey, and human plasma

Compound	Literature Values	Species	In house %remaining in 24 hour (n=2)	In house half-life (hour)
Inotersen sodium	*In vivo* plasma elimination half-life of 1 month	Human	83.1	>57.8
		Mouse	94.3	>57.8
		Monkey	108.4	>57.8
Mipomersen sodium	*In vivo* plasma elimination half-life of approximately 30 days[1]	Human	103.0	>57.8
		Mouse	101.2	>57.8
		Monkey	88.4	>57.8
Nusinersen	*In vivo* plasma half-life: 63–87 days [2]	Human	100.0	>57.8
		Mouse	95.9	>57.8
		Monkey	80.4	>57.8
Fomivirsen sodium	*In vivo* half-life of approximately 55 hours in human [3]	Human	76.0	54.3
		Mouse	57.0	31.7
		Monkey	52.0	29.6
Volanesorsen	11.7–31.2 days (over all of the doses measured)[4]	Human	94.1	>57.8
		Mouse	95.6	>57.8
		Monkey	79.5	>57.8

Table 2. Comparison of %Remaining of 2 siRNAs between the in house and literature data in rat, monkey, and human plasma

Compound	Literature Values	Species	In house %remaining in 24 hour of Antisense strand (n=2)	In house %remaining in 24 hour of Sense strand (n=2)
Patisiran	<1% left intact after 24h in serum independent of species [5]	Human	0.9	0.4
		Rat	0.8	0.5
		Monkey	0.6	0.8
Inclisiran	Relatively stable after 24 hours incubation in human serum [6]	Human	96.9	99.2
		Rat	81.8	100.0
		Monkey	72.3	98.1

Results

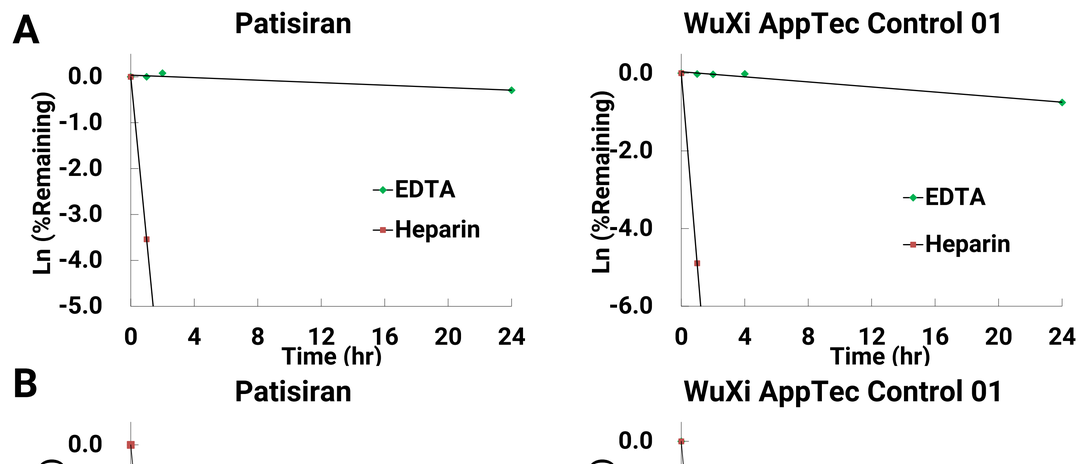

Figure 1. Comparison of the remaining data in rat plasma for Patisiran and WuXi AppTec Control 01 in (A) frozen plasma, with EDTA-K_2 and heparin as anticoagulants (B) fresh and frozen heparin anticoagulated plasma (C) frozen plasma and frozen serum.
A: The metabolism of patisiran and WuXi AppTec Control 01 in heparin anticoagulated plasma was faster than that in EDTA anticoagulated plasma.
B and C: Both patisiran and WuXi AppTec Control 01 were metabolized rapidly and there was no differences between frozen plasma and fresh plasma, or between frozen plasma and serum.

Conclusions

1. We established the plasma stability assay and verified the accuracy of this method by determining the remaining of 2 siRNA compounds (Patisiran and Inclisiran) in rat, monkey and human plasma and 5 ASOs compounds (Inotersen, Mipomersen, Nusinersen, Fomivirsen, Volanesorsen) in mouse, monkey and human plasma. The remaining values of the 2 siRNA compounds were consistent with the published data. The *in vitro* half-life of 5 ASOs were in agreement with their *in vivo* plasma half-lives.
2. Heparin anticoagulated plasma should be used in the plasma stability assay, due to the possible inhibition of nuclease activities by EDTA-K_2.
3. Freezing treatment had no significant effect on the activity of enzymes metabolizing oligos in plasma or serum.

Contact

References

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