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Development of an Exploratory Renal Toxicity Prediction Method for Oligonucleotide Therapeutics Using Human Proximal Tubule Epithelial Cells

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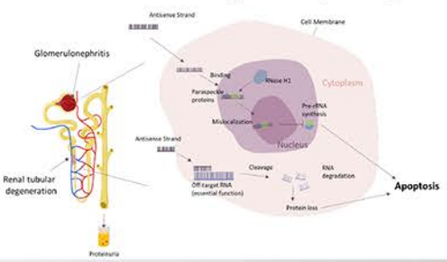
Background and Objective

- Renal toxicity is one of the most common reasons for discontinuation of oligonucleotide therapeutics, particularly antisense oligonucleotides (ASOs). As our understanding of the mechanisms that induce nephrotoxicity has advanced, the development of in vitro nephrotoxicity prediction methods has progressed.
  The conventional predictive method using human primary renal proximal tubule epithelial cells (RPTECs) with the free uptake approach has limitations for screening due to the large amount of test compounds required and the lengthy 9-day testing period*.
- To address this issue, we aimed to develop a simplified method for detecting ASO-induced renal toxicity using RPTECs with transfection reagents. We evaluated a total of 13 ASOs consisting of 7 positive and 6 negative controls.
  The positive controls included ASOs that were withdrawn from clinical trials due to the renal toxicity.

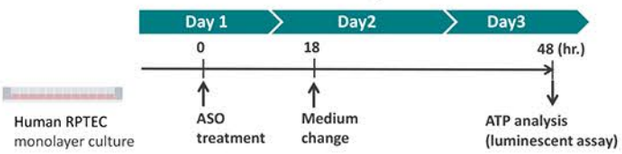
Molecular mechanisms of ASO drug-induced nephrotoxicity



Material and Methods

Cells: human RPTEC (Lonza Bioscience)
Medium: Renal epithelial cell growth Medium (Lonza Bioscience)
Plate: Multi-well plate for cell/tissue culture 96F with lid (SUMITOMO BAKELITE)
Measurement: Envision (Perkin Elmer)

Study methods:
RPTECs were seeded at 4.0 x 10^4cells/well in the 96-well plate.
The cells were cultured for about one week until they reached confluent monolayer.
ASOs were introduced into the cells via lipofection and incubated overnight at 37°C with 5% CO_2. After transfection, the medium was replaced, and the cells were further cultured for up to 48 hours.
To evaluate intracellular ATP levels, CellTiter-Glo® was added, and luminescence was measured 10 minutes later using an EnVision reader.



Information on evaluated oligonucleotides

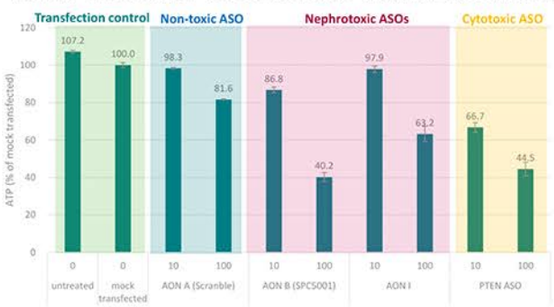
Test articles	Target gene	Type	Safety information	Manufacturer
DPBS (Vehicle control)	-	-	-	-
AON B (SPC5001)	PCSK9	LNA	The clinical trial has been terminated in view of the potential for renal toxicity	All test articles have been synthesized with commercially available phosphoramidites
AON C	PCSK9	LNA	Nephrotoxicity in rat* (High)	
ISIS388626	SGLT2	MOE	The clinical trial has been terminated in view of the potential for renal toxicity	
AON H	MYD88	LNA	Nephrotoxicity in rat* (Low/Medium)	
AON I	MYD88	LNA	Nephrotoxicity in rat* (Medium)	
AON O	BCL11A	LNA	Nephrotoxicity in rat* (Low/Medium)	
AON P	BCL11A	LNA	Nephrotoxicity in rat* (Medium/High)	
AON A	- (Scramble)	LNA	Innocuous*	
ZOREVUNERSEN	SCN1A	MOE	No safety concerns related to renal toxicity have been reported in non-clinical studies or clinical studies	
Milasen	CLN7	MOE		
BIIB080	MAPT	MOE		
Tofersen	SOD1	MOE		
Rugonersen	UBE3A	LNA		
EZN-4176	AR	LNA	Elevated AST and ALT in human clinical trials	
Mipomersen	APOB	MOE	Withdrew from the market (unacceptable risks of liver toxicity)	
Inotersen	TTR	MOE	Black box warning label (risk of liver and kidney toxicity)	
PTEN ASO	PTEN	LNA	Markedly induce caspase 3/7 activity in HepG2 cells	

*References

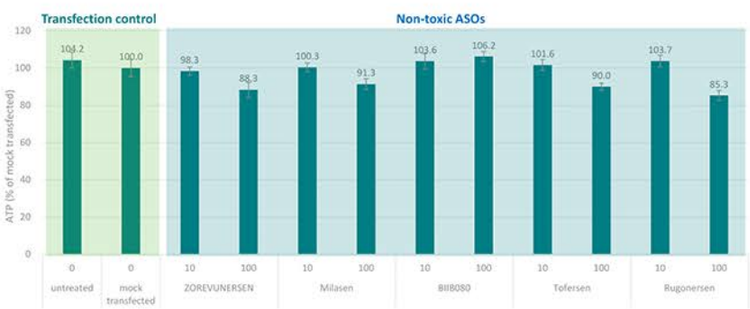
- Annie Moisan, et al. "Inhibition of EGF Uptake by Nephrotoxic Antisense Drugs In Vitro and Implications for Preclinical Safety Profiling." Molecular Therapy: Nucleic Acids Vol. 6 (2017) 89-105.

Results

Reactivity validation using nephrotoxic ASOs AON B (SPC5001) and AON I confirmed a reduction in ATP levels after exposure to 100 nM.
A similar decrease in ATP was observed with PTEN ASO, a cytotoxic ASO.



No decrease in ATP was observed at 100 nM for non-toxic ASOs.



Except for ISIS388626, all ASOs for which renal or hepatic toxicity has been reported in human clinical trials or non-clinical trials demonstrated a decrease in ATP to less than 75% at an exposure of 100 nM.

The results of this series of verification studies showed that the prediction accuracy was 92% (12/13), sensitivity was 85.7% (6/7), and specificity was 100% (6/6).

		Renal toxicity in non-clinical/clinical studies		
		+	-	Total
PTEC ATP assay	+	6	0	6
	-	1	6	7
	Total	7	6	13

Conclusion

We developed a simplified method for detecting ASO-induced renal toxicity using RPTECs with transfection reagents.
Validation studies showed a prediction accuracy of 92%, sensitivity of 85.7%, and specificity of 100%.
Compared to the conventional free-uptake method, our RPTEC assay reduced the testing period by 77% (from 9 days to 2 days) and ASO consumption by 99.9% (from 100 µM to 100 nM).
This method offers significant advantages and is suitable for exploratory screening of renal toxicity in ASO candidates.

Please contact us for any questions !
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COI Disclosure Information

Lead Presenter/Responsible Researcher
Ikuma Yoshida
I have no financial relationship to disclose.