Addressing Safety and Specificity with Aldose Reductase Inhibition: Development of AT-001 for Diabetic Cardiomyopathy

Poster #632

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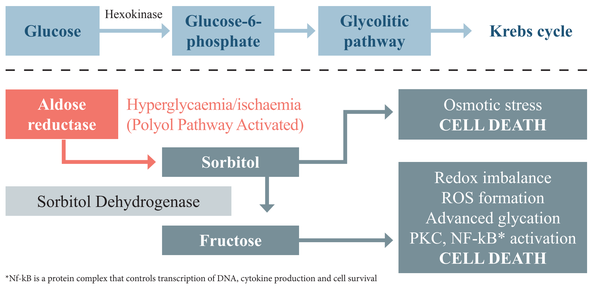
Introduction

Diabetic cardiomyopathy (DbCM) leading to overt heart failure is a common sequalae of both type 1 and type 2 diabetes. Prior attempts to develop treatments for DbCM via inhibition of Aldose Reductase (AR) demonstrated clinical efficacy.

However, first generation AR inhibitors (ARIs) presented with liver and renal safety signals. Off-target inhibition of Aldehyde Reductase, an enzyme necessary for cellular detoxification, was considered responsible for the unfavorable safety profile.

In the present study, we report the *in-vitro* safety, selectivity and specificity of AT-001, a novel small molecule ARI with optimised affinity and specificity for AR, and no off-target aldehyde reductase activity.

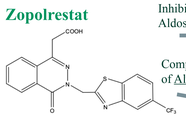
Pathogenesis of DbCM and Hyperactivation of Polyol Pathway[1,2]



*Nf-kB is a protein complex that controls transcription of DNA, cytokine production and cell survival

First Generation Aldose Reductase Inhibitor vs AT-001[3]

Zopolrestat

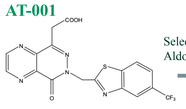


Inhibition of Aldose Reductase → Clinical Efficacy

Competitive inhibition of Aldehyde Reductase → (Off-target) Hepatotoxicity

Zopolrestat demonstrated clinical efficacy in Phase 2 studies. Off-target hepatoxicity was documented. Clinical development was terminated[3]

AT-001



Selective inhibition of Aldose Reductase → Clinical Efficacy

AT-001 developed through rational drug design to selectively inhibit AR without off-target inhibition of Aldehyde Reductase

IC_{50} of AT-001 vs zopolrestat for Aldose Reductase

Compound	IC_{50}	MTD in animals	Tissue penetration (in rats)			
			Systemic/ Heart	Nerve	Retina	CNS
AT-001	30pM	>2,000mg/kg	✓	✓	✓	X
Zopolrestat	10nM	100mg/kg	✓	✓	X	X

Methods

Cell culture: Cryopreserved human hepatocytes cultured in standard medium.

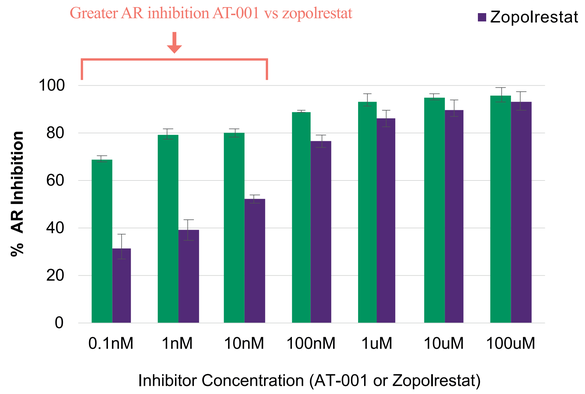
Comparative cell hepatoxicity of AT-001 vs controls: Primary human hepatocyte spheroids were cultured for 7 days. At Day 7, spheroid cultures were exposed to different concentrations of AT-001 and zopolrestat. Troglitazone (a known hepatotoxic agent) was used as a positive control. Exposure was maintained for 14 days and cell viability was assessed via ATP quantification using the CellTiter-Glo™ luminescent cell viability assay.

Liver enzyme quantitation assay: Lactate dehydrogenase (LDH) release (a measure of cell necrosis/injury), and liver injury markers alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using commercially available assay kits.

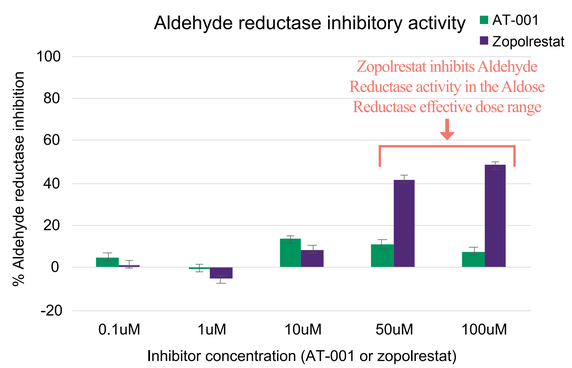
Selectivity assessment of AT-001 vs zopolrestat: Enzymatic inhibition (both on-target inhibition of AR and off-target inhibition of aldehyde reductase) was determined via spectrophotometric assay of substrate binding and nicotinamide adenine dinucleotide phosphate release.

Results

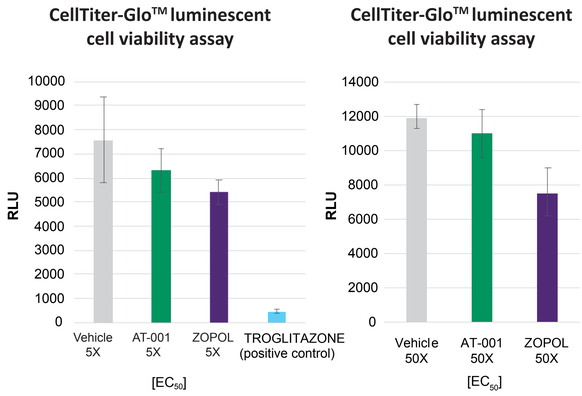
Improved inhibitory selectivity of AT-001 for Aldose Reductase



Zopolrestat (but not AT-001) Off-target Inhibits Aldehyde Reductase, an Enzyme Critical for Liver Function

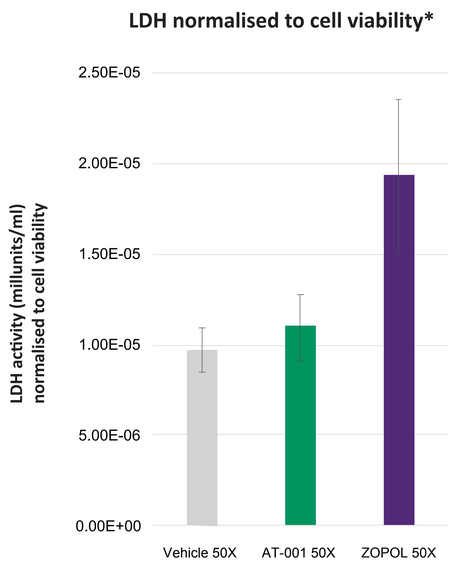


Zopolrestat (but not AT-001) Reduces Cell Viability of Cultured Hepatocytes



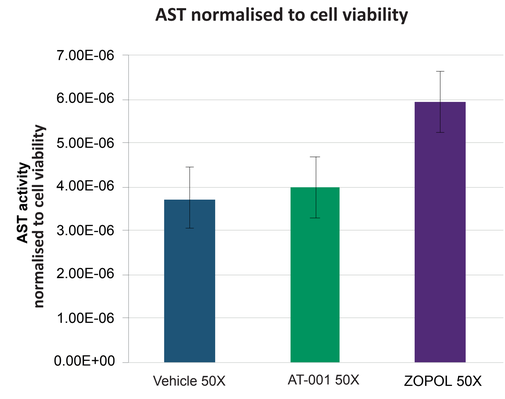
p=0.03 vs AT-001 and vehicle

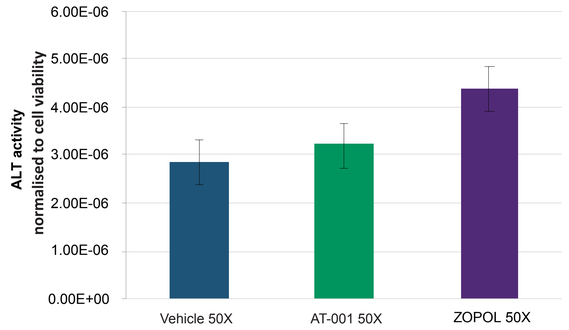
Zopolrestat (but not AT-001) Induces Cell Damage in Cultured Hepatocytes as Measured by Lactate Dehydrogenase Release



*Cell damage in cultured hepatocytes measured by lactate dehydrogenase (LDH) release; LDH is a biochemical marker for tissue injury/cellular necrosis

Zopolrestat (but not AT-001) Induces Cell Damage in Cultured Hepatocytes as Measured by AST and ALT Release





Glutamate based assay comparing AT-001 and zopolrestat at 50x EC50 concentration: AST activity reported as mmol/min/mL. One unit of AST (or ALT) is the amount of enzyme that will generate 1.0 mmol of glutamate per minute at pH 8.0 at 37 °C

Summary and Conclusions

AT-001 is a logarithmically more potent ARI than zopolrestat in inhibiting Aldose Reductase, an enzyme implicated in the development of diabetic complications including diabetic cardiomyopathy (DbCM).

Cell viability assessment at high substrate concentrations demonstrated that while zopolrestat was cytotoxic, AT-001 and vehicle were not.

The unique structure and activity of AT-001 provide selectivity for Aldose Reductase and avoids off-target inhibition of Aldehyde Reductase, which is an enzyme critical for normal detoxification processes in the liver.

Measurement of ALT, AST and LDH in *in-vitro* and *ex-vivo* systems demonstrated that zopolrestat, but not AT-001, was toxic to hepatocytes.

The *in-vitro* safety of AT-001, together with the positive safety data from the Phase 1/2 program, support the ongoing pivotal study in DbCM: ARISE-HF (NCT 04083339).

References

1. Brownlee M. Diabetes Care. 2005;54(6):1615–1625;

2. Miki T, *et al.* Heart Fail Rev. 2013;18(2):149–166;

3. Johnson, *et al.* Diabetes Care. 2004:448–454.

DISCLOSURES: RP and SS are employees of and stockholders in Applied Therapeutics Inc.

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APPLIED THERAPEUTICS