AT-001: A Next Generation Aldose Reductase Inhibitor with Improved Potency, Selectivity and Specificity Protects from Cellular Damage Associated with Hyperglycaemia

POSTER #629

Riccardo Perfetti[1], Gautham Yeppuri[2], Nosirudeen Quadri[2], Ravichandran Ramasamy[2], Ameen F Ghannan[1], Shoshana Shendelman[1].Applied Therapeutics[1] and Langone Medical Center[2], New York, NY

Introduction

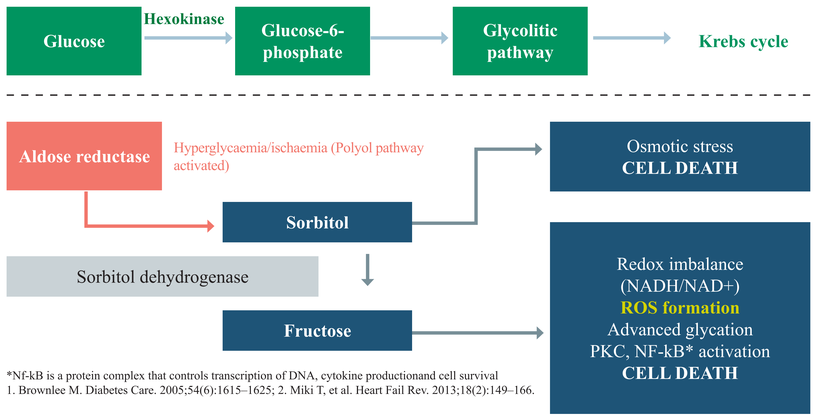
Reactive oxygen species (ROS) production resulting from chronic tissue exposure to elevated levels of glucose has been shown to lead to the development of diabetic complications. Abnormal activation of the polyol pathway converts excess glucose to sorbitol and results in generation of ROS. Aldose reductase (AR) is the first and rate-limiting enzyme in the polyol pathway.

Inhibition of AR reduces the production of sorbitol, prevents formation of ROS, and improves the ratio of nicotinamide adenine dinucleotide+ (NAD+) to nicotinamide adenine dinucleotide + hydrogen (NADH), critical for optimal energy utilisation by cells.

AT-001 is a novel, rationally designed, highly selective AR inhibitor (ARI) in clinical development for the treatment of diabetic cardiomyopathy, a fatal complication of diabetes.

The present study evaluated the effect of AT-001 in preventing cellular damage to human cells caused by oxidative stress under hyperglycaemic conditions using validated *in-vitro* assays.

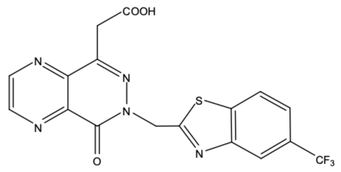
Role of aldose reductase and the polyol pathway in the formation of reactive oxygen species and cellular damage



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

1. Brownlee M. Diabetes Care. 2005;54(6):1615–1625; 2. Miki T, et al. Heart Fail Rev. 2013;18(2):149–166.

AT-001: a next generation highly selective ARI with high penetration to key target tissues for the treatment of diabetic complications



IC_{50}	MTD in animals	Tissue penetration (in rats)			
		Systemic/ heart	Nerve	Retina	CNS
30pM	>2,000mg/kg	✓	✓	✓	X

- AT-001 was developed through rational drug design, using the geometric parameters of the active site of AR determined via X-ray crystallography
- Optimal potency and target selectivity for AR was achieved, demonstrating no off-target inhibition of a structurally related enzyme, aldehyde reductase
- Aldehyde reductase plays an important role in detoxification of aldehydes in the liver. Ensuring absence of off-target aldehyde reductase activity is critical to ensuring safety of next generation ARIs, such as AT-001

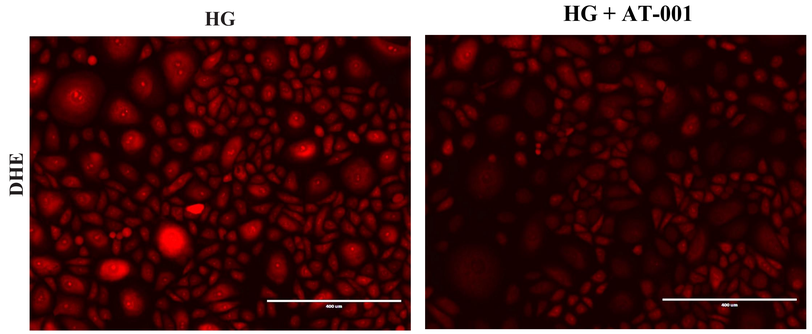
Methods

- Cultured human adult cells (NHK) were exposed to elevated glucose [25mM] to simulate hyperglycaemic conditions in diabetic tissue in the presence/absence of the ARI AT-001 [0.18nM]
- AT-001 or control compounds/vehicle were added 24 hours after introduction of high glucose conditions. Oxidative stress and senescence were evaluated at 48 hours
- Cytosolic oxidative stress was evaluated and quantified using dihydroethidium (DHE) staining and quantitated via colorimetric assessment
- Mitochondrial-specific oxidative stress ROS levels were quantitated using MitoSOX™ staining, a fluorogenic dye specifically targeted to mitochondria in live cells
- Evaluation of NHK cellular senescence was quantified via senescence-associated (SA) β-galactose staining assay

Results

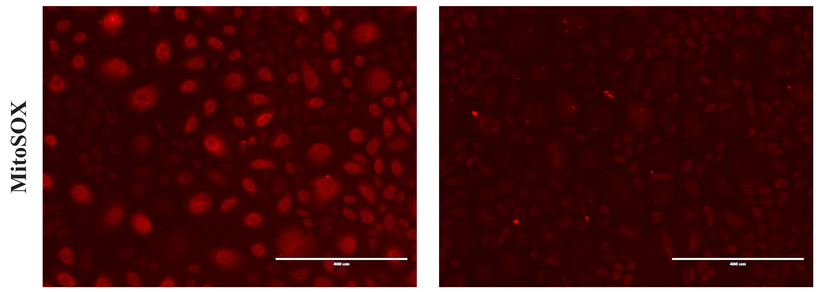
AT-001: treatment prevents cytosolic ROS generation, mitochondrial stress and aging caused by high glucose exposure

Dihydroethidium (DHE) staining for cytosolic ROS



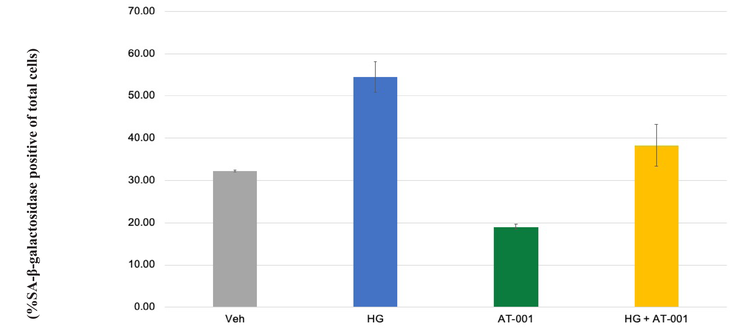
High glucose (HG) – NHK cells exposed to 25mM glucose (HG) for 48hrs
HG + AT-001 – cells treated with 0.18nM AT-001 along with above mentioned HG exposure

MitoSOX™ staining for mitochondrial ROS



High glucose (HG) – NHK cells exposed to 25mM glucose (HG) for 48hrs
HG + AT001 – cells treated with 0.18nM AT-001 along with above mentioned HG exposure

Quantitation of cell senescence via SA-β-gal staining



Vehicle (Veh) – cells treated with normal medium without excess of glucose and in absence of AT-001
HG – cells exposed to 25mM glucose (HG) for 48hrs
AT-001 – cells treated with 0.18nM AT-001 without HG
HG + AT001 – cells treated with 0.18nM AT-001 along with above mentioned HG exposure

Conclusion

In patients with diabetes, metabolism of glucose through the polyol pathway results in generation of Reactive Oxygen Species (ROS), which have been identified as key mediators of tissue damage and causal in diabetic complications. Selective inhibition of AR, the first and rate limiting enzyme in the polyol pathway reduces oxidative stress and mitigates these complications.

AT-001 prevents the production and accumulation of ROS as assessed by both DHE quantitation and MitoSOX™ staining, demonstrating effective reduction of oxidative damage in the cytosol and mitochondria of cells.

Evaluation of cellular aging via SA-β-gal staining showed less senescence in cells exposed to HG in the presence of AT-001.

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

56th EASD Annual Meeting, 21-25 September 2020
Data was first presented at ADA June 2020

APPLIED THERAPEUTICS