Beneficial effects of AT-001, an aldose reductase inhibitor, in rodent models of Diabetic Cardiomyopathy

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ABSTRACT

Background: Hyperactive flux through the polyol pathway contributes to development of diabetic complications[1]. Aldose reductase (AR) is the rate-controlling enzyme in the polyol pathway that catalyzes NADPH-dependent reduction of glucose to sorbitol. During hyperglycemic and ischemic conditions, increased polyol pathway flux causes a rise in intracellular sorbitol and consequent changes in cytosolic NAD+/NADH, oxidative stress, cell death and diabetic complications such as Diabetic Cardiomyopathy (DbCM)[2,3]. Previous efforts to target this pathway using AR inhibitors (ARIs) have been limited by unfavorable benefit risk profiles. AT-001 is a novel oral ARI with optimized specificity and affinity for AR. We assessed target engagement (Study 001) and cardioprotective activity in a cardiac ischemia reperfusion (IR) injury model (Study 002) in two rodent type 2 diabetes (DM) models.

Methods: Study 001: following a single streptozotocin (STZ) injection (100 mg/kg) to induce DM in Sprague-Dawley rats, AT-001 5 or 10 mg/kg or vehicle (V), n=5 per group, were administered orally and erythrocyte sorbitol measured over 48h. Study 002 assessed effects of AR inhibition on acute cardiac ischemia reperfusion (I/R) using an AR transgenic diabetic mouse model that expresses human levels of AR (as mice normally express 50 to 300-fold lower AR levels than humans). Transgenic mice expressing human levels of AR (Tg hAR) were treated with STZ (55 mg/kg for 5d at age 6w). Tg hAR mice with DM, defined as blood glucose levels >250 mg/dL confirmed twice for 3w were treated by oral gavage with 40 mg/kg AT-001 (n=6) or vehicle (V, n=6) for 3 days prior to I/R injury induced by in vivo ligation and reperfusion of the left anterior descending (LAD) coronary artery. Control non-DM Tg hAR mice (non-DM, n=9) were also subjected to I/R injury. Echocardiography was used to measure cardiac function, including fractional shortening (FS) prior to LAD ligation and after 48 h recovery. Mice were euthanized after 48h recovery and infarct area in hearts was assessed using 2,3,5-triphenyltetrazolium chloride staining.

Results: Study 001: Prior to treatment, sorbitol was elevated in DM vs. non-DM rats due to activation of AR and increased flux of glucose through the polyol pathway: (Mean+SD) 12.38±0.9 vs. 9.45±1.3 g/ml. AT-001 at doses of 5 and 10/mg/kg reduced sorbitol to 11.0±1.9 and 8.1±1 at 7h and 9.3±1.1 and 7.9±1.7 μg/ml 24h post treatment, respectively; *P<0.001 for both doses at 7 and 24h vs. baseline. Moreover, AT-001 normalized sorbitol to non-DM levels. Study 002: Mean (±SD) infarct area, expressed as ratio of infarcted to total left ventricular (LV) area was significantly lower in AT-001 (35.3±2.7) vs. non-DM (43±2) and vehicle (59.1±0.8); P<0.05 vs non-DM and V). FS 48 h post reperfusion, a measure of functional recovery, was greater in AT-001 vs non-DM and vehicle (38.2±2, 24±3, 21±1.5, respectively; P<0.05 vs. non-DM and vehicle). Mice were not treated during the 48h reperfusion; thus, AT-001 prevented or attenuated cardiac injury.

Conclusions: AT-001 normalized sorbitol levels relative to non-DM controls and significantly reduced cardiac damage in a mouse model of DbCM. These findings confirm biological activity of AT-001, demonstrate a role of AR inhibitors in significantly reducing or preventing cardiac damage and support clinical investigation of AT-001 in DbCM.

PURPOSE

- To confirm target engagement (Study 001) by assessing sorbitol levels
- To assess cardioprotective activity in a cardiac ischemia reperfusion (IR) injury model (Study 002) in two rodent type 2 diabetes (DM) models

METHODS

Study 1: Diabetes was induced in Sprague-Dawley rats (DM) by a single intraperitoneal injection 55 mg/kg streptozotocin. DM rats were orally gavaged with AT-001 5 mg/kg, 10mg/kg or vehicle (n=5 per group). Erythrocyte sorbitol was measured at 0, 7, 24 and 48 h after dosing.

Study 2: Transgenic mice expressing human levels of aldose reductase (Tg hAR) were treated with STZ (55 mg/kg for 5 days at age 6 weeks). Tg hAR mice with DM, defined as blood glucose >250 mg/dL confirmed twice for 3 weeks were treated with AT-001 40mg/kg (n=6) or vehicle (V, n=6) for 3 days prior to I/R injury induced by in vivo ligation and reperfusion of the left anterior descending (LAD) coronary artery. Control non-DM Tg hAR mice (non-DM, n=9) were also subjected to I/R injury. Fractional shortening fraction (FS) was assessed by echocardiography prior to LAD ligation and after 48 h recovery. Mice were euthanized after 48 h and infarct area in hearts assessed using 2,3,5-triphenyltetrazolium chloride staining and expressed as a ratio of infarcted to total LV area.

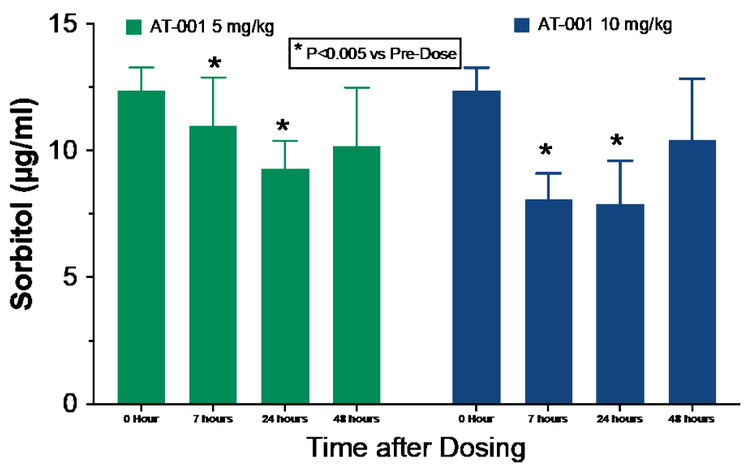
RESULTS

STUDY 1

Prior to dosing sorbitol was elevated in DM compared with non-DM rats: 12.38±0.9 vs 9.45±1.45 mg/ml. Both AT-001 doses reduced sorbitol significantly (**Figure 1**) and efficacy was sustained for 24h; however, the 10 mg/kg dose normalized sorbitol to the non-DM level.

FIGURE 1

Mean (±SEM) Erythrocyte Sorbitol at Baseline over 48-h After Dosing



STUDY 2

Group 1: Tg hAR non-DM (n=9)
Group 2 Tg hAR DM + Vehicle (n=6)
Group 3: Tg hAR DM + AT-001 (n=6)

Blood glucose was significantly lower in non-DM vs. vehicle and AT-001 treated DM rats (112±21 vs 353±76 and 426±102 mg/dL, respectively. Heart rate and FS were similar in all groups prior to LAD ligation. After 48h reperfusion functional recovery as assessed by fractional shortening (FS) was improved only in AT-001 treated hearts (**Figure 2**). AT-001 significantly reduced infarct size (**Figure 3**) and improved FS, expressed as a % of baseline (**Figure 2)** compared with both the vehicle and non-DM groups (P<0.001).

RESULTS

FIGURE 2

Mean (±SEM) Fractional Shortening

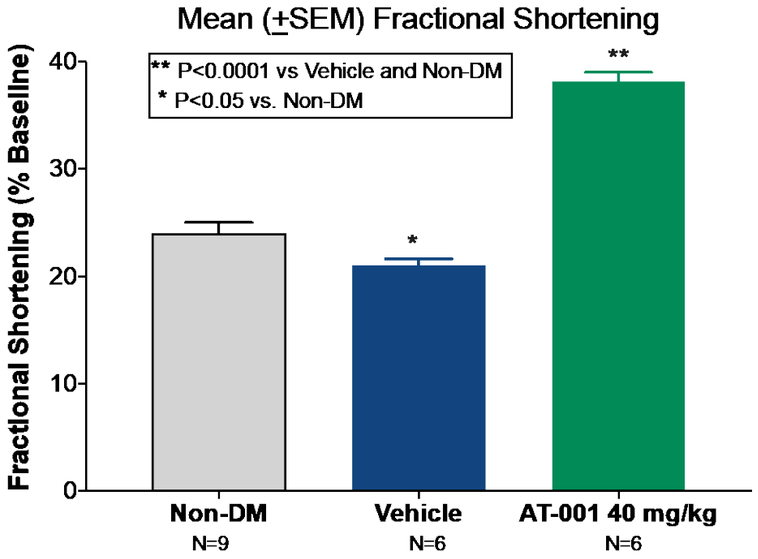
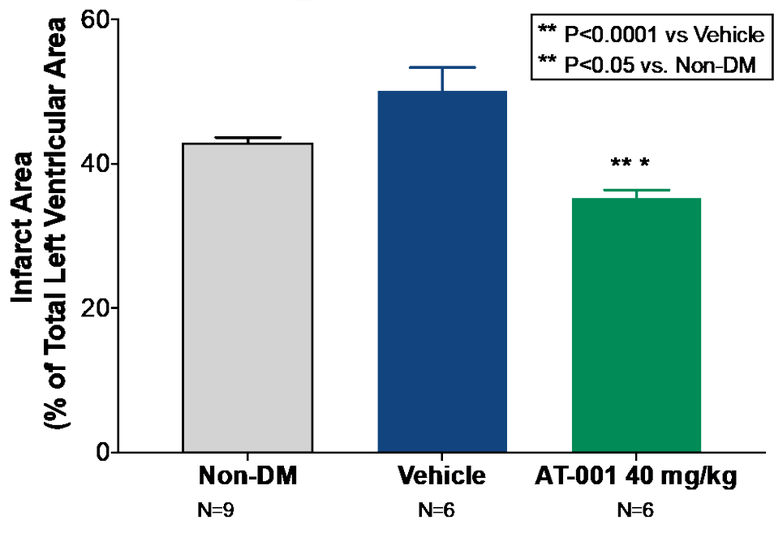


FIGURE 3

Mean (±SEM) Infarct Area



CONCLUSIONS

AT-001 normalized sorbitol levels relative to non-DM control rats (9.3±1.1 and 7.9±1.7 for AT-001 5 and 10 mg/kg vs 9.45±1.3 non-DM), demonstrating effective inhibition of AR.

AT-001 significantly reduced cardiac damage in a relevant mouse model of DbCM. At 48-h post reperfusion, both infarct area and functional recovery, defined by FS relative to baseline were improved only in AT-001 treated rats.

These findings:

- Confirm biological activity of AT-001
- Demonstrate the role of AR inhibition in significantly reducing or preventing cardiac damage
- Support clinical investigation of AT-001 in DbCM

REFERENCES

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DISCLOSURE OF INTEREST

R. Perfetti and S. Shendelman are employees of and stockholders in Applied Therapeutics Inc.