AZX100 Modulates Actin Dynamics in Hypertrophic and Keloid Myofibroblasts

Christopher C Smoke, Tyler Frye, Kim B Perkins, Emma Rousseau, Michael R Sheller
Capstone Therapeutics, 1275 W. Washington St. Tempe, AZ

Abstract

Keloid and hypertrophic scar-derived myofibroblasts are characterized by increased stress fibers and contractile function as compared to normal dermal fibroblasts. Increased stress fiber formation is characterized by increased filaments (F-actin). The present study investigated the ability of AZX100 to reduce the F-actin content of keloid dermal fibroblasts. Experimentally, keloid and hypertrophic scar-derived fibroblasts were serum starved for 24 hours, followed by treatment with Transforming Growth Factor Beta 1 (TGFβ1) at 2.5ng/ml alone or with AZX100 for 24 hours. F-actin was then separated from G-actin by ultra centrifugation and the amounts of each were determined with Western blot analysis. AZX100 treatment of hypertrophic and keloid derived fibroblasts decreased the pool of F-actin by 20% and 18%, respectively (p < 0.001). Moreover, AZX100 treatment of keloid and hypertrophic scar-derived fibroblasts visually decreased stress fiber formation and focal adhesion size as observed by immunofluorescence. In addition, treatment of TGFβ1-differentiated fibroblasts with AZX100 reduced the amount of α-smooth muscle actin mRNA, a myofibroblast marker. The data suggest that AZX100 disrupts the cytoskeleton network in persistent myofibroblasts, potentially decreasing fibrotic scar score formation.

Results

AZX100 reduces vinculin associated with stress fibers and decreases total actin in Keloid Fibroblasts at 24hrs (Figure 1)

Keloid fibroblasts (Fig. 2a-c) were plated on fibronectin coated coverslips and treated for 24hrs with or without TGFβ1 (2.5ng/ml) and AZX100 (25µM) in 10% FBS. Cells were stained with DAPI (blue), anti-vinculin Alexa Fluor 488 (green), and Phalloidin Alexa Fluor 568 (red). Images were taken at 40x magnification with Axiovert microscope (Carl Zeiss).

AZX100 reduces vinculin associated with stress fibers and decreases total actin in keloid fibroblasts at 24hrs (Figure 1)

Figure 4a and b show a significant increase in the amount of α-smooth muscle actin with 48hrs of TGFβ1 treatment. Increased α-smooth muscle actin is a key marker of fibroblasts differentiation to myofibroblasts. Co-treatment of fibroblasts with TGFβ1 and AZX100 significantly reduced ACTA2 mRNA expression compared to TGFβ1 treated cells, suggesting an AZX100-dependent decrease in myofibroblast differentiation. n=3 for all cell types.

Introduction

• 24 amino acid synthetic peptide
• Represents amino acids of the human Heat Shock Protein Beta 6 (HSPB6 or HSP20)
• N-terminal protein transduction domain (PTD)
• Depolymerizes the actin cytoskeleton

Materials and Methods

Keloid and hypertrophic scar-derived cells were plated on coverslips at 1x 10^5 cells/ml. Cells were treated with 0.5% Triton X for 10 mins. Coverslips were fixed with 4% formaldehyde for 10 mins. Cells were permeabilized with 0.5% Triton X for 10 mins. Cells were fixed with anti-vinculin (Invitrogen) (1:250) and anti-mouse Alexa Fluor 488 (Invitrogen) (1:500). DAPI (Invitrogen) (1:1000). Phalloidin Alexa Fluor 568 (Invitrogen) (1:600). Images were taken with Axiovert microscope at 40x magnification.

AZX100 reduces Filamentous actin (F-actin) in keloid and hypertrophic scar-derived fibroblasts (Figure 3)

AZX100 significantly reduces FNactin in the presence of exogenous TGFβ1 (2.5ng/ml) after 24 hrs of treatment. AZX100 (25µM) treatment for 24 hours showed visual decreases in the number of focal adhesions (vinculin) of TGFβ1 treated cells, suggesting an AZX100-dependent decrease in myofibroblast differentiation.

Summary

• AZX100 (25 µM) significantly reduces F-actin in the presence of exogenous TGFβ1 (2.5ng/ml) after 24 hrs of treatment.
• AZX100 (25 µM) treatment for 24 hours showed visual decreases in the number of focal adhesions (vinculin) of TGFβ1-differentiated myofibroblasts. Co-treatment of fibroblasts with TGFβ1 and AZX100 significantly reduced ACTA2 mRNA expression compared to TGFβ1 treated cells, suggesting an AZX100-dependent decrease in myofibroblast differentiation.

Bibliography
