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Studying the effects of Axitinib on the proliferation and migration of vascular smooth muscle cells (VSMCs) isolated from Wistar rat

Khalid H. Almutiri [1], Mohammad M. Algahtani [2], Ahmed Z. Alanazi [2]

[1] Undergraduate Pharm.D Student, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

[2] Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

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INTRODUCTION

Small tyrosine kinase inhibitors are widely used in the treatment of various types of cancer. Among these agents, Axitinib is a potent tyrosine kinase inhibitor (TKI) that inhibits vascular endothelial growth factor receptor (VEGFR-1 and -2). The FDA approved Axitinib in January 2012 for the treatment of advanced renal cell carcinoma as a second line treatment. However, several studies reported the occurrence of hypertension with Axitinib use. The effect of Axitinib on vascular smooth muscle cells is still not clear. The ultimate goal is to evaluate the effects of Axitinib on VSMCs proliferation, migration and define the underlying mechanisms on inflammation markers and apoptotic pathway.

OBJECTIVES

To investigate the effect of Axitinib on vascular smooth muscle cells proliferation. Also to evaluate the impact of Axitinib on the migration of vascular smooth muscle cells, and to define the underlying mechanisms of Axitinib on inflammation markers and induction of apoptosis.

METHODS

- **Cell culture and experimental design:**

VSMCs were extracted from Wistar rat and grown in DMEM-F12 media with appropriate supplement 10% of fetal bovine serum and antibiotics. VSMCs were seeded into 6 well plates the day before treatment. The wells were divided into two groups that was treated as the following: **Group I:** serviced as control and was treated with vehicle for 24 hr. **Group II:** was treated with increasing concertation of Axitinib for 24 hr.

- **Cell viability assay:**

methyl thiazolyl tetrazolium (MTT) assay was used to assess cell viability. VSMCs were treated for 24 hr before analyzed by MTT assay. The median inhibitory concentration (IC50) was calculated by a non-linear regression of the plot using GraphPad prism8.

- **Scratch Assay (wound healing assay):**

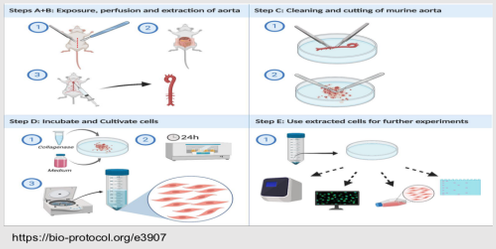
Monolayers of VSMCs were grown on 6-well plates close to 80% confluency. A single straight line scratch was made in each well using a sterile 200 µL tip, then refilled with a growth medium containing various concentrations of Axitinb (1, 5 and 10 µM).

- **Evaluation of apoptosis using flow cytometry:**

Annexin V/propidium iodide staining was used to detect apoptosis of VSMCs.

- **RNA isolation and Gene expression:**

Cells were plated in 6 well cell culture plates and treated with increasing concentration of Axitinib for RNA isolation by using Trizol Reagent. The gene expression of TNF-α, IL-6 and Bcl-2 was detected by using Real-time PCR.



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RESULTS

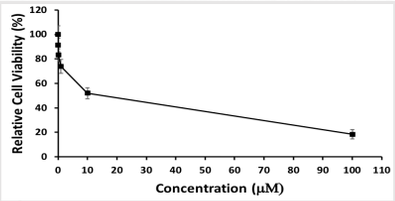

Figure (1). The inhibitory effects of Axitinib on VSMCs.
Cell growth was assessed by MTT assay after treatment with different concentrations of Axitinib (0.01 – 100 µM) for 24 hr.

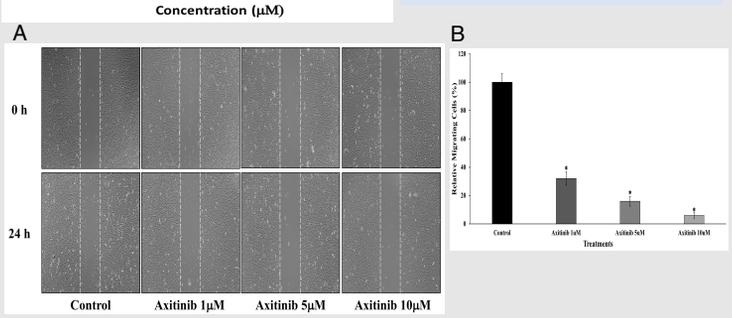

Figure (2). Inhibitory effect of Axitinib on VSM cell migration using scratch wound healing assay.
(A) Representative images of VSMCs incubated with different concentrations of Axitinib (1 µM, 5 µM and 10 µM) after 24 hr post-scratch. **(B)** Data represents the quantification of the percentage of migrated cells. The number of cells migrated to the scratch area was counted using ImageJ software. The bar graph is shown as the mean ± SD of three independent experiments (*P<0.05).

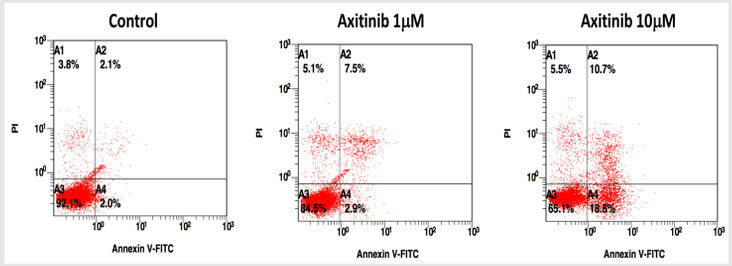

Figure (3). Axitinib-induced apoptotic cells were investigated by Annexin V/pI staining.
Cells were treated with Axitinib (1µM and 10 µM) for 24 hr.

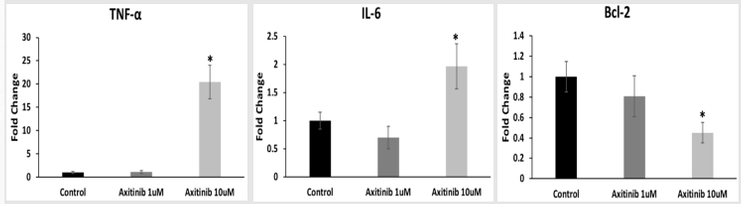

Figure (4). RT-PCR analysis of TNF-α, IL-6 and Bcl-2 mRNA expression in VSMCs after treatment with Axitinb (1µM and 10 µM) for 24 hr.
Data are shown as mean ± SD of three independent experiments (*P<0.05).

CONCLOSIONS

The ability of Axitinib to reduce VSMCs proliferation and migration and to increase the expression of the inflammatory markers may give an evidence of its propensity to induce vascular injury and remodeling as well as hypertension.

REFERENCES