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Tamsulosin Attenuates Abdominal Aortic Aneurysm Growth

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Abstract

Background—Tamsulosin, an α_{1A} -adrenergic receptor inhibitor, is prescribed to treat benign prostatic hyperplasia (BPH) in men >60 years of age, the same demographic most susceptible to abdominal aortic aneurysm (AAA). The goal of this study was to investigate the effect of tamsulosin on AAA pathogenesis.

Methods—AAAs were induced in WT C57BL/6 male mice (n=9–18/group) using an established topical elastase AAA model. Osmotic pumps were implanted in mice 5 days prior to operation to create the model administering either low dose (0.125 μ g/day tamsulosin), high dose (0.250 μ g/day tamsulosin), or vehicle treatments with and without topical application of elastase. Blood pressures were measured preoperatively and on postoperative days 0, 3, 7 and 14. On postoperative day 14, aortic diameter was measured prior to harvest. Sample aortas were prepared for histology and cytokine analysis.

Results—Measurements of systolic blood pressure were did not differ between groups. Mice treated with the low-dose and high-dose of tamsulosin showed decreased aortic diameter compared to vehicle-treated control (93% $\pm$ 24 vs. 94% $\pm$ 30 vs. 132% $\pm$ 24 respectively, p=0.0003, p=0.0003). Cytokine analysis demonstrated downregulation of pro-inflammatory cytokines in both treatment groups compared to control (p<0.05). Histology exhibited preservation of elastin in both low and high-dose tamsulosin-treated groups (p=0.0041 and p=0.0018, respectively).

Conclusions—Tamsulosin attenuates AAA formation with increased preservation of elastin and decreased production of pro-inflammatory cytokines. Further studies are necessary to elucidate the mechanism by which tamsulosin attenuates AAA pathogenesis.

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Keywords

Tamsulosin; Aortic Aneurysm; Abdominal; Receptors; Adrenergic; alpha-1; Cytokines

Introduction

Abdominal aortic aneurysms (AAA) were the primary cause of 9,863 deaths in 2014, making this disease the 10th leading cause of death for men >65 years of age in the United States.^{1,2} The risk of AAA rupture is associated with size and rate of expansion.³ AAA rupture is associated with a mortality of 50%–80%, and operative repair carries substantial morbidity.⁴ Currently, there is no medical therapy for AAA, and operative repair represents the only intervention to treat this disease.

The α_{1A} -adrenergic receptor, a G protein-coupled receptor (GPCR), is located in the prostate but also in (ELSEVIER ASK THE AUTHOR IF THEY MEAN “in the cell” or “on the cell membrane”) vascular smooth muscle cells (VSMCs), which comprise a substantial portion of the aortic wall and play an important role in the pathogenesis of AAAs.^{5,6} Activation of this GPCR ultimately leads to stimulation of muscle contraction via the inositol trisphosphate (IP3) and diacylglycerol (DAG) pathway. The receptor first sends phospholipase C to cleave phosphatidylinositol bisphosphate (PIP2), a membrane phospholipid, into IP3 and DAG. DAG remains in the membrane and later activates protein kinase C (PKC).⁷ IP3, a phosphorylated second messenger suggested to be involved in AAA pathogenesis, travels into the cell, binds to L-type calcium channels, and leads to release of intracellular Ca^{2+} from both the endoplasmic reticulum and store-operated Ca^{2+} channels further down the pathway.^{7–8} Activation of this pathway results in increased intracellular free Ca^{2+} concentration and ultimately, vascular smooth muscle contraction.

Tamsulosin is an α_{1A} -adrenergic receptor inhibitor commonly prescribed to treat benign prostatic hyperplasia (BPH) in men >60 years old, the same demographic most susceptible to AAA formation. Histologic evidence of BPH has been observed at 8% of men in their 30's and increases to more than 70% prevalence in men >60 years old.⁹ The demand for BPH treatment, such as tamsulosin, will likely increase in the US as the population ages.¹⁰ By inhibiting the α_{1A} -adrenergic receptor, tamsulosin decreases the amount of IP3 in VSMCs and the down-stream products that result from this pathway. The goal of this study was to investigate the effect of tamsulosin, an FDA-approved drug for the treatment of BPH, on AAA pathogenesis. Because it was hypothesized that it would attenuate AAA size and rate of growth in a manner independent of blood pressure.

Methods

Animal Housing

8–12 week-old WT C57BL/6 male mice (Jackson Laboratory, Bar Harbor, ME) were housed and maintained at 70°F, 50% humidity, and in 12-hour light dark cycles per institutional animal protocols. All mice were provided drinking water and fed either a minimal phytoestrogen diet (2017 Teklad Global 16% Protein Rodent Diet, Harlan Labs, Inc.,

Frederick, MD). Animal protocols were approved by the Institutional Animal Care and Use Committee (No. 3848) of the University of Virginia.

Blood Pressure Measurement

In a previously described procedure, blood pressure measurements were taken using a non-invasive, tail-cuff system under basal conditions.¹¹ In short, blood pressure was measured using the MC4000 Multi Channel Blood Pressure Analysis System (Hatteras Instruments, Cary, NC) in representative mice from each group (n=4/group) per the manufacturer recommendations. The mice were restrained and blood pressures measured 5 days preoperatively, on the day of AAA induction surgery, and on postoperative days 3, 7, and 14. For each mouse, 10 sets of systolic and diastolic measurements were taken and averaged to estimate more accurately their blood pressures during the session. Systolic blood pressure was used when comparing the groups.

Drug Administration

On preoperative day 5, osmotic infusion pumps (Alzet® 1004, Durect Corp., Cupertino, CA) continuously administering either sterile saline (0.9% NaCl)(n=10, Fig. 1A; n=18, Fig. 1B), a low dose of 0.125µg/day of tamsulosin hydrochloride (HCl) (Sigma-Aldrich, St. Louis, MO) (n=17, Fig. 1C), or a high dose of 0.250 µg/day of tamsulosin HCl (n=18, Fig. 1D) were implanted subcutaneously into 8–12 week-old C57BL/6 mice using previously described methods.^{12–15} Tamsulosin treatment started 5 days prior to the operation to allow the drug to reach a steady-state before AAA induction.¹⁶ Dosages administered to mice (25g) in the experiment are proportional by weight to the dosages prescribed clinically (0.4mg/day and 0.8mg/day) to the average human (75kg).

Topical Elastase AAA Model

Saline + Saline group (n=10, Fig. 1A)—The methods for induction of anesthesia, the operative procedure for AAA formation, and postoperative analgesia have been described previously.¹⁷ In short, on day 0, general anesthesia was established using isofluorane inhalation (2.0–2.5% for induction, 1.25–1.75% for maintenance). Once the mouse was fully anesthetized, it was placed in a supine position, and a midline incision was made. The viscera were displaced cranially, and the infrarenal aorta was dissected circumferentially from the renal arteries to the aortic bifurcation. Using a fine tip micropipette, 5µl sterile saline was applied topically to the exposed infrarenal aorta for 5 min. Then the aorta was dried using a cotton tip applicator, the viscera returned to the abdominal cavity, and the laparotomy closed in layers. The mouse was then administered analgesia (ELSEVIER HAVE THE AUTHORS DESCRIBE THE ANALGESIA) and placed under a heating lamp for recovery.

Saline + Elastase group (n=18, Fig. 1B)—The mice underwent the same induction procedure as described above but with 5µl of porcine pancreatic elastase (Sigma-Aldrich; 10.1mg protein/ml, 19U/mg protein) applied topically to the exposed segment of aorta. (ELSEVIER ASK THE AUTHORS IF THEY LEFT THE ELASTASE IN PLACE OR BLOTTED IT DRY AFTER 5 MIN AS IN THE FIRST GROUP)

Tamsulosin low-dose treated groups (n=17, Fig. 1C)—These drug-treated mice underwent the same AAA induction procedure with elastase for the topical application.

Tamsulosin High-dose treated groups (n=18 Fig. 1D)—These drug-treated mice underwent the same AAA induction procedure with elastase for the topical application.

Elastase Procedure Harvest

At postoperative day 14, mice from each group were killed humanely under anesthesia. The adhesions and scarring tissues surrounding the infrarenal aorta were cut away until the smooth wall of the aorta was reached. Using a 26-gauge needle, blood was collected from the junction of the left renal vein and the inferior vena cava. A measurement of the maximum cross-section of the infrarenal aorta was taken using video microscopy with Leica LAS V3.8 software, and a Leica DFC480 camera attached to a Leica MZ 16 microscope (Leica Inc., Annandale, NJ). The measurement was then compared to the distal suprarenal aorta as a self-control. The percentage of aortic dilation was determined using $(\text{maximal AAA diameter} - \text{self-control aortic diameter}) / \text{maximal AAA diameter} \times 100\%$.¹⁷ A dilation of 100% was considered to be positive for AAA.¹⁷ After the measurement, serum and abdominal aortic tissue were collected for cytokine analyses (n=5–9/group).

Cytokine Analysis

Using a cytokine array, 40 cytokines were screened in the sample aortic tissue. Using isolated protein from murine abdominal aortas, cytokine arrays (R&D Systems, Minneapolis, MN) were completed according to manufacturer instructions. Protein samples from each group were pooled for analysis, all samples were run in duplicate, and the mean value was used as described previously.¹⁸

Histology

Aortic tissue was fixed in 4% buffered formaldehyde for 24 h, transferred to 70% ethanol, and embedded in paraffin. Aortic cross-sections were prepared with Verhoeff-Van Gieson (VVG) for elastin and smooth muscle α -actin.^{19–20} For grading, the positive staining area of the entire aortic tissue sample was selected and measured using integrated optical density of each section.

IP3 ELISA In Vitro Experiment Using Mouse Smooth Muscle Cells

Male, non-aneurysmal, murine aortic smooth muscle cells were cultured in 10% FBS-DMEM F12 media (ELSEVIER ASK THE AUTHORS TO DEFINE WHERE THEY GOT THE DMEM F12 MEDIA) for 3 days until the population was large enough for the assay. The cells were starved, and 1×10^6 male mouse smooth muscle cells were placed in each well of a 12-well plate along with 1 mL DMEM F12 media. The plate was then placed in a CO₂ incubator at 37°C. for 24 h. After 24 h, the media is aspirated from the wells, and solutions of 0 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, and 30 ng/mL tamsulosin in DMEM F12 media were added with each concentration of tamsulosin getting (ELSEVIER ASK THE AUTHORS IF THEY MEAN “in three separate wells each” 3 wells. The cells were

incubated again for 24 h and analyzed using a mouse IP3 ELISA Kit (MyBioSource Inc, San Diego, CA).

Statistical Analysis

Prism 7 (Graphpad Software, La Jolla, CA) was used for all statistical analyses. AAA diameter, IP3 ELISA, histology, and Cytokine analysis were determined using an unpaired t-test. The data are presented as mean values and standard error, with an alpha of less than 0.05 considered statistically significant.

RESULTS

Tamsulosin did not Decrease Systolic Blood Pressure among Treated Groups

There was no difference between the systolic blood pressures at basal conditions in the Saline+Saline, Saline+Elastase, Low Dose+Elastase or High Dose+Elastase groups at any time-point ($p > 0.05$, Table 1).

Day 14 Aneurysm Formation Decreased with Tamsulosin Treatment in a Topical Elastase Model

Mice treated with the low dose of tamsulosin showed a decreased abdominal aortic diameter on day 14 compared to the Saline+Elastase group in the topical elastase model ($93\% \pm 23$ vs. $132\% \pm 24$ respectively, $p = 0.0003$, Fig 2A). Mice treated with the high dose of tamsulosin also showed a decreased abdominal aortic diameter on day 14 compared to the Saline+Elastase group in the topical elastase model ($94\% \pm 30$ vs. $132\% \pm 24$ respectively, $p = 0.0003$, Fig 2A). Histology at 10x magnification with Verhoeff-van Gieson (VVG) staining (Fig. 2B) showed that both the low and high doses of tamsulosin preserved elastin integrity in AAA samples compared to the Saline+Elastase positive control group ($p = 0.0041$, $p = 0.0018$ respectively, Fig. 2C).

Multiple Pro-inflammatory Cytokines Including IL-1 β , TNF- α , and INF- γ are Down-Regulated in Tamsulosin Treated Mice

In tamsulosin-treated aneurysm tissue, pro-inflammatory cytokines IL-1 β , TNF- α , INF- γ , IL-7, IL-17, CXCL2 and IL-1 α were downregulated in both the low and high doses ($p < 0.05$ Fig. 3, A–G). CXCL1 was down-regulated in only the low dose of tamsulosin ($p < 0.05$ Fig. 3H). No anti-inflammatory cytokines were up-regulated in the treated samples.

IP3 Formation Inhibited by α_{1A} -Adrenergic Receptor Inhibitor Treatment

The role of tamsulosin in this experiment was confirmed by measuring the levels of IP3 in aortic SMCs *in vitro*. In these *in vitro* experiments, only the greatest dose of tamsulosin administered (30 ng/mL) to the murine aortic SMCs resulted in statistically significant lesser IP3 levels from the untreated control (10.55 ± 0.89 densitometry units vs. 18.08 ± 1.99 densitometry units, $p = 0.0074$, Fig. 4).

DISCUSSION

The present study sought to evaluate the effects of tamsulosin on AAA growth. Tamsulosin treatment was then investigated in a topical elastase murine model, with resultant attenuation of AAA formation and preservation of elastin in the aortic wall. Mean systolic blood pressures did not differ between the groups. Pro-inflammatory cytokines critical to AAA pathogenesis were also downregulated by tamsulosin treatment. IP3 levels were suppressed *in vitro* in tamsulosin-treated, murine, aortic smooth muscle cells.

This study demonstrated that tamsulosin attenuated growth of AAAs by anti-inflammatory effects independent of blood pressure. This investigatory research identified a drug currently used and trusted by physicians as a potential medical treatment for AAAs that may if proven to be effective in humans result in decreased incidence of aortic rupture and the associated mortality of the disease.

α_1 -Adrenergic receptor inhibitors are used by 80% of physicians as a first line agent to treat BPH.²⁶ The use of tamsulosin is commonplace and widespread, and we sought to investigate the additional effects that the drug may have on α_1 receptors elsewhere in the body. While there exists ample research looking into the effects and pathway of tamsulosin in the prostate, little has been done to elucidate the role of α_{1A} -adrenergic receptor inhibitors in the vasculature, even though α_{1A} -adrenergic receptors are also concentrated on VSMCs. Using a murine basic science model, we showed that α_{1A} -adrenergic receptor inhibitors do in fact attenuate the growth of AAAs in mice (Fig. 4a).

Orthostatic hypotension is a known side effect of tamsulosin due to its effect on VSMCs; however, no differences in blood pressure were observed between the mouse groups, suggesting that AAA attenuation by tamsulosin derives from a mechanism independent of blood pressure. α_{1A} -Adrenergic receptors are known to be concentrated on VSMCs, which comprise a majority of the aortic wall and play a critical role in the pathogenesis of AAAs.^{5-6,27-29} These cells, when activated, have been shown to transform phenotypically and secrete pro-inflammatory cytokines, such as TNF α and IL-1 β , as well as cell adhesion molecules that propagate recruitment, migration, and differentiation of leukocytes, specifically of macrophages.³⁰ In the later stages of AAA development, VSMCs begin to go through apoptosis³¹⁻³² which leads to a decreased VSMC density and a loss of structural integrity in the aortic wall.⁶ Due to VSMCs prominent role in AAA pathogenesis, targeting α_{1A} -adrenergic receptors concentrated on these cells using tamsulosin resulted in a marked attenuation of AAA growth.

Accumulating evidence indicates that pro-inflammatory cytokines are important in AAA development in various mouse AAA models.²³⁻²⁵ Previous work by Johnston and others have found IL-1 β to be required for aortic aneurysm formation by creating a positive feedback loop of inflammation through binding its receptor, and secreting pro-inflammatory cytokines including additional IL-1 β .³⁵⁻³⁶ TNF- α exacerbates inflammatory and immunological injury, and INF- γ inhibits the production of collagen in VSMCs; both are linked to the propagation of AAA.³⁷⁻³⁹ Sharma et al showed that IL-17 produced by CD4+ T Cells plays a critical role in promoting inflammation during AAA formation as well.⁴⁰

These pro-inflammatory cytokines, known to be down-stream products of inflammatory cells, such as macrophages and T cells, are recruited by activated VSMCs.

Studies have suggested a link between excessive intracellular calcium and the IP3 DAG signaling pathway to AAA.⁷⁻⁸ IP3 is a phosphorylated second messenger involved in the pathway inhibited by tamsulosin, the α_{1A} -adrenergic receptor antagonist, which travels into the cell and binds to L-type calcium channels on the endoplasmic reticulum.⁸ From the previously described *in vitro* experiment, inhibition of the IP3 DAG signaling pathway was confirmed in murine VSMCs. Additional and more mechanistic studies are necessary in order to elucidate the mechanism by which tamsulosin suppresses inflammation and protects against AAA formation.

Limitations of the present study include the inherent lack of translatability of a small animal model to human disease. While the topical elastase model is well defined and an accepted model for AAA induction, it has been criticized as highlighting more acute rather than chronic processes in AAA pathogenesis.

In conclusion, tamsulosin treatment attenuated AAA growth in a murine model. Systolic blood pressures were not decreased with tamsulosin treatment, indicating the decrease in AAA size occurred in a manner independent of blood pressure. Elastin preservation was observed and pro-inflammatory cytokines were downregulated at postoperative day 14. IP3 was downregulated in tamsulosin-treated SMCs implicating this pathway in the decrease of AAA size. Tamsulosin-mediated AAA attenuation warrants further study in order to elucidate the mechanisms by which it exerts its effects on this disease process.

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Fig. 1. A–D. Blood pressure measurement, topical elastase and tamsulosin treatment experiment design

A. Saline + Saline group: Blood pressure measurements were taken on day –5, 0, 3, 7, and 14 for all groups. Osmotic pumps containing sterile saline were implanted in the mice and sterile saline was applied topically to the aorta. Tissue was harvested on day 14. **B. Saline + Elastase group:** Osmotic pumps containing sterile saline were implanted in the mice and elastase was used to induce AAA. **C. Low Dose + Elastase group:** Osmotic pumps administering 0.125 µg/day of tamsulosin HCl were implanted in the mice and elastase was used to induce AAA. **D. High Dose + Elastase group:** Osmotic pumps administering 0.250 µg/day of tamsulosin HCl were implanted the mice and elastase was used to induce AAA. There was no difference between any group at any time point ($p>0.05$).

Fig. 2. A–C. Tamsulosin treatment decreases the percent change in aortic diameter of treated mice and preserves elastin integrity

A. Aortic % change in diameter is decreased in the low dose of tamsulosin vs the saline + elastase group (93% vs 132%, $p=0.0003$). High dose of tamsulosin % dilation vs saline + elastase group is also different (9%, $p=0.0003$). **B.** Verhoeff-van Gieson (VVG) histologic staining of tissue harvested on day 14 showing the elastin in each group. **C.** Elastin is more present in both the low and high dose of tamsulosin treated tissue compared to the saline + elastase group ($p=0.0041$, $p=0.0018$ respectively).

Fig. 3. A–H. Pro-inflammatory cytokines IL-1 β , INF- γ , TNF α , IL-17, IL-7, CXCL1, CXCL2, IL-1 α are down-regulated in tamsulosin treated aortic tissue

A–H. IL-1 β , INF- γ , TNF α , IL-17, IL-7, CXCL2, IL-1 α are reduced in the low dose treated groups compared to saline + elastase group (p=0.0166, p=0.0021, p=0.0038, p=0.0011, p=0.0008, p=0.0244, p=0.0028, respectively). IL-1 β , INF- γ , TNF α , IL-17, IL-7, CXCL1, and CXCL2 are decreased in the high dose treated groups compared to saline + elastase group (p=0.0094, p=0.0016, p=0.0136, p=0.0076, p=0.0021, p=0.0297, p=0.0299, respectively).

Fig 4. IP3 levels are suppressed by tamsulosin treatment *in vivo* in murine vascular smooth muscle cells

The highest dose of tamsulosin (30ng/mL) decreased IP3 levels in the murine aortic smooth muscle cells ($p=0.0074$).

Table 1
There was no difference in systolic blood pressure measurements (mmHg) between any groups at all time points
At all time-points tested, there was no difference between the systolic blood pressures of any of the groups.

	Day -5	Day 0	Day 3	Day 7	Day 14
Saline+Saline	140.5 ± 30.88	143.5 ± 18.91	134.3 ± 19.82	117.5 ± 10.34	129.5 ± 14.98
Saline+Elastase	139.0 ± 12.03	125.0 ± 17.09	140.0 ± 17.93	115.8 ± 9.54	126.8 ± 18.79
Low Dose+Elastase	123.0 ± 7.70	135.0 ± 8.52	129.8 ± 11.93	123.8 ± 6.24	133.0 ± 27.28
High Dose+Elastase	119.8 ± 17.02	112.0 ± 20.77	111.8 ± 7.27	121.0 ± 19.04	124.0 ± 20.45