Treatment with the xanthine oxidase inhibitor febuxostat lowers uric acid and alleviates systemic and glomerular hypertension in experimental hyperuricaemia

Laura G. Sánchez-Lozada¹, Edilia Tapia¹, Virgilia Soto², Carmen Ávila-Casado², Martha Franco¹, Lin Zhao³ and Richard J. Johnson⁴

¹Department of Nephrology, ²Department of Pathology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico, ³TAP Pharmaceutical Products Inc., Lake Forest, IL and ⁴Department of Nephrology, Hypertension and Transplantation, University of Florida, Gainesville, FL, USA.

Abstract
Background. Experimentally-induced hyperuricaemia [due to inhibition of uricase with oxonic acid (OA)] in rats causes hypertension and renal alterations which can be prevented by lowering uric acid (UA) with allopurinol. Febuxostat (Fx), an investigational, nonpurine and selective xanthine oxidase inhibitor, is a more effective UA-lowering agent than allopurinol. We therefore tested the hypothesis that Fx might be useful in treating hyperuricemia-induced hypertension and renal damage.

Methods. Four groups of male rats were studied: OA (750 mg/kg by daily gavage) was given for 8 weeks and Fx (5–6 mg/kg/day in drinking water; OA+Fx: n = 10) or placebo (OA+P: n = 11) were administered for 4 weeks beginning at 4 weeks after initiation of the study. Two groups of normal (N) rats were studied as controls (N+P and N+Fx: n = 10/group). Systolic blood pressure (SBP) and fasting plasma UA were measured in all animals at baseline and at 4 and 8 weeks. Glomerular haemodynamics by micropuncture techniques were determined at 8 weeks followed by histological evaluation of glomerular and afferent arteriole morphologies.

Results. In OA-induced hyperuricaemic rats, Fx lowered UA and ameliorated systemic and glomerular hypertension as well as mesangial matrix expansion and the development of preglomerular arteriolar disease as indicated by a reduction of the arteriolar area and media-to-lumen ratio. In normal rats, Fx tended to lower UA and had no effect on blood pressure, renal hemodynamics and afferent arteriole morphology.

Conclusion. These results suggest that Fx merits further evaluation for the treatment of hypertension and renal alterations induced by hyperuricaemia.

Keywords: febuxostat; glomerular hypertension; hyperuricaemia; systemic hypertension; xanthine oxidase

Introduction
A growing body of experimental and clinical evidence relates increased concentrations of uric acid (UA) with the development of hypertension and renal damage [1,2]. For example, hyperuricaemia has been found in nearly 90% of adolescents with newly diagnosed essential hypertension, and blood pressure is linearly correlated (r = 0.8, P < 0.01) with serum UA in these patients [3]. Similarly, mild hyperuricaemia induced by oxonic acid (OA) administration in rats results in the development of systemic hypertension [4].

The underlying causes of hyperuricaemia-induced hypertension may relate to the development of renal disease [5–8], endothelial dysfunction [9–11] and the activation of the renin–angiotensin system (4,12). Evidence that these pathways may be operative in the setting of increased levels of UA has been reported in clinical and experimental studies [4–12].

Several in vitro studies have revealed mechanisms operating at the molecular level that may be involved in the noxious effects induced by UA. Urate, the ionized form of UA present in body fluids, enters vascular smooth muscle cells (VSMC) via an organic anion transport system, present in both human and nonhuman animal cells [13,14]; this is followed by the activation of specific MAP kinases and nuclear transcription factors, with stimulation of COX-2, PDGF A and C chain, PDGF alpha receptor and various inflammatory mediators that include C-reactive protein and monocyte chemoattractant protein-1 [15–18]. These cellular events are likely responsible for the physiological alterations observed during experimental conditions that include the rise of arterial pressure, renal arteriolopathy,
tubulointerstitial infiltration and glomerular hypertension in the setting of renal vasoconstriction [4,5,19]. In addition, urate promotes endothelial dysfunction through inactivation of nitric oxide (NO), probably secondary to an increase in oxidative stress, and halts the proliferation of endothelial cells [9]. The involvement of hyperuricaemia in the development of these alterations is supported by the fact that reducing UA levels with allopurinol or benzo-}

diarone prevents systemic hypertension, renal arteriolopa-

ty, glomerular hypertension and cortical vasoconstriction in experimental hyperuricaemia induced by OA in rats [4,5,19].

Allopurinol, a purine analog, is the only FDA-approved xanthine oxidase inhibitor for the treatment of hyperuricaemia in gout patients. Fx, a nonpurine molecule, is being developed as an inhibitor of xanthine oxidase for the treatment of hyperuricaemia in gout patients [20,21]. Fx is different from allopurinol in that it does not inhibit other enzymes in purine and pyrimidine metabolism pathways [22]. Moreover, the hypouricemic effect exerted by Fx in vitro and in vivo is more potent than that of allopurinol [23]. Studies have shown that Fx inhibited the activity of xanthine oxidase simply by obstructing substrate binding and that this inhibition was not influenced by changes in the redox status of the cofactor [24].

Since the treatment of OA-induced hyperuricaemic rats with allopurinol prevented systemic hypertension, afferent arteriolopathy, glomerular hypertension and cortical vasoconstriction [5,19], the present study evaluated the effect of the novel xanthine oxidase inhibitor Fx in this model of hypertension induced by hyperuricaemia.

Methods

Four groups of male Sprague–Dawley rats were studied for a total period of 8 weeks. To produce hyperuricaemia, 21 animals received regular diet and OA (Sigma, St. Louis, MO, USA) at a dose of 750 mg/kg body weight daily by gastric gavage. Twenty normal (N) rats were studied simultaneously as control groups. After 4 weeks, N+Fx (n = 10) and OA+Fx (n = 10) were given Fx (50 mg/L in drinking water, ∼5–6 mg/kg/day) for another 4 weeks. Two placebo (P) groups, N+P (n = 10) and OA+P (n = 11), received 5.84 mg/L of NaCl in drinking water (to maintain a salt concentration equivalent to the Fx-containing water) for 5–6 mg/kg/day) for another 4 weeks. T wo placebo (P) groups, N+P (n = 10) and OA+P (n = 11), received 5.84 mg/L of NaCl in drinking water (to maintain a salt concentration equivalent to the Fx-containing water) for 4 weeks.

Measurements

Systolic blood pressure (SBP) was measured in conscious rats by a tail cuff sphygmomanometer (XBP-1000, Kent Scientific Corp., Torrington, CT, USA). All animals were preconditioned for blood pressure measurements 1 week before each experiment. Plasma UA was measured by the uricase method (Diagnostic Chem Ltd, Charlottetown, PEI, Canada) in all animals at baseline and at the end of 4 and 8 weeks. The potential presence of OA in the plasma samples was confirmed not to interfere with the assay (data not shown).

Micro puncture

At the end of 8 weeks, the animals were anaesthetized with pentobarbital sodium (30 mg/kg i.p.) and placed on a thermoregulated table to maintain body temperature at 37°C. Trachea, jugular veins, femoral arteries and the left ureter were catheterized with polyethylene tubing (PE-240, PE-50 and PE-10). The left kidney was exposed, placed in a Lucite holder, sealed with agar and covered with Ringer’s solution. Mean arterial pressure (MAP) was monitored with a pressure transducer (model p23 db; Gould, San Juan, PR) connected to the catheter in the femoral artery and recorded on a polygraph (Grass Instruments, Quincy, MA, USA). Blood samples were taken periodically and replaced with blood from a donor rat. Rats were maintained under eu- volmic conditions by infusion of 10 mL/kg of body weight of isotonic rat plasma during surgery, followed by an infusion of 25% polyfructosan, at 2.2 mL/h (Inutest; Fresenius Kabi, Linz, Austria). After 60 min, five to seven samples of proximal tubular fluid were obtained to determine the flow rate and polyfructosan concentrations. Intratubular pressure under free-flow (FF) and stop-flow pressure (SFP) conditions and peritubular capillary pressure (Pc) were measured in other proximal tubules with a servo-null device (Servo Nulling Pressure System; Instrumentation for Physiology and Medicine, San Diego, CA, USA). Glomerular colloid osmotic pressure was estimated from protein concentrations obtained from the blood of the femoral artery (Ca) and surface efferent arterioles (Ce). Polyfructosan was measured in plasma and urine samples by the anthrone-based technique of Davidson and Sackner [25]. The whole-kidney glomerular filtration rate (GFR) was calculated using the following formula: GFR = (U × V) / P, where U is the polyfructosan concentration in urine, V is the urine flow rate and P is the polyfructosan concentration in plasma.

The volume of the fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant bore capillary tube of known internal diameter. The concentration of tubular polyfructosan was measured by the microfluorometric method of Vurek and Pegram [26]. Single-nephron GFR (SNGFR) was calculated using the formula SNGFR = (TF/P)PF × V, where (TF/P)PF is the ratio of concentration of polyfructosan in tubular fluid (TF) and plasma (PF), and V is the tubular flow rate which is obtained by timing the collection of tubular fluid [27]. Protein concentration in afferent and efferent samples was determined according to the method of Viets et al. [28]. MAP, GFR, glomerular capillary hydrostatic pressure (PGC), single-nephron plasma flow (QA), afferent (AR), efferent (ER) and total (TR) arteriolar resistances and ultrafiltration coefficient (Kf) were calculated using equations previously reported [27].

Renal histology and quantification of morphology

After the micropuncture study at the end of 8 weeks, kidneys were washed by perfusion with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde. Renal biopsies were embedded in paraffin. Four-micrometer sections of fixed tissue were stained with periodic acid
Febuxostat alleviates systemic and glomerular hypertension in hyperuricaemic rats

Schiff (PAS) reagent. Arteriolar morphology was assessed by indirect peroxidase immunostaining for alpha-smooth muscle actin (DAKO Corp., Carpinteria, CA, USA). Renal sections incubated with normal rabbit serum were used as negative controls for immunostaining against alpha smooth-muscle actin.

For each arteriole, the outline of the vessel and its internal lumen (excluding the endothelium) were generated using computer analysis to calculate the total medial area (outline–inline), in 10 arterioles per biopsy. The media–lumen ratio was calculated by the outline/inline relationship [5,19]. All analyses were performed blinded.

Glomerular size was defined as the glomerular tuft cross-sectional area and was quantified by computer-based image analysis (Image Pro-Plus version 5.0; Media Cybernetics, Silver Spring, MD, USA). Extracellular mesangial matrix expansion was identified by a PAS-positive material and was measured in all glomeruli with a vascular pole using the same image analyzer (images photographed at ×400). The area of the PAS-positive material in the mesangium was divided by the glomerular-tuft area of the same glomerulus to obtain the fraction of mesangial matrix [29]. Glomerular cellularity was measured by counting all nuclei present in the glomerulus. All analyses were performed blinded.

Statistical analysis

Values are expressed as mean ± standard error (SE). Values from the respective four treatment groups were analyzed by two-way analysis of variance (ANOVA). In the case of repeated measures (i.e. plasma UA and SBP), two-way ANOVA for repeated measures was performed. When the ANOVA P-value was <0.05, comparisons were made using the Bonferroni multiple comparisons test. Relationships between variables were determined using correlation analysis on data from the OA+P and OA+Fx groups.

Results

UA and blood pressure

Figure 1 shows values of UA and SBP at baseline, at 4 and 8 weeks of the study. Baseline values of plasma UA concentration were similar among all groups. As has been shown previously [5], OA produced a doubling of UA values at 4 weeks. Treatment with Fx beginning at 4 weeks reduced the UA levels to the normal range. Normal rats receiving Fx had a significant decrease in their UA levels relative to untreated controls. Reduction of plasma UA concentrations and SBP at Week 8.

Moreover, when both groups were analyzed together, a positive correlation (r = 0.65, P = 0.004) was found between UA and MAP at Week 8.

Glomerular hemodynamics

Six rats (two in each of the N+P, N+Fx and OA+P groups) were discarded due to complications during preparation of the micropuncture experiment. There were no changes in whole-kidney GFR; however, OA caused increases in single nephron GFR and glomerular plasma flow. There were no differences in afferent and efferent resistances among the groups (Table 1). Interestingly, Kf increased in OA+Fx rats relative to the other groups and the difference reached statistical significance relative to the OA+P group (Table 1). The Kf was negatively correlated (r = −0.53, P = 0.02) with UA levels when both OA and OA+Fx groups were analyzed.

Figure 2 shows data for glomerular pressure. As observed previously [9,10], OA-treated rats had a significant elevation in glomerular capillary pressure (N+P; 45.4 ± 1.7 mmHg; OA+P; 54.4 ± 1.5 mmHg; P < 0.001) relative to untreated controls. Reduction of plasma UA with Fx lowered glomerular pressure (OA+Fx; 46.5 ± 1.0 mmHg; P < 0.01 versus OA+P). In addition, a significant correlation (r = 0.74, P = 0.0005) was found...
Fig. 2. Arterial and glomerular pressures, afferent arteriole medial area, media-to-lumen ratio, glomerular cellularity and mesangial matrix fraction measured at Week 8 in normal (N) and oxonic acid-dosed (OA) rats treated with placebo (P) or febuxostat (Fx).

Table 1. Glomerular haemodynamics and size in normal (N) and oxonic acid-dosed (OA) rats treated with placebo (P) or febuxostat (Fx)

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>MAP (mmHg)</th>
<th>GFR (mL/min)</th>
<th>SFP (mmHg)</th>
<th>FF (mmHg)</th>
<th>Pc (mmHg)</th>
<th>PGC (mmHg)</th>
<th>SNGFR (nl/min)</th>
<th>QA (nl/min)</th>
<th>AR (s/cm²)</th>
<th>ER (s/cm²)</th>
<th>Kf (nL/s mmHg)</th>
<th>Glomerular size (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N+P</td>
<td>376 ± 6</td>
<td>118 ± 4</td>
<td>0.74 ± 0.1</td>
<td>29 ± 1</td>
<td>13 ± 1</td>
<td>45 ± 2</td>
<td>35 ± 3</td>
<td>155 ± 18</td>
<td>2.6 ± 0.9</td>
<td>1.2 ± 0.3</td>
<td>0.054 ± 0.009</td>
<td>4597 ± 117</td>
<td></td>
</tr>
<tr>
<td>N+Fx</td>
<td>378 ± 8</td>
<td>123 ± 3</td>
<td>0.91 ± 0.1</td>
<td>31 ± 1</td>
<td>13 ± 1</td>
<td>46 ± 1</td>
<td>35 ± 2</td>
<td>135 ± 11</td>
<td>2.5 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>0.046 ± 0.004</td>
<td>4420 ± 111</td>
<td></td>
</tr>
<tr>
<td>OA+P</td>
<td>385 ± 7</td>
<td>139 ± 3</td>
<td>0.83 ± 0.1</td>
<td>37 ± 1</td>
<td>12 ± 1</td>
<td>54 ± 2</td>
<td>51 ± 6</td>
<td>257 ± 49</td>
<td>1.9 ± 0.4</td>
<td>1.0 ± 0.2</td>
<td>0.041 ± 0.005</td>
<td>4573 ± 166</td>
<td></td>
</tr>
<tr>
<td>OA+Fx</td>
<td>363 ± 6</td>
<td>122 ± 5</td>
<td>0.99 ± 0.1</td>
<td>30 ± 1</td>
<td>13 ± 1</td>
<td>47 ± 1</td>
<td>46 ± 5</td>
<td>184 ± 21</td>
<td>2.0 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0.070 ± 0.011</td>
<td>4578 ± 171</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 versus N+P; °P < 0.05 versus OA+P.

BW: body weight; MAP: mean arterial pressure; GFR: glomerular filtration rate; SFP: stop flow pressure; FF: free-flow pressure; Pc: peritubular capillary pressure; PGC: glomerular capillary pressure; SNGFR: single-nephron GFR; QA: glomerular plasma flow; AR, ER: afferent and efferent arteriolar resistances; Kf: ultrafiltration coefficient.

between UA levels and glomerular pressure at 8 weeks, and a positive correlation was found between both systolic and mean arterial pressures and glomerular pressure (SBP versus PGC, r = 0.59, P = 0.01; MAP versus PGC, r = 0.76, P = 0.0003). The latter result is consistent with a previous report showing that the increased glomerular capillary pressure in hyperuricaemic rats is mediated by an anomalous autoregulatory response of preglomerular vessels to the systemic hypertension [5,19]. Finally, Fx treatment did not alter glomerular hemodynamics in normal rats.

Renal arteriolar morphology

As previously reported [5,19], chronic administration of OA was associated with thickening of the afferent arteriole, as reflected by an increase in medial area (N+P,
Febuxostat alleviates systemic and glomerular hypertension in hyperuricaemic rats

Fig. 3. Periodic acid-Schiff (PAS)-stained kidney sections after 8 weeks from normal and oxonic acid-dosed groups treated with placebo or febuxostat. The arrows indicate the vascular poles of the glomeruli (magnification ×400).

264 ± 13 μm²; OA+P, 353 ± 31 μm²; P < 0.01). Fx treatment was able to alleviate this alteration (OA+Fx, 256 ± 10 μm²; P < 0.001 versus OA+P) (Figure 2). A nonsignificant increase in the media–lumen ratio was also observed with OA (N+P, 3.0 ± 0.2; OA+P, 3.4 ± 0.2; P = ns); this was significantly reduced by Fx (OA+Fx, 2.5 ± 0.2; P < 0.001 versus OA+P) (Figure 2). Fx had no effect on arteriolar morphology in normal rats. In addition, the following correlations were noted at Week 8: plasma UA versus arteriolar area (r = 0.58, P = 0.01), and arteriolar area versus glomerular pressure (r = 0.68, P = 0.002).

Glomerular morphology

Glomerular areas were not different among the groups (Table 1). Figure 3 shows representative digitized photomicrographs of a glomerulus from each experimental group. OA induced a significant increase in glomerular cellularity (N+P, 31 ± 1 nuclei; OA+P, 44 ± 2 nuclei; P < 0.001) and diffused mesangial expansion, characterized by a 3.6-fold increase in the mesangial matrix fraction (N+P, 3.3 ± 0.2%; OA+P, 11.9 ± 0.6%; P < 0.001) as a result of the accumulation of the PAS-positive matrix (Figure 2). Fx treatment reduced matrix expansion (7.4 ± 0.3%; P < 0.001 versus OA+P) and glomerular hypercellularity (35 ± 2 nuclei; P < 0.01 versus OA+P) in OA-dosed animals (Figure 2) and maintained the normal glomerular structure in normal rats (33 ± 2 nuclei; 4.0 ± 0.1% mesangial matrix). The following parameters were found to be correlated with mesangial matrix fraction at Week 8: plasma UA (r = 0.62, P = 0.006); SBP (r = 0.49, P = 0.04); glomerular pressure (r = 0.65, P = 0.004) and arteriolar area (r = 0.52, P = 0.03).

Discussion

In the current study, we demonstrated that normalization of UA levels by Fx treatment was able to reduce both systemic and glomerular pressures and alleviated afferent arteriole thickening, mesangial matrix expansion and glomerular hypercellularity in OA-induced hyperuricaemia. We first examined the effect of Fx in normal rats. While 50 mg/L in drinking water was able to lower UA levels by ~50% without changing blood pressure in normal rats, no untoward effects were observed. Fx treatment preserved SBP within the normal range and micropuncture studies demonstrated normal glomerular haemodynamics and renal function. In addition, afferent arteriole structure and mesangial matrix fraction were unchanged in Fx-treated normal rats.

Consistent with previous studies [4,5,19], the administration of OA resulted in a rise in UA with the development of mild systolic hypertension at 4 weeks. While rats receiving OA continued to be hyperuricaemic and hypertensive after Week 4, Fx treatment initiated after the onset of hypertension in rats receiving OA caused a decrease in plasma UA concentrations and a significant reduction in systemic hypertension. Moreover, as we have shown previously [5,19], the reduction of UA levels was associated with a reduction of glomerular pressure.

The inability of the afferent arteriole to constrict in response to the increase of systemic blood pressure, as suggested by the correlations between SBP and MAP versus glomerular pressure (SBP versus PGC, r = 0.59, P = 0.01; and MAP versus PGC, r = 0.76, P = 0.0003), correlates to the presence of preglomerular arteriolar thickening (AA versus PGC, r = 0.68, P = 0.002). Importantly, Fx was able
to reverse both the systemic and glomerular hypertension as well as the preglomerular microvascular lesion.

The association of systemic hypertension and glomerular hypertension has been demonstrated previously in fawn-hooded rats [30]. In this strain, elevations in arterial pressure, renal blood flow, GFR and glomerular capillary pressure precede the development of renal damage [30]. In addition, there is a correlation between glomerular pressure and arterial pressure, suggesting that in these rats the mechanism responsible for maintaining PGC is less effective, exposing glomerular capillaries directly to variations in systemic blood pressure [31]. Furthermore, fawn-hooded rats also develop artherosclerosis of the preglomerular vessels that is characterized by media hypertrophy and is concurrent with myocyte degeneration of the innermost media layers [32]. In contrast to previous studies in rats that received OA for 5 weeks [5], renal vasoconstriction was not seen in this study after 8 weeks of OA treatment; instead there were increases in both single-nephron GFR and glomerular plasma flow. However, Fx reversed the systemic and glomerular hypertension as well as the preglomerular microvascular lesion seen in OA-treated rats. In addition, the decrease in plasma UA seen in the OA+Fx group was associated with an increase in the Kf (UA versus Kf, r = −0.53, P = 0.02); this likely contributed to the maintenance of single-nephron GFR at levels similar to those seen in the OA+P group despite the significant decrease in PGC induced by Fx treatment.

While the results of this study using Fx are very similar to those reported previously with allopurinol [5], an important difference is the absence of renal vasoconstriction in the OA-treated rats in the current study. This may be the result of a further loss of autoregulatory function due to preglomerular arteriolar injury in this model; in previous studies, the data were collected or the micropuncture was performed after 5 weeks of OA treatment when the arteriolar lesion was still mild. It is possible that the further increase in matrix within the media may effectively block the ability of the renal arterioles to vasocostrict in response to the rise in systemic pressure. Moreover, it is possible that these haemodynaemic responses are responsible for the mild glomerular lesions characterized by hypercellularity and matrix expansion that were observed in the OA-dosed (hyperuricaemic) rats.

Our prior studies have suggested that the renal vasoconstrictive effect observed in hyperuricaemic animals correlates with the plasma level of UA [16,33] and that the mechanism may be due to the ability of UA to reduce endothelial nitric oxide [9]. For example, a 5-fold increase in plasma UA was observed in rats administered both OA and UA in their diet for 10 days, and was associated with a significant decrease of GFR, suggesting severe renal vasoconstriction [34]. In the current study, UA levels after 8 weeks of treatment with OA were lower than they were in previous 5-week studies (in which there was a significant decrease in single-nephron GFR, suggesting severe renal vasoconstriction), and this difference may also account for the lack of significant renal vasoconstriction in this instance. It is possible that while OA raises UA acutely, over time, rat uricase is upregulated and UA levels end up being lower.

In summary, these studies provide additional evidence that UA may play a role in the development of hypertension. Since Fx treatment lowered the elevated systemic and glomerular pressures in this animal model of OA-induced hyperuricaemia, this novel xanthine oxidase inhibitor merits further evaluation for the treatment of hypertension and renal alterations induced by hyperuricaemia.

Acknowledgements. This study was supported by TAP Pharmaceutical Products Inc., Lake Forest, IL, USA and grant number 52021/66778 from the National Council of Science and Technology (CONACyT), Mexico. We express our gratitude to the late Jaime Herrera-Acosta, for his contributions and stimulating discussions during the conception of this project. We thank Magdalena Cristóbal, José Santamaria and Benito Chávez-Rentería for technical assistance.

Conflict of interest statement. Part of Sánchez-Lozada’s research was supported by TAP Pharmaceutical Products Inc. in the calendar years 2005 and 2006. L. Zhao is an employee of TAP Pharmaceutical Products Inc. Dr. R. J. Johnson was a consultant for TAP Pharmaceutical Products Inc. in the calendar years 2005–2006. Other authors have nothing to declare.

References


Received for publication: 2.5.07
Accepted in revised form: 8.10.07