JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 1581–1586

Received December 9, 2010
Accepted August 29, 2011
DOI
10.1111/j.2042-7158.2011.01361.x
ISSN 0022-3573

Research Paper

Ergosta-4,6,8(14),22-tetraen-3-one isolated from *Polyporus umbellatus* prevents early renal injury in aristolochic acid-induced nephropathy rats

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Abstract

Objectives Aristolochic acid (AA) nephropathy, first reported as Chinese herbs nephropathy, is a rapidly progressive tubulointerstitial nephropathy that results in severe anemia, interstitial fibrosis and end-stage renal disease. Tubulointerstitial injury was studied in a rat model of AA nephropathy to determine whether ergosta-4,6,8(14),22-tetraen-3-one (ergone) treatment prevents early renal injury in rats with aristolochic acid I-induced nephropathy.

Methods Early renal injury via renal interstitial fibrosis was induced in rats by administration of aristolochic acid I (AAI) solution intragastrically for 8 weeks. Ninety-six rats were randomly divided into four groups ($n = 24$/group): (1) control (2) AAI (3) AAI + ergone (10 mg/kg) and (4) AAI + ergone (20 mg/kg). Blood and urine samples were collected and rat were sacrificed for histological assessment of the kidneys on at the end of weeks 2, 4, 6 and 8.

Key findings AAI caused progressive elevation of blood urea nitrogen, creatinine, potassium, sodium, chlorine, proteinuria and urinary N-acetyl-β-D-glucosaminidase (NAG). Ergone suppressed elevation of blood urea, nitrogen, creatinine, proteinuria and urinary NAG to some degree, but the AAI–ergone-treated group did not differ from AAI-treated group for body weight, serum potassium, sodium and chlorine. The progress of the lesions in the kidney after AAI administration was also observed by histopathological examinations, but kidneys from rats of AAI–ergone-treated group displayed fewer lesions.

Conclusions Ergone treatment conferred protection against early renal injury in a rat model of AA nephropathy. Early administration of ergone may prevent the progression of renal injury and the subsequent renal fibrosis in AA nephropathy.

Keywords aristolochic acid I; early renal injury; ergosta-4,6,8(14),22-tetraen-3-one; renal interstitial fibrosis

Introduction

Chronic renal failure (CRF) is one of the most important public health problems, with increasing rates of incidence and prevalence.[1] Although the number of patients with end-stage renal disease has increased rapidly over the last three decades and, prior to the advent of dialysis, some treatments – including dietary control, drug therapy with a spherical carbonaceous absorbents, and antihypertensive drugs, including ACEIs (angiotensin converting enzyme inhibitors) and ARBs (angiotensin receptor blockades) – have shown some inhibition of the progression of CRF, these treatments are still not effective enough to curtail this increase. The development of new effective drugs that prevent the progression of CRF is therefore an urgent requirement. In the present article, we show the first indication that ergosta-4,6,8(14),22-tetraen-3-one (ergone) from a medical fungus *Polyporus umbellatus* could be such a candidate.

The fungus *Polyporus umbellatus* has commonly been used in Chinese traditional medicine for hundreds of years for treatment of various renal diseases. It possesses a relative diuretic effect and the ability to reinforce the functioning of water pathways capable of drain dampness, and is described as Shennong's *Herbal Classics*. It is also described as promoting

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urination and leaving out dampness, the problems caused by stagnance of dampness such as edema, scanty urine, vaginal discharge and cloudy painful urinary dysfunction.[2] Ergone is an anti-aldosteronic diuretic steroid, which is one of the main components in *Polyporus umbellatus*.[3–5] It also exists widely in other medicinal fungi, lichens and plants such as *Cordyceps sinensis*, *Vietnamese xylaria* and *Zopfiella longicaudata*.[6–8] We have recently reported that ergone also has diuretic and cytotoxic activity,[9–11] consistent with previous reports.[12,13] Our pharmacokinetic experiments have demonstrated that ergone levels are higher in faeces than in urine. Almost 57% of the loading dose is cumulative in the faeces within 24 h after oral administration, but ergone could be undetected in the urine.[14–16]

In recent years, many countries, including the USA and the UK, have realized that some Chinese herbs contain kidney-toxic aristolochic acid (AA) and have banned the use of Chinese traditional medicines containing AA in medical practice. Besides carcinogenic and mutagenic properties, AA can cause kidney damage and ultimately lead to renal interstitial fibrosis.[17–23] The mechanisms that lead to kidney damage by AA to date are not well understood, but AA-induced renal interstitial fibrosis in rats is a good model to use to study the pathogenesis of the condition.[24–26] Here we report the findings of an experimental study on ergone chemoprevention of early renal injury induced by an aristolochic acid I (AAI) from *Caulis aristolochiae manshuriensis* (Chinese: Guan-Mu-Tong).

Materials and Methods

Chemicals and animals

Ergone was synthesized by the author (Ying-Yong Zhao) and its purity (≥98.5%) was determined by HPLC. AAI (batch no. 110746–200406, purity 99.0%) was obtained from the National Institutes for Food and Drug Control (Beijing, China).

The study was conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China. All procedures and the care of the rats were in accordance with institutional guidelines for animal use in research. Male Sprague–Dawley rats were obtained from the Central Animal Breeding House of Xi'an Jiaotong University (Xi'an, China). They were maintained at a constant humidity (*c.* 60%) and temperature (*c.* 23°C) with a light/dark cycle of 12 h.

Experimental design using aristolochic acid I-induced nephropathy rats

Male rats underwent an adaptation period of several days, during which they were fed a commercial feed. The rats, weighing 190–210 g, were divided into four groups ($n = 24$/group) after measuring body weight. Groups 2, 3 and 4 then gavaged with 70 mg/kg body weight of AAI dissolved in 1% (w/v) gum acacia solution, which produced experimental renal failure in the animals after 8 weeks. Group 1 was given with an equal volume of gum acacia solution. During the period of gastric gavage of AAI, after 3 h, groups 3 and 4 were gavaged with 10 and 20 mg/kg body weight of ergone dissolved in 1% (w/v) gum acacia solution for 8 weeks, while groups 1 and 2 were similarly given an equal volume of gum acacia solution. Body weight was measured daily for all rats. At the end of weeks 2, 4, 6 and 8, six individual rats of each group selected randomly were placed in metabolic cages to obtain 24-h urine collections for the measurement of proteinuria concentrations. They were then decapitated. Trunk blood was collected and used for determination of serum creatinine (Scr) and blood urea nitrogen (BUN) concentrations. Bilateral kidneys were removed. The left kidney was stored in 10% formaldehyde solution. Specimens were embedded in paraffin and cut transversely into 5-μm-thick slices on a microtome (Leica Corp., Nussloch, Germany). The right kidney was quickly frozen for molecular studies.

Renal function and proteinuria

The rats were placed in metabolic cages for assessment of proteinuria. The concentrations of Scr, BUN and urinary protein were measured with an Olympus AU640 automatic analyser and all reagents were from Olympus Diagnostics.[27,28] The concentrations of serum potassium, sodium and chlorine were measured with an Easylyte plus Analyzer (Medica, America).[29] The concentrations of urinary N-acetyl-β-D-glucosaminidase (NAG) were measured spectrophotometrically.

Histopathological analysis

The kidneys were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin (HE) and periodic acid Schiff (PAS) reagents.

Statistical analysis

Statistical significance was determined by two-way ANOVA followed by the Duncan test to determine differences between treatment groups and by paired or unpaired *t*-tests as appropriate; $P < 0.05$ was considered significant.

Results

Body weight, renal function and urinary protein excretion

Table 1 shows pathophysiologic parameters in the four groups of rats. Table 1 shows body weight over the 8-week study period. Whereas body weight steadily increased in control rats, it increased only slightly in both AAI-treated and AAI–ergone-treated rats. In the AAI-treated group, body weights were significantly decreased compared to the control group. Body weights in the AAI–ergone-treated rats were slightly greater than those treated with AAI alone over the 8-week study period, but did not arrive at statistical significance (Table 1). The normal levels of Scr and BUN in rats are 59.1 μmol/l and 5.5 mmol/l ($n = 6$), respectively. The Scr level in only the AAI-treated rats markedly increased beyond the normal level. The administration of ergone at doses of 10 and 20 mg/kg significantly reduced Scr levels (Table 1). Similarly, the BUN level in rats treated with AAI alone increased

Table 1 Body weight, serum and urinary parameters in control rats, rats treated with aristolochic acid I (AAI) alone and rats treated with AAI plus ergone (10 or 20 mg/kg)

Parameters	AAI administration period			
	2 weeks	4 weeks	6 weeks	8weeks
Body weight (g)				
Control	262 ± 20	328 ± 21	382 ± 21	439 ± 23
AAI	202 ± 16[b]	187 ± 23[b]	189 ± 23[b]	209 ± 19[b]
AAI + ergone 10 mg/kg	203 ± 21[d]	195 ± 23[d]	217 ± 18[d]	225 ± 26[d]
AAI + ergone 20 mg/kg	196 ± 23[d]	197 ± 19[d]	221 ± 20[d]	232 ± 23[d]
Scr (μmol/l)				
Control	59.55 ± 3.84	60.74 ± 3.23	57.24 ± 3.05	58.72 ± 5.45
AAI	69.75 ± 4.80[b]	87.02 ± 3.56[b]	95.56 ± 4.07[b]	104.95 ± 5.38[b]
AAI + ergone 10 mg/kg	67.23 ± 3.25	69.57 ± 4.27[d]	79.08 ± 3.95[d]	85.56 ± 4.38[d]
AAI + ergone 20 mg/kg	68.09 ± 4.05	68.72 ± 3.95[d]	78.56 ± 4.56[d]	83.25 ± 3.96[d]
BUN (mmol/l)				
Control	5.50 ± 0.65	5.29 ± 0.72	5.65 ± 0.59	5.76 ± 0.91
AAI	8.29 ± 0.92[b]	14.71 ± 1.29[b]	23.01 ± 2.45[b]	28.62 ± 3.08[b]
AAI + ergone 10 mg/kg	6.20 ± 1.12[c]	10.76 ± 2.32[d]	16.48 ± 2.45[d]	20.63 ± 1.92[d]
AAI + ergone 20 mg/kg	6.71 ± 0.83[c]	9.75 ± 3.02[d]	15.09 ± 2.29[d]	18.62 ± 3.48[d]
Potassium (mmol/l)				
Control	5.40 ± 0.51	5.31 ± 0.98	5.50 ± 1.54	5.41 ± 0.64
AAI	5.49 ± 1.02	7.25 ± 1.21[b]	7.38 ± 1.12[b]	7.63 ± 0.78[b]
AAI + ergone 10 mg/kg	5.32 ± 0.97	6.14 ± 1.23	6.25 ± 1.08	6.91 ± 0.87
AAI + ergone 20 mg/kg	5.42 ± 0.68	6.12 ± 1.54	5.98 ± 1.12	6.45 ± 0.98[c]
Sodium (mmol/l)				
Control	139 ± 13	151 ± 14	147 ± 10	148 ± 15
AAI	138 ± 15	154 ± 18	151 ± 12	148 ± 13
AAI + ergone 10 mg/kg	137 ± 12	153 ± 16	150 ± 14	148 ± 15
AAI + ergone 20 mg/kg	140 ± 16	154 ± 17	153 ± 13	146 ± 14
Chlorine (mmol/l)				
Control	101 ± 10	102 ± 13	99 ± 9	101 ± 15
AAI	110 ± 8	115 ± 12	117 ± 15[a]	121 ± 9[a]
AAI + ergone 10 mg/kg	108 ± 12	113 ± 10	115 ± 15	120 ± 14
AAI + ergone 20 mg/kg	110 ± 10	111 ± 13	112 ± 12	117 ± 16
Proteinuria (mg/day)				
Control	5.4 ± 1.2	5.4 ± 4.6	5.8 ± 3.8	5.7 ± 4.0
AAI	15.6 ± 1.5[b]	20.7 ± 3.9[b]	22.1 ± 4.3[b]	24.5 ± 3.2[b]
AAI + ergone 10 mg/kg	13.2 ± 0.9	14.9 ± 3.5[c]	17.1 ± 4.4[c]	19.3 ± 4.3[c]
AAI + ergone 20 mg/kg	12.3 ± 1.3	12.9 ± 4.2[d]	15.7 ± 3.8[d]	18.1 ± 3.7[d]
NAG (μg/day)				
Control	2.51 ± 0.91	2.57 ± 2.09	2.61 ± 1.51	2.54 ± 1.02
AAI	5.15 ± 0.56[b]	7.54 ± 1.94[b]	9.11 ± 1.26[b]	10.68 ± 1.41[b]
AAI + ergone 10 mg/kg	4.56 ± 1.09	5.02 ± 1.81[c]	7.09 ± 1.31[c]	8.21 ± 1.53[c]
AAI + ergone 20 mg/kg	4.25 ± 0.85	5.32 ± 1.94[c]	6.76 ± 1.65[c]	8.01 ± 1.38[c]

Values are mean ± SD. The number of animals in each group was 6. [a]$P < 0.05$, [b]$P < 0.01$ vs control by ANOVA; [c]$P < 0.05$, [d]$P < 0.01$ vs AAI by ANOVA.

to *c*. 28.6 mmol/l. The administration of ergone at doses of 10 or 20 mg/kg significantly reduced BUN levels (Table 1).

Serum potassium, sodium and chlorine concentrations in the AAI–ergone-treated rats were slightly lower than those treated with AAI alone over the 8-week study period, but did not arrive at statistical significance (Table 1). Urinary protein excretion was not significantly different between AAI-treated (15.6 ± 4.6 mg/day) and AAI–ergone-treated (13.2 ± 3.8 mg/day for 10 mg/kg and 12.3 ± 4.0 mg/day for 20 mg/kg oral administration) groups at the end of week 2. However, urinary protein excretion in the AAI–ergone-treated rats was markedly decreased compared with AAI-treated rats at the end of weeks 4, 6 and 8 (Table 1). In AAI–ergone treated rats, urinary NAG excretion was 4.56 ± 1.51 μg/day for 10 mg/kg and 4.25 ± 1.02 μg/day for 20 mg/kg oral administration, values that were not significantly different from those in rats given AAI alone (5.15 ± 2.09 μg/day) at the end of week 2, but urinary protein excretion in the AAI–ergone-treated rats was markedly decreased as compared with only AAI-treated rats at the end of weeks 4, 6 and 8 (Table 1).

Histopathological changes

Figure 1 shows histopathological changes in the four groups of rats at the end of week 6. Kidney tissues were stained with HE or with PAS. No significant abnormalities were observed in the renal tissue samples obtained over the 8-week study period from control rats (Figure 1 (H1) and (P1)). In contrast, the progress of the lesions in the kidney after AAI

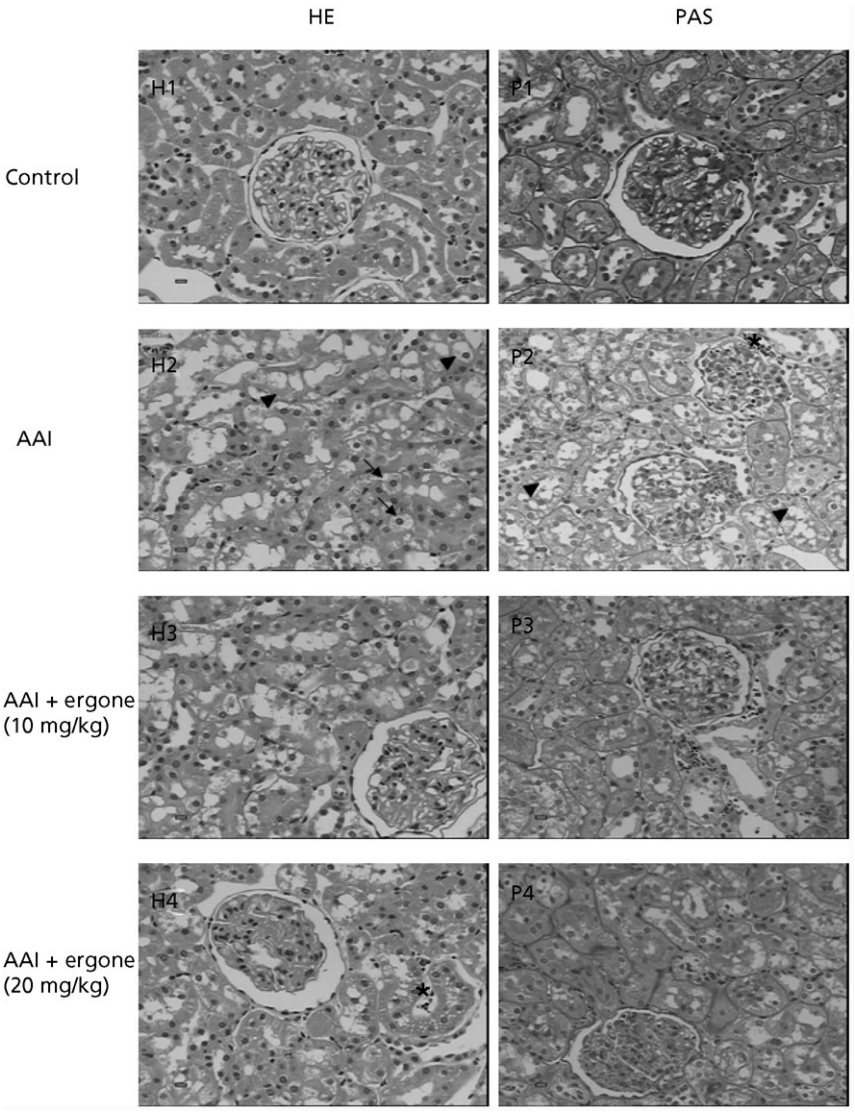

Figure 1 Kidney histological features of rat with AAI oral administration. Mice were sacrificed at the indicated time after AAI administration. Kidney tissues were stained with HE (H1–H4) or PAS (P1–P4). No morphological changes were noted for control rats (H1 and P1) at the end of week 6. Typical lesions, consisting of granular degeneration (arrows), vacuolar degeneration (arrowheads) and lymphocytic infiltrate (asterisk), were observed in both AAI- (H2, P2) and AAI–ergone-treated rats (H3, H4, P3 and P4) at the end of week 6. Paraffin-embedded sections (5 μm) were stained with haematoxylin and eosin (H1–H4), or with periodic acid Schiff (P1–P4). Magnification: ×200.

administration was observed. Slight granular degeneration of the tubular epithelium in the kidney was seen at the end of week 4 after AAI administration. Lesions of granular degeneration, vacuolar degeneration and lymphocytic infiltrate were observed at the end of week 6 after AAI administration (Figure 1 (H2) and (P2)). Lesions characterized by tubular dilation along with discrete tubular necrosis were seen in the kidney at the end of week 8 (not shown). Multiple foci of tubular necrosis and atrophy, as well as lymphocytic innltrate free of polynuclear neutrophils, were present in the deep cortex (not shown). Simultaneously, kidneys from rats of AAI–ergone-treated group displayed fewer lesions at ergone doses of 10 mg/kg (Figure 1 (H3) and (P3)) and 20 mg/kg (Figure 1 (H4) and (P4)).

Discussion

Our present study indicates that AAI induces a kidney injury with the general characteristics of the progress of renal interstitial fibrosis and that ergone has to some degree a preventative effect on the development of early renal injury. In recent years, kidney injuries caused by Chinese herbal medicines have been increasingly reported, most of which are related to *Caulis aristolochiae manshuriensis*.[30,31] Although the toxic component in *Caulis aristolochiae manshuriensis* has been identified as AA, the mechanisms and pathological features in this toxicity and how to prevent kidney injury from such a herb are only poorly understood. Our study indicates that blood (BUN, Scr) and urine (proteinuria, urinary NAG)

biochemistry indices have significant differences between the AAI–ergone-treated group and the AAI-treated group, suggesting that ergone may prevent early renal injury to a certain degree. HE (H2–H4), or PAS (P2–P4) staining shows obvious differences in the degree of early renal injury. Many studies have demonstrated that the renin–angiotensin–aldosterone system is implicated as a major mechanism in sustaining renal disease.[32,33] Recent studies in humans and experimental models have shown that aldosterone plays a pivotal role in the pathophysiology of renal injury. Aldosterone causes inflammatory response, endothelial dysfunction and fibrosis by increasing plasminogen activator inhibitor-1 (PAI-1) and TGF-β1 expression and by stimulating production of reactive oxygen species.[34] Ergone is an anti-aldosteronic compound.[12] In our and other studies on the association of aldosterone with renal fibrosis, a similar phenomenon was observed, although the detailed mechanisms are unclear.[34]

Conclusions

In conclusion, a rat model of AA nephropathy may be a powerful tool for studying the progression of tubulointerstitial nephropathy at the early stage. Long-term treatment with ergone prevented early tubular damage and thereby inhibited the progression of interstitial fibrosis in a rat model of AA nephropathy. These results suggest that early treatment with ergone, successfully used in an animal model, represents a potential new treatment to limit renal tissue damage in AA nephropathy in humans.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This study was supported in part by grants from the National Scientific Foundation of China project No. 81001622 and No. 2011ZX09401-308-34 from the Ministry of Science and Technology of the People's Republic of China and Scientific Research Program. It was also funded by the Shaanxi Provincial Education Department (No. 11JK0678).

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