Experimental and Toxicologic Pathology 68 (2016) 315–320

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Oral exposure to aristolochic acid I induces gastric histological lesions with non-specific renal injury in rat

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ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 26 February 2016
Accepted 21 March 2016

Keywords:
Aristolochic acid I
Stomach
Kidney
Histology
Toxicity
Rat

ABSTRACT

Many *Aristolochia* species herbal drugs, used for diseases treatment since antiquity, contain active component aristolochic acid mixture, which consists of aristolochic acid I and II. However, it remains unclear whether aristolochic acid I is gastrotoxic, though evidence has shown that aristolochic acid mixture is nephrotoxic, carcinogenic, and genotoxic. The present study aimed to investigate the gastrotoxicity in rats treated with aristolochic acid I alone. Four groups of rats were orally administrated with vehicle (1% $NaHCO_3$), or 30 mg, 60 mg, and 90 mg/kg/day of aristolochic acid I for twelve days. The results showed that aristolochic acid I can induce obvious body weight loss, forestomach injury characterized by necrosis, ulcer, hyperkeratosis, and hyperplasia of epithelial cells. The severity of these forestomach lesions was presented in a dose-dependent mode. Meanwhile, only non-specific, slight renal tubule degeneration, and occasionally single necrotic epithelial cell were found in aristolochic acid I-treated rats' kidney. These resulst indicated aristolochic acid I had obvious gastrotoxicity, and such aristolochic acid I-induced forestomach toxicity probably presented much prior to kidney injury. Such irritation lesions may play a partial role in gastric cancer development of rats induced by aristolochic acid. Therefore, these results expanded our understanding on the digestive system toxicity of aristolochic acid I.

1. Introduction

Aristolochic acids (AAs) are natural products derived from taxa in the Aristolochiaceae. They originate primarily from the genera *Aristolochia* and *Asarum* (NTP, 2008). Traditional Chinese Medicine containing aristolochic acid (AA) has been used extensively in hepatitis, uropoietic system and cardiovascular system (Zhang et al., 2013). Epidemiological studies showed that AA exposure is associated with a high risk of nephrotoxicity and upper urinary tract carcinoma (UUC) (De Broe, 1999; Grollman et al., 2007; Nortier et al., 2000). This disease, named aristolochic acid nephropathy (AAN) was initially reported in a Belgian cohort of more than 100 patients after the intake of slimming pills containing a Chinese herb, *Aristolochia fangchi* ((Michl et al., 2014). Subsequently, new sporadic AAN cases were regularly reported throughout the world (Debelle et al., 2008). It has been estimated that 100 million people may be at risk of developing AAN in China alone (Hu et al., 2004).

AA is a mixture containing structurally-related aristolochic acid I (AA I) and aristolochic acid II (AA II). Additionally, the content of AA I is highest among AA mixture, and it is the most representative substance in AA compound. Research showed that AA I induced tubular cell necrosis and interstitial fibrosis in the renal cortex, but AA II only resulted in minimal changes in the renal cortex of the male C3H/He mice (Shibutani et al., 2007). DNA adduct induced by AA I in stomach was significantly higher than AA II (Shibutani et al., 2007). These results indicated AA I was the main reason for kidney injury, and may play a critical role in gastric toxicity.

Previous report showed that intragastric administration of 10 mg/kg/day AA (77.24% AA I and 22.18% AA II) in rats caused isolated swollen epithelial cells only twenty-four hours after the first administration, which showed vacuolation of the cytoplasm and cystic distension of the nucleus (Mengs, 1983). Branched papilloma of the forestomach was observed after 90 days (Mengs, 1983). Later, the same researcher reported that the rats was give once the sodium salt of AA mixture by gastric intubation at a dose of 200 mg/kg, marked hyperplasia with hyperkeratosis of the

Abbreviations: AAs, aristolochic acids; AA, aristolochic acid; AA I, aristolochic acid I; AA II, aristolochic acid II; UUC, upper urinary tract carcinoma; AAN, aristolochic acid nephropathy; TP, total protein; ALB, albumin; BUN, blood urea nitrogen; CREA, creatinine; HE, hematoxylin and eosin.

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http://dx.doi.org/10.1016/j.etp.2016.03.003
0940-2993/

forestomach epithelium was developed after 14 days (Mengs, 1987). It is also reported that AA mixture causes DNA adducts formation (dA-AAI, dG-AAI, dA-AAII, dG-AAII) and is carcinogenic in rat forestomach and stomach (Stiborová et al., 1994; NTP, 2014). However, as AA mixture (AA-I and AA-II) was used in these studies, it was not possible to determine whether one or both components of AA contributed to the gastrotoxic effect.

Although there are many researches about the AA nephrotoxicity, most ones focused on AA mixture. As for digestive system toxicity, there is no report to define the effect of individual AA I on stomach. For this purpose, the effect of AA I on rat stomach was investigated via histology in the present study, and the nephrotoxic effect of AA I was evaluated as well.

2. Materials and methods

2.1. Reagents

AA I was supplied by Sigma Aldrich, and the purity is no less than 90%. Sodium bicarbonate ($NaHCO_3$) was purchase from Sinopham Chemical Reagent Co., Ltd. Water was supplied by Millipore Elix, and sterilized to use. One percent of $NaHCO_3$ solution (1% $NaHCO_3$), as a control vehicle, was prepared in sterilized water, and AA I solution was prepared in 1% $NaHCO_3$ solution.

2.2. Laboratory animals and treatment

The laboratory animals used were 8–9 weeks old male healthy Wistar rats (Shanghai Laboratory Animal Centre, Chinese Academy of Sciences), with body weight initially ranging from 220 to 230 g. All animals were used and cared in compliance with the local Institutional Animal Care and Use Committee (Centre for Drug Safety Evaluation and Research, Shanghai University of Traditional Chinese Medicine). Twelve Wistar rats were kept in plastic cages in SPF animal room(temperature: 20–25 °C, relative humidity: 40–70%). Standard food and drinking water were available ad libitum.

Rats were randomly divided into 4 groups with 3 rats per group. Three groups of rats were given AA I by gastric intubation at dose of 30, 60 and 90 mg/kg/day for 12 days, respectively. Control rats were given an equivalent amount (10 ml/kg) of 1% $NaHCO_3$ (control vehicle).

2.3. Clinical pathological data collection and detection

On day 1, 2, 4, 6, 8, 10 and 12, body weight was measured just before treatment, and the administration volume was adjusted according to the body weight. Blood was taken from tail vein, and 6 h urine was collected after each treatment on day 4, 8 and 12. All blood and urine were proceeded to detect levels of total protein (TP), albumin (ALB), blood urea nitrogen (BUN), and creatinine (CREA), using automatic biochemical analyzer (HITACHI 7080).

2.4. Histology

All rats were euthanized on day 12. Blood was collected from abdominal aorta. Kidney and stomach of each rat were removed and fixed in 10% neutral buffered formalin for at least 24 h, then dehydrated, embedded, sectioned with 4 μm in thickness, and stained with hematoxylin and eosin (HE) for histological analysis.

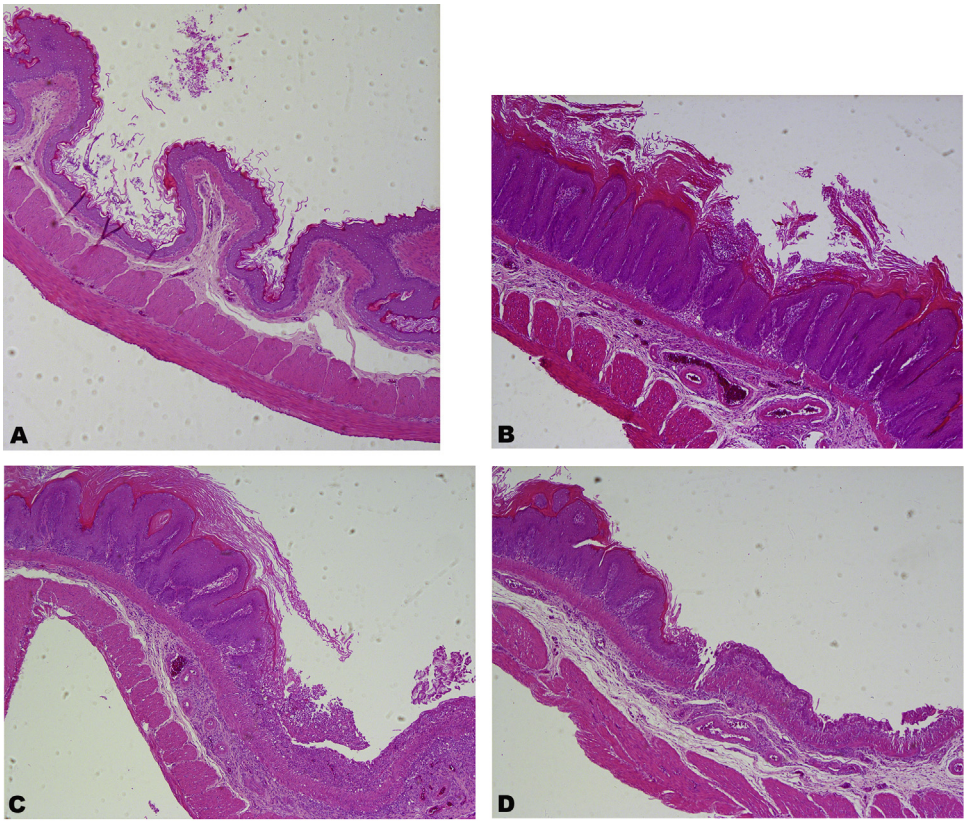

Fig. 1. Forestomach lesions induced by AA I. (A) No histological changes were founded in the stomach of control rats. (B) Focal ulceration, and marked hyperplasia with hyperkeratosis of forestomach epithelium were showed (30 mg/kg AA I). (C) Ulceration was obvious, with hyperplasia, and hyperkeratosis of forestomach epithelium (60 mg/kg AA I). (D) Massive ulceration, and marked hyperplasia with hyperkeratosis of the forestomach epithelium were presented (90 mg/kg AA I). H&E, 50×.