European Journal of Pharmacology 780 (2016) 136–141

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Effect of amlodipine on mouse renal interstitial fibrosis

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ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form
2 March 2016
Accepted 23 March 2016
Available online 28 March 2016

Keywords:
Unilateral ureteral obstruction
Amlodipine
Fibrosis
Heat shock protein 47
Type IV collagen
c-jun-N-terminal kinase (JNK)

ABSTRACT

Unilateral ureteral obstruction (UUO) is a well-established method to study interstitial fibrosis of the kidney. In this study, we investigated the effects of a calcium channel blocker, amlodipine, on UUO-induced renal interstitial fibrosis in mice. UUO significantly increased the fibrotic area in the obstructed kidney, but this change was inhibited by amlodipine (6.7 mg/kg/day in drinking water). mRNA expression of heat shock protein (HSP) 47 and type IV collagen was increased in the kidneys of UUO mice. Amlodipine reduced the expression of both HSP47 and type IV collagen mRNAs. Phosphorylation of c-jun-N-terminal kinase (JNK) was significantly increased by UUO, but the change was inhibited by amlodipine. Collectively, these results suggest that amlodipine may inhibit the expression of HSP47 and type IV collagen by reducing phosphorylation of JNK and ameliorating the renal interstitial fibrosis induced by UUO.

1. Introduction

Renal interstitial fibrosis, which is characterized by the proliferation of interstitial fibroblasts and excessive accumulation of extracellular matrix components, is a common final pathology of most progressive renal diseases. Unilateral ureteral obstruction (UUO) is a well-described model of renal fibrosis leading to tubulointerstitial fibrosis (Klahr and Morrissey, 2002). UUO is associated with the induction of ischemia, hypoxia, or oxidative stress and consequently, renal tubular epithelial cells undergo apoptotic cell death or epithelial-mesenchymal transition (Chevalier, 2006).

We previously showed that a COX-2 inhibitor, meloxicam, ameliorates UUO-induced renal interstitial fibrosis by inhibiting the expression of HSP47 and type IV collagen (Honma et al., 2014). Moreover, meloxicam reduces the increase in phosphorylation of ERK and JNK, which is observed after UUO. The expression levels of HSP47 and type I, III and IV collagens are markedly upregulated in UUO, and injection of HSP47 siRNA significantly reduces the protein expression levels and interstitial fibrosis (Xia et al., 2008). Xiao et al. (2012) reported that ERK and JNK signaling events are involved in modulating the expression of HSP47 and contribute to renal fibrosis by enhancing the synthesis and deposition of extracellular matrix proteins.

Calcium channel blockers, which target voltage-dependent calcium channels, are widely used in hypertension therapy and rank second in the pharmaceutical market for hypertension treatment. Indeed, calcium channel blockers are believed to have less serious adverse effects than other antihypertensive drugs and are reliable drugs for lowering blood pressure. Furthermore, some reports have shown that calcium channel blockers improve UUO-induced renal interstitial fibrosis, although the precise mechanisms of renal protection are still unknown (Topcu et al., 2008; Matsuda et al., 2011; Mishima et al., 2013). Therefore, in the present study, we investigated the possible role of the mitogen-activated protein kinase (MAPK) family in the protective effect of a calcium channel blocker, amlodipine, in UUO-induced renal interstitial fibrosis.

2. Materials and methods

2.1. Materials

Amlodipine was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Anti-ERK,-phospho-ERK,-JNK, and -phospho-JNK antibodies were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). Other chemicals and drugs were reagent grade or the highest quality available.

2.2. Animals

Five weeks old male BALB/cCrSlc mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). Mice were housed 3–5 per cage under a 12 h light and dark schedule for at least 1 week

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http://dx.doi.org/10.1016/j.ejphar.2016.03.041
0014-2999/

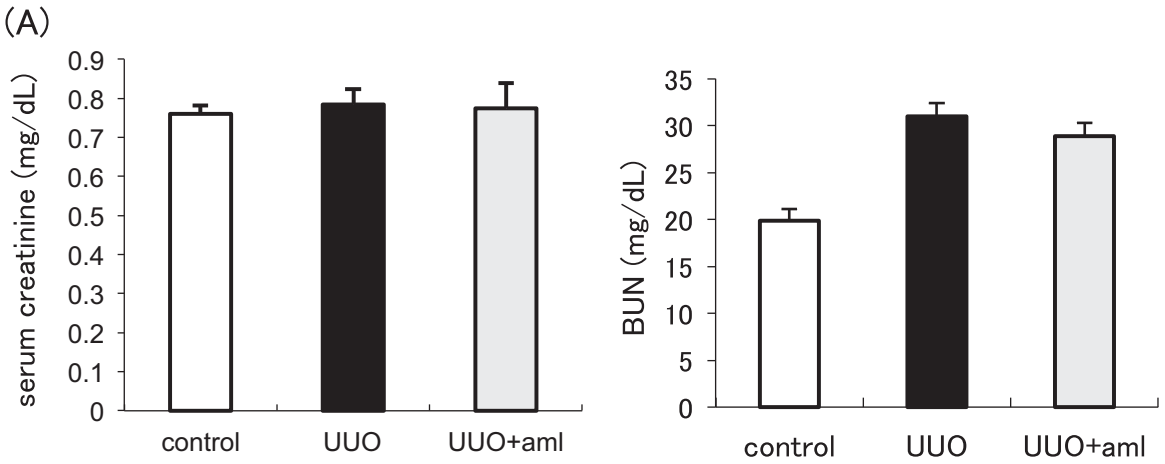

Fig. 1. Effect of amlodipine on renal functions after UUO. (A) Serum creatinine and (B) BUN were measured at 7 days after UUO in mice with a sham operation (control), UUO, or UUO and amlodipine (aml) treatment. N=6 per group. Data are presented as the mean ± S. E. M. $^{*}P < 0.05$.

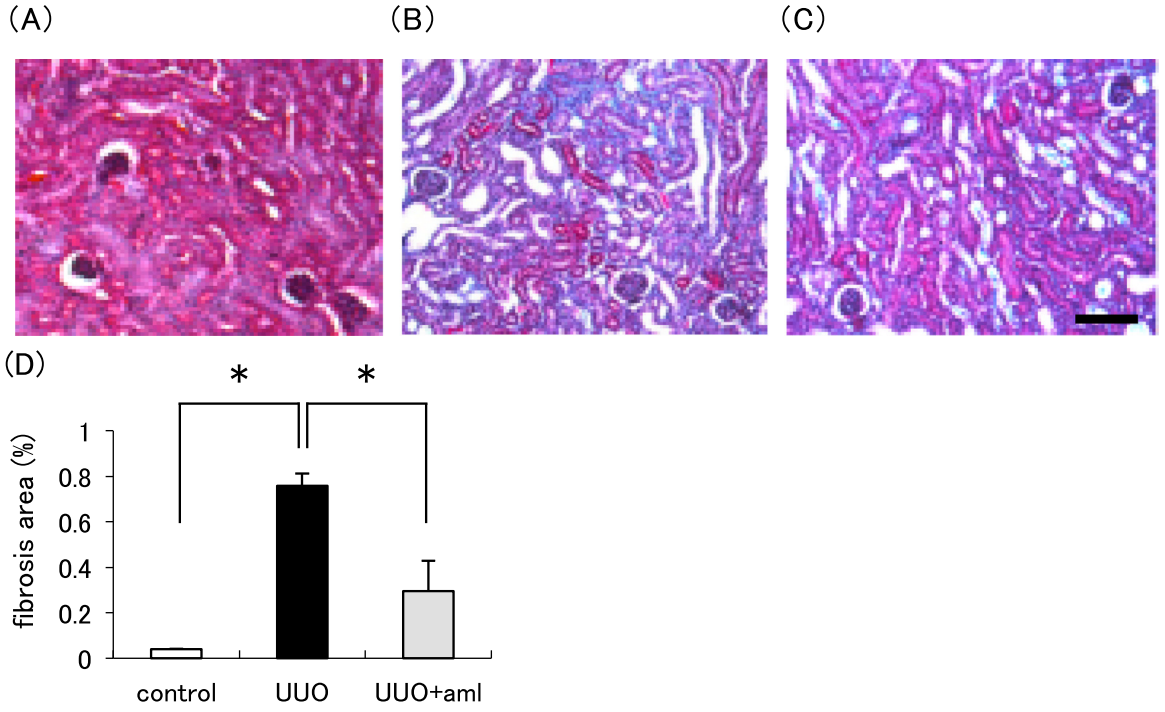

Fig. 2. Masson's trichrome staining of kidneys at 7 days after sham operation (A), UUO (B), or UUO and amlodipine treatment (C). Scale bar = 100 μm. Magnification, 200 ×. (D) Quantitative analysis of the fibrotic area. Each column represents the mean ± S. E. M. of four separate observations. $^{*}P < 0.05$.

before UUO surgery. All animal experiments were approved by the Ethics Committee for Animal Experiments in accordance with the Guidelines for Animal Experiments of Takasaki University of Health and Welfare and the Japanese Government Animal Protection and Management Law. Efforts were made to minimize suffering and reduce the number of animals used in experiments.

2.3. Experimental protocols

UUO was performed as described previously (Honma et al., 2014). In brief, a midline abdominal incision was made while the mice were under anesthesia (pentobarbital, 50 mg/kg, intraperitoneal), and the left ureter was exposed and ligated with 4–0 silk. The control group was sham-operated mice that underwent anesthesia and ureter exposure but not UUO ligation. After 7 days of UUO, each mouse was housed in a metabolic cage and urine was collected for 24 h. Blood and kidney samples were then obtained under anesthesia. To obtain serum, blood samples were incubated at room temperature for 30 min and then centrifuged at 900 × *g* for 15 min. Kidney samples were immediately stored at −80 °C until required. After UUO surgery, amlodipine was administered to mice daily via their drinking water at 6.7 mg/kg/day.

2.4. Analysis of renal functions

Serum creatinine and blood urea nitrogen (BUN) were measured by commercial kits (Wako Pure Chemical Industries Ltd.) according to the manufacturer's protocols.

2.5. Histopathological and immunohistochemical analyses

The paraffin-embedded kidney tissues were sectioned at a thickness of 1 μm. The sections were stained with Masson's trichrome to examine interstitial fibrosis. We determined the area