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Apple polyphenols reduce inflammation response of the kidneys in unilateral ureteral obstruction rats

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ABSTRACT

Renal fibrosis is the common final pathological hallmark of most progressive renal diseases. Unilateral ureteral obstruction (UUO) is a well-documented hydronephrosis model which shows interstitial inflammatory-cell infiltration and tubular dilatation, followed by tubulointerstitial fibrosis. Polyphenols possess antioxidative and anti-inflammatory properties. This study is aimed to investigate the effect of apple polyphenols (AP) on the inflammatory response and subsequent fibrotic change of the kidneys in UUO rats. After completing the UUO, 0.05, 0.1, or 0.15% of AP was given to the rats for 3 weeks. The results show that AP attenuates tubular dilatation and interstitial volume in UUO rats. It decreases the expression of inflammatory markers, ED-1 and MCP-1. AP also significantly downregulate the expression of profibrotic markers, TGF-β and α-SMA, in UUO rats. The findings show the beneficial effects of AP in UUO induced renal injury, and it is also given the alternative medical function of AP.

Chemical compounds studied in this article:

Haematoxylin (PubChem CID: 442514); eosin (PubChem CID: 11048); pentobarbital (PubChem CID: 4737); formaldehyde (PubChem CID: 712); 3,3′-diaminobenzidine (PubChem CID: 7071).

1. Introduction

Renal fibrosis is the common final pathological hallmark of most progressive renal disease regardless of the underlying disorder (Becker & Hewitson, 2000; Wu et al., 2010).

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Unilateral ureteral obstruction (UUO) is a well-documented hydronephrosis model which shows interstitial inflammatory-cell infiltration and tubular dilatation, followed by tubulointerstitial fibrosis (Wu et al., 2006). Complete UUO triggers a rapid consequence of events in the obstructed kidney and leads to reduced renal blood flow and glomerular filtration rate within 24 hours (Chevalier, Forbes, & Thornhill, 2009; Vaughan, Marion, Poppas, & Felsen, 2004). Then, hydronephrosis, interstitial inflammatory infiltration by macrophages and tubular cell death attributed to apoptosis and necrosis happen. UUO also promotes the renin–angiotensin system which produces reactive oxygen species (ROS) and nuclear factor-κB (NF-κB), and then it triggers epithelial–mesenchymal transition (EMT) which causes fibroblasts to migrate to the interstitium (Chevalier et al., 2009). The damaged tubular cells, interstitial macrophages and myofibroblasts produce cytokines and growth factors that induce an inflammatory state in the kidney, promote tubular cell apoptosis and accumulation of extracellular matrix (Grande, Pérez-Barriocanal, & López-Novoa, 2010). The continuation of hydronephrosis results in tubulo-interstitial fibrosis in the kidney, replacement of the scar tissue in the renal parenchyma, and the progressive loss of renal function (Gulmi & Felsen, 2012).

A wide variety of phenolic substances present in dietary and medicinal plants possess striking antioxidative and anti-inflammatory properties, which to some extent, contribute to their cancer chemopreventive potential (Lee et al., 2009). Polyphenols are the secondary metabolites of plants that are classified by their structure as phenolic acids derivates, flavonoids, stilbenes, or lignans (Shoji, Akazome, Kanda, & Ikeda, 2004). Apples (Rosaceae *Malus* sp.) is one of the most commonly consumed fruits in the world. Apples contain a variety of phenolic acid derivates and flavonoids, including flavan-3-ols, flavonols, procyanidins, chalcones, and anthrocyanins (Miura et al., 2008). The extracts from apples and apple polyphenol (AP) possess several biological activities such as antioxidation, anti-allergy, anti-tumour, hair growth-promotion, and obesity prevention (Akiyama et al., 2000; Gosse et al., 2005; Takahashi, Kamimura, Kagoura, Toyoda, & Morohashi, 2005; Zhao, Bomser, Joseph, & DiSilvestro, 2013). Besides, fruits containing relatively high concentrations of flavonols, anthocyanins and procyanidines were effective for reducing cardiovascular risk factors, such as anti-hypertensive effects, inhibition of platelet aggregation, increasing endothelial-dependent vasodilation, and hypocholesterolaemic effects. It is suggested that polyphenols may contribute to the cardioprotective properties of fruits (Chong, Macdonald, & Lovegrove, 2010; Sugiyama et al., 2007). Denis et al. (2013) demonstrated the beneficial actions of apple peel polyphenols on oxidative stress and inflammation in inflammatory bowel diseases. In their studies, the antioxidant and anti-inflammatory abilities of apple peel polyphenols were rendered via preventing lipopolsaccharide-induced inflammation by decreasing the expression and activity of COX-2, down-regulating the transcription factor NK-κB, and up-regulating the expression of Nrf2.

Taken together, this study was aimed to assess the effect of AP on the inflammatory response and subsequent fibrotic change of the kidneys in UUO-treated rats.

2. Materials and methods

2.1. *Apple polyphenols (AP)*

Apple polyphenols are a commercial product "Applephenon®" purchased from Asahi Co. (Tokyo, Japan). According to previous reports (Shoji et al., 2004), it was prepared from unripe apples (*Malus pumila* cv. Fuji) according to Tanabe et al. (1994). The previous study reported that unripe apples contained higher concentrations of polyphenols than ripe apples, including 63.8% procyanidins, comprised 11.1% dimers, 12.3% trimers, 8.7% tetramers, 5.9% pentamers, 4.9% hexamers and 20.9% other polymers. It also contained 12.4% flavan-3-ols (monomers), 6.5% other flavonoids and 10.8% non-flavonoids (Table 1) (Lister et al., 1994; Mayr et al., 1995). Also, the toxicity examinations have shown that Applephenon® is safe and non-toxic at average dietary level.

2.2. *Unilateral ureteral obstruction (UUO) animal model*

The animal experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee of Chung Shan Medical University (IACUC Approval No. 1165, CSMC), Taichung, Taiwan. Male Sprague-Dawley rats (180–200 g) were purchased from the National Laboratory Animal Center of Taiwan at 28 days of age and randomly divided into six groups (12 rats in each group) and kept in a room lighted 12 h each day and maintained at 22°C. The UUO rats underwent surgery as described previously (Wu et al., 2006). Briefly, the left kidney and ureter were exposed under intraperitoneal pentobarbital anaesthesia via a flank incision. The left ureter was then ligated with 4-0 silk at two points and cut between the ligatures to prevent retrograde urinary tract infection. The wound was closed in layers. After one day, AP was given via tube-feed in a concentration of 0.05, 0.1, or 0.15 % based on the weight of daily intake food for 3 weeks. Every one third of rats were randomly sacrificed at the end of 7th, 14th, or 21th day, and the blood and kidney were collected for further examinations. The drinking water was supplied unlimitedly. All groups were maintained on a control diet for the entire experimental period. Body weight and diet consumption were monitored weekly during the entire experiment.

Table 1 – Composition of apple polyphenols (AP).[a]

Procyanidins	Apple polyphenol (wt %)
Procyanidin (dimers)	11.1%
Procyanidin (trimers)	12.3%
Procyanidin (tetramers)	8.7%
Procyanidin (pentamers)	5.9%
Procyanidin (hexamers)	4.9%
Procyanidin (otherpolymers)	20.9%
Flavan-3-ols (monomers)	12.4%
Other flavonoids	6.5%
Non-flavonoids	10.8%

[a] The data are referred to Lister et al. (1994) and Mayr et al. (1995).