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Alleviative effect of fucoxanthin-containing extract from brown seaweed *Laminaria japonica* on renal tubular cell apoptosis through upregulating Na^+/H^+ exchanger NHE1 in chronic kidney disease mice

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ABSTRACT

Ethnopharmacological relevance: Brown seaweed is a common food for Asians, and the bioactive ingredient fucoxanthin exerts anti-apoptotic activities in several cell types. Renal tubular cell apoptosis is one of the common cellular events leading to renal fibrosis and chronic kidney disease (CKD). However, the influence of fucoxanthin-containing brown seaweed extract on CKD is still unknown. We intended to evaluate the inhibitory effect of fucoxanthin-containing extract from brown seaweed on renal apoptosis under CKD condition and its molecular mechanism.

Materials and methods: The fucoxanthin-containing brown seaweed extract (LJE) was prepared from *Laminaria japonica*. We investigated how LJE influences on both doxorubicin-treated rat renal tubular cells (NRK-52E) and the renal symptoms of nephrectomy-induced CKD mice.

Results: LJE inhibited doxorubicin-induced apoptosis and upregulated Na^+/H^+ exchanger isoform 1 (NHE1) expression in NRK-52E cells, which were blocked by the NHE1 inhibitor cariporide. LJE also upregulated peroxisome proliferator-activated receptor alpha (PPARα). PPARα siRNA transfection inhibited LJE-induced NHE1 expression and anti-apoptotic effect. In CKD mice, LJE increased NHE1 expression in renal tubules and reduced apoptotic renal tubular cells, but not in PPARα knockout mice. The inhibitory effect of LJE on apoptosis also reduced renal tubulointerstitial fibrosis and improved renal function in CKD mice.

Conclusion: We demonstrated that LJE inhibits renal apoptosis via NHE1 upregulation. The anti-apoptotic effect of LJE also improves renal function in CKD mice. Therefore, fucoxanthin-containing brown seaweed may have a therapeutic potential for CKD patients.

1. Introduction

Chronic kidney disease (CKD) is a common progressive disease that is becoming a global public health problem. Because there is no ideal clinical remedy for preventing and treating CKD, the incidence and cost of CKD is rising (Trivedi et al., 2002). CKD is characterized by a permanent loss of nephron and an ultimate reduction of glomerular filtration rate (Orr and Bridges, 2017). Progressive nephron loss results mainly from stress-induced cell death (i.e. apoptosis) in a glomerulus (glomerular diseases) and a tubule (Garcia-Sanchez et al., 2010; Lopez-Novoa et al., 2011). Severe nephron loss accompanies renal fibrosis, vascular rarefaction, and chronic inflammation. Recent studies have found that apoptosis is one of the common cellular events leading to renal fibrosis (Docherty et al., 2006). Prevention of stress-induced apoptosis will be beneficial for the treatment of CKD patients.

Asians has used brown seaweed as food and medicine for a long time. Traditional Chinese pharmacopoeias record that brown seaweed is used to treat edema, a symptom of kidney diseases. Dietotherapy with brown seaweed improves electrolyte and lipid imbalance in patients suffering from kidney diseases (Rudichenko et al., 2005). Therefore, brown seaweed is potentially beneficial for treatment of CKD. Brown seaweed has abundant bioactive substances, such as the sulfated

Abbreviation: CKD, Chronic kidney disease; LJE, *Laminaria japonica* extract, NHE1, Na^+/H^+ exchanger isoform 1; PPARα, Peroxisome proliferator-activated receptor alpha

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polysaccharide fucoidan (Ale et al., 2011), the carotenoid fucoxanthin (Peng et al., 2011), phlorotannin (Li et al., 2017), and polyphenolic compounds (Audibert et al., 2010). Some studies revealed that sulfated polysaccharide fucoidan alleviates the symptom of CKD and inhibits renal interstitial fibrosis in CKD mice (C.H. Chen et al., 2017; Wang et al., 2012). However, brown seaweed extracts have not been reported to express the renal protective effect.

Fucoxanthin is also one of the most important active ingredients of brown seaweed as potential chemotherapeutic or chemopreventive agents (Zorofchian Moghadamtousi et al., 2014). Recently, fucoxanthin has been found to prevent neuronal apoptosis via the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway (Yu et al., 2017). The PI3K/Akt pathway can be activated by peroxisome proliferator-activated receptor α (PPARα)-mediated Na^+/H^+ exchanger NHE1 upregulation to exert an anti-apoptotic effect in renal tubular cells (Chen et al., 2015). Therefore, the brown seaweed extracts containing fucoxanthin is potential to prevent renal cell apoptosis and improve CKD progression. In the present study, we investigated how the ethanolic extracts from *Laminaria japonica* (regarded as a synonym of *Saccharina japonica* and commonly called kelp), one of the most abundant brown seaweed in the Asian-Pacific region, influences on doxorubicin-treated renal tubular cells and the renal symptoms of nephrectomy-induced CKD mice. The protective mechanism of the brown seaweed extract was also revealed.

2. Materials and methods

2.1. Materials

The antibody against NHE1 (sc-28758) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Antibodies against Bcl-xL (2762), cleaved caspase-3 (9664), p-Akt (12178), Akt (4691) and PI3K-p85 (4257) were obtained from Cell Signaling Technology (Danvers, MA, USA). Antibodies against fibronectin (sc-9068) and NHE1 (sc-28758) were obtained from Santa Cruz (Dallas, TX, USA). The PPARα antibody was purchased from Abcam (Cambridge, MA, USA). All chemicals of reagent grade were obtained from Sigma-aldrich (St. Louis, MO, USA).

2.2. Preparation of Laminaria japonica extracts

L. japonica was verified by professor Pai-An Hwang (National Taiwan Ocean University, Taiwan). The voucher specimen was deposited in Herbarium of National Research Institute of Chinese Medicine, Taiwan (voucher numbers: NHP-00186). *L. japonica* powdered extracts were obtained from Hi-Q Marine Biotech International (Taiwan), and prepared as described in the previous report (Hwang et al., 2015). In brief, fresh *L. japonica* was collected from the coast of Penghu county, Taiwan. Washed *L. japonica* specimens were ground to flour and then dried. A hundred grams of dried *L. japonica* were shaken in 2 L of 95% ethanol at 70 °C for 6 h. Ethanolic extracts were centrifuged at 10,000 × *g* for 20 min, and supernatants were evaporated under reduced pressure at < 40 °C. *L. japonica* ethanolic extracts were analyzed by HPLC with a Hitachi L-7000 system and a Develosil ODS-UG-5 column (250 × 4.6 mm i.d., 5.0 μm particle size; Nomura Chemical Co., Japan). A mixture of methanol and acetonitrile (70:30, v/v) at a flow rate of 1.0 ml/min was used as the mobile phase. Fucoxanthin was monitored at 450 nm using a UV–Vis detector (Fig. 1). The *L. japonica* extract contained 10.0 ± 2.3% of fucoxanthin that was coated with a polysaccharide film to increase its shelf life and stability (Lin et al., 2017). Fucoidan content in the extract was less than 1%. The powdered extracts were dissolved in PBS buffer freshly for the cell and animal studies.

2.3. Cell culture

Rat renal tubular cells (NRK-52E) were purchased from the Food Industry Research and Development Institute (Taiwan). The cells were cultured in DMEM supplemented with an antibiotic/antifungal solution and 17% fetal bovine serum in a CO_2 incubator to maintain 37 °C and 95% humidity. When cells reached 70% confluence, they were then cultured in serum-free medium and incubated overnight before the experiment. Cells were used between the 20th and 40th passage counted from the day of receipt.

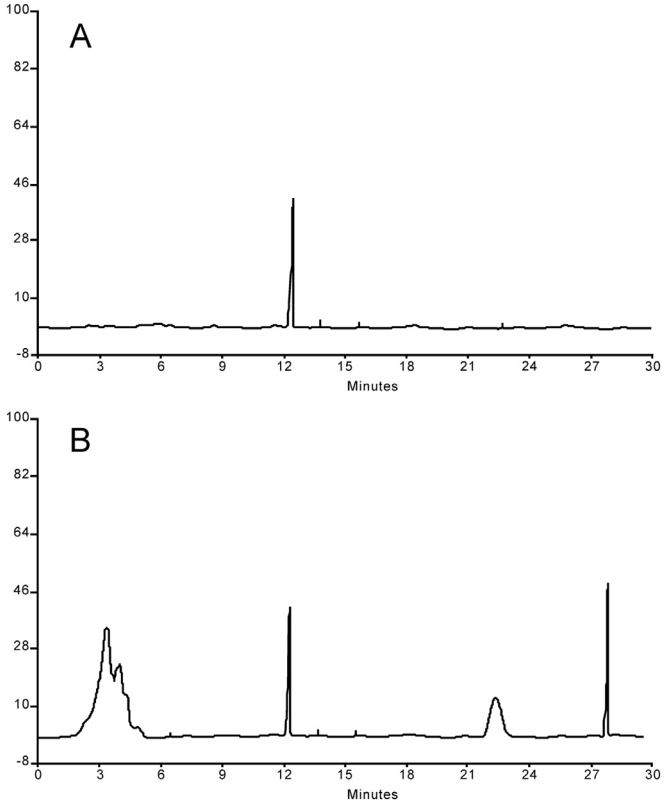

Fig. 1. HPLC chromatograms of a pure fucoxanthin standard (A) and the *L. japonica* ethanolic extract (B).

2.4. Apoptosis detection

We used Fluorescein isothiocyanate (FITC)-annexin V/propidium iodide (PI) double staining to detect apoptosis induced by doxorubicin treatment with an apoptosis detection kit (BD Biosciences-Pharmingen, San Diego, CA, USA). Treated NRK-52E cells were harvested and analyzed using flow cytometry.

2.5. Western blot analysis

Twenty micrograms of NRK-52E lysate proteins was applied to each lane and analyzed using Western blotting as described previously (D.Q. Chen et al., 2017). Relative levels of the protein bands were quantified by Quantiscan software (Biosoft, Cambridge, United Kingdom).

2.6. Mouse CKD model and brown seaweed extracts treatment

All animal experiments were approved by the Taipei Medical University Committee of Experimental Animal Care and Use (approval No. LAC-2015-0236) and performed in accordance with relevant guidelines and regulations. Male 8-week-old 129S1/SvImJ mice were obtained from Lasco Technology (Taiwan). PPARα knockout mice (B6.129S4-*Ppara*^{tm1Gonz} N12) were obtained from Taconic Biosciences (Germantown, NY, USA). The mouse CKD model was generated in 9-week-old mice (weight 21–25 g) by the surgical removal of the right