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Coptis chinensis Franch. exhibits neuroprotective properties against oxidative stress in human neuroblastoma cells

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ABSTRACT

Ethnopharmacological relevance: The dried rhizome of *Coptis chinensis* Franch. (family *Ranunculaceae*) is traditionally used in Chinese medicine for the treatment of inflammatory diseases and diabetes. Recent studies showed a variety of activities of *Coptis chinensis* Franch. alkaloids, including neuroprotective, neuroregenerative, anti-diabetic, anti-oxidative and anti-inflammatory effects. However, there is no report on the neuroprotective effect of *Coptis chinensis* Franch. watery extract against *tert*-butylhydroperoxide (*t*-BOOH) induced oxidative damage. The aim of the study is to investigate neuroprotective properties of *Coptis chinensis* Franch. rhizome watery extract (CRE) and to evaluate its potential mechanism of action.

Materials and methods: Neuroprotective properties on *t*-BOOH induced oxidative stress were investigated in SH-SY5Y human neuroblastoma cells. Cells were pretreated with CRE for 2 h or 24 h followed by 2 h of treatment with *t*-BOOH. To evaluate the neuroprotective effect of CRE, cell viability, cellular reactive oxygen species (ROS), mitochondrial membrane potential (MMP) and the apoptotic rate were determined and microarray analyses, as well as qRT-PCR analyses were conducted.

Results: Two hours of exposure to 100 μM *t*-BOOH resulted in a significant reduction of cell viability, increased apoptotic rate, declined mitochondrial membrane potential (MMP) and increased ROS production. Reduction of cell viability, increased apoptotic rate and declined mitochondrial membrane potential (MMP) could be significantly reduced in cells pretreated with CRE (100 μg/ml) for 2 h or 24 h ahead of *t*-BOOH exposure with the greatest effect after 24 h of pretreatment; however ROS production was not changed significantly. Furthermore, microarray analyses revealed that the expressions of 2 genes; thioredoxin-interacting protein (TXNIP) and mitochondrially encoded NADH dehydrogenase 1, were significantly regulated. Down regulation of TXNIP was confirmed by qRT-PCR.

Conclusion: Due to its neuroprotective properties CRE might be a potential therapeutic agent for the prevention or amelioration of diseases like diabetic neuropathy and neurodegenerative disorders like Alzheimer and Parkinsons disease.

1. Introduction

Diabetic neuropathy, neurodegenerative disease like Alzheimer or Parkinsons disease and aging are associated with oxidative damage by reactive oxygen species (ROS) (Smith et al., 1996; Finkel and Holbrook, 2000; Perry et al., 2002; Vincent et al., 2004; Perfeito et al., 2012). Cells are usually protected from oxidative damage by the endogenous antioxidant system, which consists of thioredoxin, glutathione, ascorbic acid and a variety of enzymes like superoxide dismutase, catalase, thioredoxin reductase and glutathione reductase. If the antioxidative capacity of this system is exceeded or parts of the system are damaged, ROS-induced oxidative stress will occur. ROS accumulation can lead to damage of deoxyribonucleic acid, protein oxidation, membrane lipid peroxidation, depletion of cellular thiols and release of pro-inflammatory cytokines, which can in the end elicit tissue damage

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and cell death (Gorman et al., 1996; Smith et al., 1996; Vincent et al., 2004).

In a variety of studies the membrane permeant oxidant compound *tert*-butylhydroperoxide (*t*-BOOH) was used to induce oxidative stress (Amoroso et al., 1999; Amoroso et al., 2002; Zhao et al., 2005). Results showed that after *t*-BOOH permeates the cell membrane *t*-BOOH is modified to *tert*-butoxyl radicals by way of iron-dependent reactions and induces lipid peroxidation, DNA cleavage, depletion of intracellular glutathione, protein modifications, increase of intracellular Ca^{2+} ions and as a consequence cell death (Rush and Alberts, 1986; Coleman et al., 1989; Guidarelli et al., 1997; Amoroso et al., 1999; Martin et al., 2001). Furthermore, it has been assumed that *t*-BOOH treatment leads to an opening of the mitochondrial permeability transition pore, which increases the permeability of the inner mitochondrial membrane and therefore leads to mitochondrial membrane potential depolarization (Nieminen et al., 1995, 1997; Zhao et al., 2005). By way of a downstream effect this can induce the release of cytochrome *c* (cyt *c*) into the cytosol (Kowaltowski et al., 2001). Cytosolic cyt *c* activates the caspase cascade leading to apoptotic cell death (Liu et al., 1996; Li et al., 1997). For this reason, therapeutic strategies targeting the prevention or attenuation of oxidative stress, DNA cleavage and mitochondrial membrane potential depolarization could have a major impact on the treatment of diseases associated with oxidative stress.

Coptidis rhizoma, the dried rhizome of Coptis chinensis Franch. (Chinese goldthread, commonly known as wei-lian or huang-lian), is an herb used in Traditional Chinese Medicine (TCM) for inflammatory diseases like carbuncles, aphthae or ulcers as well as for dysentery, but ancient authors like Li Shizhen also used it to treat diabetes (Li, 1999). It is used in the treatment of various diseases in TCM due to its anti-diabetic, relaxant, pyretic, antibacterial, and antiviral effects (Huang, 1999). Some of its individual compounds showed a variety of activity forms, including neuroprotective (Luo et al., 2011), neuroregenerative (Han et al., 2012), anti-apoptotic (Miura et al., 1997), anti-oxidative (Gong et al., 2012), anti-inflammatory (Marinova et al., 2000) and anti-fungal effects (Seneviratne et al., 2008). The main components of *Coptis chinensis* Franch. are berberastine, berberine, columbamine, coptisine, epiberberine, jatrorrhizine and palmatine (Chuang et al., 1996; Wang et al., 2004; Zhao et al., 2010; Ding et al., 2012). In the traditional combination *San Huang xie xin tang,* which originated in the Ming dynasty, *Coptis chinensis* Franch. is combined with *Rhei rhizoma* and *Scutellaria radix* (Dong, 2000). This recipe has been shown to have neuroprotective capacities due to its anti-inflammatory and antioxidative effects (Lo et al., 2012). However, there is no report on the neuroprotective effect of CRE against *t*-BOOH induced oxidative damage. Therefore, we investigated the neuroprotective effect of CRE oxidative stress in SH-SY5Y human neuroblastoma cells by evaluation of the cell viability, ROS production, apoptotic rate, mitochondrial membrane potential and transcriptional changes.

2. Materials and methods

2.1. Drugs

2,2-Di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH; Aldrich, Taufkirchen, Germany) was dissolved in methanol. Sodium phosphate buffer (pH 7.4) was used to prepare fluorescein and 2,2′-Azobis (2-methylpropionamidine) dihydrochlorid stock solution. Trolox (Sigma, Taufkirchen, Germany) was dissolved in 80% ethanol. *Tert*-butyl-hydroperoxide (*t*-BOOH; 48933, Fluka, Taufkirchen, Germany) working solution was prepared with RPMI 1640 medium. 2′,7′-dichlorodihydrofluorescein diacetate, Mitotracker Red CMX Ros were obtained from Life Technologies (Darmstadt, Germany) and dissolved in DMSO. Hoechst 33342 (Life Technologies) stock solution was prepared with 70% ethanol. All other reagents were purchased from Roth (Karlsruhe, Germany) or Sigma (Taufkirchen, Germany).

2.2. Herbal preparation

The rhizome of *Coptis chinensis* Franch. was purchased as a dried herb from China Medica (Ch. B. 930034; 83684 Tegernsee, Germany), and tested for identity and purity by Sebastian Kneipp research laboratory for residue analysis and organic trace analysis (Bad Wörishofen, Germany). The plant name used in this publication was verified with www.theplantlist.org on February the 10th 2014.

The rhizome of *Coptis chinensis* Franch. was ground into a fine powder and extracted twice for 30 min with boiling distilled deionized water (ratio: 1 g/10 ml). Supernatants were combined, concentrated with a rotary-vacuum evaporator (60 °C, 200 mbar; Rotavapor-R, Büchi) and dried to powder with a vacuum concentrator (Bachofer) at room temperature.

2.3. HPLC-analysis

HPLC-analysis was conducted on an Agilent HPLC 1260 infinity (Agilent Technologies, Germany) using an Alltima C18 (250 mm × 4.6 mm × 5 μm, S/N: 213100139, temperature: 25 °C) column. The mobile phase consisted of 0.1% trifluoroacetic acid (A) and acetonitrile (B). Chromatographic separation was optimized according to the method described in the Hong Kong Chinese Materia Medica Standards (Department of Health of Hong Kong, 2008). The following gradient protocol was used with a flow rate of 1.0 ml/min: 0–30 min 20–50% B for separation and 31–40 min 90% B, 41–65 min 20% B for cleaning up and equilibrium. The column pressure at equilibrium was 115 bar. 346 ± 16 nm was used as detection wavelength and 610 ± 50 nm as the reference wavelength. Coptisine, palmatine and berberine (Cfm Oskar Tropitzsch; Markdredwitz; Germany) were used as reference standard compounds. HPLC-Data were analyzed with the Agilent ChemStation for LC (Rev. B.04.03, Agilent Technologies, Germany).

2.4. Free radical scavenging activity

Antioxidant activity of CRE was determined by using the DPPH assay (Turkmen et al., 2006; Sharma and Bhat, 2009). The dried extract was dissolved in DDW to a final concentration of 56.25 μg/ml–7.2 mg/ml and 50 μl was added to each well of a 96-well-plate. Afterwards, 200 μl of 75 μM DPPH was added to each well and the plate was agitated with 650 rpm for 5 min in the dark with an AIP 4 plate shaker (Diagnostics Pasteur). After a total incubation time of 30 min, absorbance was measured 3 times at 531 nm (Thermo Multiskan SPECTRUM microplate spectrophotometer). 50 μl DDW served as the negative control and trolox as well as *N*-acetyl-L-cystein (NAC) as the positive controls. Three independent experiments were conducted and each experiment was performed in triplicate.

2.5. Peroxyl radical scavenging activity

The antioxidant capacity of CRE was determined by using the ORAC assay. The ORAC assay was carried out with slight modifications according to the method described by Gillespie et al. (2007). Briefly, 225 μl of 10 nM fluorescein solution dissolved in a 75 mM sodium phosphate buffer (SPB; pH 7.4) was pipetted into the well of a 96-well microplate and 37 μl of sodium phosphate buffer (blank), Trolox (20–80 μM) or the herbal extract (5–20 μg/ml; sample) was added in different concentrations. The microplate