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Rutin attenuates ethanol-induced neurotoxicity in hippocampal neuronal cells by increasing aldehyde dehydrogenase 2

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ABSTRACT

Rutin is derived from buckwheat, apples, and black tea. It has been shown to have beneficial anti-inflammatory and antioxidant effects. Ethanol is a central nervous system depressant and neurotoxin. Its metabolite, acetaldehyde, is critically toxic. Aldehyde dehydrogenase 2 (ALDH2) metabolizes acetaldehyde into nontoxic acetate. This study examined rutin's effects on ALDH2 activity in hippocampal neuronal cells (HT22 cells). Rutin's protective effects against acetaldehyde-based ethanol neurotoxicity were confirmed. Daidzin, an ALDH2 inhibitor, was used to clarify the mechanisms of rutin's protective effects. Cell viability was significantly increased after rutin treatment. Rutin significantly reversed ethanol-increased Bax, cytochrome c expression and caspase 3 activity, and decreased Bcl-2 and Bcl-xL protein expression in HT22 cells. Interestingly, rutin increased ALDH2 expression, while daidzin reversed this beneficial effect. Thus, this study demonstrates rutin protects HT22 cells against ethanol-induced neurotoxicity by increasing ALDH2 activity.

1. Introduction

Ethanol, a powerful neurotoxin, is particularly harmful to the developing nervous system, causing neuronal damage, probably through abnormal induction of neuronal cell death (Sun and Sun, 2001). Loss of neurons has been observed in specific regions of the central nervous system in alcoholics. Prenatal alcohol exposure causes birth defects, mental retardation and developmental disorders of the nervous system, as observed in patients suffering fetal alcohol syndrome. This includes depression of neurogenesis, delayed and aberrant neuronal migration, and anomalous structural and functional development (Goodlett and Horn, 2001; Miller, 1992; Streissguth et al., 1994).

Cytosolic enzyme alcohol dehydrogenase (ADH) metabolizes ethanol to acetaldehyde. Mitochondrial aldehyde dehydrogenase 2 (ALDH2) converts acetaldehyde to acetate (Monticone et al., 2010). ALDH2 is a key mitochondrial enzyme in ethanol metabolism and ethanol detoxification. ALDH2 is highly expressed in heart, liver, kidney and muscle (Stewart et al., 1996b). ALDH2 is also expressed in brain as well as widely expressed throughout the CNS (Stewart et al., 1996a; Zimatkin et al., 1992), however little is known about its functional role in neurons. Recently, it has been suggested that age-dependent neurodegeneration accompanies memory loss in ALDH2-deficient mice (Ohsawa et al., 2008). Moreover, a recent study indicated ALDH2 is an enzyme whose activation correlates with reduced ischemic heart damage in rodent models, probably due to removal of 4HNE (Chen et al., 2008). This raises the possibility that ALDH2 may play a role in cellular defenses against neurotoxicity induced by ethanol.

Rutin is a citrus flavonoid glycoside found in buckwheat, apples, and black tea (Kamalakkannan and Stanely Mainzen, 2006). The pharmacological properties of rutin have been well demonstrated including anti-inflammatory, antioxidant, anti-carcinogenic, anti-allergic, and anti-viral effects. Potent scavenging of superoxide radicals has also been shown (Bishnoi et al., 2007; Sheu et al., 2004). Recent studies demonstrated rutin supplementation from natural food sources, such as soba noodles or groats, might improve memory impairment and decrease hippocampal pyramidal neuronal death, such as seen in Alzheimer's disease (AD). Additionally, rutin has the ability to suppress microglial activation and proinflammatory cytokines (Koda et al., 2009). However, possible detoxification effects of rutin on ethanol-induced neuronal cell death have not been investigated. Thus, this study asked if rutin

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could improve ethanol-induced neurotoxicity in HT22 cells. ALDH2 overexpression was confirmed as a possible mechanism underlying ethanol detoxification by rutin.

2. Materials and methods

2.1. Cell culture

Hippocampal neuronal cells (HT22 cells) were maintained in Dulbecco's modified Eagle's medium (DMEM; Hyclone, UT, USA) containing 10% FBS (Hyclone, Canada) and 1% antibiotic solution (Sigma–Aldrich, St. Louis, MO, USA) in a humidified incubator with 5% CO_2 in air at 37 °C. Cells were treated with ethanol (200 mM, Sigma–Aldrich), rutin (1 μg/ml, rutin hydrate (R5143), ⩾94% (HPLC), Sigma–Aldrich) and the specific mitochondrial aldehyde dehydrogenase 2 (ALDH2) inhibitor, daidzin (60 μM, Sigma–Aldrich). All control and ethanol-treated plates were wrapped with Parafilm to prevent evaporation of ethanol.

2.2. Cell viability

Cell survival was determined with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit from Sigma. HT22 cells were replated in 48-well plates at a density of 1.0×10^4 cells per well, and incubated for 24 h. One h of pretreatment with rutin (1 μg/ml) affected the viability of HT22 cells treated with 200 mM ethanol for 24 h. After the incubation period, 10 μl of the kit solution was added to each well and incubated for 24 h at 37 °C in 5% CO_2. After this treatment, HT22 cells were incubated with MTT for 2 h at 37 °C. The resulting formazan crystals were dissolved in MTT solubilization solution. The absorbance was determined at 540 nm using a microplate reader.

2.3. Mitochondrial permeability transition analysis

HT22 cells were replated at 2.0×10^4 cells in 4-well chamber slides (Nalge Nunc, NY, USA). After overnight incubation under standard tissue culture conditions, HT22 cells were exposed to 200 mM ethanol with or without rutin (1 μg/ml). After 2 h, the cells were labeled with Mitotracker Red CMXRos (Invitrogen, OR, USA) for 15 min. Cells were washed with PBS, and fluorescence microscopy was conducted using the appropriate filters.

2.4. Western blotting

Total protein from HT22 cell lysates was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis using 12% gels, and then was electrophoretically transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were blocked in 5% skim milk in PBS then incubated with primary antibodies against Bcl-xL, Bcl-2, Cytochrome c, β-actin (Cell Signaling, Danvers, MA, USA), Bax (Santa Cruz Biotechnology, CA, USA), ADH and ALDH2 (Millipore, Bedford, MA, USA), which were diluted 1:500 in 1% skim milk in PBS overnight at 4 °C. Blots were then incubated with peroxidase-conjugated goat anti-rabbit IgG (1:5,000; Millipore, Bedford, MA, USA) for 1 h. Immunoreactions were visualized with SuperSignal West Dura Extended Duration Substrate (Thermo Scientific, San Jose, CA, USA) and analyzed using a chemiImager analyzer system (Alpha Innotech, San Leandro, CA, USA).

2.5. Caspase-3 activity analysis

Caspase-3 activation was assessed using Caspase-Glo3/7 Assays (Promega, WI, USA) as an index of apoptosis. HT22 cells were replated at 1.0×10^4 onto 48-well plates. After 24 h of incubation, HT22 cells were exposed to 200 mM ethanol with or without rutin (1 μg/ml). After this treatment, 50 μl of supernatant was transferred from each well to a new 96-well plate. Equal volumes of Caspase-Glo 3/7 reagents were added, and the plate was incubated for 30 min at room temperature before luminescence was measured.

2.6. Statistical analysis

Data were reported as means ± *SEM*. Data were analyzed with Student's *t*-tests and a repeated measure ANOVA with a Bonferroni post hoc procedure. A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Rutin protected against ethanol neurotoxicity

The effect of rutin on ethanol-induced cell death was examined. As shown in Fig. 1A, HT22 cell viability with ethanol was decreased in a dose-dependent manner. The viability of HT22 cells

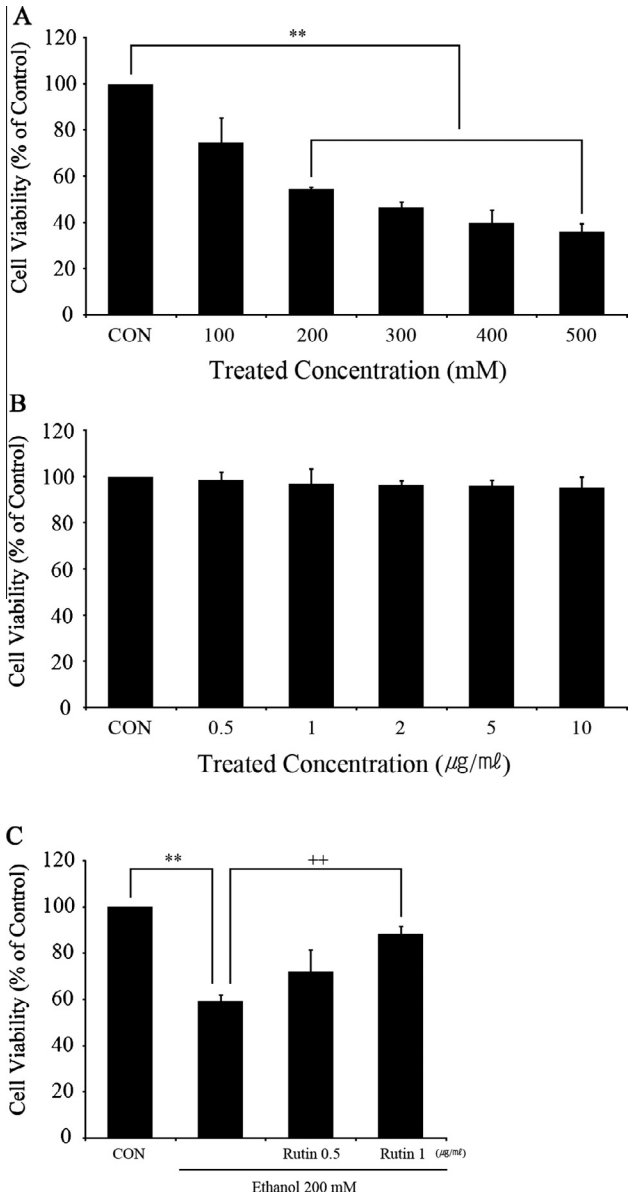

Fig. 1. Cell viability of rutin and ethanol-treated HT22 cells. HT22 cell viability was analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide analysis and expressed as a percentage of viable untreated control cells grown in a defined medium. (A) HT22 cells were treated with different concentrations of ethanol (100–500 mM). (B) Rutin (0.5–10 μg/ml) was added to culture medium and HT22 cells were incubated for 24 h. (C) Culture medium was pretreated for 1 h with rutin (0.5 and 1 μg/ml) and incubated with 200 mM ethanol for 24 h. Data are expressed as the means ± *SEM* of four to six independent experiments. $^{**}p < 0.01$, compared with control; $^{++}p < 0.01$, compared with ethanol.

maintained in 200 mM ethanol for 24 h was decreased to 59% of the control group ($p < 0.05$). Rutin efficacy in controlling ethanol toxicity in a dose-dependent manner was confirmed. All concentrations of rutin reduced cellular toxicity in HT22 cells for 24 h (Fig. 1B). Based on these results, a dose of 1 μg/ml rutin in 200 mM ethanol significantly inhibited cell death induced by ethanol (approximately 92%, $p < 0.01$) (Fig. 1C).

3.2. Effect of rutin on ADH and ALDH2

Western blots to detect ADH and ALDH2 were performed. Although rutin did not affect ADH expression in the normal condition, co-treatment with ethanol and rutin decreased ADH