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Pretreatment with *tert*-butylhydroquinone attenuates cerebral oxidative stress in mice after traumatic brain injury

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ABSTRACT

Background: Traumatic brain injury (TBI) is a worldwide health problem, identified as a major cause of death and disability. Increasing evidence has shown that oxidative stress plays an important role in TBI pathogenesis. The antioxidant transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2), is a known mediator in protection against TBI-induced brain damage. The objective of this study was to test whether *tert*-butylhydroquinone (tBHQ), a novel Nrf2 activator, can protect against TBI-induced oxidative stress.

Methods: Adult male imprinting control region mice were randomly divided into three groups: (1) sham + vehicle group; (2) TBI + vehicle group; and (3) TBI + tBHQ group. Closed-head brain injury was applied using the Feeney weight-drop method. We accessed the neurologic outcome of mice at 24 h after TBI, and subsequently measured protein levels of Nrf2 and the NOX2 subunit of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), the concentration of malondialdehyde, superoxide dismutase activity, and brain edema.

Result: The NOX2 protein level was increased fivefold in the TBI + vehicle group, whereas pretreatment with tBHQ markedly attenuated the NOX2 protein expression relative to that in the TBI + vehicle group. TBI increased Nrf2 formation by 5% compared with the sham group, whereas treatment with tBHQ further upregulated the Nrf2 protein level by 12% compared with the sham group. The level of the oxidative damage marker malondialdehyde was reduced by 29% in the TBI + tBHQ group compared with the TBI + vehicle group, Moreover, pretreatment with tBHQ significantly increased the antioxidant enzyme superoxide dismutase activity. Administration of tBHQ also significantly decreased TBI-induced brain edema and neurologic deficits.

Conclusions: Pretreatment with tBHQ effectively attenuated markers of cerebral oxidative stress after TBI, thus supporting the testing of tBHQ as a potential neuroprotectant and adjunct therapy for TBI patients.

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1. Introduction

Traumatic brain injury (TBI) is a worldwide health problem, which has become the major cause of death and disability among young adults in industrialized countries. An estimated 1.7 million people suffer traumatic brain injuries each year in the United States. Approximately 25% of these injuries result in long-term disabilities, causing significant familial, social, and economic burdens [1,2]. Despite the progress in diagnosis, neuroradiology, neurosurgical care, and treatment of TBI in recent years, the options for treatment of extensive TBI remain limited. Consequently, it is of critical importance to develop more effective treatment strategies for these patients.

Although the etiology of progressive neuropathology in TBI remains unclear, increasing evidence has shown that oxidative stress plays an important role in its development. TBI upregulates pro-oxidants, such as NADPH oxidases (NOX1–5) and inducible nitric oxide synthase, leading to the production of reactive oxygen species (ROS) and reactive nitrogen species, which cause oxidative damage of DNA, proteins, and lipid in brain tissues [3,4]. It has been generally assumed that mitochondria are the major source of ROS after brain injury. However, recent work has shown that the enzyme NADPH oxidase is a major contributor to posttraumatic cellular ROS production [5,6].

tert-Butylhydroquinone (tBHQ) is widely used as a food preservative, based on its powerful antioxidant potential. It has furthermore been shown to protect the living animal and cell lines against acute toxicity and oxidative insult, presumably through the induction of the nuclear translocation of transcription factor (nuclear factor erythroid 2-related factor 2 [Nrf2]), which in turn regulates the expression of many cytoprotective proteins, including NAD(P)H: quinone oxidoreductase 1, glutathione-S-transferase, glucuronyltransferase, and heme oxygenase-1 (HO-1) [7,8]. Novel anti-inflammatory effects of tBHQ, which have been reported in an experimental TBI model, include suppression of nuclear factor κB (NF-κB) activity, increased Nrf2 levels, inhibition of the concentrations of inflammatory cytokines (interleukin [IL]-1, IL-6, and tumor necrosis factor α), and abatement of brain edema and apoptosis index [9]. However, it remains unknown if tBHQ can suppress NADPH activity or oxidative stress in TBI. To resolve this issue, we used a moderate closed-head injury model in mice, and evaluated the effect of tBHQ pretreatment on NOX2 production, malondialdehyde (MDA), and other markers of antioxidant expression. In addition, we monitored effects of this pretreatment on the neurologic outcome and brain edema caused by TBI in the same mice.

2. Materials and methods

2.1. Animals

Imprinting control region mice were originally purchased from Animal Center of Nanjing Medical University, Nanjing, China, and were subsequently bred and raised in our laboratory. A total of 80 male mice weighing 28–32 g were housed five per cage under a constant 12h light–dark cycle. Food and water were supplied *ad libitum*. All experiments conformed to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2.2. Treatment

A total of 50 mg of tBHQ (Sigma-Aldrich Co, St Louis, MO) was dissolved in dimethyl sulfoxide, to a final concentration of 5 mg/mL in 1% DMSO-saline and intraperitoneally (i.p.) injected at a dose of 50 mg/kg, divided into three injections at intervals of 8 h before TBI. This dose and route of tBHQ administration has been used in analogous animal models described elsewhere [2]. All mice were randomly assigned to the following groups: sham + vehicle (DMSO/saline), TBI + vehicle (DMSO/saline), and TBI + tBHQ. These groups were allocated either to (1) biochemical analyses or (2) to edema and neurologic examinations, giving final groups sizes of five animals because of mortality.

2.3. Closed-head TBI injury

We used the weight-drop model with minor modifications to induce moderate closed-head TBI injury, as described previously [10,11]. After anesthesia with pentobarbital sodium (50 mg/kg i.p.; Sigma, St Louis, MO), mice were fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and the scalp was shaved and cleaned with iodophor. A midline incision was performed to expose the skull between bregma and lambda suture lines (2.0–2.5 cm), and the left lateral aspect of the skull was exposed by retracting the skin and surrounding soft tissue. Focal brain trauma was induced by dropping a 200-g steel weight with a flat end from a height of 3 cm onto the left lateral skull. Then the scalp was sutured. The free-falling weight produced a moderate contusion injury of the left parietal cortex. Sham-injured animals served as control subjects; these animals underwent scalp incision with the exception that no injury was induced. As in other studies of this type, 20%–25% of the mice died in the first seconds after the trauma, but there was no delayed mortality or prostration in the surviving mice [12]. During the surgery, rectal temperature was monitored and maintained between 36.5°C and 37.5°C with heating pads and lamps. The animals were returned to their quarters after recovery from anesthesia, and were killed at 24 h after sham or TBI.

2.4. Western blot analysis

After induction of anesthesia with pentobarbital sodium (50 mg/kg, i.p.), mice were decapitated and the left brains were rapidly dissected within 5 min, frozen, and stored at −80°C until the day of analysis ($N = 5$). The brain tissues were then thawed and mechanically lysed in the radioimmunoprecipitation assay buffer supplemented with protease inhibitors [13]. Homogenates were centrifuged at 12,000*g* for 15 min at 4°C, and supernatants were collected. The protein concentration was determined using the bicinchoninic acid method. Equal portions of protein preparations were run on 10% sodium dodecyl sulfate–polyacrylamide gels and electrotransferred to polyvinylidene difluoride membranes (ATTO Bio Instrument, Tokyo, Japan). The membranes were blocked in blocking buffer (5% skimmed milk in TBS containing 0.05% Tween 20) for 1–2 h, and then incubated with primary antibodies against NOX2 (1:5000;