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Neuropharmacology 48 (2005) 743–756
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Protective effects of ginseng saponins on 3-nitropropionic acid-induced striatal degeneration in rats

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Received 15 July 2004; received in revised form 14 October 2004; accepted 10 December 2004

Abstract

The precise cause of neuronal cell death in Huntington's disease (HD) is not known. Systemic administration of 3-nitropropionic acid (3-NP), an irreversible succinate dehydrogenase inhibitor, not only induces a cellular ATP depletions but also causes a selective striatal degeneration similar to that seen in HD. Recent accumulating reports have shown that ginseng saponins (GTS), the major active ingredients of *Panax ginseng*, have protective effects against neurotoxin insults. In the present study, we examined in vitro and in vivo effects of GTS on striatal neurotoxicity induced by repeated treatment of 3-NP in rats. Here, we report that systemic administration of GTS produced significant protections against systemic 3-NP- and intrastriatal malonate-induced lesions in rat striatum with dose-dependent manner. GTS also improved significantly 3-NP-caused behavioral impairment and extended survival. However, GTS itself had no effect on 3-NP-induced inhibition of succinate dehydrogenase activity. To explain the mechanisms underlying in vivo protective effects of GTS against 3-NP-induced striatal degeneration, we examined in vitro effect of GTS against 3-NP-caused cytotoxicity using cultured rat striatal neurons. We found that GTS inhibited 3-NP-induced intracellular Ca^{2+} elevations. GTS restored 3-NP-caused mitochondrial transmembrane potential reduction in cultured rat striatal neurons. GTS also prevented 3-NP-induced striatal neuronal cell deaths with dose-dependent manner. The EC_{50} was 12.6 ± 0.7 μg/ml. These results suggest that in vivo protective effects of GTS against 3-NP-induced rat striatal degeneration might be achieved via in vitro inhibition of 3-NP-induced intracellular Ca^{2+} elevations and cytotoxicity of striatal neurons.

Keywords: Ginseng saponins; 3-Nitropropionic acid; Striatum toxicity; Neuroprotection

1. Introduction

3-Nitropropionic acid (3-NP) is a compound found in crops contaminated with fungi (Ming, 1995) and causes neurotoxicity in both animals and human (James et al.,

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0028-3908/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2004.12.013

1980; Ludolph et al., 1991). Since treatment of 3-NP induces a selective striatal pathology similar to that seen in Huntington's disease (HD), it has been widely used as an agent for animal model study of HD (Beal et al., 1993; Borlongan et al., 1997; Brouillet et al., 1999). The primary mechanism of 3-NP-caused neurotoxicity involves the irreversible inhibition of mitochondrial succinate dehydrogenase (SDH) and leads to inhibition of ATP synthesis (Alston et al., 1977; Coles et al., 1979). ATP exhaustion by mitochondrial dysfunction also subsequently couples to the slow secondary excitotoxicity by excitatory neurotransmitter (Pang and Geddes, 1997). This secondary excitotoxicity in ATP deficient neurons is initiated by voltage-dependent Na^+ channel activation, which is coupled to membrane depolarization, Ca^{2+} channel activation, and subsequent NMDA receptor activation by relief of voltage-dependent Mg^{2+} block of the NMDA receptor (Novelli et al., 1988; Zeevalk and Nicklas, 1991). These serial cascades induced by 3-NP intoxication are also accompanied with the impaired mitochondrial Ca^{2+} homeostasis, with intracellular Ca^{2+} elevation via L-type and other types of Ca^{2+} channel activations, and with an impaired buffering capacity on intracellular Ca^{2+} in astrocytes and neurons (Deshpande et al., 1997; Fukuda et al., 1998; Calabresi et al., 2001; Nasr et al., 2003). Moreover, since 3-NP-caused elevation of intracellular calcium is known to activate calpain and caspase-9, which are involved in neuronal cell death, 3-NP-caused perturbation of calcium homeostasis in mitochondria and the following activation of these enzymes might be the main factors in 3-NP neurotoxicity in vivo (Fu et al., 1995; Brouillet et al., 1999; Bizat et al., 2003a, 2003b).

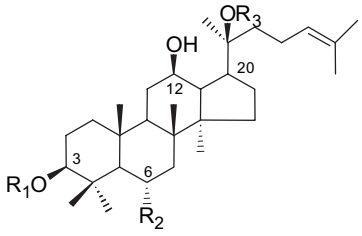
Ginseng saponins (GTS), which are also known as ginsenosides, are active ingredients isolated from *Panax ginseng* C.A. Meyer, which is a well-known tonic medicine (Nah, 1997). Recent accumulating evidences have shown that treatment of GTS not only attenuates intracellular Ca^{2+} elevation by blocking various types of Ca^{2+} channels like L-, N-, and P/Q-types and depolarization-induced Ca^{2+} influx (Nah et al., 1995; Kim et al., 1998a; Rhim et al., 2002) but also inhibits receptor agonist-induced intracellular Ca^{2+} mobilization in neurons (Jeong et al., 2004). GTS also reduces glutamate/NMDA-mediated Ca^{2+} influx in neurons (Kim et al., 1998b, 2002). Furthermore, recent reports showed that these GTS-mediated inhibitions on intracellular Ca^{2+} elevations could be the basis of in vitro or in vivo protection against excitatory amino acids- or neurotoxins-caused neuronal cell damages. For example, GTS attenuated glutamate or kainic acid-caused cortical, hippocampal, spinal cord neuron damages in rats (Chu and Chen, 1990; Kim et al., 1998b; Lee et al., 2002a; Liao et al., 2002). GTS also attenuated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 1-methyl-4-phenylpyridinium (MPP^+)-induced dopaminergic neuron deaths in rat or dopaminergic cell culture in mice (Kampen et al., 2003; Radad et al., 2004).

However, until now little is known about protective effect of GTS against progressive striatal degeneration induced by repeated neurotoxin insults, which could be linked to subsequent intracellular Ca^{2+} elevations. We therefore examined whether systemic administration of GTS could exert protective effects against systemic 3-NP- or intrastriatal injected malonate-induced rat striatal degeneration. We examined the ability of GTS to block 3-NP-induced intracellular Ca^{2+} increases, 3-NP-induced mitochondrial damage, and 3-NP-induced cytotoxicity in cultured rat striatal neurons. Herein, we present the results that in vivo protective effects of GTS against 3-NP neurotoxicity are mediated through in vitro inhibition of 3-NP-induced intracellular Ca^{2+} elevations and cytotoxicity of striatal neurons.

2. Materials and methods

2.1. Drugs

Fig. 1 shows the structures of the eight representative ginsenosides. GTS was kindly obtained from Korea Ginseng Corporation (Taejon, Korea). GTS contained Rb_1 (17.1%), Rb_2 (9.07%), Rc (9.65%), Rd (8.26%), Re (9%), Rf (3%), Rg_1 (6.4%), Rg_2 (4.2%), Rg_3 (3.8%), Ro (3.8%), Ra (2.91%) and other minor ginsenosides. GTS was diluted with bath medium or saline before use.



Ginsenosides	R_1	R_2	R_3
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc
Rb_2	-Glc_2-Glc	-H	-Glu_6-Ara(pyr)
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)
Rd	-Glc_2-Glc	-H	-Glc
Re	-H	-O-Glc_2-Rha	-Glc
Rf	-H	-O-Glc_2-Glc	-H
Rg_1	-H	-O-Glc	-Glc
Rg_2	-H	-O-Glc_2-Rha	-H
Rg_3	-Glc_2-Glc	-H	-H

Fig. 1. Structures of the eight representative ginsenosides. They differ at three side chains attached to the common steroid ring. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara (pyr), arabinopyranoside; Rha, rhamnopyranoside. Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.