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Experimental Neurology 184 (2003) 521–529

Experimental Neurology

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Neuroprotective actions of the ginseng extract G115 in two rodent models of Parkinson's disease

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Received 22 May 2003; revised 22 July 2003; accepted 5 August 2003

Abstract

The herbal remedy, ginseng, has recently been demonstrated to possess neurotrophic and neuroprotective properties, which may be useful in preventing various forms of neuronal cell loss including the nigrostriatal degeneration seen in Parkinson's disease (PD). In these studies, we examine the potential neuroprotective actions of the ginseng extract, G115, in two rodent models of PD. Animals received oral administration of G115 prior to and/or following exposure to the parkinsonism-inducing neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), in mice, or its toxic metabolite, 1-methyl-4-phenylpyridinium (MPP^+), in rats. Such treatment significantly and dramatically blocked tyrosine hydroxylase-positive cell loss in the substantia nigra and reduced the appearance of locomotor dysfunction. Thus, oral administration of ginseng appears to provide protection against neurotoxicity in rodent models of PD. Further examination of the neuroprotective actions of ginseng and its various elements may provide a potential means of slowing the progress of PD.

Keywords: Ginseng; Parkinson; MPTP; MPP^+; Rat; G115; Neuroprotection; Rotation

Introduction

Ginseng has been used in traditional Chinese medicine for the treatment of various disorders for thousands of years (Lee, 1992), but it is only more recently that we have begun to understand its actions, observing definite and measurable pharmacological effects in the central nervous system. Included among these effects is the modulation of several putative biochemical markers important to the initiation and progression of Parkinson's disease (PD).

PD is a neurodegenerative disorder characterized by the progressive depletion of dopamine in the caudate/putamen (striatum) resulting from the progressive loss of neurons in the substantia nigra pars compacta (SNc) (Calne, 1992). While the pathogenesis of PD remains unclear, evidence suggests that oxidative damage evoked by free radicals may be involved (Fahn and Cohen, 1992; Foley and Riederer, 2000). Free radicals, including superoxide and hydroxyl radicals, are normal by-products of certain biochemical reactions within the cell. These free radicals are maintained at low levels by endogenous antioxidant processes including reactions with vitamins E and C or enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase (Cadet and Brannock, 1998; Mattson and Liu, 2002). Any disruption in the balance between free radical production and antioxidant processes can lead to oxidative stress. Postmortem studies have revealed indications of increased lipid peroxidation, a sign of oxidative damage, in the SNc of PD patients (Dexter et al., 1989a). This may result, in part, from elevations in iron (Dexter et al., 1989b; Hirsch et al., 1991; Berg et al., 2002), disruptions in mitochondrial complex I activity (Schapira et al., 1990; Hattori et al., 1991), or enhanced nitric oxide (NO) production (Bockelmann et al., 1994; Hunot et al., 1999; Knott et al., 2000), each known to contribute to free radical generation and each demonstrated in postmortem PD brains. In addition, parkinsonian brains exhibit reductions in vital antioxidant enzymes (Ambani et

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doi:10.1016/j.expneurol.2003.08.002

al., 1975; Kish et al., 1985; Poirier et al., 1994), suggesting a reduced capacity for detoxification in these patients. Thus, the neurodegeneration observed in PD may involve a combination of mitochondrial defects, elevations in free radical generation, and reduced antioxidant processes.

The root of *Panax ginseng*, C. A. Meyer, has been shown to have a number of actions in the central nervous system including stimulatory and depressive actions, anticonvulsant activity, antipsychotic activity, analgesic activity, antifatigue activity, and enhanced cognitive performance (Saito, 1990). It has also been suggested that certain herbal remedies including ginseng may provide protection against various forms of neuronal damage such as that seen in PD. Ginseng and its components, ginsenosides, reduce oxidative stress in various tissues (Kitts et al., 2000), including brain tissue (Siddique et al., 2000), and have been shown to prevent neuronal loss in models of cerebral ischemia (Lim et al., 1997), spinal cord injury (Liao et al., 2002), and amyotrophic lateral sclerosis (Jiang et al., 2000). Several of the neuroprotective mechanisms ascribed to ginseng in these models may also have beneficial effects in PD.

The purpose of the present study was to investigate the potential of orally administered ginseng extract to influence the neurotoxic potential of MPTP and its neurotoxic metabolite, MPP^+, in two rodent models of PD. Sprague-Dawley rats and C57B16 mice were administered the ginseng extract, G115, in the drinking water prior to and/or following neurotoxin exposure. This extract is standardized as to its ginsenoside content, is the most widely studied in the scientific literature, and is readily available as an over-the-counter supplement. Animals were then evaluated for lesion severity and locomotor dysfunction.

Materials and methods

Animals

Female Sprague-Dawley rats (250–275 g) and C57B16 mice (23–26 g) were obtained from Charles River Inc. (Montreal, Quebec). Animals were housed individually in a temperature-controlled environment with a 12-h-light/dark cycle (lights on at 0700) and ad libitum access to standard rat chow and water. All procedures used in this study were approved by the Dalhousie University Council on Animal Care. All efforts were made to minimize animal suffering and reduce the number of animals used.

Ginseng

For the MPTP mouse model, C57 B16 mice were monitored for their drinking habits for a period of 2 weeks prior to treatment. Animals ($n = 8$) were then administered the ginseng extract, G115 (Pharmaton), in their drinking water (0, 25, 75, 200, or 500 mg/kg/day; based on 5 ml/day approximate intake) for 10 days prior to, and 10 days following, MPP^+ intoxication. For the MPP^+ rat model, Sprague-Dawley rats ($n = 8$) were administered the ginseng extract in their drinking water (100 mg/kg/day; based on 30 ml/day approximate intake) for 10 days prior to, and 2 weeks following, neurotoxin infusion. Ginseng treatment was terminated 24 h prior to behavioural testing. For time course studies in rats, ginseng treatment (100 mg/kg/day) was terminated 0, 1, 2, 3, or 7 days prior to MPP^+ intoxication ($n = 8$). Alternatively, ginseng treatment was initiated 0, 1, 2, 3, or 7 days following exposure to the neurotoxin ($n = 4$).

Neurotoxin administration

For the MPTP mouse model, animals received 4 injections of the dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg, i.p.; Sigma), spaced 2 h apart. For the MPP^+ rat model, animals were anaesthetized using halothane (2%) and placed in a Kopf stereotaxic frame. An infusion cannula was connected by polyethylene tubing to a 50-μl Hamilton syringe driven by a Harvard pump. The neurotoxic metabolite 1-methyl-4-phenylpyridinium iodide (MPP^+) (4 μg in 2 μl; Sigma) (0.5 μl/min.) was infused unilaterally into the right medial forebrain bundle just anterior to the substantia nigra according to coordinates derived from Paxinos and Watson (1986) (A. P. −4.30, M. L. −1.70, D. V. −8.40).

Behaviour

Two weeks following MPP^+ infusion, rats were tested for apomorphine-induced rotations. Animals were habituated to cylindrical test chambers for 30 min prior to testing. Immediately following administration of *R*(−)-apomorphine (0.3 mg/kg, s.c.; Sigma), rotations were recorded for 1 h.

Immunohistochemistry

Twelve hours following behavioural testing, animals were anaesthetized with sodium pentobarbitol and transcardially perfused with 4% paraformaldehyde. Their brains were postfixed for 24 h followed by cryoprotection in 30% sucrose for a minimum of 24 h. Symmetrical 30-μm-thick coronal sections were cut on a freezing microtome and stored in a Millonig's solution. Every 12th section was stained with a monoclonal antibody against either tyrosine hydroxylase (TH) or the dopamine transporter (DAT) (Chemicon). Sections were incubated free floating at room temperature for 3 × 10 min with Tris-buffered saline (TBS) containing 0.25% Triton X-100, 30 min with 3% horse serum in TBS, 16 h with primary antibody (1:10,000) in TBS/serum/Triton X-100, 1 h with horse anti-mouse IgG (Vector Laboratories) in TBS/serum, 1 h with avidin biotin complex (ABC Elite, Vector Laboratories) in TBS, and, finally, 10 min with a mixture of 0.04% 3′3-diaminobenzi-

dine tetrahydrochloride/0.06% $NiCl_2$/0.02% H_2O_2 in Tris-HCl. In between steps, sections were washed for 3 × 10 min in TBS. Stained sections were mounted on gelatin-subbed glass slides, air-dried, incubated for 30 s in 0.005% OsO_4, dehydrated in ethanol, cleared in xylene, and coverslipped in Permount. For the counting of substanta nigra neurons on both sides of the brain, the compacta regions were defined by the distribution of the TH-positive neurons and a set of clear anatomical landmarks/boundaries. The number of TH-immunopositive pars compacta neurons having a maximal cell body diameter of 10 μm was determined by counting at 250×. Optical density measurements were obtained by first capturing the image using a Zeiss Axiophot light microscope (Carl Zeiss, West Germany) and transporting the image using a SPOT Digital Microscope Camera. Relative optical density measurements were calculated in three sections per animal using Scion Image software. The light density of the overlying motor cortex was assigned an optical density value of zero to account for background labeling due to the DAB reaction product. Cell counts and density measurements were obtained under blind conditions.

Statistical analysis

Data were analyzed using either a one-way (TREATMENT) or a two-way (TREATMENT × HEMISPHERE) analysis of variance. Where significant F values were found, planned pairwise comparisons were made using a Newman-Keuls test.

Results

Ginseng effects in MPTP-treated mice

Acute administration of the dopaminergic neurotoxin MPTP significantly reduced (45%) the density of immunohistochemical staining for TH in the striatum of C57B16 mice ($F_{1,60} = 51.92$, $P < 0.0001$, LESION main effect) (Fig. 1). Prolonged oral administration of the ginseng extract G115 dose-dependently blocked reductions in striatal TH immunoreactivity ($F_{4,60} = 3.08$, $P = 0.0226$, GINSENG main effect), without affecting basal levels ($F_{4,60} = 4.51$, $P = 0.003$, LESION × GINSENG interaction effect). At the lowest dose of 25 mg/kg/day, ginseng treatment did not affect MPTP-induced striatal TH loss (41%). However, significant partial preservation of striatal TH was observed with the next higher dose of 75 mg/kg/day (28% loss) and no significant loss of TH immunoreactivity was seen in those animals receiving the highest doses of ginseng, 200 and 500 mg/kg/day (13% and 6%, respectively), suggesting near-total neuroprotection at these doses.

Ginseng effects in MPP^+ infused rats

Two weeks following unilateral MPP^+ infusion, ginseng-treated Sprague-Dawley rats displayed significantly fewer contralateral rotations in response to apomorphine than did non-ginseng-treated controls ($F_{1,12} = 12.58$, $P = 0.004$) (Fig. 2) indicating significantly less functional asymmetry in these animals and suggesting that the ginseng-treated animals sustained significantly less damage as a result of MPP^+ exposure. This was subsequently confirmed by immunohistochemistry. Oral ginseng treatment effectively abolished the loss of TH-positive substantia nigra pars compacta (*SNc*) cells induced by MPP^+ (Fig. 3). Approximately 92% ($P < 0.001$) of TH-positive SNc cells were lost ipsilateral to MPP^+ infusion in those animals that did not receive ginseng. Remarkably, nigral TH staining was almost completely preserved in those animals treated with ginseng, showing only a nonsignificant (18%) decrease in TH-positive SNc cell counts. Ginseng did not alter basal levels of TH expression ($F_{1,14} = 12.45$, $P = 0.003$, GINSENG main effect; $F_{1,16} = 44.03$, $P < 0.0001$, GINSENG × HEMISPHERE interaction effect). Striatal DAT staining was similarly affected, with near-total loss ipsilateral to MPP^+ infusion in those animals who did not receive ginseng compared to a significant preservation of staining in those who were so treated ($F_{1,14} = 34.96$, $P < 0.0001$, GINSENG main effect; $F_{1,16} = 25.65$, $P = 0.0002$, HEMISPHERE main effect; $F_{1,16} = 6.72$, $P = 0.0213$, GINSENG × HEMISPHERE interaction effect) (Fig. 4).

Time course of ginseng effects in MPP^+ infused rats

Pretreatment

In Sprague-Dawley rats, unilateral infusion of MPP^+ resulted in a significant (96.5%) decrease in ipsilateral TH-positive SNc cell counts (Fig. 5A). Pretreatment with ginseng for 10 days effectively attenuated this TH-positive cell loss in a time-dependent manner. When ginseng treatment was terminated 0, 1, or 2 days prior to MPP^+ intoxication, no significant reductions in TH-positive cell counts were evident (15%, 31%, and 35%, respectively). By contrast, ginseng failed to alter MPP^+-induced cell loss when administration was terminated as early as 3 or 7 days prior to MPP^+ infusion (79% and 85% reductions, respectively) ($F_{5,18} = 5.19$, $P = 0.004$, GINSENG main effect; $F_{1,24} = 78.38$, $P < 0.0001$, HEMISPHERE main effect; $F_{5,24} = 4.44$, $P = 0.0082$, GINSENG × HEMISPHERE interaction effect). Significantly fewer apomorphine-induced rotations were observed in those animals treated with ginseng up to 0, 1, or 2 days prior to MPP^+ infusion when compared to those receiving plain drinking water ($F_{5,18} = 14.49$, $P < 0.0001$) (Fig. 5B).

Posttreatment

In Sprague-Dawley rats, unilateral infusion of MPP^+ resulted in a significant (81.6%) decrease in ipsilateral TH-

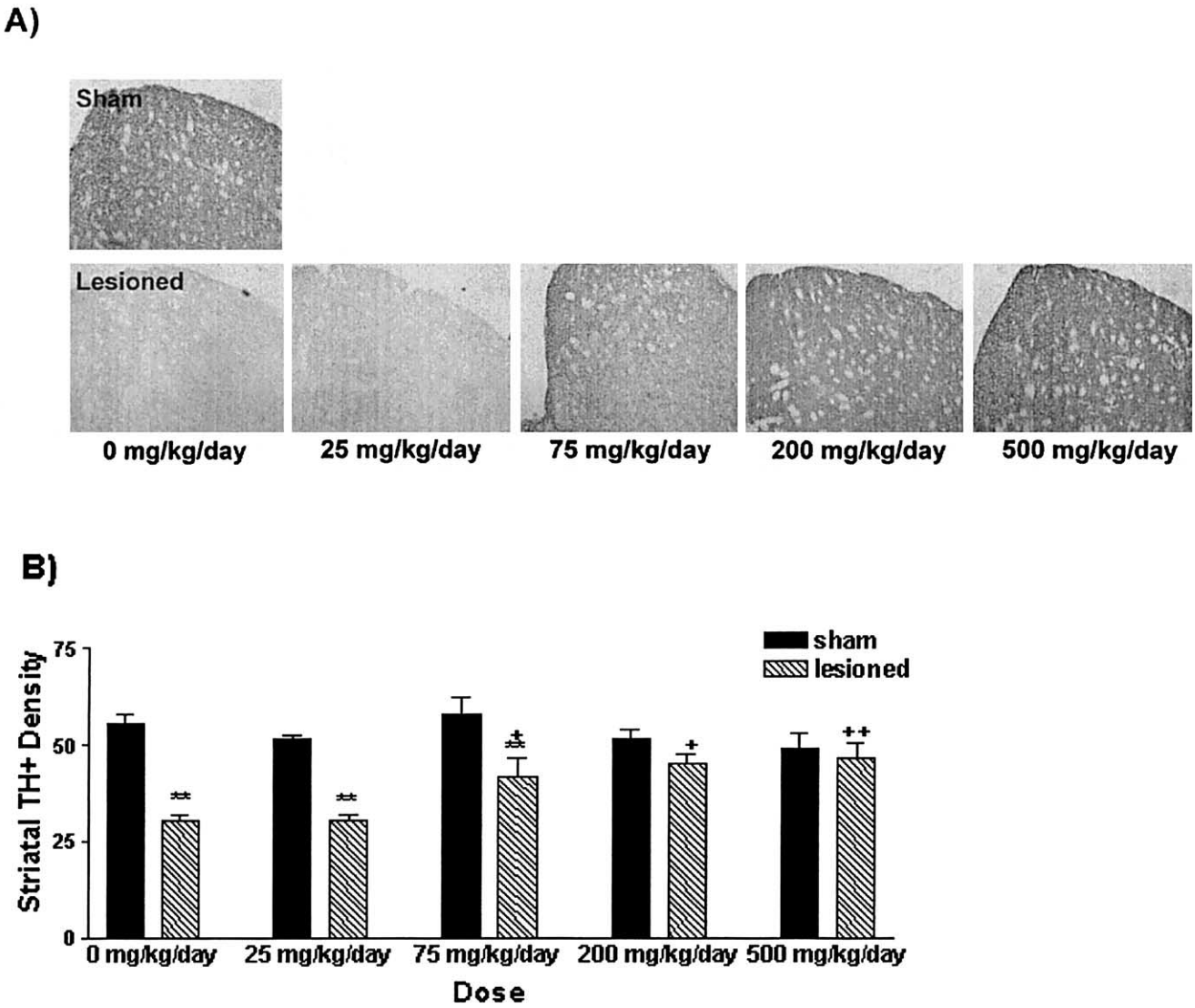

Fig. 1. Prolonged oral administration of ginseng significantly attenuates MPTP-induced reductions in TH immunoreactivity. (A) Representative coronal sections through the striatum immunostained for TH (scale = 100 μm). (B) In those animals receiving plain drinking water, MPTP administration significantly reduced striatal TH immunostaining 10 days following intoxication, but no such loss was seen in those animals treated with ginseng at a dose of 200 or 500 mg/kg/day. Each bar represents the mean (±SEM) ($n = 7$) relative density in three sections through the striatum. **Significantly diff. from sham control, $P < 0.001$. $^{++}$Significantly diff. from vehicle control, $P < 0.001$; $^{+}P < 0.01$.

positive SNc cell counts ($F_{1,60} = 714.68$, $P < 0.001$, HEMISPHERE main effect) (Fig. 6A). Prolonged oral treatment with ginseng significantly attenuated this cell loss in a time-dependent manner. Thus, when ginseng treatment was initiated immediately following MPP^+ intoxication, no significant reductions (9.5%) in TH-positive cell counts were observed. Partial preservation of TH-positive SNc cells (37% and 65% reductions, respectively) was seen when ginseng treatment was initiated 1 and 2 days following neurotoxin exposure, while initiation following 3 and 7 days resulted in cell counts that were not significantly different from those seen in animals receiving plain drinking water (76% and 88% reductions, respectively) ($F_{4,50} = 9.14$; $P < 0.0001$; TIME main effect; $F_{1,50} = 47.90$; $P < 0.0001$, GINSENG main effect; $F_{4,60} = 5.21$, $P = 0.0014$, TIME ×

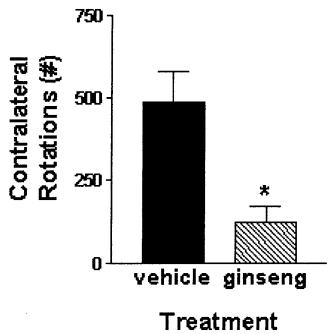

Fig. 2. Ginseng-treated animals display significantly fewer apomorphine-induced contralateral rotations than non-ginseng-treated controls. Each bar represents the mean (± SEM) ($n = 8$) number of contralateral rotations recorded over a 1-h period. *Significantly diff. from vehicle-treated control, $P < 0.01$.

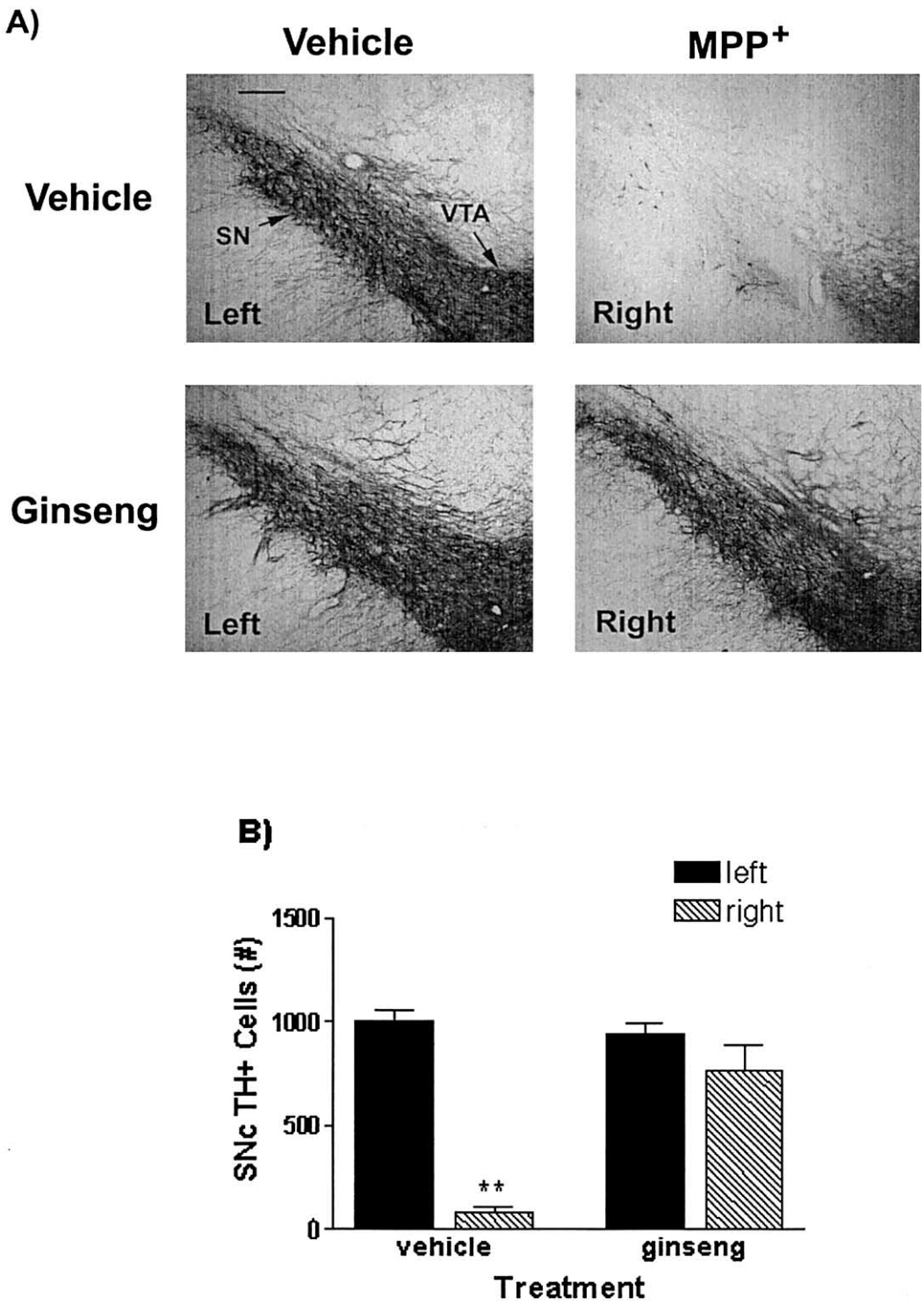

Fig. 3. Prolonged oral administration of ginseng significantly attenuates dopamine cell loss induced by MPP^+. (A) Representative coronal sections through the substantia nigra immunostained for TH (scale = 100 μm). (B) Ipsilateral SNc TH-positive cell counts were significantly reduced following MPP^+ infusion in control animals, but no such loss was seen in those animals treated with ginseng. Each bar represents the "total" number ($\pm$ SEM) ($n = 8$) of TH-positive cells counted in six sections through the nigral complex. **Significantly diff. from left hemisphere, $P < 0.001$. SN, substantia nigra; VTA, ventral tegmental area.

GINSENG $\times$ HEMISPHERE interaction effect). Significantly fewer apomorphine-induced rotations were seen in those animals treated with ginseng when ginseng treatment was initiated 0, 1, or 2 days following MPP^+ intoxication (Fig. 6B). Ginseng failed to significantly affect apomorphine-induced rotations when treatment was initiated 3 or 7

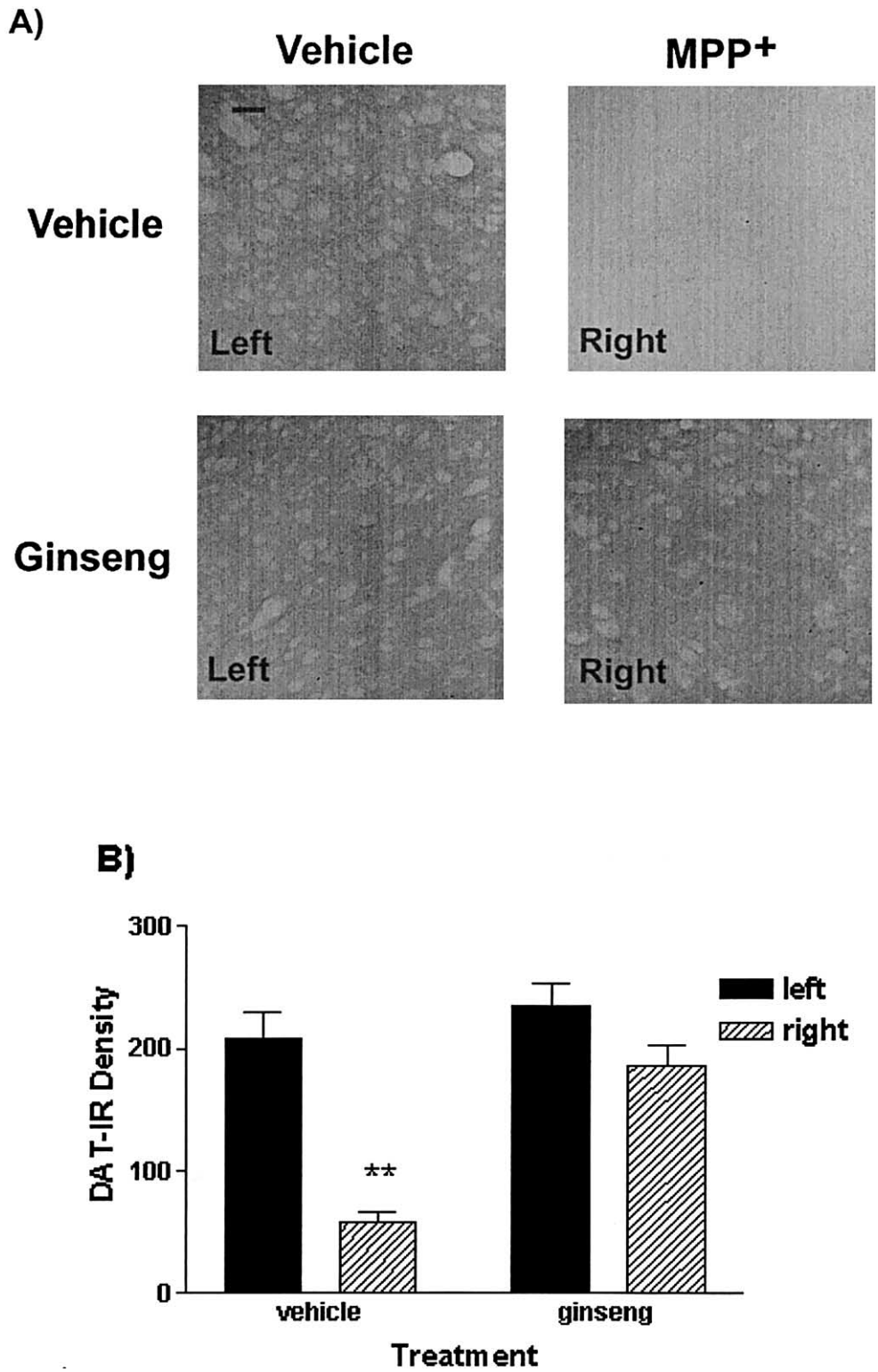

Fig. 4. Prolonged oral administration of ginseng significantly attenuates dopamine terminal loss induced by MPP^+. (A) Representative coronal sections through the striatum immunostained for DAT (scale = 50 μm). (B) Ipsilateral striatal DAT-positive immunostaining was significantly reduced following MPP^+ infusion in control animals, but no such loss was seen in those animals treated with ginseng. Each bar represents the mean ($\pm$SEM) ($n = 8$) relative density of immunostaining in six sections through the striatum. **Significantly diff. from left hemisphere, $P < 0.001$.

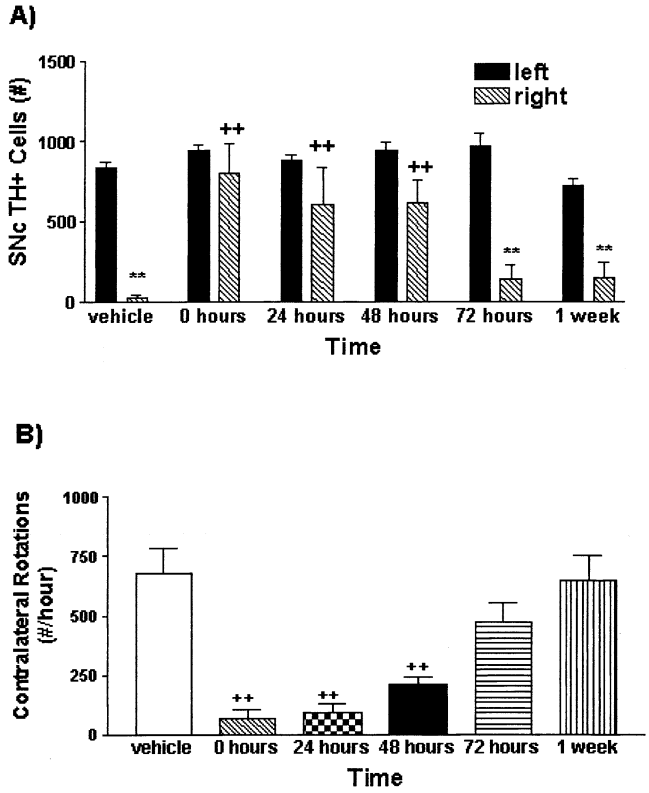

Fig. 5. The timing of ginseng pretreatment significantly influences its neuroprotective actions against MPP^+ toxicity. (A) Ipsilateral SNc TH-positive cell counts were significantly reduced following MPP^+ infusion in control animals. In animals pretreated with ginseng for 10 days, however, TH-positive cell counts were not significantly reduced when ginseng treatment was terminated within 48 h prior to MPP^+ intoxication, when compared to vehicle-treated animals. Thus, ginseng treatment significantly attenuated MPP^+-induced nigral dopaminergic cell loss when treatment was terminated 0, 24, or 48 h prior to MPP^+ intoxication. Each bar represents the mean (± SEM) ($n = 4$) of TH-positive cells counted in six sections through the nigral complex. (B) Two weeks following MPP^+ infusion, apomorphine induced contralateral rotations in those animals receiving plain drinking water. Animals pretreated with ginseng displayed significantly fewer rotations when ginseng treatment was terminated within 48 h prior to MPP^+ intoxication. Each bar represents the mean (±SEM) ($n = 4$) number of contralateral rotations recorded over a 1-h period. **Significantly diff. from left hemisphere, $P < 0.001$. $^{++}$ Significantly diff. from vehicle control, $P < 0.001$.

days following neurotoxin exposure ($F_{4,50} = 8.55$, $P < 0.0001$, TIME main effect; $F_{1,50} = 34.80$, $P < 0.0001$, GINSENG main effect; $F_{4,50} = 6.11$, $P = 0.0004$, TIME × GINSENG interaction effect).

Discussion

These findings demonstrate that ginseng treatment provides protection against the neurotoxic effects of a parkinsonism-inducing agent in rodents. The mechanism involved in this effect remains to be delineated. However, several possibilities exist. The neurotoxic action of MPTP involves a cascade of events beginning with the dopamine transporter, which is thought to play an important role in the uptake of its neurotoxic metabolite, MPP^+ (Javitch et al., 1985), possibly accounting for its selectivity as a toxin for dopaminergic neurons. The neurotoxic effects of MPP^+ can be blocked following pretreatment with dopamine uptake blockers (Giros and Caron, 1993) or reductions in dopamine

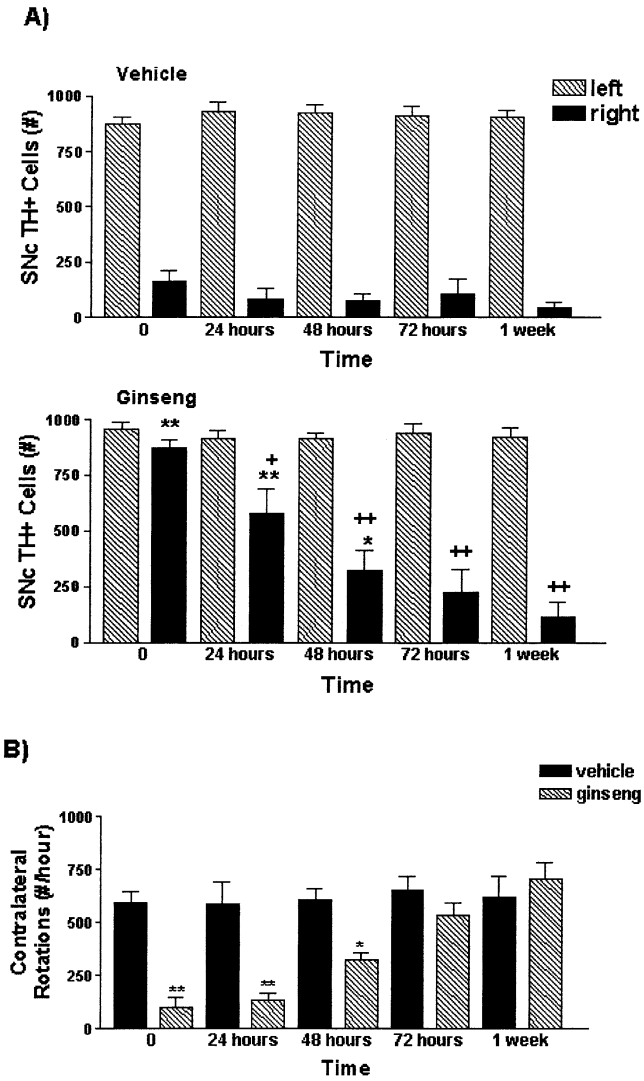

Fig. 6. The timing of posttreatment with ginseng significantly influences its neuroprotective actions following MPP^+ infusion. (A) Ipsilateral SNc TH-positive cell counts were significantly reduced following MPP^+ infusion in all animals receiving plain drinking water. In those animals treated with ginseng for 2 weeks, significantly fewer TH-positive cells were lost when treatment was initiated within 48 h of MPP^+ intoxication, when compared to vehicle-treated animals. Thus, ginseng treatment significantly attenuated MPP^+-induced nigral dopaminergic cell loss when treatment was initiated 0, 24, or 48 h following MPP^+ intoxication. (B) Apomorphine-induced contralateral rotations were significantly lower in those animals treated with ginseng for 2 weeks when administration was initiated within 48 h following MPP^+ intoxication. Each bar represents the mean (±SEM) ($n = 4$) number of contralateral rotations recorded over a 1-h period. **Significantly diff. from vehicle control, $P < 0.001$; *$P < 0.01$. $^{++}$Significantly diff. from left hemisphere, $P < 0.001$; $^{+}P < 0.01$.

transporter expression (Gainetdinov et al., 1997; Van Kampen et al., 2000). Certain ginsenosides have been shown to inhibit the uptake of dopamine into rat synaptosomes (Tsang et al., 1985). Thus, ginseng could potentially provide neuroprotection in this model through blockade of MPP^+ uptake rather than any potential antioxidant activity. However, the findings reported here, suggest this is not the case. Following cerebral infusion of MPP^+, this neurotoxin is rapidly taken up by the DAT, being cleared from the extracellular fluid in a relatively short time (Zimmer et al., 2000). Thus, the neuroprotective actions of ginseng administered up to 48 h following MPP^+ infusion are not likely due to blockade of neurotoxin uptake.

Once inside the cell, MPP^+ impairs mitochondrial respiration through complex I inhibition, ultimately resulting in excitotoxicity due to a consequent elevation in glutamate release (Beal et al., 1993) and removal of Mg^{2+} blockade of NMDA receptors (Schulz et al., 1997). Indeed, NMDA antagonists are capable of attenuating the neurotoxic effects of MPTP (Brouillet and Beal, 1993; Lange et al., 1993). Certain ginsenosides similarly display neuroprotective capabilities against glutamate-induced neurodegeneration (Liu and Zhang, 1995; Kim et al., 1998). Enhanced glutamate receptor activation, in turn, triggers an influx of Ca^{2+} resulting in elevations in intracellular Ca^{2+} stores and activation of Ca^{2+}-dependent enzymes including nitric oxide synthase, which produces nitric oxide (NO). NO further impairs mitochondrial function and promotes the generation of free radicals resulting in oxidative damage (Schulz et al., 1997). Ginseng reduces glutamate-induced Ca^{2+} influx, thereby decreasing cytosolic Ca^{2+} (Kim et al., 1998) and subsequent NO production. Ginseng also reduces oxidative stress by altering antioxidant enzyme activities required to eliminate free radicals (Kitts et al., 2000).

NO production is also enhanced by inflammatory cytokines such as interleukin 1-beta, shown to be elevated in the striatum of Parkinson's patients and animal models of PD (Nagatsu et al., 2000). Anti-inflammatory drugs such as dexamethasone (Kurkowska-Jastrzebska et al., 1999) and minocycline (Du et al., 2001) reduce the inflammatory response to MPTP in mice, blocking production of cytokines and NO, and reducing cell loss. Similary, ginseng blocks lipopolysaccharide-induced cytokine and NO production in cell culture (Wang et al., 2000) and reverses cytokine elevations associated with aging in the rat (Yu and Li, 2000). Thus, ginseng may reduce toxicity in this model by reducing the inflammatory response to MPP^+.

Perhaps the most interesting possibility involves nerve growth factor (NGF). The neurotrophic factor NGF stimulates the outgrowth of neurites in neuronal cells and plays an important role in the survival and maintenance of neurons in the central nervous system (Fukunaga and Miyamoto, 1998). NGF levels are reduced in the striatum of PD patients and experimental animal models of PD (Nagatsu et al., 2000). The addition of NGF to cell cultures can both prevent (Shimoke and Chiba, 2001) and reverse (Rudakewich et al., 2001) MPTP-induced cell death in an in vitro model system of PD. Ginsenosides, particularly Rb_1 and Rg_1, elevate NGF mRNA expression in rat brain (Salim et al., 1997) and potentiate NGF-induced neurite outgrowth in cell cultures (Rudakewich et al., 2001). Thus, ginseng may prevent or reverse the neurotoxic effects of MPP^+ through alterations in NGF expression. This remains to be investigated.

Regardless of the physiological mechanism involved, these findings provide concrete evidence that ginseng extract provides neuroprotection in rodent models of PD and may provide a potential means of slowing the progression of this debilitating disease. It is interesting to speculate whether geographic variations in PD prevalence might reflect ginseng consumption. In North America, PD occurs in approximately 200 cases per 100,000 persons compared to only 44 cases per 100,000 in Chinese cities (Tanner and Ben-Shlomo, 1999) where ginseng consumption is high. While many advances have been made in the symptomatic treatment of PD, little progress has been made in preventing or slowing the progression of this disease. A further understanding of how ginseng prevents neuronal loss in this model may have profound implications for the treatment and prevention of PD.

Conclusion

Prolonged oral administration of the ginseng extract G115 significantly attenuates measures of functional asymmetry and dopaminergic cell loss following exposure to a parkinsonism-inducing neurotoxin in two animal models of PD. These findings are in agreement with a growing body of evidence that ginseng and its individual components provide protection against neuronal cell death.

Acknowledgments

Supported by the Canadian Institutes of Health Research and the Nova Scotia Health Research Foundation. J.V.K. is a postdoctoral fellow of the Parkinson Society of Canada.

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