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Effect of berberine on pentylenetetrazol-induced seizures in rats

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Abstract

Objective: Antiepileptic drugs (AEDs) that are usually used for treatment of epilepsy have substantial side effects and about 30% of patients continue to have seizures with current AEDs therapy. Some herbs which traditionally used in the management of seizures of many rural areas of the developing countries have shown anticonvulsant activity in modern pharmacological bioassays. The aim of the present study was to evaluate the effects of berberine, an alkaloid from *Berberis vulgaris*, on seizures induced by pentylenetetrazol (PTZ) in rats.

Material and Methods: Rats (n=6-7) received berberine (100, 200 and 400 mg/kg, i.p.), diazepam (4mg/kg, i.p. as positive control), and vehicle (saline) and then 30 min later PTZ (110mg/kg, i.p.) were injected . Behavioral responses of the animals to PTZ administration were evaluated using these criteria: latency to first minimal clonic seizure (MCS), incidence of MCS, latency to the first generalized tonic–clonic seizures (GTCS), incidence of GTCS, protection against GTCS and mortality.

Results: Intraperitoneal administration of lower doses of berberine (100 and 200 mg/kg) had no significant effects on minimal clonic seizures (MCS) and generalized tonic-clonic seizures (GTCS) latencies, while injection of 400 mg/kg caused significant increase in both MCS and GTCS latencies ($p<0.05$). In this study diazepam, (4 mg/kg) 30 min prior to PTZ, significantly increased GTCS latency. Berberine at tested doses had no protection against mortality following PTZ administration.

Conclusion: It can be concluded that berberine at high doses could be a useful protective agent in PTZ-induced epileptic seizures in rats.

Keywords: Berberine, *Berberis vulgaris*, Pentylenetetrazol, Seizure, Diazepam

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Introduction

Epilepsy is a neurological disorder characterized by abnormal recurrent seizures (Giblinand Blumenfeld, 2010). It is one of the most common disorders affecting approximately 0.5-2% of the global population (Naseer et al., 2009). Berberine is a plant alkaloid with a long history of medicinal use in both Ayurvedic and Chinese medicine. The various plant sources of berberine include *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric) (Imanshahidi and Hosseinzadeh, 2008; Zhou et al., 2008). The pharmacologic actions of berberine include metabolic inhibition of certain organisms, inhibition of bacterial enterotoxin formation, inhibition of intestinal fluid accumulation and ion secretion, inhibition of smooth muscle contraction, reduction of inflammation, inhibition of platelet aggregation, platelet count elevation in certain types of thrombocytopenia, stimulation of bile and bilirubin secretion, and inhibition of ventricular tachy-arrhythmias. It also has been known as antitumor and antioxidant agent (Imanshahidi and Hosseinzadeh, 2008; Pereira et al., 2008). Berberine has been shown to block potassium channels of hippocampal CA1 neurons, leading to suppression of apoptosis and a substantial increase in the cell survival rate under anoxic/ischemic injury (Zhou et al., 2008). A recent study showed that berberine had no significant effect on seizures induced by pentylenetetrazol in mice (Bhutada et al., 2010).

Pentylenetetrazol (PTZ) is the most popular chemoconvulsant agent used for epileptogenesis and assessment of antiepileptic drugs (AEDs) (Hosseinzadeh and Sadeghnia, 2007; Mohammad et al., 2009). A sufficiently high dose of PTZ can produce a continuum of seizure activity that progress from mild myoclonic jerks to face and forelimbs clonus without loss of righting reflex (which is known as minimal clonic seizure, MCS), to clonic seizures of limbs with loss of righting reflex, to full tonic extension of both forelimbs and hind limbs (generalized tonic-clonic seizures, GTCS) (Hosseinzadeh and Sadeghnia, 2007).

AEDs that are usually used for treatment of epilepsy have substantial side effects and about 30% of patients continue to have seizures with current AEDs therapy (Hosseinzadeh and Sadeghnia, 2007; Sahin et al., 2009). Some herbs traditionally used in the management of seizures of many rural areas of the developing countries, have shown anticonvulsant activity in modern pharmacological bioassays without severe side effects (Hosseinzadeh and Sadeghnia, 2007, Ching et al., 2009).

The aim of the present study was investigation on the effect of berberine on seizures induced by PTZ in rats.

Materials and Methods

Chemicals

PTZ and berberine hydrochloride were purchased from Sigma-Aldrich Co (St. Louis, MO). Diazepam was obtained from Tolidaru Pharmaceuticals (Tehran, Iran).

Animals

Adult male Wistar rats weighing 200-300 g were used throughout the study. All of them were housed in the same room under a constant temperature (22±2°C) and illuminated 7:00 a.m. to 7:00 p.m., with food pellets and water available ad libitum.

PTZ-induced seizures

Rats (seven to eight per group) were intraperitoneally administrated with berberine (100, 200 and 400 mg/kg), diazepam (4mg/kg, positive control), and vehicle (saline) and then 30 min later were injected with PTZ (110mg/kg, i.p.). The animals were observed for 60 min after PTZ

administration. Behavioral responses of the animals to PTZ administration were evaluated using these criteria: latency to first minimal clonic seizure (MCS), incidence of MCS, latency to the first generalized tonic–clonic seizures (GTCS), incidence of GTCS, protection against GTCS and mortality (Hosseinzadeh and Sadeghnia, 2007).

Statistical analysis

Data are expressed as mean ± S.E.M. Fisher's exact probability test, as well as analysis of variance (ANOVA), followed by Tukey's test, were used for statistical evaluation. P-values less than 0.05 were considered to be statistically significant.

Results:

Animals in all groups showed MCS and GTCS after PTZ injection. Intraperitoneal (i.p.) administration of berberine (100 and 200 mg/kg) had no effect on MCS and GTCS latency while i.p. injection of berberine with dose of 400mg/kg ($p<0.05$) caused significant increase in MCS and GTCS latencies (Tables 1 and 2). In this study, diazepam (4 mg/kg, 30 min prior to PTZ) significantly increased GTCS latency (Table 2). Berberine at tested doses had no protection against mortality following PTZ administration, while protection against mortality for diazepam was 100% (Table 3).

Table 1. Effect of berberine on minimal clonic seizures (MCS) induced by PTZ.

Treatment dose (mg/kg)	# of animal with MCS	Protection (%)	MCS Latency (sec)
Vehicle (saline)	8/8	0	49.63 ± 5.37
Diazepam 4	5/7	33.33	121.8 ± 4.61*
Berberine 100	7/7	0	47.20 ± 3.26
Berberine 200	7/7	0	67.29 ± 10.09
Berberine 400	8/8	0	149.3 ± 18.41*

All animals were injected with PTZ (110 mg/kg), 30 min after berberine administration. All drugs were administrated intraperitoneally. Latency periods are expressed as mean ± SEM. *$p<0.05$ as compared to vehicle (Tukey's test).

Table 2. Effect of berberine on generalized tonic-clonic seizures (GTCS) induced by PTZ.

Treatment dose (mg/kg)	# of animal with GTCS	Protection (%)	GTCS Latency (sec)
Vehicle (saline)	8/8	0	63.88 ± 4.61
Diazepam 4	5/7	33.33	152.8 ± 17.84*
Berberine 100	7/7	0	57.20 ± 3.32
Berberine 200	7/7	0	84.57 ± 17.72
Berberine 400	8/8	0	142.0 ± 22.31*

All animals were injected with PTZ (110 mg/kg), 30 min later after berberine administration. All drugs were administrated intraperitoneally. Latency periods are expressed as mean ±SEM. *$p<0.05$ as compared to vehicle (Tukey's test).

Table 3. Effect of berberine on rat survival after generalized tonic-clonic seizures (GTCS) induced by PTZ.

Treatment dose (mg/kg)	# of surviving animals	Mortality protection (%)
Vehicle (saline)	0	0
Diazepam 4	6	100 ###
Berberine 100	0	0
Berberine 200	0	0
Berberine 400	0	0

All animals were injected with PTZ (110 mg/kg), 30 min later after berberine pre-treatment. All drugs were administrated intraperitoneally. ###$p<0.0001$ as compared to vehicle (Fisher's exact probability test)

Discussion

AEDs with few side effects and high ability to control all the seizures are ideal to use. Since AEDs that are currently prescribed for epileptic patients have side effects and fail to manage epilepsy in some patients, we investigated the anticonvulsant effect of berberine as an herbal drug with low side effects (Goldenberg, 2010). Our results demonstrated that berberine dose-dependently affects epileptic seizures induced by PTZ, which was evident by lengthened latency period of induced

seizures. On the other hand, berberine failed to protect against seizure incidence or mortality upon PTZ administration. In this study, diazepam also significantly inhibited PTZ-induced seizures as well as mortality.

Berberine has multiple therapeutic actions (Imanshahidi and Hosseinzadeh, 2008). It was shown that following systemic administration, berberine can easily cross the blood brain barrier (Imanshahidi and Hosseinzadeh, 2008). Preclinical evidences have suggested its utility in various neurodegenerative and neuropsychiatric disorders (Imanshahidi and Hosseinzadeh, 2008; Zhou et al., 2008). The alkaloid is effective (200 mg/kg/day) in ameliorating diabetic nephropathy in rats (Tang et al., 2006). Berberine (0.012, 0.006 mmol/L) could also protect the injured cerebral microvascular endothelial cells from hypoxia/hypoglycemia (Benaissa et al., 2009). In another study, berberine (50 mg/kg) when administered intragastrically to rats, significantly reversed the amyloid peptide Aβ (1-40)-induced memory damage when assessed in the Morris water maze (Asai et al., 2007). Recent studies have indicated the beneficial neuroprotective roles of barberry (*Berberis vulgaris*) extract and berberine in ischemic insults, in vitro and in vivo (Yoo et al., 2006; Cui et al., 2009). The protective action may be due to the antagonistic activity of berberine on N-methyl-D-aspartate receptors (Cui et al., 2009). It is also suggested that berberine (30 μM) blocks the voltage-dependent potassium currents in acutely isolated CA1 pyramidal neurons of rat hippocampus (Wang et al., 2004). Also, berberine (1-100 μM) reduced 6-hydroxydopamine-induced neurotoxicity and free calcium concentration elevation in cultured brain cells (Kwon et al., 2010). It has been shown that berberine improved neurological outcome and reduced ischemia/reperfusion (I/R)-induced cerebral infarction 48h after middle cerebral artery occlusion (MCAO) in rats. Berberine can also protect PC12 cells against oxygen-glucose deprivation (OGD)-induced injury (Zhou et al., 2008).

A recent study revealed that in maximal electroshock (MES)-induced seizures model, berberine (10 and 20 mg/kg, i.p.) decreased duration of tonic hind limb extension and as well as mortality. Moreover, these doses of berberine also protected mice against KA-induced clonic convulsions and decreased mortality, however at the tested doses; berberine did not significantly influence the latency period, duration of myoclonic jerks, and frequency of myoclonic jerks or mortality following PTZ administration (Bhutada et al., 2010). In this study we used high dose of berberine (400 mg/kg) which significantly increased MCS and GTCS latency upon PTZ administration.

It can be concluded that berberine at high doses could be a useful protective agent in PTZ-induced epileptic seizures in rats.

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