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Review

Neuropharmacological and pharmacokinetic properties of berberine: a review of recent research

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Abstract

Objectives This review summarizes recent research on the neuropharmacological and pharmacokinetic properties of berberine, an isoquinoline alkaloid extracted from *Coptidis rhizoma*.

Key findings Berberine has multiple neuropharmacological properties, such as neuroprection, anti-neuronal apoptosis, improvement of cerebral microcirculation and anti-Alzheimer's disease, and so on. The pharmacokinetic characteristics of berberine are that it is not easily absorbed and it is not stable in the gastrointestinal tract of animals or humans.

Summary Further studies need to be carried out to develop berberine as a drug for nervous system diseases, such as brain ischaemia and Alzheimer's disease, that has favorable pharmacokinetic properties.

Keywords Alzheimer's disease; berberine; neuropharmacology; pharmacokinetics

Introduction

Berberine (BBR), a yellow plant isoquinoline alkaloid (see Figure 1) with yellow fluorescence under ultraviolet light, is found in the root, rhizome and stem bark of many plants, such as *Berberis*, *Hydrastis canadensis* and *Coptidis rhizoma*, which have all been used as herbal drugs in traditional Chinese medicine. BBR has had several different bioactivities reported, including anti-inflammatory,[1] cardioprotective,[2] antitumor,[3] antimalarial,[4] antioxidative[5] and cerebroprotective effects.[6] These properties were summarized in two recent and excellent reviews.[7,8] In clinical use, BBR chloride or BBR sulfate are the generally applied formulations.

In view of increasing numbers of demonstrations of BBR's pharmacological effects in the nervous system and its potential application in the therapy of nervous system diseases such as brain ischaemia and Alzheimer's disease, this article provides an overview of recent research into BBR's neuropharmacology and its pharmacokinetics in animals and humans.

The following databases were used in searching key literature: Medline (1982 to December 2008), Scifinder (1982 to December 2008) and Full Text Database of Journals Published in Chinese (1994 to December 2008).

Neuropharmacology of berberine

Berberine and brain ischaemia

Neuroprotection

Studies of ischaemia-induced cell damage have revealed a complex mechanism involving glutamate excitoxicity, intracellular calcium increase and free radical production. The production of free radicals correlates with intracellular calcium elevation. Calcium is considered a mediator of ischaemic brain damage from global or focal ischaemia. Glutamate receptor blockage can result in decreased free radical production and markedly diminished intracellular calcium accumulation.

BBR exerts a protective effect against neuronal injury due to the neurotoxicity of excitatory amino acids, such as glutamate, during ischaemia. Wu *et al.* showed that the cell death rate of neurons treated with BBR (5 μmol/l) is significantly lower than that of non-treated neurons.[9] No pathological and morphological changes were found in BBR-treated neurons, whose shapes were similar to those of normal ones.

BBR protects the hippocampal CA1 region from ischaemic injury by inhibiting *N*-methyl-D-aspartate receptor 1 immunoactivity in ischaemic gerbil brains,[10] and Fan

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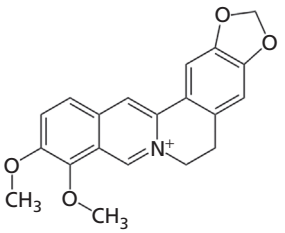

Figure 1 Chemical structure of berberine

et al. demonstrated that it can also potentially protect the brain tissue of acute hypoxic mice.[11] It can inhibit the increase of intracellular calcium concentration ($[Ca^{2+}]_i$) by reducing glutamate, serotonin and noradrenaline (norepinephrine),[12] and it can alleviate neuronal injury from oxygen and glucose deprivation, improve superoxide dismutase (SOD) activity, reduce melondialdehyde (MDA) generation[13] and increase the content of glutathione. BBR (5–30 μmol/l) was observed by Wu *et al.* to significantly reduce cell death rate, lactate dehydrogenase leakage rates and MDA generation, and to elevate activity of SOD in cortical neurons exposed to H_2O_2.[14] These activities of BBR may be the reason why $ONOO^{(-)}$-induced damage in an in-vitro system was potently blocked by BBR (10–20 mg/kg per day for 10 days).[15]

Anti-neuronal apoptosis

Neuronal apoptosis plays an important role in the pathogenesis of cerebral ischaemia. While damaged neurons often die from necrosis, apoptosis contributes significantly to cell death subsequent to cerebral ischaemia and is predominant when the excitoxic insult is relatively mild.[16]

It has been reported that BBR can inhibit delayed neuronal death (DND), a kind of cell apoptosis in the hippocampal CA1 region after an ischaemic insult, which is characterized by the dramatic shrinkage and loss of the fibre bundle. Ultrastructural changes of neurons treated with BBR show clearly that DND is significantly alleviated or delayed by BBR, and that the morphology of neurons in the CA1 region is normal compared with that in animals treated with placebo.[17] In addition, after short/long-term ischaemic reperfusion in the hippocampus, the morphology of neurons in the CA1, CA2 and CA3 regions was also protected by BBR (30 mg/kg, 30 min before ischaemia and 8 mg/kg 24 h and 48 h after reperfusion), possibly due to a protective effect on the hippocampal pyramidal cells caused by improving the resistance of the mitochondria, rough endoplasmic reticulum and the Golgi body to ischaemia in the early stages of ischaemic reperfusion.[18]

Pathologic conditions such as hypoxia or ischaemia have been reported to induce cellular apoptosis as well as to regulate hypoxia-inducible factor-1α (HIF-1α). In ischaemic conditions, HIF-1α induces pro-apoptotic BNIP3 (Bcl-2/adenovirus E1B interacting protein 3), Nix gene expression through binding with p53, and then promotes the expression of inducible nitric oxide synthase (iNOS) and the generation of nitric oxide (NO), leading to apoptosis. BBR inhibits neuronal apoptosis by reducing the expression of HIF-lα, which plays an important role in maintaining oxygen equilibrium. The mechanism by which HIF-1α expression is reduced is based on the enhancement of lysine acetylation and proteolysis.[19] Furthermore, BBR (1 mg/kg) can enhance the expression of the Bcl-2 gene and lower the Bax gene expression in the hippocampal CA3 region after cerebral ischaemia in mice. Bcl-2 is an anti-apoptotic protein while Bax is a proapoptotic one, therefore BBR reduces the occurrence of neuronal apoptosis.[20]

In addition, excessive K^+ efflux and intracellular K^+ depletion have been hypothesized to be key steps in the apoptotic cascade of many cells, including central neurons. BBR (1–300 mmol/l) can block the potassium channels of hippocampal CA1 neurons. This is beneficial for the cation balance of neurons after ischaemic injury, and leads to the suppression of apoptosis.[21]

Improving cerebral microcirculation in the ischaemic brain

Microcirculation dysfunction is an important pathophysiological change in ischaemic cerebrovascular disease, therefore improvement of cerebral pial microcirculation is crucial for the maintenance of cerebral blood flow and the treatment of symptoms of cerebral ischaemia.[22]

BBR has a favourable effect on vasodilatation, markedly dilating the peripheral arteries of the cerebral pial microcirculation, accelerating microcirculation blood flow and maintaining cerebral blood flow and the basic metabolism of nerve cells.[23] It has been reported that BBR (20 mg/kg per day, i.p., 5 days) can inhibit the elevated platelet adhesion and aggregation rate induced by adenosine diphosphate, collagen or arachidonic acid after cerebral ischaemia.[24,25] In addition, BBR can decrease lesions of cerebral vascular endothelial cells, which are relevant to the occurrence and the development of cerebral vascular diseases, particularly in the early pathological changes of cerebral ischaemia.[26] A possible mechanism may be that BBR can modulate the expression of intercellular adhesion molecule 1 and partially modulate expression of vascular cell adhesion molecule 1 and other adhesion molecules, by lowering the nuclear factor (NF)-κB light chain gene enhancer of activated B cell gene expression.[26]

Berberine and Alzheimer's disease

Alzheimer's disease (AD), the most common form of dementia, is recognized as a progressive neurodegenerative disease of the brain, which causes cognitive dysfunction and memory loss related to hippocampal damage. There are several hypotheses to explain the underlying mechanisms of AD, including the cholinergic hypothesis, the amyloid protein (Aβ)-toxicity hypothesis and the oxidative stress hypothesis.[27,28] These hypotheses are very useful as a guide in the search for novel strategies against AD or to explain the effects of drugs for AD.

The results of both postmortem and antemortem studies in the aged and in AD patients, as well as animal experiments, suggest that a host of cholinergic abnormalities, including alterations in choline transport, acetylcholine release,

nicotinic and muscarinic receptor expression, neurotrophin support and perhaps axonal transport, may all contribute to cognitive abnormalities in AD. Multiple lines of evidence demonstrate that oxidative stress occurs prior to cytopathology and therefore may play a key pathogenic role in AD.

The Aβ hypothesis is a particularly well-known description of the pathogenesis of AD. Aβ accumulation and extracellular Aβ deposition are toxic to neurons. Inhibition of Aβ generation and aggregation, enhancement of extracellular Aβ removal and Aβ vaccination are therefore currently under investigation as possible treatments.

Recently, several studies have found that BBR is an excellent reversible acetylcholinesterase inhibitor.[29–32] Shi *et al.* found that it can improve learning and memory disorders induced by scopolamine in mice.[33] However, Guo *et al.* showed that it did not improve learning–memory dysfunction induced by cycloheximide, indicating that the improvement in learning and memory induced by BBR (4.0 μg) may be related to inhibition of central cholinesterase but that there is no relation to the synthesis and metabolism of proteins.[34]

The neuroprotective effects of BBR have also been observed by assaying learning and memory in animals with post-cerebral ischaemia.[35] Differences in the deficiency of learning and memory in different ischaemic-reperfused rats were used to determine the extent of damage to the hippocampus, in which neurons of regions CA2, CA3 and CA4 play a crucial role in animal spatial learning and memory. Obviously, this protective effect of BBR is based on maintaining the morphological structure of the hippocampus in reperfused animals.[17]

Many cytokines and proteins, such as Bcl-2 and tumour necrosis factor α (TNF-α), are involved in the development of AD.[36] It is very interesting that BBR was found to improve cognitive ability in aged people with a high risk of developing dementia, alongside increasing the whole blood Bcl-2 level and decreasing the level of serum TNF-α. These features may be indicators of neuroprotection in the blood of patients with chronic cerebropathy.[37] Because of the small size of the sample, further research should be carried out to confirm the function of BBR. In addition, BBR has significantly ameliorated spatial memory impairment in a rat model of AD, although at the same time it increased the expression of two inflammatory factors, interleukin-1β (IL-1β) and iNOS.[38]

Interestingly, recent findings have indicated that BBR decreases Aβ levels in order to protect neurons. Its 50% inhibition concentration (IC50) for extracellular Aβ production is around 5 μM. This effect of BBR is due to modulation of amyloid precursor protein processing at a non-neurotoxic concentration, suggesting that BBR may be a promising candidate for the treatment of AD.[39] BBR (10 μg/ml) was also found to have nerve growth factor (NGF)-potentiating activity, which could increase NGF-induced neurite outgrowth in a dose-dependent manner without cytotoxicity in rat phaeochromocytoma cells (PC12 cells). This NGF-potentiating activity of BBR was not associated with its inhibition of acetylcholinestrase (AChE) and/or the accumulation of acetylcholine.[40]

Other pharmacological activities

Improving diabetic neuropathy

Diabetic neuropathy (DN) has become one of the most common chronic complications of diabetes. Control of blood glucose is one of the most effective methods of preventing the formation and development of DN.

BBR (100 mg/kg) can significantly decrease the concentration of fasting blood glucose in diabetic rats, and a large dosage of BBR can ameliorate nerve pain in DN rats to some extent.[41] It also significantly increases the nerve conduction velocity in diabetes complicated with DN in rats.[42] In addition, BBR is reported to inhibit glycosylation, in particular glycosylation in brain tissue, reducing the formation of advanced glycation end-products in brain tissue and inhibiting calcium overload to reduce the damage to nerve cells that these induce. BBR particularly protects the mitochondria of the hippocampus, and this might be the basis of its prevention of DN.[43] It is worth noting that AChE and butyrylcholinesterase activity was significantly higher in the serum of type 2 diabetes rats complicated by AD compared with normal rats.[44] BBR may therefore have important clinical applications in the prevention and treatment of type 2 diabetes accompanied by AD.

Antidepressant and anxiolytic effects

BBR (10–20 mg/kg, p.o. or 5–20 mg/kg i.p.) exerts an antidepressant-like effect in two models of depression. The mechanism may be the modulation of noradrenaline, serotonin and dopamine levels in the hippocampus and frontal cortex.[45,46] However, the antidepressant-like effect of BBR is not dose-dependent. Moreover, BBR (IC50, 126 μM) is reported to have an inhibitory effect on monoamine oxidase enzymes, particularly monoamine oxidase-A.[47]

BBR (100 mg/kg) exerts a significant anxiolytic effect, which may be related to increased turnover rates of monoamines in the brain stem and to decreased serotonergic system activity, by activating 5-HT1A receptors and inhibiting postsynaptic 5-HT1A and 5-HT2 receptors.[48]

Attenuation of repeated nicotine-induced behavioural sensitization

Repeated injections of nicotine can produce an increase in locomotor activity and the expression of the immediate-early gene, c-fos, in the central dopaminergic areas. Pretreatment with BBR (100 mg/kg, i.p.) significantly inhibited the nicotine-induced locomotor activity and the expression of c-Fos in the striatum and nucleus accumbens in rats. These results suggest that BBR inhibits nicotine-induced behavioural sensitization, possibly by reducing postsynaptic neuronal activation in the central dopaminergic system.[49]

The pharmacokinetics of berberine

BBR has been used clinically for several decades.[7,8] Apart from its pharmacological properties, many pharmacokinetic studies have been conducted in animals and in human beings (see Table 1).[50]

Table 1 The pharmacokinetic parameters of berberine in animals and humans

Species	Dosage	Method of administration	PK parameters	Findings	References
Rabbit	50 mg/kg	Oral	$t_{1/2\alpha}$: 0.14 h, $t_{1/2\beta}$: 3.11 h, C_{max}: 92.7 μg/l, t_{max}: 0.63 h, AUC: 491.70 μg/h/l	Single-compartment model; oryzanol affects the absorption of BBR in rabbits	[51]
Rabbit	46.25 MBq/kg	i.g.	$t_{1/2\alpha}$: 1.41 h, $t_{1/2\beta}$: 35.3 h, V_d: 20 l/kg, K_α: 2.45/h	Open two-compartment model; rapid absorption, distributes extensively and eliminated slowly	[53]
Rabbit	25.9 MBq/kg	i.v.	$t_{1/2\alpha}$: 1.03 h, $t_{1/2\beta}$: 35.8 h, V_d: 22.1 l/kg	Open two-compartment model; fast absorption and extensive distribution; highest concentration in lung; inhibition to heart less likely	[53]
Rat	40 mg/kg	p.o.	C_{max}: 10 g/ml, AUC: 37.42 μgh/l, MTT: 10.52 h	Rapid absorption and fast metabolism; liver and intestinal bacteria participate in the metabolism and disposition of BBR *in vivo*	[52]
Rat	10.2 mg/kg *C. rhizoma* extract containing 3 mg/kg BBR	i.v.	Hippocampus: $t_{1/2\alpha}$: 0.22 h, C_{max}: 272 ng/g, t_{max}: 3.67 h, $t_{1/2\beta}$: 12.0 h, AUC: 6940 ng/h/l Plasma: AUC: 473 ng·h/l, $t_{1/2\alpha}$: 0.23 h, $t_{1/2\beta}$: 1,13 h, V_d: 2400 ml/kg	Kinetic characteristics of BBR are different in the plasma and hippocampus: eliminated rapidly in the plasma, increases rapidly in the hippocampus Direct action on neuron and accumulation in the hippocampus	[54]
Dog	100 mg/kg	i.v.	$t_{1/2\alpha}$: 0.15 h, $t_{1/2\beta}$: 12.59 h, CL: 60.70 l h, AUC: 1979.31 μg/h/l, V_d: 699.53 l	Two-compartment model, distributes extensively	[50]
Dog	280 mg/kg	oral	$t_{1/2\alpha}$: 0.63 h, t_{max}: 3. 71 h, V_d: 125.4 l C_{max}: 15.46 μg/l, AUC: 777.29 μg/h/l, CL: 2.64 l/h	Distributes extensively, eliminated slowly	[50]
Human	300 mg/kg	oral	$t_{1/2\alpha}$: 0.869 h, t_{max}: 2.37 h, C_{max}: 394.7 μg/l, AUC: 2799 μg/h/l	Single-compartment model; rapid absorption, distributes extensively	[58]
Human	300 mg/kg	oral	$t_{1/2\alpha}$: 0.87 h, t_{max}: 2.37 h, C_{max}: 394.8 μg/l, AUC: 3028.30 μg/h/l	Single-compartment model; oryzanol promotes the absorption of BBR and has no effect on absorption rate in humans	[51]

PK, pharmacokinetic; i.g., intragastric; i.v., intravenous; p.o., per os; $t_{1/2}$, half-life; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, elimination half-life; AUC, area under curve; C_{max}, maximum thalamus concentration; t_{max}, time to peak concentration; MTT, mean transit time; V_d, volume of distribution; CL, body clearance.

The pharmacokinetic characteristics of berberine in animals

BBR is difficult to absorb because of its poor absorption rate by the gut wall. After oral adminstration of 50 mg/kg BBR a maximum concentration of only 92.7 μg/l is attained.[51] The absorption ratio of BBR can reach about 33.6% within 1 h using an in-situ intestinal loop, but the concentration peak value of BBR was only 10 ng/ml at 2 h after oral administration.[52] Subsequently 40 mg/kg BBR was eliminated within 12 h, and then a very low plasma concentration was maintained for 48 h.

BBR distributes itself extensively in animal bodies. ^{3}H-berberine administered intravenously to rabbits distributed rapidly to the organs, with the highest radioactivity found in the lungs, followed by the liver, spleen and heart.[53] In addition, BBR can penetrate the blood–brain barrier, with a rapid increase in the hippocampus after intravenous administration, followed by slow elimination. This suggests that BBR can act directly on neurons and accumulate in the hippocampus.[54]

The limited number of metabolic studies of BBR in animal bodies suggests that BBR is metabolized rapidly in the body, and that the liver is the main metabolic site. After BBR is absorbed, clearance of BBR from the blood is very fast and, in rats at least, it is quickly transferred to the liver and bile through active transportation and than rapidly biotransformed.[50,55] Once absorbed by the body, BBR is frequently metabolized completely. It was shown that only 4.93% and 0.5% of an i.v. dose of 2 mg/kg BBR was eliminated from the urine and bile.[56] In a study on rats, after a single oral dose of 12 g/kg xie xin decoction (containing 32.7 mg/g BBR), the amount of BBR excreted from urine over the following 72 h was only 0.036% of the original dose.[57]

BBR is metabolized in the rat liver via phase I demethylation and phase II glucuronidation, and it is then apparently excreted through the duodenum in bile. Metabolites of BBR also circulate in the body, and in rats the liver and intestinal bacteria may participate in the metabolism and disposition, and may therefore affect the bioavailability.[52] The main metabolites are phase I demethylberberine (see Figure 2) and phase II conjugation products.

In animals, BBR is mainly excreted by the hepatobiliary system and kidney in the form of metabolites. After administration of BBR (10 mg/kg) through the femoral vein, BBR and its main metabolites were excreted by the hepatobiliary system and detected by high-performance liquid chromatography coupled to microdialysis.[55] After i.v. administration of ^{3}H-berberine in rats over 6 days, 73% of the BBR given was detected in the urine.[51] Similar results have been reported in rabbits.[56]

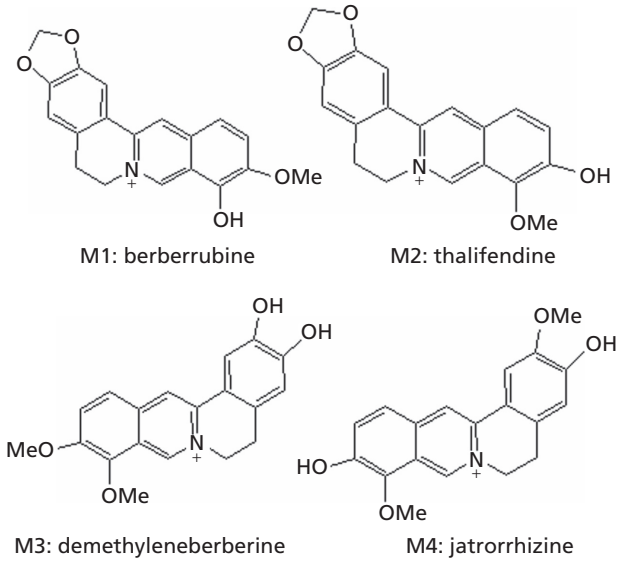

Figure 2 Structures of phase I metabolites of BBR in rat plasma after oral BBR[52]

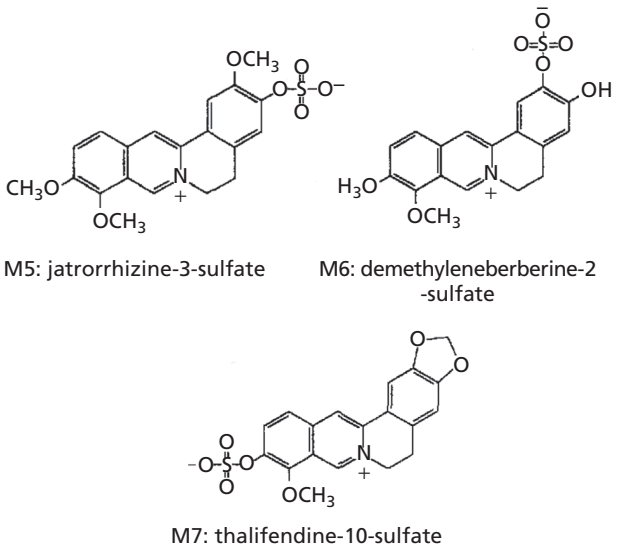

Figure 3 Structures of phase II metabolites (sulfaction) in human urine after oral administration[59]

The pharmacokinetic characteristics of berberine in humans

The available clinical data suggest that, with oral administration, BBR can be absorbed by the gut wall and reach an effective treatment concentration. Bao showed that after oral administration of BBR chloride (300 mg, single dose), a maximum concentration of 0.39 mg/l was reached, sufficient for healing cardiac arrhythmia.[58] This study demonstrates that BBR can be absorbed by humans.

The metabolism of BBR in the body is related to its chemical components. BBR is known to be a quaternary amine alkaloid, which binds easily to proteins, affecting disposition and action intensity.[59] Tan and Xie demonstrated that BBR hydrochlorate can bind strongly to human serum albumin.[60] In addition, human multidrug resistance protein 1 and multidrug resistance-associated protein 1 directly efflux BBR as their substrate and thus reduce accumulation of BBR in cells.[61]

The metabolism of BBR in humans may mainly be based on phase I demethylation and phase II glucuronidation and/or sulfation. Pan *et al.* identified three sulfate-conjugated metabolites of BBR chloride in human urine after oral BBR administration. These are the phase II metabolites jatrorrhizine-3-sulfate (M5), demethyleneBer-2-sulfate (M6) and thalifendine-10-sulfate (M7). M6 was the major metabolite (see Figure 3).[59] Recently, Qiu and colleagues have fully isolated and identified urinary metabolites of berberine in rats and human beings, including phase I demethylberberine and phase II conjugation products.[62]

Summary

BBR, an isoquinoline alkaloid, can protect neurons against damage induced by ischaemia and/or oxidative stress. It can be used coupled with other drugs for treatment of brain ischaemia. In addition to traditional functions such as lowering cholesterol and glucose, BBR has diverse functions in the nervous system, including improvement of memory and learning, an antidepressant-like effect and an anxiolytic effect. Pharmacokinetic studies of BBR in the body indicate that BBR is not easily absorbed and is not stable in the gastrointestinal tract. In order to cure diseases of the central nervous system, adequate bioavailability of the drug in the brain is very important. However, current findings indicate that the toxicity of BBR is mainly associated with intravenous administration. Thus, one crucial step towards more widespread use of BBR is to increase its bioavailability. A promising recent study by Lu achieved bioavailability levels 6.47 times greater than that of berberine tablet suspensions.[63] In addition, structural modification of BBR could be another way to develop drugs for diseases of the nervous system.[8] In summary, BBR is a promising candidate for the treatment of neurological disorders, although further research is still required.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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