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Review

Recent pharmacological studies on natural products in China

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Abstract

Natural products have been used as medicinal agents for many years. In addition, these compounds have also served as the starting points for semisynthetic analogs with improved properties. This review focuses on recent advances in the pharmacological studies on natural products mainly performed and published in China. Emphasis will be placed on those compounds that show the greatest promise clinically such as huperzine A (9-amino-13-ethylidene-11-methyl-4-azatricyclo[7.3.1.0(3.8)]trideca-3(8),6,11-trien-5-one), *s*-(−)-3-*n*-butylphthalide (*s*-(−)-3-butyl-1(3H)-isobenzofuranone), (−)-clausenamide (3-hydroxy-4-phenyl-5*a*-hydroxybenzyl-*N*-methyl-γ-lactam) and *Ginkgo biloba* extract and its active components.

Keywords: Natural product; Huperzine A; *s*-(−)-3-*n*-butylphthalide; (−)-Clausenamide; *Ginkgo biloba* extract

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1. Introduction

Natural products have been used as medicinal agents for many years in China. Through a large number of chemical and pharmacological research works, numerous bioactive compounds have been found from Chinese medicinal plants and some of them have been used clinically. This review is not intended to be comprehensive, but rather focuses on recent advances in the pharmacological studies on natural products mainly performed and published in China. Emphasis will be placed on those compounds that show the greatest promise clinically.

2. Huperzine A

Huperzine A (9-amino-13-ethylidene-11-methyl-4-azatricyclo [7.3.1.0(3.8)] trideca-3(8),6,11-trien-5-one) (Fig. 1) is a lycopodium alkaloid isolated from the moss *Huperzia*

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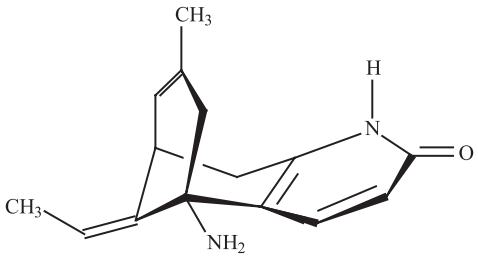

Fig. 1. Structure of huperzine A.

serrate. Its chemistry and pharmacology were first studied by Chinese scientists in 1980s and several review articles have been published (Bai et al., 2000; Jiang et al., 2003; Tang, 1996; Zangara, 2003). In the last several years, huperzine A has been further studied in various aspects including its chemical synthesis, structural modification, structure–activity relationship as well as its pharmacological effects.

Huperzine A acts as a potent, highly specific and reversible inhibitor of acetylcholinesterase. It crosses the blood–brain barrier easily. Its potency of acetylcholinesterase inhibition has been compared in vitro with other three acethylcholinesterase inhibitors, galanthamine, donepezil and tacrine, which have been approved for Alzheimer's disease in the United States and some European countries (Wang and Tang, 1998). The inhibition of acetylcholinesterase activity of huperzine A was more effective than that of tacrine and galanthamine, but less than that of donepezil. Evidence has demonstrated that acetylcholinesterase inhibitors may vary in their selectivity for acetylcholinesterase versus butyrylcholinesterase. The IC_{50} ratios of huperzine A, donepezil and tacrine for butyrylcholinesterase/acetylcholinesterase inhibition were 884.57, 489.05, and 0.80, respectively, suggesting that huperzine A was the most selective acetylcholinesterase inhibitor among these compounds (Cheng et al., 1996). The high selectivity of huperzine A for acetylcholinesterase may contribute to the clinically favourable tolerability profile of huperzine A in Alzheimer's disease patients (Scott and Gao, 2000). However, several studies have suggest that a stronger inhibition of butyrylcholinesterase may also be important in the later stage of Alzheimer's disease (Ballard, 2002), and offer more protection over Aβ amyloid plaque deposition (Guillozet et al., 1997). Effects of huperzine A on different molecular forms of acetylcholinesterase in rat cerebral cortex, hippocampus and striatum have been studied recently. The results revealed that huperzine A preferentially inhibited tetrameric acetylcholinesterase (G4 form), while tacrine and rivastigmine preferentially inhibited monomeric acetylcholinesterase (G1 form) (Zhao and Tang, 2002). Although the potency of huperzine A on acetylcholinesterase was less than that of donepezil in vitro, huperzine A produced a greater acetylcholinesterase inhibition in the rat brain than donepezil when these two drugs were administrated orally, indicating that huperzine A has greater bioavailability, and penetrates the blood–brain barrier more easily than donepezil (Cheng and Tang, 1998).

In addition to its acetylcholinesterase inhibitory effect, huperzine A may have neuroprotective effects. Pretreatment of primary neuronal cells with huperzine A reduced glutamate-induced toxicity and decreased neuronal death (Ved et al., 1997). Huperzine A protected PC12 cells against β-amyloid- and H_2O_2-induced cell death (Xiao et al., 1999, 2000a,b, 2002). Huperzine A may protect neurons against β-amyloid-induced apoptosis by the inhibition of reactive oxygen species formation and caspase-3 activity (Xiao et al., 2002). Huperzine A also attenuated serum deprivation- and staurosporine-induced apoptosis in primary cultures of rat cortical neurons (Zhou and Tang, 2002; Zhang and Tang, 2003) and nitric oxide (NO)-induced neurotoxicity in human neuroblastoma cell line SK-N-SH (Zhao and Li, 2002). In vivo, huperzine A alleviated the cognitive dysfunction induced by intracerebroventricular infusion of β-amyloid protein-(1–40) in rats (Wang et al., 2001). While (−)-huperzine A is more potent than (+)-huperzine A on acetylcholinesterase, these two isomers protected cells with similar potency, suggesting that the neuroprotective effects of huperzine A is not related to its acetylcholinesterase inhibitory property (Zhang et al., 2002a,b).

Huperzine A has been found to be an effective cognition enhancer in several animal models such as the escape task of water maze, passive footshock avoidance and spatial discrimination of radial arm maze in rodents (see review by Tang, 1996) and delayed response performance in monkeys (Ye et al., 1999). The performance improvement of scopolamine-treated rats and monkeys by huperzine A suggest that a cholinergic mechanism is involved. Huperzine A may also improve working memory by an adrenergic mechanism since huperzine A improved the reserpine- or yohimbine-induced memory impairments (Ou et al., 2001). Daily intraperitoneal administration of huperzine A for 12 consecutive days also produced significant reversal of the β amyloid-induced deficit in a water maze task test. Huperzine A treatment attenuated β-amyloid protein-(1–40)-induced loss of choline acetyltransferase activity and neuronal cells in cerebral cortex. β-amyloid protein-(1–40)-induced down-regulation of Bcl-2 and up-regulation of Bax and P53 were reversed when animals were treated with huperzine A (Wang et al., 2001). In brain ischemia model, the memory impairment and neuronal degeneration in the CA1 region were attenuated and the choline acetyltransferase activity was restored in the hippocampus by subchronic oral administration of huperzine A, suggesting that huperzine A may have beneficial effect in cerebrovascular type dementia (Zhou et al., 2001). Similar results have been obtained in neonatal rats (Wang et al., 2002).

Attempts have been made recently to observe acute effects of huperzine A on rat liver (Ma et al., 2003a,b). Although both huperzine A and tacrine induced increases in

the activities of serum aspartate aminotransferase and alanine aminotransferase, only tacrine produced histopathologic changes. The liver effects of huperzine A, but not tacrine, could be redressed by atropine. The metabolism of huperzine A in rat liver microsomes is primarily mediated by cytochrome P450 (CYP) 1A2, with a probable secondary contribution of CYP3A1/2, since 76.2% of huperzine A metabolism was inhibited by CYP1A2 antibody and 17.8% by CYP3A1/2 antibody, respectively. CYP2C11 and 2E1 antibodies had only minor effect on the metabolism of huperzine A. Phenacetin, a CYP1A2 substrate showed an inhibitory effect on huperzine A metabolism, further supporting the notion that the metabolism of huperzine A is mainly mediated by CYP1A2 (Ma et al., 2003a). To predict possible drug interaction, the effects of huperzine A on expressions and activities of CYP were also examined in rats. The activity and expression of liver CYP isoenzymes were not affected by pharmacological dose of huperzine A (0.1 mg/kg). However, huperzine A at toxicological dose (1 or 2 mg/kg) elicited an inductive response of CYP1A2 (Ma et al., 2003a,b). Since CYP1A2 is involved in the metabolism of many commonly used drugs, further studies are required to assess whether huperzine A causes a clinically relevant interaction with other CYP1A2 substrates and/or inhibitors.

Several clinical trials have been conducted in China. Huperzine A enhanced the memory and learning performance of adolescent students (Sun et al., 1999). A double blind clinical trial on 120 patients of age-associated memory impairment with memory quotient <100 has shown an effective rate of 68.3% in huperzine A group and 26.4% in control group, respectively. Recently, a multicenter, double blind, randomised and placebo-controlled clinical trial on huperzine A was conducted in patients meeting the DSM IV criteria for possible or probable Alzheimer's disease (Zhang et al., 2002a,b). In this trail, 202 patients aged between 50 and 80 years were enrolled from 15 centers in five cities in China. The ability of daily life, Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), ADAS-non-Cog, the Mini-Mental State Examination (MMSE), and Clinicians' Interview-Based Impression of Change-Plus (CIBIC-plus) scales were measured in the trail. Safety checks including vital signs, physical, neurology and laboratory tests were carried out every 6 weeks. In comparison with the baseline data, the cognitive functions (MMSE, ADAS-Cog), noncognitive function (mood and behavior-ADAS-non-Cog) and ability of daily life were all improved significantly at Week 6, and particularly at Week 12 in the huperzine A-treated patients. The results suggest that huperzine A is a safe and effective treatment for Alzheimer's disease. However, larger and longer clinical trials might be needed (Zhang et al., 2002a,b). Currently huperzine A is in phase III trials in China for the treatment of patients with Alzheimer's disease. Clinical trials of huperzine A in elderly patients with age-associated memory loss are also underway in the US.

3. *s*-(−)-3-*n*-butylphthalide

s-(−)-3-*n*-butylphthalide (*s*-(−)-3-butyl-1(3H)-isobenzofuranone, *s*-(−)-NBP) (Fig. 2) is a compound isolated from seeds of *Apium graveolens* Linn. Pharmacological studies have shown that *s*-(−)-NBP as well as synthesized (±)-NBP has anti-ischemic effects. In rats subjected to focal ischemia, the infarct area and the score of neurological deficits were significantly reduced when rats were pre- or post-treated with (±)-NBP. Treatment with (±)-NBP 15 min and even 2 h after middle cerebral artery occlusion markedly reduced the infarct area and the neurological deficits score. No effect was found when (±)-NBP was injected intraperitoneally 4 h after middle cerebral artery occlusion (Liu and Feng, 1995). Similar results were obtained in the stroke prone spontaneously hypertensive rats (Zhang and Feng, 1996). When these rats were pre-treated with (±)-NBP, the onset of stroke was delayed, life span prolonged and the score of neurological deficit decreased. Since no change was found in blood pressure or heart rate when (±)-NBP was given, the protective effect of (±)-NBP could not be explained by its possible effect on blood pressure. However, (±)-NBP enhanced regional blood flow in middle cerebral artery occlusion and subarachnoid hemorrhage model (Yan et al., 1998; Chong and Feng, 1998) and improved microcirculation in pial arterioles in middle cerebral artery occlusion rats (Xu and Feng, 1999). (±)-NBP also ameliorated brain edema and blood–brain barrier damage in middle cerebral artery occlusion rats (Deng and Feng, 1997; Chong and Feng, 1999). Mitochondria have been assumed to be involved in apoptosis. (±)-NBP may have neuroprotective effects since it improved mitochondria dysfunction (Xiong and Feng, 1999, 2000). In a transient focal cerebral ischemia model, a decrease in the DNA fragmentation, cytochrome *c* release and caspase-3 activity were found when rats were treated with *s*-(−)-NBP or (±)-NBP. These attenuating effects were mainly associated with *s*-(−)-NBP since the same dose of *r*-(+)-NBP showed much less effects (Chang and Wang, 2003). Recently, the effects of *s*-(−)-NBP and *r*-(+)-NBP on extracellular NO levels and intracellular cyclic GMP (cGMP) levels were studied in primary cultured rat cortical neuronal cells. *r*-(+)-NBP induced a significant increase in the extracellular NO levels and intracellular cGMP levels in cultured neurons when exposed to hypoxic/hypoglycemic, *N*-methyl-D-aspartate (NMDA), or KCl media for 10 h. However, *s*-(−)-NBP significantly decreased

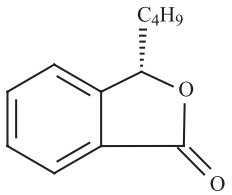

Fig. 2. Structure of *s*-(−)-3-*n*-butylphthalide.

extracellular NO levels and intracellular cGMP levels (Yan and Feng, 1998).

s-(−)-NBP and (±)-NBP, but not *r*-(+)-NBP, also exhibited an inhibitory effect on thrombus formation in rats. *s*-(−)-NBP, *r*-(+)-NBP and (±)-NBP inhibited ADP-, collagen- and arachidonic acid-induced platelet-rich plasma aggregation in vitro. However, *s*-(−)-NBP, *r*-(+)-NBP and (±)-NBP showed no effect on thrombin-induced platelet aggregation. *s*-(−)-NBP in high concentration also decreased platelet thromboxin A_2 level. In addition, *s*-(−)-NBP, but not (±)- and *r*-(+)-NBP, inhibited 5-HT release from platelets. The results suggest that the antithrombotic and antiplatelet activities of NBP might be related to its regulatory effects on 5-HT release (Xu and Feng, 2001).

At present, (±)-NBP is developed as an anti-cerebral ischemic drug and phase III clinical trial has recently been completed in China.

4. (−)-Clausenamide

(−)-Clausenamide (3-hydroxy-4-phenyl-5*a*-hydroxybenzyl-*N*-methyl-g-lactam, Fig. 3) is a compound isolated from *Clausena lansium (lour)* Skeel, with a chemical structure similar to piracetam. Early studies found that this compound prolonged the survival time after $NaNO_2$ administration (Liu et al., 1991). This compound also inhibited the contraction of basilar artery caused by 5-HT, prostaglandin $F_2\alpha$ ($PGF_2\alpha$)? and arachidonic acid, suggesting that this compound may have cerebral protective effects (Liu et al., 1991). The neuroprotective effects of (−)-clausenamide has been studied in anisodine-induced amnesia model (Duan and Zhang, 1998). It was found that (−)-clausenamide ameliorated anisodine-induced memory deficits in step-through test in mice. Pretreatment with (−)-clausenamide significantly ameliorated anisodine-induced reduction of acetylcholine in a dose-dependent manner in the frontal cortex, hippocampus and striatum. However, (+)-clausenamide had no effect on anisodine-induced acetylcholine reduction in all brain regions examined. The effects of (−)-clausenamide on spatial cognitive functions and hippocampal long-term potentiation (LTP) after transient focal cerebral ischemia in rats have been studied (Tang and Zhang, 2002). Four weeks after middle cerebral artery occlusion, Morris water maze tasks demonstrated that 2 h of transient forebrain ischemia resulted in a significant decrease in spatial discrimination performance. The escape latency at 4 and 5 days of acquisition trial was lower in the ischemic rats than in sham-operated rats. Chronic treatment with (−)-clausenamide significantly improved the impairment. When changes in population spike amplitude were recorded as an index of LTP in the perforant path-dentate gyrus synapses, no difference in population spike amplitude was found between the sham-operated and vehicle-treated animals, whereas the fractional increase of population spike 20–50 min after tetanus was significantly larger in (−)-clausenamide-treated group. Histopathological analysis also revealed that this compound had neuroprotective effect in cortex and striatum. In cultured cortex neurons, (−)-clausenamide increased the activity of choline acetyltransferase and stimulated proliferation of neuronal cells (Duan et al., 1998). The effects of (−)-clausenamide on Ca^{2+} signaling in primary cultures of rat cortical neurons has recently been studied by using laser confocal microscopy (Tang and Zhang, 2004). The mean amplitude of (−)-clausenamide (1 μM)-induced Ca^{2+}transient was similar in extracellular solution with or without Ca^{2+}. (−)-Clausenamide failed to trigger Ca^{2+}transient after treatment with endoplasmic reticulum Ca^{2+}pumps inhibitor 2,5-di(*tert*-butyl)-1,4-benzohydroquinone (BHQ) to exhaust intracellular Ca^{2+}stores. This result suggested that the primary source of (−)-clausenamide-induced Ca^{2+}transient was from internal stores. Application of inositol-1,3,4-triphosphate (IP_3) receptor inhibitor $MgCl_2$ and phospholipase C-γ (PLC-γ) inhibitor U73122 ((1-[6-{[17-3-methoxyestra-1,2,3 (10)-trien-17-yl] amino} hexyl]-1H-pyrrole-2,5-dione) suppressed (−)-clausenamide-induced Ca^{2+} transient, suggesting that the major source of (−)-clausenamide-induced Ca^{2+} transient was from IP_3 receptor pathway. The distinctive spatial and temporal characteristic of (−)-clausenamide-induced Ca^{2+} transient may play an important role in its action.

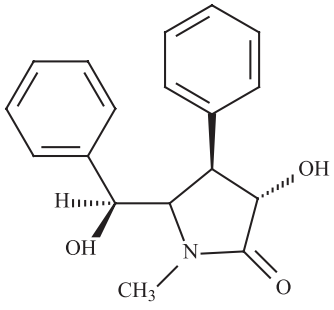

Fig. 3. Structure of (−)-clausenamide.

Recently, the pharmacokinetics of clausenamide enantiomers and their metabolites were investigated in Wistar rat (Zhu and Zhang, 2003a,b). Stereoselective differences in pharmacokinetics were found. The mean plasma levels of (+)-clausenamide were higher than those of (−)-clausenamide. The absorption, distribution, and elimination of (−)-clausenamide were more rapid than those of (+)-clausenamide. Similar findings for (−)-7-OH-clausenamide, the major metabolite of (−)-clausenamide, and (+)-4-OH-clausenamide, the major metabolite of (+)-clausenamide, were also found in rat plasma. The stereoselective pharmacokinetics of clausenamide enantiomers might be related to their differences in plasma protein binding, first-pass metabolism and interaction with CYP enzymes.

To identify which CYP isoform(s) are responsible for the metabolism of clausenamide enantiomers in rats, effects of CYP inducers and inhibitors on the formation of clausena-

mide metabolites were investigated in liver microsomes (Zhu and Zhang, 2003a). In rat liver microsomes incubation, clausenamide enantiomers were mainly converted to 4-hydroxy, 5-hydroxy, and 7-hydroxy-metabolites. 4-OH-clausenamide was the major metabolite of (+)-3*R*, 4*S*, 5*S*, 6*R*-clausenamide, while 7-OH-clausenamide was the major one of (−)-3*S*, 4*R*, 5*R*, 6*S*-clausenamide. A marked increase in the rate of metabolism of clausenamide enantiomers was observed in microsomes of dexamethasone-treated rats. Dexamethasone and rifampicin also increased the amount of (+)-5- and (+)-7-OH-clausenamide. These results suggested that inducible CYP3A1 was involved in the hydroxylation of clausenamide enantiomers. In inhibition studies, ketoconazone completely inhibited the production of main metabolites of (−)-clausenamide (100%) and (+)-clausenamide (97%). Triacetyloleandomycin strongly inhibited the corresponding metabolites by 34–85%. These findings indicated that CYP3A2 played an important role in the hydroxylation of clausenamide enantiomers. The results suggest that CYP3A is the predominant isoform responsible for the metabolism of clausenamide enantiomers.

5. *Ginkgo biloba* extracts and its active components

Ginkgo biloba extracts has been therapeutically used for several decades to increase peripheral and cerebral blood flow as well as for the treatment of dementia (see review by Ahlemeyer and Krieglstein, 2003). Placebo-controlled, double-blind, and randomized trials have demonstrated that EGb 761, a standard extract from the leaves of *Ginkgo biloba* (EGb) was effective in mild-to-moderate dementia of the Alzheimer's disease patients (Le bars et al., 1997; Maurer et al., 1997). Although pharmacological studies have shown that EGb improves cerebral blood flow in models of focal ischemia (Oberpichler et al., 1988; Krieglstein et al., 1995); exerts neuroprotective effect during ischemia (Smith et al., 1996); and attenuates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced nigrostriatal dopaminergic neurotoxicity in C57 mice (Wu and Zhu, 1999), the mechanism underlying its neuroprotective effect remains unclear. EGb is a complex mixture containing a number of flavonoids (e.g., kaempferol, quercetin, and isorhamnetin derivatives), terpenes (e.g., ginkgolides A, B, C, and J, and bilobalide), and other various constituents (DeFeudis, 1991). The early experiments of Oberpichler et al. (1988) showed that the nonflavone fraction, and not the flavonoid glycosides, protected brain tissue against hypoxic damage. Krieglstein et al. (1995) demonstrated that ginkgolides A and B and bilobalide reduced the infarct volume after focal ischemia in rodents and that ginkgolide B and bilobalide reduced the number of damaged neurons in culture after glutamate treatment or hypoxia. Recently, we demonstrated that bilobalide, the major constituent of EGb, could protect against β-amyloid toxicity (Zhou et al., 2000). Evidence also has shown that EGb and some of its constituents could inhibit serum deprivation- or staurosporine-induced apoptosis and that bilobalide was the most potent constituent (Ahlemeyer et al., 1999). Apoptosis is an active process of cell destruction. A number of genes and their proteins such as *bcl-2* family, *bax*, *bcl-xS*, *bad*, *bak*, *bik*, p53, c-Myc and caspases. Clarification of the effect of EGb and its components on the expression of these molecules involved in apoptosis may give us new insight into the mechanism of neuroprotection by bilobalide and other components of EGb. Reactive oxygen species plays a critical role in glutamate-, β-amyloid, and nerve growth factor- or serum-deprivation-induced apoptosis (Greenlund et al., 1995; Reynolds and Hastings, 1995; Butterfield, 1997). Apoptosis associated with increased generation of reactive oxygen species has been demonstrated by direct exposure of neuronal cells to oxidative stress, such as hydrogen peroxide, lipid hydroperoxide, and superoxide anion (Aoshima et al., 1997; Satoh et al., 1997). Recently, the reactive oxygen species-induced changes in c-Myc, p53, and *Bcl-2* family expression and caspase-1 and -3 activities were measured in PC12 cells. At the same time, the effects of bilobalide, the main constituent of the nonflavone fraction of EGb; on the reactive oxygen species-induced apoptosis; the expression of c-Myc, p53, and *Bcl-2* family; and the caspase-1 and -3 activities were studied. It was found that exposure of cells to xanthine (100 mM)/xanthine oxidase (150 mU/ml) (reactive oxygen species producer) resulted in a characteristic DNA fragmentation and an increase in the apoptosis rate. When p53, c-Myc, *Bcl-2*, *Bcl-xL*, and *Bax* were measured by flow cytometry and the activities of caspase-1- and caspase-3-like protease determined with Ac-YVAD-AMC or Ac-DEVD-AMC as substrates, the profile of reactive oxygen species-induced changes in these apoptosis regulatory and effector proteins suggests that elevation of c-Myc, p53, and *Bax* and activation of caspase-3 play an important role in the apoptosis. When cells were treated with reactive oxygen species and bilobalide simultaneously, a dose-dependent reduction in the apoptotic rate was found. The percentage of cells with positive staining for c-Myc and p53 decreased when bilobalide was present. Bilobalide also reduced reactive oxygen species-induced elevation of *Bax* and activation of caspase-3 effectively. The results provide the direct evidence that bilobalide can protect neurons against oxidative stress. Bilobalide may block the apoptosis in the early stage and then attenuate the elevation of c-Myc, p53, and *Bax* and activation of caspase-3 in cells (Zhou and Zhu, 2000). In addition, bilobalide as well as Ginkgolide A and B were neuroprotective against NO- and β-amyloid-induced toxicity in vitro (Song et al., 2000; Zhao and Li, 2002; Zhou et al., 2000).

6. (−)-Stepholidine

(−)-Stepholidine (3,9-dimethoxy-5,8,13,13*a*-tetrahydro-6H-isoquino[3,2-*a*] isoquinoline-2,10-idol), a compound isolated from *Stephania*, was found to be a dopamine D1 receptor agonist and a dopamine D2 receptor antagonist.

This dual action was demonstrated in behavioral, electrophysiological, biochemical and immunohistochemical studies (Jin et al., 2002). Evidence has shown that (−)-stepholidine had a direct D1 receptor agonist effect on neurons in neostriatum and substantia nigra pars reticulata (Zhang et al., 1999). However, (−)-stepholidine blocked dopamine D2 receptors in substantia nigra pars compacta (SNc) and neostriatum. In 6-hydroxydopamine (6-OHDA)-lesioned rats, the D1 receptor agonist effect of (−)-stepholidine counteracted its dopamine D2 receptor blocking effect, resulting in rotation towards the intact side (Jin et al., 1992; Ding et al., 2000). (−)-Stepholidine had different effects on ventral tegmental area. At low doses, (−)-stepholidine increased the firing activity of dopamine-containing neurons in ventral tegmental area and SNc of rats and reversed the inhibition of firing activity of dopamine-containing neurons induced by the dopamine receptor agonist apomorphine (Huang and Jin, 1992; Sun and Jin, 1992). However, at high doses, (−)-stepholidine selectively inhibited the firing activity of dopamine-containing neurons in the ventral tegmental area with no effect on SNc (Zhang et al., 1997). Chronic treatment with (−)-stepholidine decreased the number of active dopamine-containing neurons in the ventral tegmental area but not in the SNc (Zhang et al., 1997). (−)-Stepholidine has dual effects on nucleus accumbens neuronal firing. When rat was given (−)-stepholidine systematically, opposite effects on the firing activity of nucleus accumbens neurons were observed (Zhu et al., 2000a). While low doses of (−)-stepholidine inhibited the activity of nucleus accumbens neurons, high doses of (−)-stepholidine produced an initial inhibition of firing followed by a marked excitation (Zhu et al., 2000a,b). The latter effect is reversed by the selective D1 receptor antagonist SCH23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine), suggesting that (−)-stepholidine act as an agonist at D1 receptors. However, when applied directly to nucleus accumbens neurons using microiontophoresis, (−)-stepholidine produces no effect on nucleus accumbens cells. In addition, (−)-stepholidine has direct effect on D1 receptors in medial prefrontal cortex. Microiontophoretic application of (−)-stepholidine inhibited the activity of medial prefrontal cortex cells (Zhu et al., 2000c). This inhibition was blocked by SCH23390, indicating that the effect is mediated by D1 receptors. Evidence also suggests that D1 receptor-mediated effect of (−)-stepholidine in the medial prefrontal cortex is responsible for systemic (−)-stepholidine-induced excitation of nucleus accumbens neurons. Dysfunction of dopamine D1 receptors in medial prefrontal cortex accompanied by secondary dopamine D2 receptor hyperactivity in subcortical regions such as ventral tegmental area and nucleus accumbens has been thought to occur in the sufferers of schizophrenia. D1 receptor dysfunction is suggested to be responsible for the negative symptoms of schizophrenia, whereas the dopamine D2 receptor hyperactivity might result in the positive symptoms of this disorder. On the basis of this hypothesis, an effective antipsychotic drug should have both D1 receptor agonist and dopamine D2 receptor antagonist effects. (−)-stepholidine might represent a compound that matches with this hypothesis (Jin et al., 2002).

7. Triptolide

Extracts of the herb *Tripterygium wilfordii* Hook F have been used for more than two centuries in traditional Chinese medicine to treat a variety of autoimmune and inflammatory diseases including rheumatoid arthritis. Triptolide (Fig. 4) was identified as one of the major components responsible for the immunosuppressive and anti-inflammatory effects of this herb (Zheng, 1991). Its immunosuppressive activities have been demonstrated in animal models of autoimmune disease, organ transplantation and chronic inflammatory disease.

In animal model of autoimmune disease, such as rat adjuvant-induced arthritis model, triptolide significantly reduced joint acute edema and secondary inflammatory swelling (Lin et al., 1998b). Oral administration of triptolide also ameliorated renal dysfunction and histological progression in rat anti-glomerular basement membrane nephritis model (Dai et al., 2000). In this model, urinary protein excretion, glomerular hypercellularity and crescents decreased significantly at the end of the experiments. In experimental autoimmune uveoretinitis model, triptolide treatment suppressed experimental autoimmune uveoretinitis induction and Th1-cytokines production during the whole period (Wu et al., 2003). In multiple low-dose streptozotocin-induced diabetes mice, the scores of insulitis were significantly reduced when triptolide was used (Li et al., 1994).

In animal models of organ transplantation, such as heterotopic renal allograft experiment, triptolide produced significant increase in the survival time of the allograft (Yang et al., 1997a). The allograft average survival times were significantly prolonged when cyclosporine was used together with triptolide (Yang et al., 1997a). Similar results were also obtained in the heterotopic cardiac transplantation experiment (Lin et al., 1998a) and the skin allograft experiments (Yang et al., 1997b). The survival time of

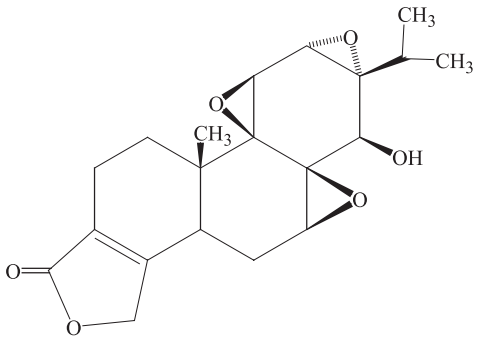

Fig. 4. Structure of triptolide.

allograft was significantly prolonged in triptolide-treated animals compared with control animals. The combination of cyclosporine and triptolide was more effective than cyclosporine or triptolide given alone (Yang et al., 1997a,b). In the nerve grafting experiment, the graft nerve contained few axons with many T cells and macrophages. Oral application of triptolide resulted in an extensive axonal regrowth and a reduced cellular infiltration into the allografts (Ding et al., 1999).

In animal models of chronic inflammatory disease such as guinea pig model of asthma, the effects of triptolide on airway inflammation was tested. When Guinea pig was actively sensitized by aerosolized ovalbumin, acute bronchoconstriction was induced. The treatment with triptolide significantly reduced ovalbumin-induced increases in hyperdense eosinophils in bronchoalveolar lavage fluid, the level of viability enhancing activity and airway hyperresponsiveness to inhaled histamine (Wang and Guo, 1996). Triptolide also effectively prevented the development and progression of experimental pulmonary hypertension in rats (Faul et al., 2000).

Rapid progress has also been made in understanding the mechanism of the immunosuppressive and anti-inflammatory actions of triptolide. Evidence has demonstrated that triptolide potently inhibited lymphocyte activation in vivo and in vitro (Pu and Zhang, 1990; Yang et al., 1998; Chan et al., 1999). The inhibitory effect of triptolide on T-cell activation was stronger than cyclosporine A (Lu et al., 1999) and tacrolimus (FK506) (Chan et al., 1999). Triptolide inhibited interleukin(IL)-2 expression by normal human peripheral blood lymphocytes stimulated with phorbol 12-myristate 13-acetate (PMA) and antibody to CD3, and with PMA and ionomycin(Iono) (Qiu et al., 1999). In Jurkat T-cells, triptolide completely inhibited interleukin-2 transcriptional activation at the purine-box/ARRE/nuclear factor (NF)-AT and NF-kappaB target DNA sequences triggered by PMA, PMA/Iono and tumor necrosis factor-alpha. Triptolide also inhibited PMA-stimulated activation of a chimeric transcription factor in which the C-terminal TA1 transactivation domain of NF-kappaB p65 is fused to the DNA binding domain of GAL4 (Qiu et al., 1999). In 16HBE human bronchial epithelial cells, interleukin-8 expression is regulated predominantly by NF-kappaB, and triptolide but not cyclosporin A can completely inhibit expression of interleukin-8. Thus, the mechanism of triptolide inhibition of cytokine gene expression differs from cyclosporin A (Qiu et al., 1999; Zhao et al., 2000). Triptolide inhibited vascular endothelial growth factor expression and secretion in endothelial cells treated by 12-*O*-tetradecanoylphorbol 13-acetate dose-dependently. This effect may be one of the mechanisms underlying the therapeutic effects of triptolide on rheumatoid arthritis (Hu et al., 2001, 2002). Triptolide induced reduction of prostaglandin E_2 production in human monocytes and rheumatoid arthritis synovial fibroblasts (RSF) (Tao et al., 1998) and inhibition of promatrix metalloproteinase-1 and -3 mRNA expressions (Lin et al., 2001). Triptolide decreased viability, inhibited proliferation, and induced apoptosis of rheumatoid synovial fibroblasts (RSF) in a concentration-dependent manner at very low (nM) concentrations. Caspase-3 activity was increased by treatment with triptolide. Although peroxisome proliferator-activated receptors-gamma (PPARγ) activation was induced by 15-deoxy-.12,14-prostaglandin J_2, but not by triptolide under the same experimental conditions. The results suggest that triptolide may have disease-modifying effect in patients with rheumatoid arthritis (Kusunoki et al., 2004).

Recent studies on the action of triptolide revealed many properties relevant not only to anti-inflammatory activity but also to anticancer activity. Antiproliferative and proapoptotic activity of triptolide has been shown with many different types of cancer cells in vitro and in vivo (Shamon et al., 1997; Wei and Adachi, 1991). Triptolide also potentiates the activities of other agents and therefore may be useful not only as a single compound but also in combination with other cytotoxic drugs for cancer treatment. For example, triptolide sensitized tumor necrosis factor α-resistant tumor cells to tumor necrosis factor α-induced apoptosis (Lee et al., 1999) and showed cooperative proapoptotic effects with doxorubicin. In the latter studies, triptolide was found to block doxorubicin-mediated induction of p21WAF1/CIP1 and accumulation of cells in G2-M (Chang et al., 2001). The investigators proposed that triptolide, by blocking p21WAF1/CIP1-mediated growth arrest, created conflicting cell cycle checkpoints that enhanced apoptosis in tumor cells.

The main disadvantages of triptolide are its poor water solubility and toxic effects. The organic systems affected by triptolide include gastrointestinal, urogenital, cardiovascular, blood circulatory system, bone marrow as well as skin. Adverse events have been reported on the extracts of the herb *T. wilfordii* Hook F or triptolide, such as nausea, vomiting, bellyache, diarrhea, duodenal ulcer, and gastrointestinal bleeding. The development of new derivatives of triptolide with less adverse effects and novel types of delivery systems are undergoing in several research institutes in China.

PG490-88, a succinate salt water-soluble derivative of PG490 that is converted to triptolide in the serum (Krishna et al., 2001), has recently been approved for Phase I clinical trials (Fidler et al., 2003).

8. Conclusion

In summary, natural products have been used as medicinal agents for many years. At the same time, these compounds have also served as the starting points for semisynthetic analogs with improved properties. In the present review, we give some examples with scientific and experimental evidence about their effective constituents, pharmacological actions, pharmacokinetics and toxicities as well as clinical trials. We think that natural products have

been and will continue providing a unique element of molecular diversity and biological functionality in drug research and development.

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