Ascomycin and FK506: Pharmacology and Therapeutic Potential as Anticonvulsants and Neuroprotectants

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Keywords

Anticonvulsants; Ascomycin; Calcineurin; Epilepsy; FK506; Neuroprotection.

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doi: 10.1111/j.1527-3458.2008.00036.x

Ascomycin and FK506 are powerful calcium-dependent serine/threonine protein phosphatase (calcineurin [CaN], protein phosphatase 2B) inhibitors. Their mechanism of action involves the formation of a molecular complex with the intracellular FK506-binding protein-12 (FKBP12), thereby acquiring the ability to interact with CaN and to interfere with the dephosphorylation of various substrates. Pharmacological studies of ascomycin, FK506, and derivatives have mainly been focused on their action as immunosuppressants and therapeutic use in inflammatory skin diseases, both in animal studies and in humans. CaN inhibitors have been also proposed for the treatment of inflammatory and degenerative brain diseases. Preclinical studies suggest, however, that ascomycin and its derivatives exhibit additional pharmacological activities. Ascomycin has been shown to have anticonvulsant activity when perfused into the rat hippocampus via microdialysis probes, and ascomycin derivatives may be useful in preventing ischemic brain damage and neuronal death. Their pharmacological action in the brain may involve CaN-mediated regulation of gamma aminobutyric acid (GABA) and glutamate receptor channels, neuronal cytoskeleton and dendritic spine morphology, as well as of the inflammatory responses in glial cells. FK506 and ascomycin inhibit signaling pathways in astrocytes and change the pattern of cytokine and neurotrophin gene expression. However, brain-specific mechanisms of action other than CaN inhibition cannot be excluded. CaN is a likely potential target molecule in the treatment of central nervous system (CNS) diseases, so that the therapeutic potential of ascomycin, FK506, and nonimmunosuppressant ascomycin derivatives as CNS drugs should be further explored.

Introduction

Ascomycin and FK506 are 23-member ring macrolide lactones (Fig. 1). Ascomycin was originally isolated in the early 1960s from the fermentation product of *Streptomyces hygroscopicus* var *ascomyceticus* (Arai et al. 1962), and initially described as an antifungal antibiotic (Arai et al. 1962). The immunosuppressant properties of ascomycin were recognized by Dumont et al. (1992) when searching for less toxic FK506 analogs (Dumont 2000). FK506 was discovered in 1984 in the fermentation broth of the filamentous bacterium, *Streptomyces tsukubaensis* (Kino et al. 1987).

Several ascomycin and FK506 derivatives were shown to be systemically effective as powerful and clinically useful immunosuppressants in organ transplantation (Dumont 2000; Scott et al. 2003; Spencer et al. 1997). Topical administration of these compounds was shown to be effective both in animal models of cutaneous inflammation and in inflammatory skin diseases in humans (Bornhove et al. 2001; Griffiths 2001).

Ascomycin acts through disruption of signaling events mediated by the calcium-dependent serine/threonine protein phosphatase, calcineurin (CaN, protein phosphatase 2B). Its mechanism of action involves the formation of a molecular complex with

Figure 1 Chemical structures of ascomycin and FK506.

the intracellular FK506-binding protein-12 (FKBP12), thereby acquiring the ability to interact with CaN and to interfere with its access to and dephosphorylation of various substrates. Among the CaN substrates, whose activity is altered by ascomycin, are the nuclear factors of activated T cells (NFAT), a family of transcription factors that regulate lymphokine gene expression and play a prominent role in ascomycin-induced immunosuppression (Dumont 2000).

In the central nervous system (CNS), ascomycin and FK506 have been used to inhibit CaN activity both *in vitro* and *in vivo*. CaN is highly enriched in neurons and glial cells (Pallen and Wang 1985), and CaN-mediated dephosphorylation is an important modulatory factor in several cellular processes, including development of learning and memory (Riedel 1999), regulation of neuronal plasticity (Groth et al. 2003), cytoskeletal stability (Halpain et al. 1998), and induction of apoptosis (Springer et al. 2000). Additionally, CaN may regulate the activity of the GABA_A receptor (Amico et al. 1998; Huang and Dillon 1998), NMDA (Shi et al. 2000; Tong et al. 1995), and AMPA (Beatie et al. 2000; Lin et al. 2000) glutamate receptors. GABA transporter activity has been found to be regulated by CaN (Gonçalves et al. 1999). Within neurons, CaN is predominantly found at the postsynaptic densities and cell soma (Groth et al. 2003), but the particular targets of CaN dephosphorylation following synaptic activity depend upon the subcellular localization of the different pools of CaN in relation to the Ca²⁺ signal. Modifications in CaN activity have been associated with several neurological diseases such as epilepsy (Kurz et al. 2001; Lie et al. 1998; Vázquez-López et al. 2006), neurotrauma (Kurz et al. 2005; Sharkey et al. 2000) and Alzheimer disease (Norris et al. 2005).

The pharmacology of ascomycin, FK506, and its numerous analogs as immunosuppressants and dermatological drugs has been extensively reviewed (Bornhovd et al. 2001; Dumont 2000). The focus of this review is on the CNS pharmacology of ascomycin and FK506 and evaluation of their therapeutic potential as anticonvulsants and neuroprotectants.

Pharmacodynamics

The elucidation of the mechanism of immunosuppressant action of ascomycin and other FK506 analogs generated an enormous interest (Clipstone and Crabtree 1992). Liu et al. (1991) showed that upon formation of a complex with the intracellular protein FK506-binding protein 12 (FKBP12), FK506 selectively inhibits the enzymatic activity of the calcium/calmodulin-dependent protein phosphatase CaN (Klee et al. 1998). This mechanism has been demonstrated for ascomycin and other FK506 analogs with immunosuppressant action.

The immunophilin FKBP12 is one of the most abundant and conserved proteins in biology. It is the primary receptor for the immunosuppressant actions of ascomycin in whose presence FKBP12 binds to and inhibits CaN, disrupting interleukin (IL) formation in lymphocytes (Dumont 2000). However, the physiological functions of FKBP12 in the brain are less clear. The protein has been demonstrated to physiologically interact with the inositol 1,4,5-trisphosphate receptor (IP3R), the ryanodine receptor, and the type 1 transforming growth factor beta receptor (Cameron et al. 1996). FKBP12 binds the IP3R at residues 1400–1401, a leucyl-prolyl dipeptide epitope that structurally resembles FK506. Binding to IP3R at this site enables FKBP12 to interact with CaN,

presumably to anchor the phosphatase to IP3R and modulate the receptor's phosphorylation status. Ascomycin may promote an FKBP12–CaN interaction by mimicking structurally similar dipeptide epitopes present within proteins that use FKBP12 to anchor CaN to the appropriate physiologic substrates (Cameron et al. 1997).

Given its essential role in modulating neuronal excitability and its ability to regulate the architecture of the neuronal cytoskeleton, brain CaN inhibition may have many pharmacological consequences. Due to its high affinity for calcium ions and relatively low dissociation constant for Ca^{2+} (Klee et al. 1998), CaN is ideally placed for the regulation of intracellular free calcium via negative feedback on several systems, including several types of voltage-gated ion channels (Armstrong 1989; Burley and Shira 2000; Zhu and Yakel 1997), NMDA receptors (Lieberman and Mody 1994), and endoplasmic reticulum calcium stores (Cameron et al. 1995). Furthermore, the postsynaptic density is rich in CaN, and the NMDA receptor, the AMPA/kainate glutamate receptor, and the GABA_A receptor have been identified as either direct CaN substrates or as being indirectly regulated by CaN. CaN is highly bound to scaffolding proteins, including A-kinase anchoring proteon 79/150 (AKAP 79/150) (Dodge and Scott 2003) and microtubule associated protein-2 (MAP-2). AKAP 79/150 holds CaN in a complex with PKA near AMPA receptors, facilitating the regulation of these receptors (Dodge and Scott 2003). CaN plays a role in NMDA receptor-triggered endocytosis of AMPA receptors, thus indirectly limiting AMPA receptor activity (Lin et al. 2000). Several studies have demonstrated a downregulation of GABA_A receptor function by CaN (Amico et al. 1998; Chen and Wong 1995; Lu et al. 2000). Modulation of GABA_A receptor function may occur through direct dephosphorylation of receptor subunits by CaN (Lu et al. 2000). Finally, CaN has been shown to dephosphorylate several microtubule-associated proteins, including MAP2 and tau (Goto et al. 1985), and is capable of modulating actin stability and dendritic spine morphology (Halpain et al. 1998; Zhou et al. 2004).

However, other pharmacological effects of FK506, distinct from CaN inhibition, cannot be excluded, and may in fact lead to novel therapeutic applications of this drug or its derivatives. This is certainly the case in respect to the action of FK506 on nerve regeneration (Gold et al. 1994; Snyder et al. 1998). By systemic administration to rats, FK506 has been shown to accelerate the functional recovery of crushed sciatic nerves (Gold 1997). Most interestingly, a novel FK506 derivative, L-685818, proved to be as effective as FK506 in such a system (Snyder et al. 1998), suggesting that this neurotrophic effect is not mediated by an inhibition of CaN (Dumont

2000). Indeed, novel nonimmunosuppressive FKBP ligands have been synthesized, which facilitate the recovery of damaged sciatic nerves (Gold 1997) and of chemically lesioned brain neurons (Snyder et al. 1998). Interestingly, this neurotrophic action of FKBP ligands may involve FKBP52 and not FKBP12 (Gold et al. 1999).

Ascomycin and FK506 as Anticonvulsants in Preclinical Studies

The role of CaN in the development and propagation of epileptic seizures is still controversial. Moia et al. (1994) showed that the concentration of CaN in the brain of rats, as detected immunohistochemically, increases after completion of electrical kindling, and two CaN inhibitors, FK506 and cyclosporine A (CsA), inhibit progression of electrical kindling in rats. Kurz et al. (2001) have reported a significant increase in CaN activity in the rat pilocarpine model of status epilepticus, occurring through a NMDA-dependent mechanism. Sanchez et al. (2005) have shown that in the developing rat brain, GABAergic synaptic transmission is downregulated by CaN after seizures. However, Suzuki et al. (2001) reported a facilitation in pentylenetetrazole (PTZ)-induced chemical kindling in rats treated with FK506. Also, a decreased expression of CaN mRNA in the mouse hippocampus after kainic acid-induced seizures has been reported (Solá et al. 1998). Recently, Misonou et al. (2004) have shown that in the kainate model of continuous seizures in the rat, a CaN-dependent loss of voltage-dependent $\text{K}_v2.1$ potassium channel clustering is observed, suggesting an important link between calcium influx leading to CaN activation and the intrinsic excitability of pyramidal neurons. In chemical kindling induced in mice by chronic administration of a subconvulsant dose of PTZ (40 mg/kg), FK506 dose-dependently (0.5–1 mg/kg, p.o.) decreased the kindling score. Pretreatment with L-arginine (50–100 mg/kg, i.p.) potentiated the PTZ-induced kindling, whereas N^{ω} -nitro-L-arginine methyl ester (L-NAME) (10–20 mg/kg, i.p.) showed a protective effect. When given in combination, L-NAME potentiated the protective effect of a lower dose of FK506 (0.5 mg/kg) on PTZ-induced kindling. L-Arginine (50–100 mg/kg) reversed the protective effect of FK506 (1 mg/kg) and L-NAME (20 mg/kg), suggesting that a possible protective mechanism of FK506 involves the reduced formation of free radicals either directly or indirectly by NOS inhibition, thereby reducing NO formation (Singh et al. 2003). Ascomycin, at 50 or 100 μM , had anticonvulsant effect against picrotoxin-induced seizures when infused into the rat hippocampus (Vázquez-López et al. 2006).

CaN enzymatic activity has been related to epileptic seizures in several animal models. Kurz et al. (2001) demonstrated a significant increase in CaN activity in cortical and hippocampal homogenates during status epilepticus induced by pilocarpine. CaN is a calcium/calmodulin-stimulated enzyme (Klee et al. 1998) and would be stimulated by increased intracellular calcium concentrations. An increase in intracellular free calcium has been found during and after status epilepticus (Pal et al. 1999). The increased intracellular calcium associated with status epilepticus could be responsible for activating CaN above its normal physiological level, because status epilepticus induces a loss of function of the endoplasmic reticulum Mg^{2+}/Ca^{2+} ATPase (Parsons et al. 2000). This enzyme sequesters calcium ions in the microsomes of the smooth endoplasmic reticulum, providing a high-affinity mechanism for regulating intracellular calcium concentration (Carafoli 1987; Miller 1991). After status epilepticus, ATPase-mediated uptake of calcium into the microsomes is less efficient (Parsons et al. 2000), which could potentially result in higher than normal resting calcium concentrations inside the cell, affecting the status epilepticus-induced increase in CaN dephosphorylation.

One important CaN-mediated mechanism is modulation of the GABA_A receptor. GABA_A receptors are the primary receptors responsible for the fast inhibitory response in neuronal tissue (Macdonald and Olsen 1994), and play a major role in preventing the neuronal hyperexcitability associated with epilepsy. Several recent studies have demonstrated an inhibitory modulation of GABA_A receptor function by CaN (Amico et al. 1998; Chen and Wong 1995; Lu et al. 2000). CaN activity might be involved in the biochemical changes leading to picrotoxin-induced epileptic seizures, because picrotoxin binding to GABA_A receptors results in a net disinhibition of cellular excitability and may lead to increased dephosphorylation. Thus, CaN inhibition might favor GABA_A receptor activation antagonizing the effect of picrotoxin (Vazquez-López et al. 2006).

Furthermore, in accordance with the data of Suzuki et al. (2001), CaN may play a role in regulating the long-term changes leading to epileptogenesis. CaN inhibitors might acutely potentiate GABAergic transmission transiently increasing threshold of picrotoxin-induced seizures (Vazquez-López et al. 2006), but when excessive excitatory activity induces massive Ca^{2+} entry through NMDA or Ca^{2+} -permeable AMPA receptors (Sanchez et al. 2005), CaN might be also involved in the activation of long-term molecular cellular mechanisms (Lieberman and Mody 1994) leading to sustained recurrent excitatory activity, which induces late spontaneous seizures. CaN activity was significantly increased in the short- and long

term after actin depolymerization with latrunculin A. The increase in CaN activity was reversed by continuous MK-801 microperfusion in the rat hippocampus, both in acute and chronic animals (unpublished data). The increase in CaN activity observed after the *in vivo* microperfusion of latrunculin A in the rat hippocampus may be related to cytoskeletal reorganization induced by F-actin depolymerization followed by an increase in NMDA receptor activation, with implications for epileptogenesis.

The antiinflammatory effect of ascomycin and FK506 may be also involved in their anticonvulsant action. Proinflammatory and antiinflammatory cytokines and related molecules have been described in CNS and plasma in experimental models of seizures and in clinical cases of epilepsy (Vezzani and Granata 2005). Experimental studies in rodents have shown that inflammatory reactions in the brain can enhance neuronal excitability, impair cell survival, and increase the permeability of the blood-brain barrier to blood-borne molecules and cells (Vezzani and Granata 2005).

Finally, it has been also shown that CaN plays a major role in astrocyte activation and proliferation (Norris et al. 2005). CaN overexpression is sufficient for triggering the full activation cascade and phenotype, and CaN upregulation is consistently present in activated astrocytes in aging animals (Norris et al. 2005). Since astrocyte overactivation has been recently linked to epileptic activity (Tian et al. 2005), it is conceivable that astromycin produces its anticonvulsant effect by a combined action on neurons and glial cells.

Possible Role of CaN Inhibitors in the Treatment of Neurodegeneration

CaN upregulation both in neurons and glia has been observed in experimental models of several CNS diseases. CaN inhibitors such as FK506 and CsA have shown neuroprotectant effect in neurodegenerative disorders associated with acute brain ischemia. Adenovirus-mediated, high-level forced activity of CaN-induced cytochrome c/caspase-3-dependent apoptosis in cortical neurons and preincubation with the CaN inhibitors, CsA or FK506, reduced their susceptibility to apoptosis (Asai et al. 1999). In rat focal ischemia models, neuroprotection can be elicited by a single injection of FK506 given up to 72 h before or up to 2 h after the insult (Bochelen et al. 1999; Sharkey et al. 2000). These neuroprotective properties are shared by other CaN inhibitors, such as cyclosporine, while immunosuppressants acting by different mechanisms, such as rapamycin, are not neuroprotective in the models of focal ischaemia, but can effectively inhibit the neuroprotective effect of FK506 (Sharkey et al. 2000).

However, CaN may play a dual role during neuronal ischemia and excitotoxicity. CaN may mediate aspects of calcium-induced neuronal death, but also may inhibit neuronal excitation and calcium overload following ischemia (Siesjo et al. 1995) by negatively regulating Ca^{2+} channels and inhibiting calcium-dependent release of glutamate from presynaptic vesicles (Victor et al. 1995) and desensitizing NMDA receptors and IP3R (Cameron et al. 1995; Tong et al. 1995). Intracellular localization of CaN may determine the outcome of ischemia or other calcium-dependent cellular pathologies. *In vitro* studies of human brain cells suggest that Bcl-2, an antiapoptotic and neuroprotective protein, shuttles CaN to substrates such as IP3R and protects against ischemic damage. These data suggest that Bcl-2 modulates neuroprotective effects of CaN and that CaN inhibitors may increase ischemic neuronal damage (Erin et al. 2003). Takamatsu et al. (2001) showed a powerful neuroprotective effect of FK506 in a nonhuman primate model of stroke. A single dose of FK506 (0.1 mg/kg) was injected intravenously to Cynomolgus monkeys at 5 or 175 min after 3 h of occlusion followed by 5 h of reperfusion of the right middle cerebral artery. Eight hours after ischemia, a neuropathologic study was performed and the volume of ischemic damage was determined using PET scans to measure local cerebral blood flow, the cerebral metabolic rate of oxygen, and the oxygen extraction fraction. The treatment significantly reduced cortical damage 8 h after ischemia by 82% and 73%, respectively. In PET studies, FK506 did not affect cerebral blood flow or physiologic parameters.

Immunophilins are intracellular binding proteins for FK506 and ascomycin, but they also act as peptidylprolyl-cis-trans-isomerases (PPIases) that facilitate protein folding and are essential for protein–protein interactions. Since ascomycin and FK506 inhibit PPIase activity of immunophilins, as well as of CaN, the question remains whether inhibition of immunophilins alone might contribute to the neuroprotective effect of drugs. One postulated mechanism refers to inhibition of the mitochondrial permeability transition pore (mPTP) opening triggered by increase of Ca^{2+} level in mitochondrial matrix (Kaminska et al. 2006).

Mitochondrial dysfunction and damage have been proposed as key factors in the pathogenesis of disease resulting from brain ischemia and excitotoxicity. Mitochondrial calcium uptake plays a primary role as a sink for the large calcium load induced by intense glutamate receptor stimulation (Stout et al. 1998). Opening of a high-conductance channel, the mitochondrial permeability transition, affects normal mitochondrial function and results in the release of proteins involved in the initiation of a cell death cascade (Nicholls and Budd 2000).

Pore opening can be inhibited by a variety of agents, including CsA, and recent experiments have proposed that CsA and FK506 can prevent the sequelae of neuronal cell death observed after such perturbations as ischemia–reperfusion injury (Okonkwo et al. 1999; Uchino et al. 1998), traumatic brain injury (Singleton et al. 2001), and glutamate excitotoxicity in cultured cells (Shinder et al. 1996). The mechanism of protection more likely involves maintenance of the mitochondrial membrane potential (Bernardi et al. 1998), and is not the result of the immunosuppressant properties of CsA. However, administration of FK506, which lacks an effect on the MPT, had no effect on modification of susceptibility to kainate-induced cell death in either strain (Santos and Shauwecker 2003).

Cyclophilin D is one of the main components of mPTP along with voltage-dependent anion channel (VDAC), adenine nucleotide translocator (ANT), hexokinase, mitochondrial creatinine kinase, glycerol kinase, and pro- and antiapoptotic proteins of Bcl-2 family (Crompton 1999). *In vitro*, CsA in micromolar concentrations inhibits formation of mPTP, preventing changes in permeability of mitochondrial membranes, and thus impeding subsequent events leading to apoptosis (Lin and Lechleiter 2002; Waldeimer et al. 2002). However, FK506 does not bind cyclophilin D nor it inhibits mPTP opening (Friberg et al. 1998; Gold et al. 1999). Rapamycin, which binds with a similar affinity to FKBP12 but does not inhibit CaN, has no protective effect and even abolished neuroprotective effects of FK506 when coadministered (Sharkey and Butcher 1994), suggesting that CaN inhibition is indispensable for neuroprotective effects of FK506, at least in brain ischemia.

Recent microarray studies, validated by extensive statistical analyses, showed that CaN overexpression induced genes and cellular pathways representing most major markers associated with astrocyte activation and recapitulated numerous changes in gene expression found previously in the hippocampus of normally aging rats or in Alzheimer disease (Norris et al. 2005), identifying CaN upregulation in astrocytes as a novel candidate for an intracellular trigger of astrogliosis, particularly in aging and Alzheimer disease. One promising therapeutic strategy may be neuroprotection achieved through inhibition of proinflammatory response of microglia and astrocytes and modulation of neurotrophic factor expression. These data suggest that targeting CaN expression in astrocytes might represent a potentially important new therapeutic strategy against neurodegenerative processes.

Tissue damage in ischemia is the result of a complex pathophysiological cascade, which comprises a variety of distinct pathological events. Primary neuronal death occurring shortly after brain damage is probably due

to necrosis, while delayed neuronal death taking place over days and months bears many features of apoptosis (Dirnagl et al. 1999). After ischemia, astrocytes proliferate and become hypertrophic, while microglial cells transform into activated microglia (Morioka et al. 1993). Activated microglia release neurotoxic and proinflammatory cytokines such as: IL-1 β (Schielke et al. 1998), TNF- α (Wilde et al. 2000), FasL (Rosebaum et al. 2000), INF- γ , and numerous chemokines (Minami and Satoh 2003). Cytokines produced by microglia may further activate astrocytes that in turn become a source of neurotoxic cytokines (Benveniste 1998). Growing evidence indicate that the inhibition of secretion or activity of IL-1 β and TNF- α , with neutralizing antibodies or soluble cytokine receptors, leads to decrease in neuronal damage (Martin-Villalba et al. 2001; Lutsep and Clark 2001). Reactive gliosis is a widespread response to damage of neurons, and astrocytes are a source of proinflammatory cytokines (IL-1, IL-6) and cytotoxic cytokines (Fas L, TNF- α , and TGF- β) (Yu and Lau 2000). Activated astrocytes also produce toxic molecules such as reactive oxygen species and NO (Endoh et al. 1994).

Adult astrocytes express CaN regulatory subunit mRNA, and FK506 inhibits activation of glial cells and cytokine expression *in vitro* (Pyrzynska et al. 2001; Zawadzka and Kaminska 2005). FK506 treatment inhibits growth and hypertrophy of primary cortical astrocytes, downregulates the expression of genes coding for proinflammatory/cytotoxic cytokines, such as IL-1 β and TNF- α , while increases the expression of neuroprotective BDNF (Zawadzka and Kaminska 2005). Increase in BDNF expression could possibly have neuroprotective role, since injection of this cytokine was shown to be protective, leading to decrease in lesion grade and improvement in neurologic functions in models of global and focal ischemia (Kiprianova et al. 1999). In a model of transient middle cerebral artery occlusion, a single injection of FK506 (1mg/kg, administered 1 h after ischemia) produced a significant reduction in the brain damage and neurologic deficits. The neuroprotective effect maintained for 48 and 72 h. (Zawadzka and Kaminska 2005). In FK506-treated animals, a necrotic death of neurons in the striatum was not inhibited, which rather excludes a direct effect of FK506 on excitotoxic neuronal death. Administration of FK506 resulted in the decrease in the number of GFAP- and lectin B4-positive cells in the ischemic cortex, consistent with the inhibition of microglia and astrocyte activation. Moreover, FK506 decreased the levels of mRNA encoding TNF- α and IL-1 β in astrocytes *in vitro*, while the levels of TGF- β 1 and IL-6 were unaffected (Kaminska et al. 2006; Zawadzka and Kaminska 2005). An inhibition of microglia and astrocyte activation accompanied by a decrease in the lesion volume induced

by global ischemia in animals treated with FK506 has also been observed (Wakita et al. 1998).

Recent findings demonstrate that microglial cultures stimulated with lipopolysaccharide (LPS) show rapid (1–3 h) activation of three different MAPKs (p38, ERK1/2, and JNK) and a later increase in IL-1 β , TNF- α , and IL-6 mRNA levels, consistent with a possible relationship between MAPK and proinflammatory cytokine expression (Kaminska et al. 2006). FK506 strongly inhibits the activation of p38 MAPK and JNK induced by LPS in microglial cells, which results in blocking of IL-1 β mRNA and protein expression. Recent studies suggest that inhibition of p38 MAPK activation might have protective effects in experimental models of nervous system injury and neurodegeneration (Zhang and Stanimirovic 2002), and immunosuppressants may trigger and modulate signaling pathways critical for inflammation (Kaminska et al. 2004; 2006).

Pharmacokinetics

Tacrolimus is metabolized primarily in the liver and intestinal mucosa by the cytochrome P4503A4 enzyme, and is eliminated through biliary excretion. (Plosker and Foster 2000; Lampen et al. 1995; Nakazawa et al. 1998; Sattler et al. 1992; Vincent et al. 1992).

In clinical trials, as immunosuppressor, FK506 was administered either orally or by intravenous (i.v.) infusion. After single-dose i.v. infusion (20 μ g/kg per 4 h) and oral (80 μ g/kg) administration to 6 nondialysis patients awaiting renal transplantation, whole-blood FK506 levels were determined using a standard, two-step, nonspecific enzyme immunoassay. Mean $\pm$ standard deviation (S.D.) distribution half-life was 0.9 ± 0.2 h, elimination half-life ($t_{1/2\beta}$) 33 ± 8 h, total body clearance (CL) 2.4 ± 1.1 L/h, and bioavailability $14 \pm 12\%$. It was found that FK506 is incompletely and erratically absorbed after oral administration, and is rapidly distributed outside the blood compartment after i.v. dosing (Gruber et al. 1994). In patients receiving i.v. FK506, 0.03 mg/kg per day by continuous infusion, a pharmacokinetic estimate of clearance of 5.22 L/h was found (Jacobson et al. 2001). FK506 pharmacokinetics was age-dependent within the pediatric population. Mean clearance in children of <6, 6–12, and >12 years of age was 0.159 ± 0.082 , 0.109 ± 0.053 , and 0.104 ± 0.068 L/h per kg, respectively (Przepiorka et al. 2000).

In a preclinical trial, cats received FK506 (0.375 mg/kg, p.o., every 12 h) for 14 days. Blood levels of FK506 were measured by a high-performance liquid chromatography–mass spectrometry assay. Dosage was modified to maintain a target blood concentration of 5–

10 ng/mL. Mean ($\pm$ S.D.) values of elimination half-life, time to maximum concentration, maximum blood concentration, and area under the concentration versus time curve from the last dose of FK506 to 12 h later were 20.5 ± 9.8 h, 0.77 ± 0.37 h, 275 ± 31.8 ng/mL, and 161 ± 168 h $\times$ ng/mL, respectively (Kyles et al. 2003).

Recently, FK506 levels were successfully measured in anesthetized monkey brain and blood using ^{11}C -FK506 PET, a potentially useful method to measure FK506 concentrations in human brain. FK506 (0.1 mg/kg) containing [^{11}C]FK506 was intravenously injected into the monkeys, and dynamic PET images were acquired for 30 min afterward. Seven minutes after administration, the radioactivity in the brain became constant and was maintained up to 30 min. Fifteen minutes after FK506 (0.1 mg/kg) administration, the concentrations in the cortex and striatum were 20.0 ± 1.7 ng/g and 14.1 ± 1.7 ng/g, respectively (Murakami et al. 2004).

Adverse Effects and Toxicity

The greatest limitation to the therapeutic potential of ascomycin and FK506 in the CNS comes from its immunosuppressant effect and from its toxic side effects by chronic administration, including nephrotoxicity, diabetogenicity, and gastrointestinal disturbances (Peters et al. 1993; Spencer et al. 1997). The pathophysiological mechanisms of ascomycin and FK506 toxicity may arise from the same biochemical mechanisms that underlie its immunosuppressant effects, namely an inhibition of CaN activity in various tissues.

A toxicity study in rats showed that at 1–2 mg/kg per week i.m. or i.v. for 3 months FK506 develops certain toxic effects (hyperglycemia, hyperkalemia, and nephrotoxicity), but the animals responded well to dose reduction. However, no toxic effects were observed when the drug was administered topically or intravitreally (Akar et al. 2005). In our experiments, no toxic effects were observed when 100 μM ascomycin was infused into the rat hippocampus for 3 h (Vazquez-López et al. 2006).

FKBP12-binding analogs of FK506 that do not inhibit CaN function do not show the same toxic effects (Dumont et al. 1992; Dumont 2000), and the antagonist of FK506-induced immunosuppression, L-685818, can block FK506-induced toxicity in animal models (Dumont et al. 1992; Mollison et al. 1997). However, a different toxicity profile of FK506 compared with that of CsA suggests that CaN inhibition does not mediate all toxic effects of FK506 (Rodriguez-Hernandez et al. 2003).

Conclusions

Ascomycin, FK506, and several of their novel derivatives have been proposed as a treatment of inflammatory and degenerative brain diseases, including prevention of ischemic brain damage and Alzheimer disease. Ascomycin shows an excellent anticonvulsant activity when infused into the rat hippocampus via microdialysis probes. By systemic administration, it prevents PTZ-induced chemical kindling in mice. The pharmacological action of ascomycin and FK506 in the brain may involve CaN-mediated regulation of GABA and glutamate receptors, neuronal cytoskeleton and dendritic spine morphology, as well as of inflammatory responses in glial cells. However, other brain-specific mechanisms of action of FK506 that are distinct from CaN inhibition cannot not be excluded. Systemic immunosuppressant effects and toxicity at high doses by chronic administration (as required for the treatment of chronic CNS diseases) are the main obstacles for the development of ascomycin-derived CaN inhibitors as CNS drugs. However, CaN remains a potential target molecule in the search of new approaches to the treatment of epilepsy, neuroinflammation, or stroke. The therapeutic potential of ascomycin, FK506, and non-immunosuppressant ascomycin derivatives as CNS drugs should be further explored.

Conflict of Interest

The authors have no conflict of interest.

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