Pharmacological Properties of BN82451: A Novel Multitargeting Neuroprotective Agent

Pierre Etienne Chabrier and Michel Auguet

Ipsen; Institut Henri Beaufour, Les Ulis, France

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ABSTRACT

BN82451 belongs to a new family of small molecules designated as multitargeting or hybrid molecules. BN82451 is orally active, has good central nervous system penetration, and elicits potent neuronal protection and antiinflammatory properties. Neuronal protection is due to Na⁺ channel blockade, antioxidant properties, and mitochondria-protecting activity, whereas inhibition of cyclooxygenases is mostly responsible for its antiinflammatory activity. BN82451 has been shown to exert a potent neuroprotective effect in various *in vitro* and *in vivo* animal models. BN82451 was found to exert a significant protection in experimental animal models mimicking aspects of cerebral ischemia, Parkinson disease, Huntington disease, and more particularly amyotrophic lateral sclerosis. Collectively, its pharmacological properties designate BN82451 as a promising neuroprotective agent.

INTRODUCTION

BN82451 has been selected from a new family of small molecules designated as multitargeting or hybrid molecules. This compound was designed as a neuroprotective-disease-modifying agent for treatment of amyotrophic lateral sclerosis (ALS), Parkinson disease (PD), Huntington disease (HD), and other neurodegenerative diseases such as Alzheimer disease (AD). BN82451 acts via three major pathways involved in neuronal death: excitotoxicity, oxidative stress, and inflammation, and is also a mitochondrial protective agent.

Address correspondence and reprint requests to: Dr. P.E. Chabrier, Institut Henri Beaufour, 5, Avenue du Canada, 91966 LES ULIS CEDEX – France. Tel.: +33-1-60-92-20-55; Fax: 83 169073802; E-mail: pierre.chabrier@ipsen.com

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Selective or generalized neuronal loss is a major feature of neurodegenerative diseases. The cascade of events leading to neuronal death is multifactorial. Factors involved include metabolic disturbance, acidosis, excitotoxicity, oxidative stress, neuroinflammation, and mitochondrial dysfunction (Beal 1997, 1998; Andersen 2004; von Lewinski and Keller 2005; Marchetti and Abbracchio 2005). These alterations are not mutually exclusive and cooperate in the vicious cycle of toxicity leading to neuronal dysfunction and cell death, which may be apoptotic or necrotic depending on the severity of the insult. Different neuroprotective strategies have been aimed at limiting these neurotoxic processes to halt or delay disease progression by interfering with one of the specific events involved in such neurotoxic cascade. However, owing to the lack of early markers of disease and late diagnosis, treatment is initiated long after the neurodegeneration has started when all perpetrators of neuronal death are activated. This may explain the disappointing results obtained in clinical trials with agents that specifically interfere with only one neurotoxic pathway. It has thus been postulated that a multidrug approach may be a better strategy to fight these devastating diseases. This is particularly exemplified in ALS where the association of several drugs with different mechanisms of action have shown dramatic increase of efficacy in preclinical models mimicking the disease (Desnuelle et al. 2001; Kriz et al. 2003; Nagano et al. 2003; Snow et al. 2003; Zhang et al. 2003; Klivenyi et al. 2004).

An alternative strategy to a cocktail of drugs is to conceive single compounds endowing different biological properties. This approach may result not only in a greater efficacy but also avoid the pharmacokinetic interactions between the different associated drugs and may improve patient compliance. Conception of multitargeting drugs can be achieved by pharmacophore combination using either conjugated pharmacophores (Chabrier et al. 1999; Auvin et al. 2004) or integrated pharmacophores (De Lombaert et al. 1994). This latter procedure was used and led to BN82451, which interferes with excitotoxicity (Na^+ channel blocker), oxidative stress (antioxidant), and inflammation (cyclooxygenase inhibitor). This strategy contrasts with the classical monotargeting approach of previous neuroprotective molecules that act typically through only one mechanism.

The present review describes the pharmacological properties of BN82451.

CHEMISTRY

BN82451 (4-[2(aminomethyl)-1,3-thiazol-4-yl]-2,6 di-*tert*-butylphenol), mono or dihydrochlorhydrate salt, is a white crystalline solid with a molecular weight of 318.48 Da (for the base, $\text{C}_{18}\text{H}_{26}\text{N}_2\text{OS}$) (Fig. 1) (Harnett et al. 2004).

PHARMACOLOGY

The pharmacology of BN82451 has been investigated in both *in vitro* and *in vivo* systems. Because BN82451 is a multitargeting agent, each of its specific sites of action has been

FIG. 1. Chemical structure of BN82451.

extensively evaluated, namely, neuronal excitotoxicity (sodium channel blocker), oxidative stress (antioxidant), neuroinflammation (cyclooxygenase inhibitor), and mitochondrial dysfunction (mitochondria-protective properties).

Excitotoxicity and Sodium Channel

Considerable evidence supports a role for excitotoxicity in acute as well as in chronic neurological disorders. Excessive release of glutamate, the major excitatory neurotransmitter, leads to cell death. An initial step in glutamate release is an increase in intracellular Na^+ concentration, cellular depolarization, and elevation of intracellular Ca^{2+} concentration. Prolonged opening of voltage-dependent Na^+ channels in neuronal cell cultures results in glutamate release and cell degeneration (Abita et al. 1977; Pauwels et al. 1989). BN82451 was evaluated on binding of [^3H]batrachotoxinin A 20- α -benzoate to neurotoxin receptor site 2 in rat cortex membrane preparations (veratridine site). In this assay, BN82451 inhibits the binding of [^3H]batrachotoxinin A in a concentration-dependent manner. The IC_{50} value for BN82451 is 74 nM (95% confidence interval: 40.7–139; $n = 3$).

The functional interaction of BN82451 with Na^+ channels was further explored *in vitro* in human neuroblastoma SH-SY5Y cells exposed for 3 h to veratridine (100 μM). Veratridine induces cell degeneration, which is measured by a colorimetric indicator, Alamar blue. In this system, BN82451 exerts a complete and concentration-dependent protection with an IC_{50} of 2.9 μM (95% confidence interval: 1.04–7.93; $n = 3$). Finally, in cultured rat cerebellar granule cells inhibition of the release of glutamate evoked by depolarization with 10 μM veratridine was used to evaluate the effect of BN82451 (Fig. 2).

Taken together, these results show that BN82451 effectively blocks neuronal sodium channels and suggests that this biological property may contribute to the therapeutic neuroprotection offered by this compound.

FIG. 2. Effect on veratridine-induced glutamate release from cultured rat cerebellar granule cells. BN82451 or vehicle control had been added for 30 min before introducing the media containing veratridine, 10 μM . After 5 min, the media were collected and glutamate release was quantified using Amplex red glutamic acid oxidase kit (Molecular Probes). Data are expressed as arbitrary unit (AU) after subtraction of blank values. Mean $\pm$ S.E.M.

Lipid Peroxidation and Oxidative Stress

BN82451 possesses hindered phenolic structure of lipophilic antioxidants and thus acts as a peroxyl radical trapping, chain-breaking antioxidant (Gavino et al. 1981; Barclay and Vinquist 2000). Lipid peroxidation induced by Fe^{2+} in lipid vesicles is due to free radicals produced by the Fenton reaction and can be easily assessed by malonaldehyde formation using a colorimetric method (Esterbauer et al. 1991). BN82451 inhibits lipid peroxidation in rat brain microsomes in a concentration-dependent manner with an IC_{50} value of $0.54 \mu\text{M}$ (95% confidence interval: $0.29\text{--}1.03 \mu\text{M}$; $n = 3$). The functional significance of this effect is emphasized by the neuroprotection afforded by BN82451 against glutamate-induced oxidative stress in an immortalized murine hippocampal cell line, HT-22. HT-22 cells are currently used to assess the antioxidant properties of various compounds (Behl et al. 1997), because these cells are exquisitely sensitive to oxidative stress induced by glutamate (Davis and Maher 1994). Because HT-22 cells do not possess glutamate receptors, the classical excitotoxic pathway is not involved in these cells. In fact, at high concentrations (5 mM), glutamate competes with cystine uptake, which is reduced in cysteamine, a precursor of glutathione (Murphy et al. 1989), resulting in a depletion of glutathione content, accumulation of reactive oxygen species, and ultimately cell death (Tan et al. 1998). In this model, a close correlation between the potency of the compounds to prevent glutamate-induced HT-22 cell death and their effect on membrane lipid peroxidation has been observed (Demerle-Pallardy et al. 2000). In HT-22 cells exposed to glutamate (5 mM) for 24 h, BN82451 prevents cell death, which is determined by WST-1 labeling of living cells. Its protective effect is concentration-dependent with an IC_{50} : $0.34 \mu\text{M}$ (95% confidence interval: $0.201\text{--}0.511 \mu\text{M}$, $n = 3$).

Cyclooxygenase Inhibition and Inflammation

In the last 10 years, the role of neuroinflammation has gained much attention in the initiation as well as perpetration of neurodegenerative diseases (Hirsch et al. 1998; McGeer and McGeer 1998; Floyd 1999; Gonzalez-Scarano and Baltuch 1999). Activated microglia and astrocytes have the potential to damage contiguous neurons by secreting toxic substance including free radicals, prostaglandins, inflammatory cytokines, and proteolytic enzymes. Neuroinflammation participates, therefore, in the vicious cycle of toxicity leading to neuronal dysfunction and has emerged as an important target for neuroprotection, notably through cyclooxygenase (COX). Inhibition of human platelet COX activity, a COX-1 selective assay, and of the HUV-EC-C line activated by phorbol 12-myristate 13-acetate, a COX-2 assay (Miralpeix et al. 1997), have been used to evaluate the nonsteroidal antiinflammatory potency of BN82451. The IC_{50} values for BN82451 are 0.08 and $0.05 \mu\text{M}$ for COX-1 and COX-2, respectively. The *in vivo* antiinflammatory activity was assessed in the rat model of carrageenan-induced paw edema, the most commonly used experimental model of inflammation (Winter et al. 1962). The study was performed on male Sprague-Dawley rats. BN82451 administered orally 1 h before a subplantar injection of carrageenan ($1 \text{ mg}/0.1 \text{ mL}$) in the right hind paw of the rats reduces the edema in a dose-dependent manner; $\text{ED}_{50} = 17.3 \text{ mg/kg}$ (1.5 h after carrageenan injection). The antiinflammatory effect of BN82451 is significant up to 6 h after carrageenan injection. With the same experimental conditions, the ED_{50} value for ibuprofen was 8.3 mg/kg p.o. It should be noted that

BN82451 is also able to alleviate the mechanical hypersensitivity induced by carrageenan and offers a protection of 68% when tested at the dose of 30 mg/kg p.o.

Mitochondrial Protection

Mitochondrial dysfunction is frequently associated with neurodegenerative disorders. Disruption of mitochondrial function plays an important role in cell death; this includes loss of mitochondrial membrane potential, opening of the mitochondrial permeability transition (MPT) pore, and matrix swelling and rupture of the outer mitochondrial membrane, allowing the release of proapoptotic molecules. Preservation of the mitochondrial membrane potential and protection of mitochondria from swelling are, therefore, considered to be essential processes in rescuing neurons in a number of neurodegenerative diseases (Tatton and Chalmers-Redman 1998; Hall 1993; Kristal et al. 2004; Jemmerson et al. 2005). The mitochondrial properties of BN82451 were examined in three experimental paradigms: isolated mitochondria, neuronal cell culture, and *in vivo* animal models of mitochondrial intoxication.

In rat isolated liver and brain mitochondria, BN82451 inhibits mitochondrial swelling. Addition of 1-methyl-4-phenylpyridinium (MPP⁺, 1 mM) to isolated liver mitochondria inhibits complex I of the respiratory chain impairing ATP synthesis and produces toxic free radicals that lead to swelling and cytochrome c release (Cassarino et al. 1999). BN82451 effectively decreases both the extent of mitochondrial swelling and the release of cytochrome c (Fig. 3). In rat brain mitochondria, excessive calcium accumulation triggers the opening of the MPT pore (Krieger and Duchen 2002) and a partial swelling (Friberg et al. 1999; Kristian et al. 2000, 2002). At these experimental conditions, the maximal extent of swelling was 40–50% of that obtained by subsequent addition of alamethicin, a “nonselective” pore-forming hydrophobic peptide. The swelling of brain mitochondria sensitive to the MPT pore blocker cyclosporin A was found to be inhibited by BN82451 (Delaflotte et al. 2004) (Fig. 3C).

In human neuroblastoma SH-SY5Y cells, *tert*-butylhydroperoxide (*t*-BH; 200 μ M/2 h) induces a fall ($47 \pm 0.7\%$) in mitochondrial membrane potential ($\Delta\Psi_m$) and a 1.7-fold increase of caspase-9 cleavage activity. Pretreatment (0.5 h) with BN82451 (25 μ M) inhibits the decrease in mitochondrial membrane potential and maintains the caspase-9 activity at the control level, thus preventing apoptosis induction. Indeed, cell death induced by *t*-BH (200 μ M) is prevented by BN82451 in a dose-dependent manner ($EC_{50} = 3 \mu$ M, maximal protection $61 \pm 7.6\%$) (Chabrier et al. 2002).

In vivo, experimental animal models of mitochondrial dysfunction mimicking some aspects of neurodegenerative diseases are based on mitochondrial toxin administration such as MPTP and malonate, which inhibit complexes I and II of the mitochondrial respiratory chain, respectively (Nicklas et al. 1985; Greene et al. 1993; Schulz and Beal 1994; Cornet et al. 2004). BN82451 tested in these two animal models effectively affords protection.

MPTP-treated mice ($n = 6$) exhibit a marked decrease (63% depletion) in striatal dopamine level in comparison to control animals ($n = 8$); 5.20 ± 0.47 and 14.21 ± 1.07 ng/mg of tissue, respectively. BN82451 at the doses of 3, 10, and 30 mg/kg p.o. significantly reduces the decrease in dopamine level by 2%, 43.5%, and 102%, respectively ($n = 6$). The protective effect of BN82451 is dose-dependent ($ED_{50} = 9.8$ mg/kg) and the dose of 30

FIG. 3. (A) Effects of 10 and 25 μ M BN82451 on swelling induced by 1 mM MPP⁺ in isolated liver mitochondria. The insert shows the effect of cyclosporin A (CyA 1 μ M). Mean $\pm$ SEM; $n = 3-4$. (B) Western blot analysis of supernatant fraction from isolated liver mitochondria subjected to MPP⁺. Representative Western blot from six different experiments using three different rats. Cytochrome c immunoreactivity is evident as a single band of molecular mass (15 kDa). Ca²⁺/Pi (control); MPP⁺ (1 mM) BN82451 (25 μ M); cyclosporin A 1 μ M. (C) Effect of BN82451 (10–20 μ M) on calcium (50 μ M) induced swelling in isolated rat brain mitochondria. The insert shows the effect of cyclosporin A 1 μ M. Mean $\pm$ S.E.M. ($n = 3-5$).

mg/kg provides a total preservation (Fig. 4A). This protective effect is not accompanied by a modification in the striatal levels of MPP⁺, the monoamine oxidase B-derived active metabolite of MPTP, excluding an interaction of BN82451 with MPTP metabolism or brain uptake (Fig. 4B).

Direct intracerebral injections of micromolar quantities of malonate elicit focal necrotic lesions in the striatum of the adult rat. The mechanisms of neuronal cell death involve secondary excitotoxicity and the generation of reactive oxygen species. BN82451 protected against malonate toxicity. A dose effect of BN82451 on striatal lesion induced by malonate was performed using TTC staining. Forty-eight hours after intrastriatal injection of 2 μ mol of malonate, the volume of the lesion was 73 ± 4.36 mm³ in the striatum of control rats ($n = 10$). Treatment with 1, 6, 10, 30, and 60 mg/kg, p.o. of BN82451 significantly reduces the striatal lesion by 32%, 37%, 30%, 48%, and 54%, respectively (Fig. 5).

The protection afforded by BN82451 against mitochondrial toxins was further strengthened by the dramatic potency of BN82451 in prevention of lethality induced by potassium cyanide (KCN). The lethal effect of KCN is largely due to inhibition of mitochondrial complex IV (cytochrome oxidase) of the respiratory chain and subsequent breakdown of cellular metabolism. The brain has been considered as the primary organ of cyanide intoxication (Ikegaya et al. 2001) and the main site of cytochrome c inhibition (Pearce et al. 2003). Oral administration of BN82451 (3–30 mg/kg) 1 h before a bolus injection of a lethal dose of

FIG. 4. (A) Dose-effect of BN82451 on dopamine level reduction induced by MPTP in striatum of C57BL/6 mice. Mice were injected (+) or not (0) with MPTP (3×20 mg/kg, i.p. at 2-h intervals). BN82451 or sterile water were administered 60 min before each injection of MPTP, 90 min after the last injection of MPTP, and 24 h after the first administration of sterile water or BN82451. A second group treated with sterile water was the control MPTP group. The other four groups were injected with different doses of BN82451 (3, 10, and 30 mg/kg, p.o.). Results are expressed as means $\pm$ S.E.M. ($n = 6$). "a" indicates comparison with control group (two-tailed Student t test). "b" indicates comparison with MPTP group (two-tailed Dunnett test). Statistically significant difference: $*P < 0.05$; $**P < 0.01$; and $***P < 0.001$. (B) Effect of oral administration of 30 mg/kg of BN82451 in mice on MPP⁺ striatal levels. MPP⁺ levels were determined 90 min after administration of 20 mg/kg MPTP (i.p.). Mean $\pm$ S.E.M.; $n = 6$.

KCN (3.25 mg/kg, i.v.) to male CD1 mice results in a dose-dependent decrease in lethality. At the dose of 30 mg/kg, BN82451 provides a complete protection, and the calculated ED₅₀ value was 4.1 mg/kg.

Collectively, these results show that BN82451 exhibits neuroprotection *in vitro* and *in vivo* against mitochondrial poisoning induced by the different toxins that shut down the aerobic oxidative phosphorylation and ATP synthesis. Using the same experimental paradigm based

FIG. 5. Dose-effect of BN82451 on striatal lesion 48 h after intrastriatal injection of malonate (2 μ mol) in rats. BN82451 (1, 6, 10, 30, and 60 mg/kg, i.p.) or water was administered 1 h before and 5 h after malonate injection. The volume of lesion was measured after TTC staining (2%). Results are expressed as means $\pm$ S.E.M. ($n = 8$). $***P < 0.001$ significantly different from control group (one-way analysis of variance).

on *in vivo* models of mitochondrial toxin administrations and on *in vitro* models of isolated mitochondria we recently showed that the neuroprotective action of minocycline was more related to its antiinflammatory action than to a mitochondrial protective property (Cornet et al. 2004).

Neuroprotection

In vitro

Rat primary cultured cortical neurons undergoing serum deprivation are a well-defined *in vitro* model system for apoptosis and neuronal death. Serum-deprivation induces energy depletion leading to mitochondrial dysfunction and generation of reactive oxygen species.

After 8 days of culture, cortical neurons were cultured in medium deprived in serum with or without BN82451. Cell death was evaluated by measuring the release of lactate dehydrogenase (LDH) in the culture supernatant 1 and 6 days after treatment. Serum deprivation results in about twofold increase in LDH release when compared to serum supplemented control. BN82451 exerts a concentration-dependent neuroprotective effect: IC_{50} is 142 nM 1 day after serum deprivation. Six days after treatment and without medium renewal within the 6 days, BN82451 is still effective, suggesting a potent and long-lasting neuroprotective effect on primary cortical neurons (Fig. 6).

In vivo—ischemia models

The Mongolian gerbil is extensively used as a model for cerebral ischemia and infarction. In gerbils, a transient bilateral carotid occlusion for 10 min produces delayed neuronal death in the hippocampus. An indirect index of neuronal damage was used with the measurement

FIG. 6. Concentration-dependent and long-lasting efficiency of BN82451 in preventing primary cortical neurons from serum deprivation-induced LDH release. Means $\pm$ S.E.M. of LDH values were processed from optical density values cleared from blank values issued from two experiments, done in triplicate and in quadruplicate, respectively ($n = 7$).

FIG. 7. Effect of BN82451 on global ischemia in gerbils. Mean $\pm$ S.E.M.; $n = 5, 3, 6, 8$, and 8 for the groups: sham saline, sham BN82451, ischemic saline, ischemic BN82451 1 mg/kg , and ischemic BN82451 3 mg/kg , respectively. Brains were removed 7 days postocclusion. Hippocampi were homogenized and aliquots of $50 \mu\text{L}$ of homogenate were incubated with 2 nM of $[^3\text{H}]\text{PK11195}$ (75 Ci/mmol) in 1 mL Tris-saline buffer for 1 h at 25°C . Bound radioactivity was recovered and counted by liquid scintillation spectrometry. Nonspecific binding was measured in the presence of $1 \mu\text{M}$ PK11195. Binding was done in triplicate. The statistical significance of the ischemia-induced changes in PTBBS density was performed using one-way analysis of variance followed by Student test (homogenous variance). The different groups of ischemic gerbils were compared using Dunnett test.

7 days postocclusion of the total peripheral-type benzodiazepine binding site densities (PTBBS) with a specific ligand $[^3\text{H}]\text{PK11195}$ (Benavides et al. 1990; Demerle-Pallardy et al. 1991). The interest of this approach is based on the fact that a glial reaction and macrophage invasion (cell types that in contrast to neurons are richly endowed with PTBBS) are the unavoidable consequences of neuronal injury. In these conditions, 10 minutes of carotid artery occlusion followed by 7 days of recirculation induced a 52% ($P = 0.0001$) increase in PTBBS density in the hippocampus. Treatment with BN82451, 10 min and 5, 24, and 48 h after bilateral occlusion at 1 and 3 mg/kg i.v. reduces the increase of PTBBS by 29% and 43% ($P = 0.0199$ at the highest dose; Fig. 7). Treatment with 3 mg/kg i.v. of BN82451 does not affect the basal level of PTBBS in sham-operated gerbils (fmol/mg tissue: 94.97 ± 2.41 vs. 94.52 ± 2.32 for saline and BN-treated sham-operated gerbils, respectively).

In a second model of ischemia, the effect of BN82451 was investigated on transient focal cerebral ischemia induced by occlusion of middle cerebral artery (MCA) in rats. In animals subjected to transient (90 min) MCA occlusion followed by reperfusion, BN82451 (30 and 60 mg/kg p.o. at 90 min and 24 h after occlusion) was administered orally. Infarct volume was measured 48 h after the onset of ischemia using 2,3,5-triphenyl-tetrazolium chloride technique. In vehicle-treated rats, MCA occlusion causes infarction mainly located in the cerebral cortex and striatum. BN82451 (30 mg/kg) reduces total infarct volume by 22% ($270.48 \pm 20.45 \text{ mm}^3$ for control and $210.06 \pm 30.85 \text{ mm}^3$ for BN-treated group, $P = 0.1221$). BN82451 (60 mg/kg) significantly reduces total infarct volume by 42% ($275.26 \pm 17.22 \text{ mm}^3$ for control and $158.29 \pm 27.06 \text{ mm}^3$ for BN-treated group, $P = 0.005$). Reduction of

the infarct is evident throughout the hemisphere, and is statistically significant in all coronal sections.

This result obtained by posttreatment and using the oral route highlights the neuroprotective potential of BN82451.

In vivo—chronic physiopathological models

ALS is a rapidly progressive neurodegenerative disease that affects about 100,000 individuals worldwide. It is a fatal disease with a mean survival of 3 to 5 years. It is characterized by a loss of both upper and lower motor neurons, resulting in progressive muscle loss and paralysis. Possible mechanisms that cause death of motor neurons include intense productions of ROS due to SOD1 mutations, excitotoxicity, mitochondrial dysfunction, apoptosis, protein aggregation, and inflammation. Considering its multiple mode of action, BN82451 may well address this pathology.

Two different animal models of motor neuron disease provide the opportunity of testing potential therapeutic strategies for ALS treatment: the progressive motor neuropathy (*pmn*) mouse with a hereditary autosomal recessive wasting disease and SOD1 (G93A) transgenic mice carrying a mutation in the gene encoding human Cu/Zn superoxide dismutase [B6SJL-TgN(SOD1-G93A)1Gur] (Pioro and Mitsumoto 1995; Doble and Kennel 2000).

The progressive motor neuropathy (*pmn*) mouse is a genetic model of a degenerative motor neuron disease, involving a dying-back process with distal axon degeneration and relative preservation of proximal axons and cell bodies. *pmn* mice were treated by oral administration from day 7 to death every day with vehicle (0.9% sterile aqueous solution of sodium chloride) or BN82451 at 10 mg/kg.

In SOD1 transgenic mice, two different studies have been performed by two independent teams to measure the effect of BN82451 on the lifespan of animals. In the first experiment in SOD1 mice (Study 1), animals were treated by oral administration from day 70 until death with vehicle (0.9% sterile aqueous solution of sodium chloride) or BN82451 at 30 mg/kg. For the second experiment (Study 2), mice were treated by oral administration from day 70 until death with vehicle (sterile water) or BN82451 at a dosage of 20 mg/kg/day (5 days/week) under a volume of 10 mL/kg and during the weekend BN82451 was included into the animal chow (0.03% w/w). Mice were observed daily and survival time recorded. In the first model of motor neuron disease, the *pmn* mice, the prolongation of the mean survival time by 5 days corresponds to a 26% increase in the lifespan of the mice (Table 1). The prolongation of the mean survival time of SOD1 mice by 22 days in Study 1 and by 14.5 days in Study 2 corresponds to a 27% and 11.2% increase in the lifespan of the mice, respectively (Table 1).

These neuroprotective effects are also observed on the R6/2 transgenic mouse model of HD. In this model, lab chow diets supplemented with 0.015% BN82451 improves motor performance and increased survival of the R6/2 transgenic mice by 15% (Klivenyi et al. 2003). The mean survival increased from 92.6 ± 3.5 days to 106.8 ± 1.1 days with BN82451 ($P < 0.001$). The treated mice have significantly better motor performance from 57 to 67 days of age than mice fed unsupplemented diets. Oral administration of BN82451 significantly reduces gross brain atrophy, neuronal atrophy, and the number of neuronal intranuclear inclusions at 90 days of age. In this model, the selective antioxidant lipoic acid (100 mg/kg/day) induced a significant prolongation of mouse lifespan (7.1%),

TABLE 1. Effect of BN82451 on survival time in two different models of motor neuron diseases (*pnn* and *SOD1* mice)

Mouse	Treatment mg/kg/day	Age at the start of treatment (days)	Survival time (days $\pm$ S.E.M.)	<i>n</i>	<i>P</i> value
<i>pnn</i> control	–	7	19 $\pm$ 1.2	15	0.02
<i>pnn</i> treated	10	7	24 $\pm$ 2.1	15	
Study 1					
SOD1 control	–	70	80 $\pm$ 3.0	9	0.002
SOD1 treated	30	70	102 $\pm$ 6.1	10	
Study 2					
SOD1 control	–	70	129 $\pm$ 2.5	11	0.03
SOD1 treated	20	70	144 $\pm$ 5.9	12	

BN82451 was administrated orally every day until death. The study has been analyzed on a Per Protocol Population (exclusion of outliers identified with a Dixon test) using a log-rank test [one- (*pnn*) or two-tailed (*SOD1*)] for comparison between mean survival times of control and treated groups.

whereas the radical scavenger SPBN (120 mg/kg/day) was inactive (Andreassen et al. 2001)

Although the underlying neurodegenerative mechanisms in these physiopathological models displaying different features are multiple, mitochondrial defect appears to be primarily involved in both pathologies (Brouillet et al. 2005; von Lewinski and Keller 2005).

EARLY SAFETY

BN82451 does not demonstrate mutagenic activity in the bacterial mutation assay *in vitro*, or clastogenic activity in the human lymphocyte chromosome aberration assay *in vitro*, suggesting it is unlikely to be genotoxic.

In vitro inhibition of radioligand binding to neurotransmitters, neuropeptides, and ionic ligands shows the selectivity and specificity of BN82451 (Table 2).

Studies monitoring mouse central nervous system activity did not reveal any major clinically relevant adverse effects at the dose of 60 mg/kg p.o. Moreover, the results indicate that relatively high doses of BN82451 elicit antiepileptogenic actions in the maximal electroshock seizure (MES) test and to a lesser extent delays lethality in the model of NMDA. Such an effect may be attributed to the interaction of BN82451 with sodium channel. The rota-rod test is used to assess the effect of drugs on motor coordination and thus allows the identification of ataxic, myorelaxant, neuroleptic, or sedative activities. Mice motor coordination is affected only by high doses of BN82451 (ED₅₀ = 224 mg/kg).

Many inflammatory drugs have been reported to cause gastrointestinal damage. Oral administration of BN82451 for 5 days had no statistically significant effect on the gastric mucosa in rats at a dosage of up to 30 mg/kg/day. Cardiovascular studies in conscious rats do not show significant modification in heart rate, systolic, diastolic, and mean arterial blood pressures after BN82451 at a dose of 60 mg/kg p.o.

A tolerance study was performed to evaluate the effects of a 4-week oral treatment with BN82451 at 3 and 30 mg/kg/day. The good general tolerance of BN82451 allows daily oral administration to the male mouse for at least 4 weeks.

TABLE 2. Receptor binding profiling

Receptor	BN82451 (IC ₅₀ μ M)	Tissue
A ₁ (<i>h</i>)	—	Human cDNA
A _{2a} (<i>h</i>)	—	Human cDNA
A ₃ (<i>h</i>)	—	Human cDNA
α_1 (nonselective)	—	Rat brain
α_2 (nonselective)	—	Rat brain
β_1 (<i>h</i>)	—	Human cDNA
β_2 (<i>h</i>)	(91)*	Human cDNA
NE uptake (<i>h</i>)	—	Human cDNA
AT ₁ (<i>h</i>)	—	Human cDNA
AT ₂ (<i>h</i>)	—	Human cDNA
ANP	—	Guinea pig cerebellum
BZD (central)	—	Rat brain
BZD (peripheral)	—	Rat heart
Bombesin	—	Rat brain
B ₂ (<i>h</i>)	—	Human cDNA
CGRP (<i>h</i>)	—	Human cells
CB1 (<i>h</i>) (central)	—	Human cDNA
CB2 (<i>h</i>) (peripheral)	—	Human cDNA
CCK _A (<i>h</i>)	—	Human cDNA
CCK _B (<i>h</i>)	—	Human cDNA
D ₁ (<i>h</i>)	—	Human cDNA
D ₂ (<i>h</i>)	—	Human cDNA
D ₃ (<i>h</i>)	—	Human cDNA
D ₄ (<i>h</i>)	—	Human cDNA
D ₅ (<i>h</i>)	—	Human cDNA
DA uptake (<i>h</i>)	—	Human cDNA
ET _A (<i>h</i>)	—	Human cDNA
ET _B (<i>h</i>)	—	Human cDNA
GABA (nonselective)	—	Rat brain
GAL1 (<i>h</i>)	—	Human cDNA
PDGF	—	Mouse cells
IL-8A (<i>h</i>) (CXCR1)	—	Human cDNA
TNF- α (<i>h</i>)	—	Human cells
CCR1 (<i>h</i>)	—	Human cDNA
H ₁ (central)	—	Guinea pig brain
H ₂	2.28	Guinea pig brain
ML1	—	Chicken brain
M ₁ (<i>h</i>)	—	Human cDNA
M ₂ (<i>h</i>)	—	Human cDNA
M ₃ (<i>h</i>)	—	Human cDNA
M ₄ (<i>h</i>)	—	Human cDNA
M ₅ (<i>h</i>)	—	Human cDNA
NK ₁ (<i>h</i>)	—	Human cDNA
NK ₂ (<i>h</i>)	—	Human cDNA
NK ₃ (<i>h</i>)	—	Human cDNA
Y ₁ (<i>h</i>)	—	Human cDNA
Y ₂ (<i>h</i>)	—	Human cells

Continued.

TABLE 2. Continued.

Receptor	BN82451 (IC ₅₀ μ M)	Tissue
NT ₁ (<i>h</i>)	—	Human cDNA
δ (<i>h</i>)	—	Human cDNA
κ (<i>h</i>)	—	Human cDNA
μ (<i>h</i>)	—	Human cDNA
ORL-1 (<i>h</i>)	—	Human cDNA
PACAP	—	Rat brain
PCP	—	Rat brain
TXA ₂ /PGH ₂ (<i>h</i>)	—	Human platelets
PGI ₂ (<i>h</i>)	—	Human platelets
P _{2X}	—	Rat urinary bladder
P _{2Y}	—	Rat brain
5-HT _{1A} (<i>h</i>)	—	Human cDNA
5-HT _{1B}	18.1	Rat brain
5-HT _{2A} (<i>h</i>)	—	Human cDNA
5-HT _{2C} (<i>h</i>)	1.41	Human cDNA
5-HT ₃ (<i>h</i>)	—	Human cDNA
5-HT _{5A} (<i>h</i>)	—	Human cDNA
5-HT ₆ (<i>h</i>)	0.771	Human cDNA
5-HT ₇ (<i>h</i>)	2.02	Human cDNA
σ_1	2.09	Guinea pig brain
σ_2	0.114	Rat brain
Somatostatin	—	Mouse cells
VIP (<i>h</i>)	—	Human cells
V _{1a} (<i>h</i>)	—	Human cDNA
Ca ²⁺ channel (L, verapamil site)	3.8	Rat brain
Ca ²⁺ channel (N)	—	Rat brain
K ⁺ channel (voltage-dependent)	—	Rat brain
K ⁺ channel (Ca ²⁺ -dependent)	—	Rat brain
Cl ⁻ ionophore	—	Rat brain

Data from CEREP. Mean values; *n* = 2. The symbol—indicates inhibition of less than 70% at 10 μ M of tested drug. *% inhibition at 10 μ M.

CONCLUSIONS

Associations or cocktail of drugs have been proposed for treating neurological diseases such as ALS. An alternative strategy to drug combination is to use a single compound endowed with multiple pharmacological properties targeting different events involved in neuronal cell death. For this purpose, BN82451 was designed as a multitargeting agent to interact with neuronal excitotoxicity (sodium channel blocker), oxidative stress (antioxidant), and neuroinflammation (cyclooxygenase inhibitor) and was selected for its unique mitochondrial protective properties. *In vitro* studies have confirmed its multiple modes of action and in different experimental animal models and more particularly ALS models, BN82451 was found to exert a highly significant protection. The pharmacological efficacy is associated with favorable early safety and toxicology data that designate BN82451

as a promising candidate for clinical development in the treatment of neurodegenerative diseases.

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