Transworld Research Network
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences II, 2012: 15-28 ISBN: 978-81-7895-569-8
Editors: Diego Muñoz-Torrero, Diego Haro and Joan Vallès

2. Role of JNK in neurodegenerative diseases

Fèlix Junyent[1,2], Ester Verdaguer[3], Jaume Folch[2], Carlos Beas-Zarate[4]
Mercè Pallàs[1], Carme Auladell[3] and Antoni Camins[1]

[1]Unitat de Farmacologia I Farmacognòsia, Facultat de Farmàcia, Institut de Biomedicina (IBUB), Centros de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Universitat de Barcelona, Spain; [2]Unitat de Bioquimica i Biotecnología, Facultat de Medicina i Ciències de la Salut Universitat Rovira i Virgili, Reus, Tarragona, Spain; [3]Departament de Biologia Cel•lular, Facultat de Biologia Universitat de Barcelona, Barcelona, Spain; [4]Departamento de Biología Celular y Molecular, CUCBA Guadalajara. División de Neurociencias, Centro de Investigación Biomédica de Occidentes IMSS, Guadalajara, Mexico

Abstract. The c-Jun N-terminal kinases (JNK) are members of the MAPK family and can be activated by different stimuli such as cellular stress, heat shock and ultra-violet irradiation. JNKs have different physiological functions and they have been linked to apoptosis in different cell types. Therefore, the JNK signalling pathway is an important target to prevent cell death. In the present chapter, the role of JNKs in neurodegenerative diseases will be discussed, as well as the pharmacological compounds that inhibit this signalling pathway as therapeutic intervention to prevent neuronal death.

Introduction

Mitogen-activated protein kinases (MAPKs) are part of a signalling cascade that allows the cell to respond to exogenous and endogenous stimuli.

Correspondence/Reprint request: Dr. Antoni Camins, Unitat de Farmacologia I Farmacognòsia, Facultat de Farmàcia, Institut de Biomedicina (IBUB), Centros de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Universitat de Barcelona, Barcelona, Avda/Joan XXIII, Spain
E-mail: camins@ub.edu

MAPK translates and integrates signals into complex cytoplasmic and nuclear processes and it is involved in organized cellular responses such as proliferation, differentiation and apoptosis. Currently, three major constituents of the MAPK family have been characterized in mammals: the extracellular signal-regulated kinases (ERKs) (ERK1/2, p42/p44), the c-Jun N-terminal kinases (JNKs) (JNK1, 2 and 3), and the p38 MAPKs consisting of four isoforms (α, β, γ and δ). MAPK pathways are activated either as a result of a series of interactions between the kinase components or through the formation of a signalling complex that contains multiple kinases, driven by a scaffold protein. c-JNKs are activated by phosphorylation of Thr and Tyr residues in the activation loop by mitogen-activated protein kinase kinase 4 (MKK4) and MKK7. Interactions between proteins and scaffold proteins are crucial for MAPK function and regulation in the cell. For instance, kinase suppressor of Ras-1 (KSR) and MEK partner 1 (MP1) act as scaffold proteins for the ERK signalling pathway, whereas JNK-interacting proteins (JIPs) serve as scaffold proteins for the c-JNK pathway. Likewise, β-arrestin 2 acts as a scaffold protein for both the ERK and JNK signalling pathways.

Following activation, c-JNKs can phosphorylate multiple targets and have more than 50 substrates [1]. The substrates identified that are phosphorylated in the nucleus include some hormone receptors, as well as transcription factors such as the activator protein-1 (AP-1), the family of Jun factors (c-Jun, JunB, Jund), Elk-1, p53, the anti-activation transcription factor-2 (ATF-2), JDP2, c-Myc, the NAFT family, the STAT family and the PAX family [1]. For many of these factors, such as c-Jun, ATF-2, Elk-1 and p53, phosphorylation increases its activity and induces transcriptional gene expression [1]. Currently, the most studied substrate for JNK is c-Jun; however, it is not known which isoform is responsible for its phosphorylation. High expression of both the gene and protein of c-Jun precedes or coincides with periods of cell death, such as that occurring during embryonic development [2], after trauma [3], cerebral ischaemia [4] and seizures [5].

c-JNKs have attracted enormous interest because their pathway has been linked to numerous physiological processes, including neuronal death in several neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. In mammals, three genes have been characterized that encode c-JNKs (JNK1, JNK2 and JNK3). These proteins exhibit several differences in their localisation; JNK1 and JNK2 have a wide tissue distribution in the whole body, whereas JNK3 is primarily localized in the central nervous system (CNS), making it an attractive CNS drug target to prevent neurodegenerative diseases. In addition, JNK3 is also found in the heart and testis at low levels.

In this chapter we reviewed the progress in understanding the role of c-JNKs in the pathophysiology of neurodegenerative diseases and we presented current knowledge of JNK inhibitors in clinical trials to treat neurodegenerative disorders.

1. Role of the JNK pathway in apoptosis: Genetically modified experimental models

It is impossible to study the role of c-Jun *in vivo* in the nervous system because knockout mice for c-Jun die of liver failure during embryonic development [6]. However, knockouts for the mammalian Jnk genes *Jnk1*, *Jnk2* and *Jnk3* are viable and have enabled the study of the physiological and pathological roles of the JNK pathway. *Jnk3* (-/-) knockout mice remain viable during development and show normal brain structure [7]. The first evidence of the role of *Jnk3* in neurotoxicity was provided by Yang and colleagues, who demonstrated that in comparison to wild-type mice, *Jnk3* (-/-) mice were less sensitive to seizures induced by kainic acid and neuronal death in the hippocampal CA1 and CA3 areas. Therefore, it was concluded that JNK3 is a suitable target to prevent both neuronal cell death and seizures elicited by KA. In contrast, loss of *Jnk1* or *Jnk2* did not show any effects against KA treatment [8].

Recently, we demonstrated that *Jnk3* (-/-) mice had increased p110 beta protein levels and PI3K activity because of an upregulation of the *pik3cb* gene [9]. Accordingly, the AKT pathway was seen to be activated in *Jnk3* (-/-) mice. However, we did not find any changes in AKT activity in *Jnk1* (-/-) null mice. Therefore, the lack of *Jnk3* selectively increases neuroprotective pathways, but it is unknown how the PI3K/Akt signalling pathway is activated in the absence of JNK3.

All these experimental data suggest that JNK3 is required to induce neuronal stress and apoptosis in adult hippocampi. The disruption of *Jnk1* or *Jnk2* does not affect the nervous system, but double knockout *Jnk1* (-/-) *Jnk2* (-/-) mice die during embryonic development with major alterations in the neural phenotype [10,11]. This is because the neural tube fails to close due to a deficiency in apoptosis. However, the opposite effect has been observed in the developing cortex brain, where there is an increase in apoptosis [10,11]. This indicates that Jnk1 and Jnk2 are necessary for the development of cell death in the neural tube and, in turn, for promoting cell survival during cerebral cortex development. Thus, although c-JNK and c-Jun proteins are pro-apoptotic in different cell types, they may have other functions, as already mentioned. Furthermore, induction of axonal regeneration in axotomised peripheral neurons in an

adult organism appears to be associated with increased expression of c-Jun, suggesting that this transcription factor regulates the expression of genes related to regeneration [12].

Several mechanisms have been proposed to explain how the c-JNK pathway governs the whole process of neuronal death by apoptosis. For example, c-JNKs directly phosphorylate and regulate the pro- and anti-apoptotic activity of members of the B-cell lymphoma 2 (Bcl-2) families. c-JNKs phosphorylate and decrease the anti-apoptotic activity of both Bcl-2 and Bcl-XL. In addition, these kinases phosphorylate the pro-apoptotic protein BAD at Ser-128, thereby potentiating its pro-apoptotic effect [13]. Similarly, c-JNKs phosphorylate the pro-apoptotic proteins Bim and Bcl-2-modifying factor (Bmf), causing their release and translocation to the mitochondria, where they promote the release of mitochondrial proteins such as cytochrome *c,* apoptosis-inducing factor (AIF) and other mitochondrial pro-apoptotic death mediators. An apoptotic process eventually occurs and could be caspase-dependent or independent.

As discussed, the release of cytochrome c and other pro-apoptotic proteins from the mitochondria is regulated by the Bcl-2 protein family. The multi-domain pro-apoptotic Bcl-2 family member Bcl-2-associated X protein (Bax) is essential for cytochrome c release and cell death in neurons in response to different apoptotic stimuli. In contrast, the anti-apoptotic proteins Bcl-2 and Bcl-xL, which can form heterodimers with Bax, inhibit cytochrome c release and protect against cell death. Interestingly, several pro-apoptotic BH3-only members are expressed in neurons and the c-JNK pathway could be involved in the expression of dp5, Bim and PUMA following an apoptotic stimulus. These BH3-only proteins may promote neuronal apoptosis by binding to the anti-apoptotic members of the Bcl-2 family, which would then be unable to interact with Bax. Thus, by acting through both transcriptional and non-transcriptional mechanisms, c-JNKs are a key mediator of neuronal cell death.

It has been widely demonstrated that oxidative stress is involved in the onset of neurodegenerative disorders and favours the activation of MAPKs and the induction of apoptosis in a variety of experimental models [14, 15]. A potential link between oxidative stress and the c-JNK pathway could be by means of apoptosis signal regulating kinase 1 (ASK1). Hence, it has been reported that treatment of embryonic fibroblasts from *ASK1*−/− mice with H_2O_2 does not activate JNK and the cells are resistant to oxidative stress-induced apoptosis [16]. It has also been reported that oxidative stress induced by H_2O_2 in COS7 cells promotes the dissociation of the 14-3-3 protein from ASK1, which leads to the dephosphorylation of ASK1 at Ser-967, resulting in ASK1 and JNK activation [15, 17].

Therefore, it seems that the c-JNK pathway is strongly involved in the process of neuronal death. This hypothesis is further supported by another study which showed that cdk5 activation in experimental models of Alzheimer's disease was associated with reactive oxygen species (ROS) increase and c-JNK activation.

Given the above, it is clear that the inhibition of c-JNKs could be a potent neuroprotective tool. However, suppressing the c-JNK pathway has limitations due to the biological functions involved. For example, it has been shown that c-JNK inhibitors can rescue axotomised neurons, but prevent its regeneration [18]. A possible alternative would be to develop direct targets against specific molecules of the c-JNK pathway; however, this requires more information about the individual actions of the different c-JNK isoforms. In this sense, the work of Zhao and Herdegen not only provided new data on the differential activity of different c-JNK isoforms, but also analysed mitochondria after ischaemic insult [19]. This study suggested that inhibiting the mitochondrial complex MKK: JNK3 attenuated apoptosis without affecting physiological functions.

Likewise, the study of Björkblom and colleagues suggests that the nuclear localisation of c-JNKs is the main factor responsible for cell death, while the cytoplasmic localization is responsible for its physiological functions [20]. This is an important factor to consider when designing drugs to treat neurodegenerative diseases.

2. Control of the JNK3 pathway and neuroprotection

As we have commented above, the first evidence of the involvement of c-JNKs in excitotoxicity was derived from the reduction of seizures activity and prevention of apoptosis in JNK3-deficient mice treated with kainic acid [7]. In addition, mice with an inactive form of the *c-jun* gene (Jun AA: alanine instead of serine at positions 63 and 73) showed resistance to excitotoxic neuronal death. These data opened the study of a specific target to protect against neurodegenerative disorders. Thus, blocking the access of c-JNKs to their substrate c-Jun may offer a suitable target in neuroprotection [21].

2.1. Role of JNK3 and ischaemia

Pirianov et al. were the first to describe the neuroprotective role of the specific isoform of JNK3 in a model of hypoxic–ischaemic injury [22]. They demonstrated that the deletion of JNK3 had a neuroprotective role by reducing ATF-2 phosphorylation, which was associated with the size of the

infarct. Therefore, it was proposed that the JNK3/c-Jun/ATF-2 pathway was likely to be the main route in neural cell death induced by hypoxic–ischaemic injury. Moreover, the authors demonstrated that after the hypoxic–ischaemic injury, JNK3 activation phosphorylated c-Jun, which has been shown to trigger the transcription of a large number of death genes including the pro-apoptotic Bcl-2 family member, Bim, and the death receptors TNFR (p55) and CD95/Fas [23, 24]. Furthermore, JNK3 signalling is implicated in the mitochondrial release of cytochrome c, leading to caspase-3 activation either via a Bim-dependent mechanism or through direct targeting of the mitochondria [23, 24, 25]. *Jnk3* knockout in perinatal brain injury has been linked to a decrease in caspase-3 activity, as well as a reduction in the levels of the pro-apoptotic proteins PUMA and Bim.

The postsynaptic density protein 95 (PSD-95) is a scaffold protein characterized by the presence of several protein-binding domains, including three N-terminal PDZ domains, a signal Src homology region 3 domain and a C-terminal guanylate kinase-like domain. Moreover, the PDZ domains bind to the C-terminus of the NMDA receptor NR2 and KA receptor GluR6 subunit, which is crucial for the grouping of NMDA receptors and KA receptors in the postsynaptic membrane. It has been reported that brain ischaemia alters the GluR6-PSD-95-MLK3 complex in the hippocampus, which affects JNK3 phosphorylation. Moreover, the neuroprotective role of GABA against ischaemic injury occurs through the inhibition of the JNK3 signalling pathway.

2.2. Role of JNK3 in experimental models of Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease. The mechanisms involved in its pathogenesis include oxidative stress production, mitochondrial dysfunction and protein aggregation, which promote the loss of dopaminergic neurons in the substantia nigra pars compacta. Currently, the main problem of PD and all neurodegenerative diseases is that therapy is focused on symptomatic relief. It is necessary to develop neuroprotective therapies that will slow disease progression. The investigation of cell death mechanisms common to several models of experimental PD may identify new drug targets for treatment.

It has been observed that in mice exposed to MPTP, a PD neurotoxin that inhibits mitochondrial complex I, dopaminergic neurons degenerate in the substantia nigra [26, 27]. Inhibiting c-JNKs or their upstream signals may reduce dopamine-mediated neuronal death induced by MPTP, suggesting a possible therapeutic application for c-JNK inhibitors in PD [28]. Pharmacological inhibition of mixed lineage kinases that are upstream of JNK by CEP-1347 attenuates MPTP-mediated nigrostriatal dopaminergic

neuronal loss and MPTP-induced c-JNK activation. Additionally, dopaminergic neuronal death induced by MPTP, rotenone, paraquat and 6-hydroxydopamine all require JNK3 activation. Therefore, JNK3 is a critical and common mediator of dopaminergic neuronal death in PD experimental models.

Hunot and colleagues demonstrated that mice treated with MPTP showed increased COX-2 expression that was mediated by JNK [29]. Interestingly, COX-2 expression is upregulated in PD brains and is generally induced by stress stimuli. Moreover, COX-2 localizes in neurons and its expression is upregulated in numerous pathological conditions, including Alzheimer's disease. Therefore, COX-2 induction might represent an important step in the cascade of molecular events leading to PD neurodegeneration.

Disappointingly, a clinical trial using CEP1347 to treat PD was terminated because it failed to produce significant improvements. The main problem of this compound is its poor selectivity against JNK3, the main target involved in apoptosis. As we previously discussed, JNK1 and 2 are ubiquitously expressed in adult tissues and have important physiological functions; hence, the side effects associated with inhibiting these enzymes limit the tolerable doses of JNK inhibitors. Accordingly, general inhibition of all JNK isoforms, such as that achieved by CEP1347, may be of limited benefit to treat neurodegenerative diseases. On the other hand, JNK3 is neural-specific and does not exhibit high basal activity in the brain. Therefore, selective or specific inhibition of the JNK3 isoform may be more specific to slow down PD progression.

2.3. Role of JNK3 in Alzheimer’s disease

Alzheimer's disease (AD) is currently the leading global cause of dementia in the elderly. At the initial stages, AD is characterised by a mild loss of memory and then progresses to a severe loss of cognitive performance in the advanced stages [30]. From histological images, AD is characterised by a series of markers that include neurofibrillary tangles, senile plaques and a large loss of neurons. Apart from these markers, the loss of neurons is associated with apoptosis, which is probably mediated by several inducers such as reactive oxygen species, β-amyloid, mitochondrial alteration and an inflammatory process that induces microglial activation in the AD brain. In this process of neuronal demise in AD, different signalling pathways are activated and among them, the JNK pathway plays a prominent role. This is based on several lines of evidence: (a) JNK can phosphorylate tau; (b) β-amyloid activates the JNK pathway that then promotes neuronal loss and this activation mediates Aβ toxicity and its adverse effect on long-term potential in the hippocampus and (c) JNK translocates from the nucleus to the cytoplasm

following the course of disease severity [31], and its upstream activator, MEK1, is also activated in vulnerable neurons in AD [32]. Furthermore, it has been previously reported that expression of c-Jun increases in the AD brain and neurons from c-Jun-null mice are resistant to β-amyloid toxicity [33]. A recent study reported that c-Jun is specifically phosphorylated at Ser63 in AD, whereas c-Jun phosphorylation at Ser73 does not change, suggesting it is differential phosphorylation that underlies cell death in AD [34].

Morishima et al. were the first to demonstrate that neuronal hippocampal and cortical cultures of JNK3 knockout mice were partially protected from neuronal apoptosis mediated by β-amyloid [35]. However, c-Jun phosphorylation was not completely inhibited, indicating that JNK1 or JNK2 may be involved in this phosphorylation [35]. On the other hand, a very important point in AD is the formation of β-amyloid fragments that are derived from amyloid precursor protein (APP) after cleavage by beta/gamma secretase. The C-terminal intracellular region (AICD) of APP plays an important functional role in regulating APP metabolism. AICD contains eight potential phosphorylation sites, but one of them, specifically T668, is phosphorylated by several kinases including GSK3β, JNK3, Cdc2 and Cdk5. Likewise, these kinases are associated with neurotoxicity and have been implicated in neurodegenerative diseases. Interestingly, JNK3 is specifically involved in the physiological regulation of AICD during neuronal differentiation, suggesting a role of JNK3 in synaptogenesis [36].

Moreover, Colombo and colleagues used the JNK inhibitor peptide (D-JNKI1) to demonstrate that JNK plays a prominent role in APP production and that the extracellular β-amyloid fragments are also reduced [37]. It has been observed that β-secretase (BACE1) is regulated by BACE1 gene transcription through the JNK/c-Jun signalling pathway. This is important because it has been hypothesized that β-amyloid fragments are mainly responsible for the neurodegeneration in AD.

Studies performed in neuronal cell cultures have shown that JNK3 is involved in the apoptotic process mediated by β- amyloid. This process involves MLK3–MKK7–JNK3 activation, as well as downstream events including p-JNK nuclear localization, c-Jun phosphorylation and Bad translocation to the mitochondria, with the mitochondria then releasing pro-apoptotic proteins.

2.4. JNK and Huntington's disease

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by an autosomal dominant mutation in either of the two copies of the huntingtin gene. Specifically, this disorder is caused by an abnormal expansion of a CAG codon in exon-1 of the gene [38].

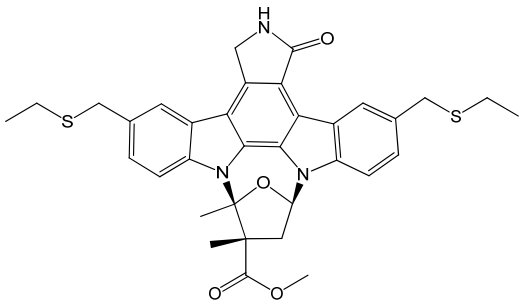
Systemic administration of mitochondrial toxin 3-nitropropionic acid (3-NPA) to experimental animals, such as non-human primates and rodents, produces symptoms similar to those of human HD. Thus, 3-NPA is used in the laboratory to replicate neurodegenerative conditions [39]. The toxin irreversibly inhibits the succinate dehydrogenase (SDH) enzyme, the main constituent of the mitochondrial respiratory chain complex (MCC) II [40].

Treatment of rats and *in vitro* primary striatal cultures with 3-NPA activates the JNK pathway and contributes to neuronal death [41]. This cell loss depends on c-Jun because expression of dominant negative c-Jun protects striatal neurons from cell death mediated by this complex II inhibitor. Likewise, JNK activation appears to be a major factor in the apoptotic death of HN33 cells induced by polyglutamine-expanded huntingtin. Mutated huntingtin with 48 or 89 polyglutamine repeats enhances JNK activation and may trigger apoptosis, while normal huntingtin with 16 repeats fails to activate the JNK pathway.

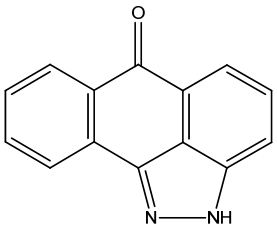
However, a recent study demonstrated that the intraperitoneal administration of 3-NPA to *Jnk3* (-/-) mice was not neuroprotective in contrast to the neurotoxin KA [42]. This suggests that although the JNK pathway may be activated in this model, JNK3 is probably not mainly responsible for neuronal death and other pathways may be involved in neuronal loss.

3. Neuroprotective effects of JNK inhibitors

As already discussed, since the activation of the c-JNK pathway may be a common step in various neurodegenerative diseases, the pharmacological inhibition of JNK is a suitable strategy to protect against neuronal death. In this sense, we highlight three chemical inhibitors: CEP-1347, an inhibitor of the MLK family of the JNK pathway, and SP600125 and AS601245, both selective inhibitors of JNK activity.



CEP-1347



SP600125

Figure 1. Molecular structures of the c-JNK inhibitors CEP-1347 and SP600125.

The main limitation of the CEP-1347 inhibitor is its poor selectivity because it acts upstream of JNK activators, namely the MLKs [43]. This compound has shown neuroprotective effects both *in vitro* and *in vivo* against β-amyloid toxicity, trophic withdrawal in PC12 cells, MPP+ exposure and apoptosis in cerebellar granule cells following serum and potassium deprivation. Moreover, in addition to inhibiting the pro-apoptotic JNK pathway, this drug activates neurotrophic pathways, including the neurotrophin BDNF in a mouse model of HD. Specifically, CEP-1347 increases BDNF mRNA levels in the brain compared to vehicle, which correlates with a reduction of disease progression in a pilot pre-clinical trial in R6/2 mice, an experimental model of HD.

However, although the PRECEPT clinical trial showed that CEP-1347 was safe and well-tolerated in a randomised placebo-controlled study in PD subjects, it was concluded that this drug was not effective to treat PD. Nevertheless, further studies are needed to assess why this drug was not effective.

SP600125 is a reversible ATP-competitive inhibitor that can inhibit JNK, including JNK-1, -2 and -3 isoforms, with high selectivity. The neuroprotective effect of SP600125 has been seen in ischaemic processes [44]. Other studies have further demonstrated that SP600125 can inhibit the phosphorylation of c-Jun and prevent the expression of IL-2, IFN-γ, TNF-α and COX-2, while inactivating Bcl-2 and blocking IL-1-induced accumulation of p-Jun and inducing c-Jun transcription [44, 45, 46, 47].

SP600125 exerts neuroprotective effects against MPTP-induced neurotoxicity in mice, inhibiting JNK signalling and also reducing COX-2 expression [48]. Moreover, SP600125 displays neuroprotective functions in β-amyloid-injected rats, as it has potent memory-enhancing effects and blocks learning deficits induced by β-amyloid [49]. In addition to the neuroprotective properties of SP600125, this compound also improves neuroplasticity. Effectively, this JNK inhibitor increases synaptic transmission in the hippocampus after treatment with β-amyloid in the CA1 area, suggesting a role of JNK in regulating short-term memory formation. More studies are required to evaluate the effects of SP600125 in β-amyloid production in AD models; however, its low water solubility limits its usefulness in human treatment.

AS601245 is also a reversible ATP-competitive inhibitor that inhibits the three JNK isoforms, with a higher affinity for JNK3. This compound exhibits neuroprotective effects in models of ischaemia [50]. It also decreases microglial activation and exerts beneficial effects on memory deficits.

As mentioned above, JNK activity can be regulated by JNK-interacting proteins. JIP-1 is a protein that integrates the positive and negative regulators

of JNK, facilitating the activity of the JNK signalling pathway. In mice, JIP-1 contains a JNK-binding domain (JBD) that mediates the sequestration of JNK in the cytoplasm, thus inhibiting the expression of genes that are activated via the JNK signalling pathway and acts as a functional inhibitor of JNK. Therefore, in addition to chemical inhibitors, JIP-derived peptides have been developed to inhibit JNK activity based on the properties of the protein JIP-1. The most studied peptide is XG-102, also called D-JNK-permeable peptide 1 (D-JNKI). It exerts neuroprotective effects against different models of excitotoxicity *in vitro* and has also been shown to play a neuroprotective role in experimental models of ischaemia, preventing cell death by apoptosis.

Thus, rather than inhibiting the enzymatic activity of JNKs as classical chemical inhibitors do, XG-102 selectively blocks the access of JNK to different substrates, preventing protein-protein interactions without interfering with its activation. Moreover, XG-102 has been observed to show beneficial effects on both hair cell death and the permanent loss of hearing induced by sound trauma [51]. A phase I/II clinical study with XG-102 is currently underway to evaluate the efficacy of this compound in patients with acute acoustic trauma; the study will be completed in 2012. Therefore, in the coming years, more clinical data will shed light on the neuroprotective potential of these compounds.

4. Future perspectives of inhibiting the JNK pathway in the treatment of neurodegenerative diseases

The increase in life span is expected to see an increase in neurodegenerative diseases, with the most important being Alzheimer's disease. The development of neuroprotective drugs is undoubtedly an area of increasing relevance due to the high incidence and prevalence of neurodegenerative diseases and the lack of effective treatments. However, since the exact mechanism of neuronal cell death in neurodegenerative diseases is not known, this limits the success in searching for effective drugs. Given that the process of neuronal death is complex, to at least find a drug that effectively blocks a pathway involved in cell death or delays the progress of AD, PD or HD is considered a success. Apart from c-JNK activation in neurodegenerative diseases, other biochemical parameters such as oxidative stress, mitochondrial alteration, cell cycle re-entry, cytoskeletal alteration, an increase in GSK-3 activation and inhibition of pro-survival pathways (such as the AKT pathway) might also contribute to the neurodegenerative process. Therefore, targeting the c-JNK pathway with effective inhibitors at least provides a powerful way to experimentally achieve neuroprotection, as well as preserving cognitive function, inhibiting apoptosis and having a trophic

function. Unfortunately, clinical studies with CEP-1347 in PD have failed, but the loss of drug efficacy could have been due to multiple causes, such as whether the clinical trial (selected patients) for the specific compound was well designed or not. Other possible causes could have been a failure of the dose, administering the drug when the neurons were already dead, or the drug rescuing non-functioning neurons that could not perform their physiological roles. Therefore, it is necessary to conduct more clinical studies with inhibitors of the c-JNK pathway. It would probably be interesting to consider clinical trials with two drugs, such as an antioxidant, a GSK3β inhibitor or other c-JNK antagonists, since more than one pathway may be involved in neuronal death and this might be more effective in treating neurodegenerative diseases.

Acknowledgements

We thank the Language Advisory Service of the University of Barcelona for revising the manuscript. This study was supported by the grants SAF-2009-13093 and BFU2010-19119/BFI from the Ministerio de Educación y Ciencia, PI080400 from the Instituto de Salud Carlos III, 2009/SGR00853 from the Generalitat de Catalunya and grant 610RT0405 from the Programa Iberoamericano de Ciencia y Tecnologia para el Desarrollo (CYTED).

References

1. Bogoyevitch, MA., Kobe, B. 2006, *Microbiol. Mol. Biol. Rev.*, 70(4), 1061.
2. Sun, W., Gould, TW., Newbern, J., Milligan, C., Choi, SY., Kim, H., Oppenheim, RW. 2005, *J. Neurosci.*, 25(23), 5595.
3. Raivich, G., Bohatschek, M., Da Costa, C., Iwata, O., Galiano, M., Hristova, M., Nateri, AS., Makwana, M., Riera-Sans, L., Wolfer, DP., Lipp, HP., Aguzzi, A., Wagner, EF., Behrens, A. 2004, *Neuron,* 43(1), 57.
4. Wessel, TC., Joh, TH., Volpe, BT. 1991, *Brain Res.,* 567(2), 231.
5. Morgan, JI., Curran, T. 1988, *Cell Calcium,* 9(5-6), 303.
6. Hilberg, F., Aguzzi, A., Howells, N., Wagner, EF. 1993, *Nature,* 365(6442), 179.
7. Yang, DD., Kuan, CY., Whitmarsh, AJ., Rincón, M., Zheng, TS., Davis, RJ., Rakic, P., Flavell, RA. 1997, *Nature,* 389(6653), 865.
8. Brecht, S., Kirchhof, R., Chromik, A., Willesen, M., Nicolaus, T., Raivich, G., Wessig, J., Waetzig, V., Goetz, M., Claussen, M., Pearse, D., Kuan, CY., Vaudano, E., Behrens, A., Wagner, E., Flavell, RA., Davis, RJ., Herdegen, T. 2005, *Eur. J. Neurosci.*, 21(2), 363.
9. Junyent, F., de Lemos, L., Verdaguer, E., Folch, J., Ferrer, I., Ortuño-Sahagún, D., Beas-Zárate, C., Romero, R., Pallàs, M., Auladell, C., Camins, A. 2011, *J. Neurochem.*, 117(2), 244.
10. Kuan, CY., Yand, DD., Samanta Roy, DR., Davis, RJ., Rakic, P., Flavell, RA. 1999, *Neuron*, 22(4), 667.

11. Sabapathy, K., Jochum, W., Hochedlinger, K., Chang, L., Karin, M., Wagner, EF. 1999, *Mech. Dev.*, 89(1-2), 115.
12. Herdegen, T., Skene, P., Bähr, M. 1997, *Trends Neurosci.*, 20(5), 227.
13. Donovan, N., Becker, EB., Konishi, Y., Bonni, A. 2002, *J. Biol. Chem.*, 277(43), 40944.
14. Bhat, NR., Zhang, P. 1999, *J. Neurochem.*, 72(1), 112.
15. Goldman, EH., Chen, L., Fu, H. 2004, *J. Biol. Chem.*, 279(11), 10442.
16. Tobiume, K., Matsuzawa, A., Takahashi, T., Nishitoh, H., Morita, K., Takeda, K., Minowa, O., Miyazono, K., Noda, T., Ichijo, H. 2001, *EMBO Rep.*, 2(3), 222.
17. Zhang, L., Chen, J., Fu, H. 1999, *Proc. Natl. Acad. Sci. USA.*, 96(15), 8511.
18. Newbern, J., Taylor, A., Robinson, M., Lively, MO., Milligan, CE. 2007, *Neuroscience*, 147(3), 680.
19. Zhao, Y., Herdegen, T. 2009, *Mol. Cell. Neurosci.*, 41(2), 186.
20. Björklom, B., Vainio, JC., Hongisto, V., Herdegen, T., Courtney, MJ., Coffey, ET. 2008, *J. Biol. Chem.*, 283(28), 19704.
21. Behrens, A., Sibilla, M., Wagner, EF. 1999, *Nat. Genet.*, 21(3), 326.
22. Pirianov, G., Brywe, KG., Mallard, C., Edwards, AD., Flavell, RA., Hagberg, H.,Mehmet, H. 2007, *J. Cereb. Blood Flow Metab.*, 27(5), 1022.
23. Whitfield, J., Neame, SJ., Paquet, L., Bernard, O., Ham, J. 2001, *Neuron*, 29(3), 629.
24. Kuan, CY., Whitmarsh, AJ., Yang, DD., Liao, G., Schloemer, AJ., Dong, C., Bao, J., Banasiak, KJ., Haddad, GG., Flavell, RA., Davis, DJ., Rakic, P. 2003, *Proc. Natl. Acad. Sci. USA*, 100(25), 15184.
25. Borsello, T., Clarke, PG., Hirt, L., Vercelli, A., Repici, M., Schorderet, DF., Bogousslavsky, J., Bonny, C. 2003, *Nat. Med.*, 9(9), 1180.
26. Oo, TF., Henchcliffe, C., James, D., Burke, RE. 1999, *J. Neurochem.*, 72(2), 557.
27. Saporito, MS., Hudkins, RL., Maroney, AC. 2002, *Prog. Med. Chem.*, 40, 23.
28. Borsello, T., Forloni, G. 2007, *Curr. Pharm. Des.*, 13(18), 1875.
29. Hunot, S., Vila, M., Teismann, P., Davis, RJ., Hirsch, EC., Przedborski, S., Rakic, P., Flavell, RA. 2004, *Proc. Natl. Acad. Sci. USA.*, 101(2), 665.
30. Smith, MA. 1998, *Int. Rev. Neurobiol.*, 42, 1.
31. Zhu, X., Raina, AK., Rottkamp, CA., Aliev, G., Perry, G., Boux, H., Smith, MA. 2001, *J. Neurochem.*, 76, 435.
32. Zhu, X., Sun, X., Lee, HG., Siedlak, SL., Perry, G., Smith, MA. 2003, *J. Neurochem.*, 86(1), 136.
33. Anderson, AJ., Su, JH., Cotman, CW. 1996, *J. Neurosci.*, 16(5), 1710.
34. Pearson, AG., Byrne, UT., MacGibbon, GA., Faull, RL., Dragunow, M. 2006, *Neurosci. Lett.*, 398(3), 246.
35. Morishima, Y., Gotoh, Y., Zieg, J., Barrett, T., Takano, H., Flavell, R., Davis, RJ., Shirasaki, Y., Greenberg, ME. 2001, *J. Neurosci.*, 21(19), 7551.
36. Kimberly, WT., Zheng, JB., Town, T., Flavell, RA., Selkoe, DJ. 2005, *J. Neurosci.*, 25(23), 5533.
37. Colombo, A., Bastone, A., Ploia, C., Sclip, A., Salmona, M., Forloni, G., Borsello, T. 2009, *Neurobiol. Dis.*, 33(3), 518.
38. No authors listed. 1993, *Cell*, 72, 971.
39. Brouillet, E., Jacquard, C., Bizat, N., Blum, D. 2005, *J. Neurochem.*, 95(6), 1521.

40. Brouillet, E., Guyot, MC., Mittoux, V., Altairac, S., Condé, F., Palfi, S., Hantraye, P. 1998, *J. Neurochem.*, 70(2), 794.
41. Garcia, M., Vanhoutte, P., Pages, C., Besson, MJ., Brouillet, E., Caboche, J. 2002, *J. Neurosci.*, 22(6), 2174794.
42. Junyent, F., de Lemos, L., Verdaguer, E., Pallàs, M., Folch, J., Beas-Zárate, C., Camins, A., Auladell, C. 2011, *Neuropathol. Appl. Neurobiol.*, doi: 10.1111/j.1365-2990.2011.01214.x.
43. Saporito, MS., Hudkins, RL., Maroney, AC. 2002, *Prog. Med. Chem.*, 40, 23.
44. Guan, QH., Pei, DS., Zhang, QG., Hao, ZB., Xu, TL., Zhang, GY. 2005, *Brain Res.*, 1035, 51.
45. Bennett, BL., Sasaki, DT., Murray, BW., O'Leary, EC., Sakata, ST., Xu, W., Leisten, JC., Motiwala, A., Pierce, S., Satoh, Y., Bhagwat, SS., Manning, AM., Anderson, DW. 2001, *Proc. Natl. Acad. Sci. USA.*, 98(24), 13681.
46. Shin, M., Yan, C., Boyd, D. 2002, *Biochim. Biophys. Acta*, 1589(3), 311.
47. Han, Z., Boyle, DL., Chang, L., Bennett, B., Karin, M., Yang, L., Manning, AM., Firestein, GS. 2001, *J. Clin. Invest.*, 108(1), 73.
48. Whang, Y., Zhang, Y., Wei, Z., Li, H., Zhou, H., Zhang, Z., Zhang, Z. 2009, *J. Neurol. Sci.*, 285(1-2), 172.
49. Ramin, M., Azizi, P., Motamedi, F., Haghparast, A., Khodagholi, F. 2011, *Behav. Brain Res.*, 217(2), 424.
50. Carboni, S., Hiver, A., Szyndralewiez, C., Gaillard, P., Gotteland, JP., Vitte, PA. 2004, *J. Pharmacol. Exp. Ther.*, 310(1), 25.
51. Whang, J., Ruel, J., Ladrech, S., Bonny, C., van de Water, TR., Puel, JL. 2007, *Mol. Pharmacol.*, 71, 654.