Targeting Stress Activated Protein Kinases, JNK and p38, as New Therapeutic Approach for Neurodegenerative Diseases

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Abstract: Signal transduction pathways involving the activation of c-Jun N-terminal kinases (JNK) and p38 mitogen-activated protein kinase (p38MAPK), also called stress-activated protein kinases, have been implicated in many cellular processes such as proliferation, differentiation and death of a variety of cell populations. Growing evidence indicates that these pathways can strongly contribute to the neuronal death associated to neurodegenerative diseases such as Alzheimer's, Parkinson's, amyotrophic lateral sclerosis and cerebral ischemia . These kinases can be activated by a variety of toxic stimuli such as oxidative stress, excitotoxicity, inflammatory cytokines through different signalling cascades. Once activated these kinase cascades may induce alterations in the cellular function through transcriptional activity, alterations of cytoskeletal proteins and production and release of inflammatory molecules, all factors highly implicated in the neurodegenerative processes.

Thus considerable effort is being addressed to the manipulation of these signal transduction pathways as a potential strategy for therapeutic interventions in neurodegenerative disorders. In this review we will examine the role of JNK and p38MAPK in neurodegeneration and we will illustrate the progresses in the development of inhibitors targeting these stress activated protein kinase pathways as therapeutic approach to neurodegenerative disorders.

Keywords: Stress activated protein kinases, c-Jun N terminal kinase (JNK), p38 mitogen activated protein kinase (p38MAPK), neurodegeneration, Stroke, Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease(PD).

INTRODUCTION

Mitogen activated protein kinases (MAPK) are highly conserved signal transduction modules whose function and regulation have been conserved during evolution from unicellular organisms to complex organisms including humans [1]. In mammalian cells the MAPK family consists of three main kinase subfamilies, the c-Jun-N-terminal kinases (JNKs), the p38 kinases (p38MAPK) and the extracellular signal-regulated kinases (ERKs). All these kinases are implicated in many cellular processes such as proliferation, differentiation and death of a variety of cell populations by regulating cellular activities ranging from gene expression, mitosis, movement and programmed death. MAPKs phopshorylate specific serines and threonines of target protein substrates that include other protein kinases, phospholipases, transcription factors and cytoskeletal proteins. In addition, they propagate and amplify the external stimuli from the plasma membrane to the cytoplasm and finally to the nucleus with a regulated mechanism of phosphorylation and dephosphorylation which is organised by specific MAPKs kinases and phosphatases.

The JNKs (JNK1, 2 and 3) are critical regulators of gene transcription playing a role in the degenerative processes that depend on de novo protein synthesis. The p38MAPKs represent a family of four homologous kinases which derive from different genes (p38 , , and). These kinases are rapidly activated by inflammatory cytokines and environmental stresses and are considered relevant for the cell death process Fig. (**1**). The ERK family which includes two members, ERK1 and ERK2 is instead mainly involved in the control of cell proliferation and differentiation by regulating meiosis, mitosis and postmitotic functions. The MAPKs are widely expressed in many tissues including the nervous tissue. Growing evidence indicates that these pathways, in particular JNKs and p38MAPK, may strongly contribute to the neuronal death associated to neurodegenerative diseases such as Alzheimer's, Parkinson's, amyotrophic lateral sclerosis and cerebral ischemia. This review will focus on the mechanisms of activation of these two intracellular signalling cascades in the central nervous system and their implications for the development of neuroprotective therapies.

REGULATION OF JNK SIGNALLING PATHWAY : STIMULI AND SUBSTRATES

The c-Jun N-terminal Kinase (JNK) is a MAPK that responds more heavily to a variety of stimuli collectively designated as "stress-signals". These include UV, irradiation, osmotic stress, heat shock, oncogenic transformation, inflammatory responses and an excessive release of neurotransmitter [2]. Many of these environmental, physical and chemical insults can induce neuronal death.

In mammals JNK1, 2 and 3 are encoded by three different genes: jnk1, jnk2 and jnk3. JNK1 and 2 are ubiquitously expressed while the JNK3 is selectively expressed in the

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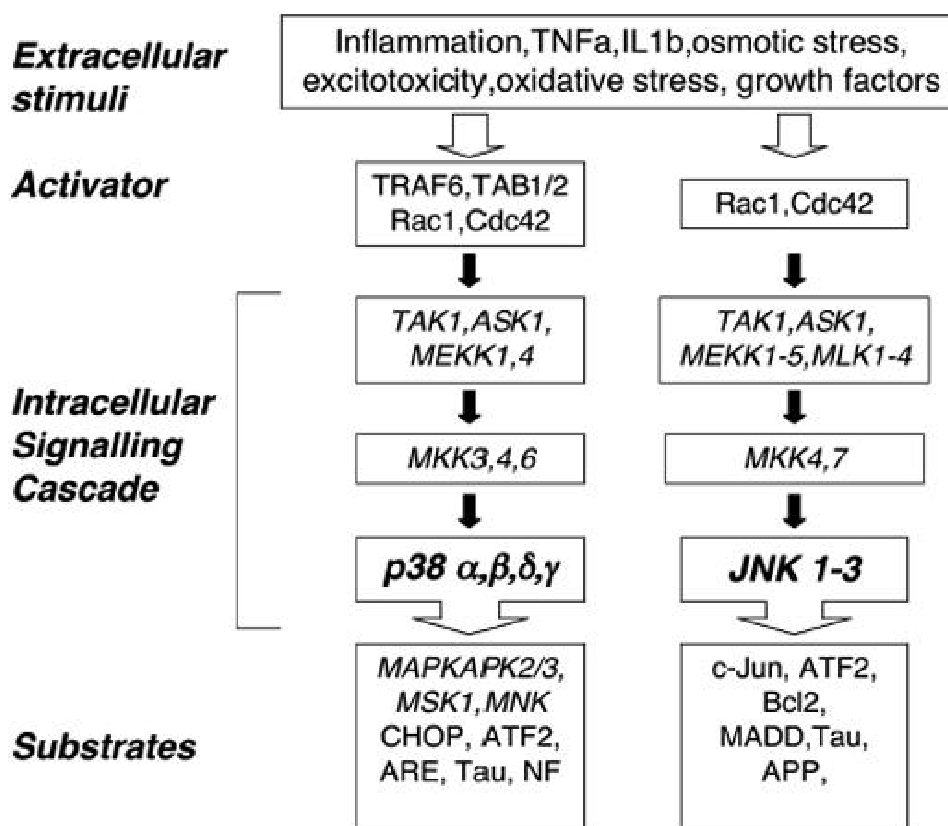

Fig. (1). MAPK signalling pathways. Schematic diagram of the major putative activators and downstream targets of p38MAPK and JNK. Inflammatory cytokines, i.e. TNF and IL1 , cell stressful stimuli such as osmotic or oxitadive stress, growth factors,i.e.TGF ,NGF, excessive stimulation of glutamate receptors activate a cascade of iuntracellular kinases (shown in italic) leading to the phosphorylation of different substrates. Selected examples of target substrates including transcription factors (c-Jun, ATF2, ARE, CHOP), cytoskeletal proteins (Tau, Neurofilaments), mitochondria proteins (Bcl2) and other cytosolic proteins (APP) are shown.

brain, heart and testis [3]. An alternative splicing of the transcripts can lead to two isoforms for each product of appreciatively 46-49 kDa and 55-57kDa.

The different isoforms inside the JNK family further increase diversity by having different behavior towards the substrates that are target of JNK [4, 5]. In fact the presence of tissue-specific JNK isoforms (JNK3/brain) is certainly an important determinant that induces two different cell-types to produce a different cell-response when activated by an identical stimulus.

Regulation of the JNK pathway is extremely complex and is influenced by many MAPK kinases. The mechanism of JNK activation involves dual phosphorylation on Thr183 and Tyr185 residues [6],[7]. This phosphorylation is directly mediated by two upstream kinases: MKK4 and MKK7 which are in turn phosphorylated by other MAPKKKs, that include ASK1, TAK1, MEKK1 and MEKK2/3. JNK phosphorylates a variety of nuclear factors such as c-Jun, ATF2 and Elk but also cytoplasmic substrates such as cytoskeletal proteins, mitochondrial proteins like Bcl-2 and Bcl-xl, the glucocorticoid receptor, Tau or the amyloid precursor protein (APP) membrane protein.

Another important element that regulates the signal specificity of JNK pathway is the scaffold proteins that create multienzyme complexes. The scaffold proteins contribute to physically ordered protein-protein interactions accelerating the signal and to focus the pathway in a particular ntracellular compartments. This is of major importance and contribute to control the spatial-temporal regulation of JNK signal and its specificity [8].

The JIPs (JNK-interacting) proteins aggregate upstream activating elements of the JNK cascade [9] and they create a functional signalling module that highly controls the specificity of signal pathway. Recent studies demonstrate that also the beta-arrestin-2 protein can bind components of the JNK pathway [10, 11] and this group of proteins can have important functions in the modular organization of this signalling complex [12]. JNK cascade can result both in changes of gene expression and in functional regulation of pre-existing proteins. Since many of these events are associated with pathological functions in the Central Nervous System (CNS), JNK pathway offers many opportunities for design inhibitors with attractive therapeutic interest.

REGULATION OF P38MAPK: STIMULI AND SUBSTRATES

The p38MAPK family comprises four isoforms: , , and which differ in their tissue expression and affinity for upstream activators and downstream effectors. The and

are widely expressed in different tissues and are the major isoforms within the CNS whereas e isoforms have more limited distribution, the being expressed exclusively in the muscle [13]. In neurons the p38 is distributed in the cytoplasm and the nucleus whereas is confined to the nucleus [14]. All the isoforms are activated by dual phosphorylation of threonine 180 and tyrosine 182 residues in a Thr-Gly-Tyr motif near the active site [13]. This occurs for all isoforms by the same upstream kinases, MAP kinase kinase (MKK) 3 and 6, in response to inflammatory and stressful stimuli, tipically tumor necrosis factor- (TNF), interleukin 1 (IL1), heat shock, UV irradiation [13].

MKK4 which was originally known to phosphorylate mainly JNK, has been recently shown to activate also the p38MAPK suggesting an interaction among intracellular cascades. All these MKKinases are in turn activated (phosphorylated) by a bulk of other kinases (MKKKinases) which include ASK1, TAK1, MEKK4, MLK (mixed lineage kinases) and that function as first up-stream signal evoked by diverse extracellular stimuli [15]. Although interfering with each of these upstream kinases could potentially inhibits both JNK and p38MAPK pathways at the same time, is still quite unknown how they are distributed and how they can possibly interact to activate the two different pathways. In fact, we recently observed that activation of one of these upstream kinases (ASK1) occurs in degenerating motor neurons of a mouse model of amyotrophic lateral sclerosis and this effect was associated with the p38MAPK but not with JNK-activation [16].

However, p38 can also be activated by MAPKK-independent mechanisms. For example, TAB1 (transforming growth factor– -activated protein kinase 1 (TAK-1)–binding protein) binds and activates TAK1, a MAP3K that can phopshorylate both the JNK and p38MAPK [17]. Interaction between TAB1 and p38 was also reported, which allows p38 to autophosphorylate and activate itself [18]. However, this mechanism has not been confirmed by other groups [17]. TAB1 is also a physiological substrate of p38 and once it becomes phosphorylated it may downregulate TAK1. This results in a feedback control mechanism that limits the activation of the pathway [17]. Another way of activation of p38MAPK upstream of MAP kinases occur through the interaction of the low molecular weight GTP-binding proteins in the Rho family as Rac1 and Cdc42 with MKK1 or MLK1 and both result in the activation of p38MAPK via MKK3 [19]. Thus, activation mechanisms of p38MAPK can vary in different cells and under various physiopathological conditions. Strategies aimed to intercept the p38MAPK pathway at different levels in the upstream cascade could therefore provide a more specific intervention to counteract the pathological process without compromise the normal physiology.

The activity of p38MAPK can be downregulated by dephosphorylation mediated by various protein phophatases such as protein phosphatase 1 or protein phosphatase 2A. *In vitro* studies have shown that p38MAPK can also be dephosphorylated by MAPK phosphatases MKP1, MKP7 and to a lesser extent by MKP5 [20-22]. These phosphatases are, in some cases, activated by phopshorylated p38MAPK as self control for its activation suggesting a mechanism for tight regulation of active p38MAPK.

The substrates of p38MAPKs include other kinases, cytosolic proteins and transcription factors [13, 23]. A major substrate of p38 and is MAPK-activated protein kinase (MPKAPK)-2. This kinase has been shown to activate various substrates including small heat shock protein 27 (Hsp27), the cAMP response element binding protein (CREB) and transcription factor ATF1 [24]. Other types of substrates for p38 are cytoskeletal proteins such as the neurofilaments and the protein tau, the phosphorylation of which is known to play an important role in neuropathologies such as amyotrophic lateral sclerosis and Alzheimer disease [25, 26]. The p38 and forms are less effective at activating the downstream kinases and better at phosphorylating transcription factors such as activating–transcription factor (ATF)2, Elk1, CHOP, MEF-2 and SAP-1. Moreover, numerous gene families are regulated by the p38MAPK pathway and they include cytokines, transcription factors and cell surface receptors. p38MAPK is also known to regulate the expression and production of cytokines at the post-transcriptional level through the phorphorylation of the AU rich element in their 3' untraslated regions (UTR) which results in the release, stabilisation and translation of their mRNAs [27, 28]. All these downstream activities of p38MAPK phosphorylation are frequently cell-type specific.

ROLE OF JNK IN NEURODEGENERATION

Numerous studies have demonstrated that JNK pathway might be crucial in various CNS diseases states. In fact JNK phosphorylates numerous substrates related to neuronal death. In particularly JNK3 is implicated in excitotoxicity, ischemia but also in Parkinson (PD) and Alzheimer diseases (AD) [29-34].

JNK could act both at transcriptional or at post-transductional level. At the transcriptional level, JNK acts on several members of the AP-1 group of transcription factors, the most important of which is c-Jun, activated by phosphorylation on Ser 63 and 73 [35] and strongly related to neuronal death. Regarding cytoplasmic substrates (post-transductional level) JNK phosphorylates a lot of proteins associated to cell death such as Bcl-2 family protein [36],[37], involved in the release of cytochrome c from mitochondria. DENN/MADD was also identified as a substrate for JNK3 [38]. Increasing evidence supports a strong correlation between low MADD/DENN expression and neuronal loss [39, 40]. The meaning of MADD phosphorylation by JNK is still unclear, but recently it has been shown that MADD down-regulation correlates with neuronal cell death in AD brain and hippocampal neurons [39]. Finally Tau and APP, two proteins strongly related to neurodegenerative diseases, are also substrates of JNK. A recent study reported that all three JNK isoforms phosphorylate Tau at many serine/threonine-prolines [41]. Tau phosphorylation reduces ability of Tau to promote microtubule assembly, which in turn compromise neuronal transport and function.

APP is phosphorylated by JNK3 both *in vitro* [42] and *in vivo* [43] and APP phosphorylation by JNK is enhanced by the association of APP with scaffold protein JIP-1b [44].

Moreover *in vivo* experiments demonstrated that in AD brains JNK co-localize with Tau pathology and that the JNK activity is increased over control brains [45]. For these reasons activation of JNK pathway in AD has been investigated and in post-mortem pathological brain phosphorylated c-Jun staining were found only in the affected regions of the disease,cerebral cortex and hippocampus but not in the other unaffected regions [45, 46] of the brain. All the results suggested a strong implication of JNK pathway in the pathology of AD.

ROLE OF p38MAPK IN NEURODEGENERATION

In vitro and *in vivo* studies have demonstrated that p38MAPK pathway plays a pivotal role in neuronal death occurring upon a variety of harmful stimuli such as oxidative stress, excitotoxicity and inflammation that are implicated in various neurodegenerative disorders such as PD, AD and amyotrophic lateral sclerosis (ALS) Fig. (**2**). For example, studies *in vitro* have demonstrated that activation of p38MAPK is part of the intracellular cascade leading to the apoptotic death induced by reactive oxygen and nitric oxide species in primary mesencephalic dopaminergic neurons or dopaminergic cell lines that are used as experimental paradigms for PD [47, 48].

In the cultured primary motor neurons, that are the specific cellular targets of ALS, the Fas-triggered death pathway involved the activation of the Daxx, ASK1 and p38MAPK cascade which eventually lead to cell death through the induction of neuronal nitric oxide synthase (NOS) [49]. That p38MAPK might play a role in the pathogenesis of ALS, has also been suggested by a series of *in vivo* studies reporting a prominent activation of p38MAPK in the spinal cord of $SOD1^{G93A}$ mice, one of the most representative model of ALS [26, 50, 51]. In particular, a selective increase of phosphorylated p38MAPK was found in spinal motor neurons before the onset of clinical symptoms suggesting a primary role in the development of the disease [51]. This was associated to the activation of upstream kinases MKK3-6, MKK4 and ASK1 and the concomitant upregulation of the TNF receptors (TNFR1 and TNFR2) indicating a possible functional link between TNF and p38MAPK pathway in determining motor neuron death [16]. Interestingly, we found that in motor neurons of sporadic ALS patients, the abnormal intracellular perikaryal aggregates containing ubiquitin and neurofilaments, hallmarks of this pathology, were also strongly immunostained for phospho-p38MAPK [52]. Since the isoform of p38MAPK is known to phosphorylate the side-arm domains of middle and heavy neurofilament chains, it is likely that activation of p38MAPK might contribute to their accumulation in motor neuron perikarya and axons leading to cell dysfunction and death[26].

Another cytoskeleton substrate of p38MAPK is the protein Tau [25] whose accumulation in its phosphorylated form represents a pathological hallmark of AD. In line with this, the immunoreactivity for phospho-p38MAPK was enhanced in hippocampal neurons of AD patients containing phosphotau at early stage of neurofibrillary degeneration [53]. The activation of p38MAPK in these patients has been recently associated also with the phosphorylation of MSK1 in Thr581, providing further insight in the role of this death pathway in AD [54]. Another potential down stream mechanism of p38MAPK activation involved in AD cell death is related to the neurotoxicity of the beta-amyloid. In fact, the neuronal damage triggered by the interaction of beta-amyloid peptide with p75NTR in a neuroblastoma cell line and produced through NF-kB translocation and p53 activation, was mediated by activation of p38MAPK and JNK[55].

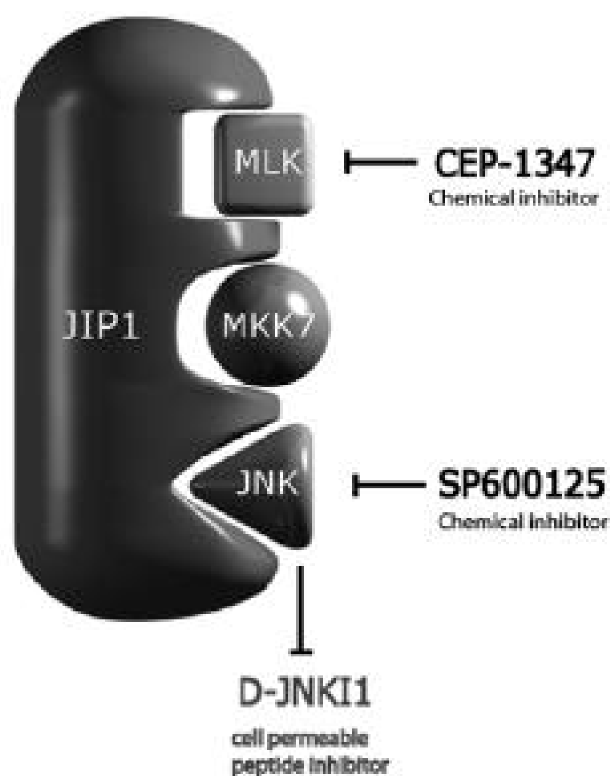

Fig. (2). Multifunctional role of p38MAPK in the neurodegeneration.
Different toxic stimuli such as an excess of glutamate, cytokines, cell death receptor activators, intracellular or extracellular NO and ROS may induce the activation (phosphorylation) of p38MAPK in neurons through a upstream kinase cascade which includes Ask1 and MKK3-6/MKK4. The activity of p38MAPK in reactive astrocytes and microglia may regulate the release of these toxic mediators. Phosphorylated p38MAPK may play a role in neuron degeneration through different potential mechanisms: i) hyperphosphorylating the neurofilaments, causing their aberrant accumulation; ii) increasing intracellular levels of NO with consequent increase in protein nitration; iii) altering the mitochondrial function which may induce release of cytochrome C followed by activation of caspase cascade and cell death; iiii) activating several transcriptional factors which regulate cell death and survival.

A potential role of p38MAPK has been proposed also for other neurological disorders such as prion protein related diseases or HIV-associated dementia. Activation of p38MAPK, and protective effects of its inhibitors, have been observed in SH-SY5Y human neuroblastoma undergoing apoptotic death when exposed to the peptide corresponding to the sequence 106-126 of prion protein [56, 57]. Moreover, HIV-1 glycoprotein gp120 and stromal cell-derived factors

(SDF) induced neuronal apoptosis in mixed cortical co-cultures via p38MAPK activation [58].

The excitotoxicity is a mechanism common to many CNS degenerative disorders and there are increasing evidence that aberrant activation of glutamate receptors can cause neuron degeneration via p38MAPK activation. This effect is likely mediated by the increase of intracellular free calcium via signalling molecules such as PYK, Src and Ras-GRF that trasduce Ca^{2+} signal to MAPK cascade [59, 60]. The inhibition of p38MAPK protects cultured neurones against excitotoxic injury in different experimental models [61-64] and this may provides useful indications for the clinical utility of these compounds in the CNS diseases. *In vivo* studies, also have shown that activation of glutamate receptors through the intracortical injection of the glutamate analogue quinolinic acid in rats causes neuron death preceded by transient increase of p38MAPK immunoreactivity in neurones and astrocytes surrounding the injection site [65]. Although intraneuronal p38MAPK activation is likely responsible of the neurodegenerative process, activation of p38MAPK in glial cells can also contribute to neurodegeneration through the release of toxic factors. In fact, it is well known that p38MAPK pathway is implicated in the production and release of cytokines such as TNF and IL-1 by astrocytes and microglia [66]. These cytokines in turn can activate p38MAPK to induce the generation of other mediators toxic to neurons like nitric oxide determining a vicious cycle that likely contribute to propagate the damage to adjacent cells. In fact, inhibitors of p38MAPK pathway abolish inducible nitric oxide synthase(iNOS) expression and nitric oxide release induced by combined TNF /IL-1 stimulation in cultured astrocytes [67] and protect neuron death induced by LPS treatment in neuron-glia co-cultures [68]. Thus, activation of p38MAPK pathway in both neuronal and glial cells may play a key role in the development and progression of the CNS disorders.

INHIBITORS OF JNK

JNK inhibitors could be divided in two classes: chemical compounds and cell permeable peptide inhibitors.

Chemical Compounds

This compounds that are competitive inhibitors of the ATP-binding site of the kinase and for this reason they have only a moderate specificity.

Two of them, CEP-1347 and SP600125 have been the subjects of peer-reviewed studies as described in Bozyczko-Coyne and Bogoyevitch reviews [69, 70] but the published information on their efficacy in clinical trials remains limited.

CEP-1347 is an inhibitor of the MLK group of MAPKKK Fig. (3). CEP-1347 blocks the upstream component in the JNK pathway in fact it competes with ATP to bind MLKs family [71]. It has been shown that CEP-1347 is able to prevent neuronal cell death *in vivo* models of Alzheimer's disease, Parkinson's disease and cochlear hair cell death [72, 73].

SP600125 is another ATP competitive inhibitor of JNK pathway Fig. (3). However this compound inhibits at a different level of the cascade: SP600125 prevents JNK activation. However it is not specific for JNK; in fact has an inhibitory activity against ERK and p38MAPK [74]. Interesting SP600125 protected dopaminergic neurons in the MPTP model of Parkinson's disease [73] and attenuated cell death in a model of Alzheimer's disease neurotoxicity [75]. Moreover, this compound inhibited leukocyte recruitment in a rat inflamed lung model [76] and was active in the rat rheumatoid arthritis model [77]

Cell Permeable Peptide Inhibitor

The cell-permeable JNK inhibitor is an efficient and specific inhibitor of JNK action. The inhibitory action of D-JNKI-1 (cell-permeable JNK inhibitor) peptide is fundamentally different from that of classical small chemical inhibitors [31, 78]. D-JNKI-1 does not inhibit JNK's enzymatic activity, but selectively blocks access to many of its substrates by a competitive mechanism and prevents protein-protein interactions [31, 78, 79] Fig. (**3**). Furthermore this peptide did not interfere with the activities of the other kinases [31, 78], proving its exceptional superiority. D-JNKI-1 peptide (which is the D-retro-inverso form, made of aminoacids in reversed sequence order) has been engineered by linking the 20 amino acid JNK-inhibitory sequence of IB1/JIP-1 scaffold protein (JBD_{20}) to the 10 amino acid HIV-transporter sequence [79]. In another study, Barr and collaborators [80] found that a shorter peptide sequence (RPKRPTTLNLF=TI-JIP) based on amino acids 143-153 on the JBD of JIP-1, partly overlapped with the sequence described by Bonny [79] and was also able to prevent c-Jun phosphorylation.

This inhibitor offers numerous therapeutic opportunity in preventing neuronal death in the CNS. In a recent study we showed that the D-JNKI-1 peptide completely inhibited excitotoxicity in primary culture and strongly prevented neuronal loss against two different models, transient and permanent, of middle cerebral artery occlusion (MCAO) [31]. However other authors have demonstrated powerful protection in different models of CNS injury: D-JNKI-1 protects against toxic drug and acoustic trauma induced auditory hair cell death [81] and against retinal ganglion cells death following optic nerve crush [82].

INHIBITORS OF p38MAPK

Early after discovering that the p38MAPK signalling pathways played an important role in cytokines production, pharmaceutical development has been focused on molecules that inhibit this kinase for the treatment of inflammatory disorders. Since the initial bicyclic pyridynil imidazole (SKF 86002) empirically found to inhibit the production of cytokines induced by lipopolysaccharide (LPS) in cell lines [83], several structurally related p38MAPK inhibitors have been developed to improve kinase selectivity and potency. The prototype of this class of compounds is the SB203580 [15, 84]. Recent works have described the X-ray crystal structures of p38 MAPK complexed with pyridinyl/ pyrimidinyl imidazole compounds , i.e. SB220025 and SB203580, demonstrating that these inhibitors bind to the inactive enzyme in an ATP-competitive manner [15, 85] preventing its phosphorylation. In particular, the inhibitors bind to the region

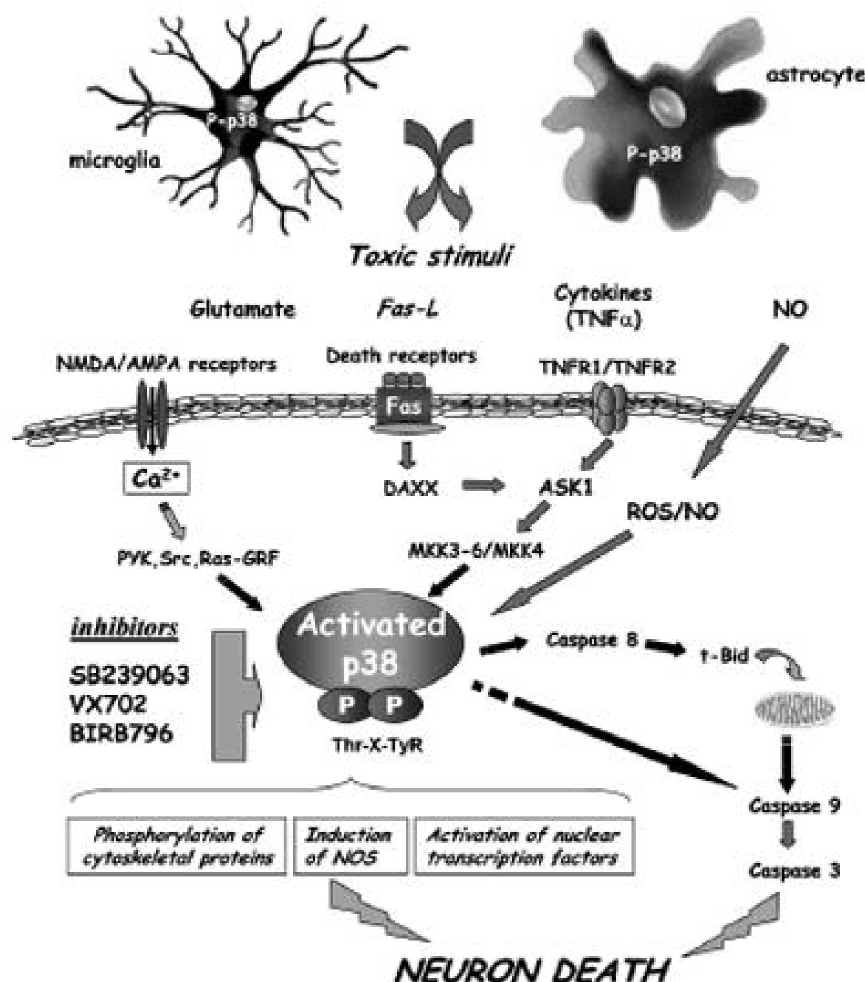

Fig. (3). Modular organization of the JNK signaling pathway.

JIP-1 scaffold protein binds to JNK, MKK7, one of the two JNK activators, and to members of the mixed-lineage protein kinases (MLK) group. The assembly of the JNK module by a scaffold may lead to a more efficient activation of JNK. The two small chemical inhibitors of the JNK pathway are acting at different levels of the cascade; whilst CEP-1347 inhibits MLK, SP600125 inhibits JNK activation. Whereas the cell permeable peptide D-JNKI-1 prevents JNK action without interfere with JNK activation/phosphorylation.

containing the residue threonine Thr 106 present in the hydrophobic pocket I of p38 MAPK and p38 MAPK isoforms adjacent to the ATP-binding cleft. The presence of a larger residue like methionine in the same position as observed in the and isoforms of p38MAPK, makes these two kinase isoforms insensitive to the SB203580. However, SB203580 also shows a high binding affinity for other kinases such as JNK-1 and cRaf, demonstrating the limited selectivity of this compound. A second generation of p38MAPK inhibitors was then developed by Glaxo Smith Kline, the prototype of which is the SB239063, showing higher selectivity for p38MAPK and and exhibiting a significant reduction in brain injury and neurological deficits induced by cerebral focal ischemia in rodents even after oral administration [86]. The mechanism of neuroprotection from focal stroke injury is likely due to the effect of SB239063 in blocking the production of inflammatory cytokines and other inflammatory mediators in the ischemic area. However this compound also protected the hippocampal neurons of organotypic hippocampal slice culture undergoing *in vitro* ischemia indicating that this compound can also have a direct neuroprotective activity on neurons. The fact that SB239063 displayed its neuroprotective action following oral administration in rodents is of great importance for the potential use of this compound in chronic diseases requiring repeated treatments. However, its penetration in the brain tissue is very limited, about 4-5% of plasma levels. Only in the hemispheric forebrain undergoing focal ischemia, but not in the control hemisphere, the drug levels increased to about 31-36% of plasma levels as result of the local damage of the brain blood barrier (BBB) occurring under these conditions [86].

Administration of the compounds intracerebroventricularly might therefore be an useful approach to inhibit these kinases in the central nervous system in order to define better their role in the neurodegeneration.

Other more recently developed urea-containing p38 inhibitors, i.e. BIRB 796 from Boehringer, were reported to bind at a site remote from the ATP pocket and to induce a major conformational change in the enzyme. Specifically the movement of residue Phe169 in the p38MAPK induced by the interaction with the inhibitors may prevent the ATP binding to the inactive kinase [87]. In principle, the inhibitors that are non ATP competitors should exhibit a higher selectivity. Indeed, BIRB 796 which is active at low nanomolar concentrations, has a selectivity for the p38MAPK that is 330 to over 1000 fold higher than that showed for other kinases [87]. However, so far no published data are available on the potential effect of this compound in preclinical models of neurodegeneration.

Other p38MAPK inhibitors have been developed in different companies and many of them are already in the phase 2 clinical trial for different pathologies, mainly rheumatoid arthritis and other inflammatory diseases. None of these compounds, except for the SB239063 which has been tested preclinically on the focal cerebral ischemia in rats (see above), has been proposed as therapy for neurological disorders. This is probably due to their poor distribution in the central nervous system. One p38MAPK inhibitor developed by Vertex, VX-745, that crosses the BBB was reported to produce adverse neurological effects in animals when administered at high doses [15]. Nevertheless, whether such adverse effects are strictly related to the inhibition of p38MAPK in the CNS has not been demonstrated.

CONCLUSIONS

Growing evidence indicates that many neurotoxic stimuli including glutamate, inflammatory cytokines and oxidative stress products can converge on the intracellular pathways modulated by JNK and p38MAPK to produce neuronal death. The specific involvement of one or other of these signalling pathways may depend on the prevalence of a specific neurotoxic stimulus and/or by the cell type involved. Thus, if the inhibition of p38MAPK rather than that of JNK may potentially rescue motor neurons in ALS, the inhibition of both kinases might exhibit an additive effect in the treatment of stroke and Alzheimer's disease.

On the other hand, it is necessary to consider that these intracellular pathways can be regulated by a complex cross talk among the kinases upstream to JNK or p38MAPK and the inhibition of one kinase might cause the upregulation of the other. For example, the inhibition of p38MAPK can abolish the feedback control of TAK1 by TAB1 and activated TAK1 in turn cause the upregulation of the JNK pathway [17] leading to adverse effects. Thus, an alternative approach is to develop compounds that intercept these kinase pathways at different levels of the cascade.

The identification of the JNK permeable peptide inhibitor that, by modifying JNK action rather than JNK activation, leads to prevention of neuronal death in several experimental conditions offers new strategies for selectively inhibiting these signalling cascades and preventing unwanted effects.

Finally, the possibility to develop strategies that allow to selectively inhibit a specific kinase in specific cell populations exposed to the injuries should also be explored. A new promising approach is represented by the use of RNA interference–mediated silencing of specific targets in combination with viral vectors driven to the CNS. This strategy was successfully used in reducing the progression of pathology in transgenic mouse models of familial ALS by silencing the transcript of mutant SOD1 in the motor neurons [88, 89].

The potential opportunity of selectively inhibiting JNK and/or p38MAPK homologous into the neuron exposed to injuries may be of great impact for the treatment of many devastating neurodegenerative disorders that still lack of effective therapies.

ACKNOWLEDGMENTS

C.B. and M.T. are supported by grants from Fondazione Monzino, Fondazione Cariplo, the Italian Ministry for Health (RF Malattie neurodegenerative), the Italian Ministry for University and Research (MIUR, FIRB Negoziali, Protocol RBNEO1B5WW 004) and Telethon Grants GP0222Y01. T.B. is supported by grants from the the Botnar Foundation grant and FNRS (Swiss National Science Foundation) grants 310000-107888. Special thanks to Architettura Laboratorio Communication for the graphic figure (www.archilab.it).

LIST OF ABBREVIATIONS

AD = Alzheimer's Disease

ALS = Amyotrophic lateral sclerosis

AMPA = -Amino-3-hydroxy-5-methylisoxazole-4-propionic acid

APP = Amyloid precursor protein

ARE = AU rich element

ASK1 = Apoptosis-signal regulating kinase 1

ATF = Activating transcription factor

CHOP = CAAT enhancer binding protein-homologous protein

CNS = Central Nervous System

CREB = cAMP response element binding protein

ERKs = Extracellular signal-regulated kinases

Hsp = Heat shock protein

IL1 = Interleukin 1

JIP = JNK-interacting protein

JNK = c-Jun N-terminal kinases

LPS = Lipopolysaccharide

MADD = Mitogen activated kinase activating death domain protein

MAPK = Mitogen-activated protein kinase

MAPKAPK2 or MK2 = MAP kinase-activated protein kinase 2

MEF-2 = Myocite enhanced factor 2

MEKK or MKKK = MAPK-extracellular signal-regulated kinase kinase

MLK	=	Mixed lineage kinases
MKK	=	MAP kinase kinase
MKKK or MEKK	=	MAP Kinase Kinase kinase
MKP	=	MAP kinase phosphatase
MNK	=	MAPK signal integrating protein kinase
NMDA	=	N-methyl-D-aspartate
SAP1A	=	Signalling lymphocytic activation molecule-associated protein 1A
TAK-1	=	Transforming growth factor–beta-activated protein kinase 1
TAB1	=	TAK1–binding protein
TNF	=	Tumor necrosis factor
TNFR	=	Tumor necrosis factor receptor
TRAF	=	Tumor necrosis factor receptor-associated factor

MAP kinase inhibitors mentioned in the text (formula and source)

p38 inhibitors

SKF 86002	=	6-(4-Fluorophenyl)-2,3-dihydro-5-(4-pyridyl)imidazo-[2,1***b***]-thiazole (Glaxo Smith Kline)
SB-203580	=	4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole (Glaxo Smith Kline)
SB239063	=	trans-1-(4-Hydroxycyclohexyl)-4-(4-fluorophenyl)-5-(2-methoxypyridimidin-4-yl)imidazole (Glaxo Smith Kline)
BIRB-796	=	1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3(4-(2-morpholin-4-yl-ethoxy)naphthalen-1-yl)urea (Boehringer)
VX-745	=	triazanapthalenones (Vertex)

JNK inhibitors

CEP-1347	=	9,12-Epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4i][1,6]benzodiazocine-10-carboxylic acid, 5,16-bis[(ethylthio)methyl]-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, methylester, (9S,10R,12R)- (Chephalon)
SP600125	**=**	Anthra(1,9-cd)pyrazol-6(2H)-one1,9-Pyrazoloanthrone (A.G. Scentific, Inc.)

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Received: ?????????? Revised: ???????????? Accepted: ??????????????