Pharmacology & Therapeutics 160 (2016) 159–179

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate Editor: J. Turgeon

A beacon of hope in stroke therapy—Blockade of pathologically activated cellular events in excitotoxic neuronal death as potential neuroprotective strategies

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ARTICLE INFO

Available online 17 February 2016

Keywords:
Excitotoxicity
Stroke
Neuron death
NMDAR
Calpain
nNOS

ABSTRACT

Excitotoxicity, a pathological process caused by over-stimulation of ionotropic glutamate receptors, is a major cause of neuronal loss in acute and chronic neurological conditions such as ischaemic stroke, Alzheimer's and Huntington's diseases. Effective neuroprotective drugs to reduce excitotoxic neuronal loss in patients suffering from these neurological conditions are urgently needed. One avenue to achieve this goal is to clearly define the intracellular events mediating the neurotoxic signals originating from the over-stimulated glutamate receptors in neurons. In this review, we first focus on the key cellular events directing neuronal death but not involved in normal physiological processes in the neurotoxic signalling pathways. These events, referred to as pathologically activated events, are potential targets for the development of neuroprotectant therapeutics. Inhibitors blocking some of the known pathologically activated cellular events have been proven to be effective in reducing stroke-induced brain damage in animal models. Notable examples are inhibitors suppressing the ion channel activity of neurotoxic glutamate receptors and those disrupting interactions of specific cellular proteins occurring only in neurons undergoing excitotoxic cell death. Among them, Tat-NR2B9c and memantine are clinically effective in reducing brain damage caused by some acute and chronic neurological conditions. Our second focus is evaluation of the suitability of the other inhibitors for use as neuroprotective therapeutics. We also discuss the experimental approaches suitable for bridging our knowledge gap in our current understanding of the excitotoxic signalling mechanism in neurons and discovery of new pathologically activated cellular events as potential targets for neuroprotection.

Contents

Abbreviations: AIF, Apoptosis-inducing factor; AMPA, α-amino-3- hydroxy-5-methyl-4-isoxazolepropionic acid; Aβo, amyloid β oligomers; Bax, Bcl-2-associated X-protein; CaMKIV, Calcium-calmodulin-dependent kinase IV; CaM, Calmodulin; CaN, Calcineurin; Cdk5, Cyclin-dependent protein kinase 5; CREB, cAMP-responsive element-binding protein; CRMP3, Collapsin response mediator protein 3; HDAC, Histone deacetylase enzymes subfamily C; JNK, c-Jun N-terminal kinase; Kidins220/ARMS, Kinase D-interacting substrate of 220 kDa/ankyrin repeat-rich membrane spanning; mGluRα-1, Metabotropic glutamate receptor α-1; NCX, Sodium–calcium exchanger; NMDA, N-methyl-D-aspartate; nNOS, Nitiric oxide synthase; NO, Nitric oxide, NOX2, NADPH oxidase 2; PSD-95, Postsynaptic density protein 95; PTEN, Phosphatase and tensin homolog; REST, Repressor element-1 transcription factor; Sin3A, Paired amphipathic helix protein Sin3a; STEP, Striatal-enriched protein tyrosine phosphatase.

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http://dx.doi.org/10.1016/j.pharmthera.2016.02.009
0163-7258/

1. Introduction

Cerebral stroke is the second leading cause of mortality and the leading cause of morbidity in the developed world (Mortality GBD and Causes of Death, 2015). There are two types of cerebral stroke: ischaemic stroke and haemorrhagic stroke, which account for around 87% and 13% of the stroke cases, respectively (Donnan et al., 2008). Effective pharmacological agents for the treatment of haemorrhagic stroke are not available. Current treatment strategies for ischaemic stroke focus on reducing ischaemic damage (infarct size) and rescuing dying cells after the ischaemic attack by restoring blood flow to the affected regions. Recombinant tissue plasminogen activator (rt-PA), which increases blood flow, is the only FDA-approved drug used in ischaemic stroke patients (NINDS tSG, 1995). For patients admitted to hospital 4.5 h after the onset of ischaemic stroke, rt-PA may cause haemorrhagic complications. Thus, it is used for the treatment in less than 10% of the ischaemic stroke patients (reviewed in Miller et al., 2011). Despite years of intensive research, therapeutics that directly protect against neuronal loss to reduce stroke-induced brain damage are not available for clinical use (O'Collins et al., 2006; Savitz & Fisher, 2007). This pessimistic scenario has been challenged by the promising clinical trial success of a cell membrane-permeable peptide Tat-NR2B9c. Tat-NR2B9c, an inhibitor of a key intracellular neurotoxic signalling pathway, could effectively reduce brain damage caused by small, procedurally induced ischaemic strokes in patients undergoing treatment to repair ruptured cerebral aneurysm (Hill et al., 2012). In this treatment scenario, Tat-NR2B9c was delivered after the onset of stroke. Although the clinical trial success at this stage is limited to only a small subset of ischaemic stroke patients, the success proves that neuroprotection to reduce brain damage in human acute ischaemic stroke is achievable. The neuroprotective effect of Tat-NR2B9c is attributed to its ability to inhibit a key pathological cellular event that directs stroke-induced neuronal death, confirming that investigation of the mechanism of stroke-induced neuronal death is one of the best avenues to develop therapeutic strategies to reduce stroke-induced brain damage.

Excitotoxicity, a pathological process caused by over-stimulation of ionotropic glutamate receptors, is a major cause of neuronal loss in acute neurological conditions such as ischaemic stroke, traumatic brain injury, prolonged seizure in stroke, and neurodegenerative diseases such as Alzheimer's and Huntington's diseases. Despite the efforts by many researchers, exactly how neurons die in excitotoxicity remains unclear. There are several excellent reviews summarising the mechanisms of this neuronal death process. The most elegant ones are by Moskowitz et al. (2010), Lai et al. (2011, 2014), Fujikawa (2015), Lau and Tymianski (2010), Fatokun et al. (2014), Hardingham and Bading (2010), and Ofengeim et al. (2011). Instead of emulating these authors in giving a comprehensive review of the current state of knowledge of neuronal death, we review the functional consequences and the temporal and spatial aspects of regulation of several cytotoxic cellular events in neurons undergoing excitotoxic cell death in the hope of identifying those that can be exploited to develop neuroprotective therapeutic drugs.

Stuart Lipton proposed that neuroprotective drugs interacting with their cellular targets only during states of pathological activation but not under physiological condition are clinically well tolerated. He reasoned that these drugs, referred to as the pathologically activated therapeutic drugs, would exhibit no or little interference with the physiological functions of the targets (Lipton, 2007). Using this approach, a small-molecule compound called memantine was developed as a neuroprotectant to slow down excitotoxic neuronal loss in Alzheimer's disease patients (reviewed in Lipton, 2007; Nakamura & Lipton, 2016). Since memantine can also enhance recovery from stroke in a mouse model when applied more than 2 h after ischaemic stroke (Lopez-Valdes et al., 2014), clinical trials of memantine for the treatment of ischemic stroke are currently under way (Clinical Trials.gov identifier: NCT02144584). In this manuscript, we focus on the known pathologically activated cellular events in neurons undergoing excitotoxic cell death and evaluate their suitability for development of new neuroprotective drugs to reduce brain damage in acute and chronic neurological disorders.

Recent advances in quantitative proteomics approaches suggest that these approaches can be employed to clearly define the mechanisms of excitotoxic neuronal death. In this review, we discuss how these approaches can be applied to identify the new pathological cellular events directing neuronal death, and decipher how these pathologically activated cellular events interplay temporally and spatially in neurons to direct cell death.

2. Stroke-induced neuronal loss is the culmination of many cellular processes

Pathologically, stroke is the combined outcome of many cellular processes. Brain cells succumb to ischaemic attack due to the loss of energy supplies, excessive intracellular calcium accumulation, oxidative stress, and potentiation of inflammatory responses. These events in neurons initiate a complex sequence of signalling cascades that ultimately lead to cell death. The concurrent steps that occur during an ischaemic attack include depletion of energy supply, acidosis, influx of ions, generation of free radicals, disruption of the blood–brain barrier, oedema, and inflammation. Brain tissues affected by ischaemic stroke can be divided into two regions: ischaemic core and ischaemic penumbra. Cells in the ischaemic core, which are directly supported by the occluded blood vessel, die immediately after the onset of cerebral ischaemia. The ischaemic core is surrounded by a zone of less severely affected tissue with less blood flow and contains brain cells that are alive and metabolically active (Astrup et al., 1981; reviewed in Broughton et al., 2009). Most of the live brain cells in this region, referred to as the ischaemic penumbra, undergo cell death several hours or even days after the onset of ischaemic attack. Thus they are salvageable if effective neuroprotective treatment is applied within this period (Fisher, 2004; Mehta et al., 2007; Nakka et al., 2008; Zheng & Yenari, 2004). Since ischaemic penumbra represents as much as half of the infarct volume in the initial phase of ischaemic stroke, neurons in this region are the target for the possible neuroprotective treatment to reduce brain damage after stroke (Ginsberg, 1997).

Neuroprotectants capable of reducing neuronal loss in ischaemic penumbra are urgently needed for the treatment of stroke patients. Owing to the complex and multifactorial nature of cerebral stroke, many lead compounds, albeit effective in reducing brain damage in animal models under experimental conditions, failed in clinical studies (Kalia et al., 2008). Our poor understanding of the mechanism of neuronal death in adults is one the causes of the clinical trial failure of these compounds. In the past few decades, researchers have identified multiple signalling pathways responsible for stroke-induced neuronal death.