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REVIEW ARTICLE

Inhibition of Caspase 3 and Caspase 9 Mediated Apoptosis: A Multimodal Therapeutic Target in Traumatic Brain Injury

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Abstract: Traumatic brain injury (TBI) is one of the significant causes of death and morbidity, and it is hence a focus of translational research. Apoptosis plays an essential part in the pathophysiology of TBI, and its inhibition may help overcome TBI's negative consequences and improve functional recovery. Although physiological neuronal death is necessary for appropriate embryologic development and adult cell turnover, it can also drive neurodegeneration. Caspases are principal mediators of cell death due to apoptosis and are critical for the required cleavage of intracellular proteins of cells committed to die. Caspase-3 is the major executioner Caspase of apoptosis and is regulated by a range of cellular components during physiological and pathological conditions. Activation of Caspase-3 causes proteolyzation of DNA repair proteins, cytoskeletal proteins, and the inhibitor of Caspase-activated DNase (ICAD) during programmed cell death, resulting in morphological alterations and DNA damage that define apoptosis. Caspase-9 is an additional crucial part of the intrinsic pathway, activated in response to several stimuli. Caspases can be altered post-translationally or by modulatory elements interacting with the zymogenic or active form of a Caspase, preventing their activation. The necessity of Caspase-9 and -3 in diverse apoptotic situations suggests that mammalian cells have at least four distinct apoptotic pathways. Continued investigation of these processes is anticipated to disclose new Caspase regulatory mechanisms with consequences far beyond apoptotic cell death control. The present review discusses various Caspase-dependent apoptotic pathways and the treatment strategies to inhibit the Caspases potentially.

Keywords: Traumatic brain injury, apoptosis, caspase-3, caspase-9, caspase-dependent apoptosis, caspase inhibitors.

1. INTRODUCTION

Traumatic brain injury (TBI) is defined as impairment of neural activity or other signs of neurological disorder resulting from an external, tangible force. It is predicted to affect 50 million people globally each year, which means that over 50% of the worldwide population experiences TBI at some point in their lives. Furthermore, low- and middle-income countries (LMIa Cs) have a considerably greater prevalence of illness and death. The condition impacts the world's economy by around $400 billion each year, or 0.5% of global GDP [1]. It is the main ground of injury-related mortality and morbidity worldwide, putting a tremendous strain on the families of the patients and society. The growing incidence of TBI caused by increased traffic congestion events disproportionately afflicts young people in LMICs [2]. Posttraumatic epilepsy [3, 4], chronic traumatic encephalopathy (CTE) [5], Parkinson's disorders [6], and Alzheimer's are potential long-term neurological consequences associated with TBI [7-9]. The molecular and cellular mechanisms that cause these disorders to originate and advance after a brain trauma challenge are not well understood.

TBI results from primary and secondary brain injury, which results in the activation of microglia, neuronal death, and astrocytic gliosis, leading to complicated neurological diseases. Whilst primary brain injury is permanent and triggered by mechanical stress during the initial blow, secondary brain damage is induced by prolonged neurobiological processes and intracellular signal transduction pathways and is recoverable [10]. Apoptosis, axonal degeneration, excitotoxicity (overactivation of glutamate), lipid peroxidation, mitochondrial failure, neurological inflammation, and oxidative stress are all variables that lead to secondary injuries [11].

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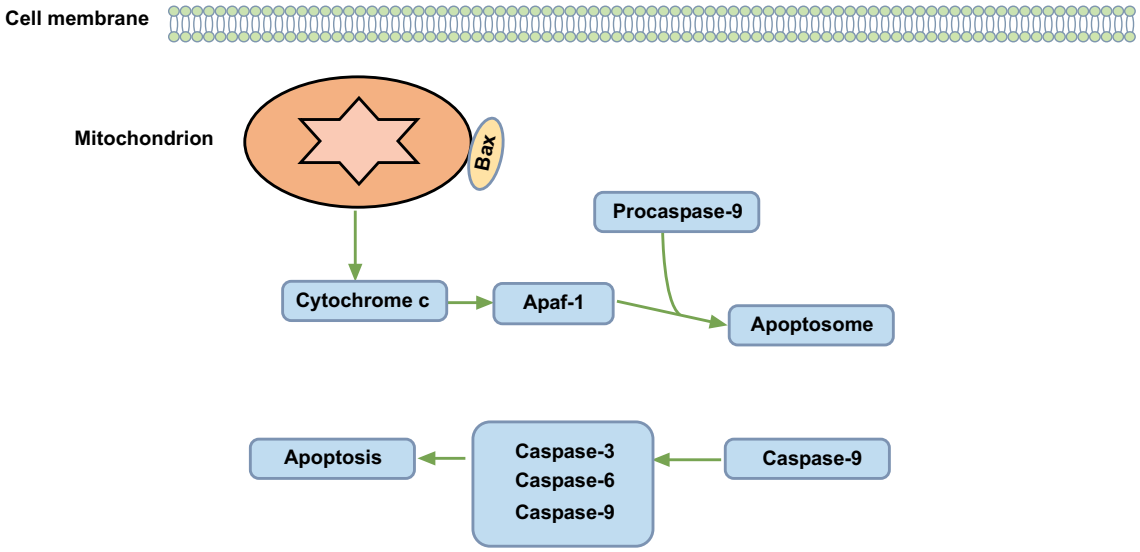

Fig. (1). Intrinsic apoptotic pathway. Bax is triggered in response to apoptotic stimuli and attaches to the mitochondrial membrane to promote its permeabilisation, thus releasing cytochrome c. When cytochrome c is released into the cytosol, it forms an apoptosome with apoptotic protein-activating factor-1 (Apaf-1) and procaspase-9, which activates caspase-9 and leads to caspase-3-dependent apoptosis. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

Caspase-3 and Caspase-9 overexpression in neuronal and glial cells has been linked to TBI pathophysiology in humans and animal models, according to research articles [12]. A study found, after a severe TBI, the concentrations of four important apoptotic molecules, cytochrome c, caspase-9, [13] caspase-3 and sFas [14], along with caspase-3 related protein breakdown product, Caspase-3-cleaved cytokeratin18 (CCCK18), in serum to be dramatically elevated in the CSF of patients [15]. Clinical studies show that levels of various αII-spectrin breakdown products (SBDPs) are raised in the cerebrospinal fluid (CSF) of patients for 4-5 days after injury, incluing 120 kDa αII spectrin breakdown product (SBDP120), a specialised proteolytic product involved in the activity of Caspase-3 [16]. One more notable TBI biomarker is the 150 kDa αII-spectrin breakdown product (SBDP150) [16-18], which is found to be linked to calpain as well as Caspase-3 protease activity [19]. Another well-known substrate for proteolytic activity of Caspase 3 is tau, which results in the formation of two different cleavage products [20]. Elevated concentrations of Caspase-3 cleaved tau have been detected in brain extracts of individuals with CTE [21] and the serum of patients with AD and TBI [22, 23]. The breakage and activation of downstream effector caspases (caspases-3, -6 and -7) to promote cell apoptosis is the most well-known result of caspase-9 activation. In cerebral ischemia, cleavage of caspase-6 by caspase-9 results in axon degeneration. In epithelial cell apoptosis, caspase-9 cleaves and suppresses the activity of Major Vault Protein (MVP), which is another biological substrate for caspase-9 [24]. Lastly, experimental evidence suggests that Caspase-3 activation leads to cell death along with brain tissue loss, and that inhibiting this pathway could be a proper TBI therapeutic strategy [25].

2. APOPTOSIS

The intrinsic pathway (Fig. **1**) and the extrinsic pathway (Fig. **2**) can initiate apoptosis. DNA damage activates the intrinsic (mitochondrial) pathway, which stimulates mitochondria to generate apoptotic factors, like the second mitochondrial activator of Caspases (SMAC), cytochrome-c, endonuclease G, direct IAP binding protein with low pI (DIABLO), and apoptosis-inducing factors (AIF). These can stimulate the initiator Caspase (Caspase-9), which sends death signals to the effector Caspase (Caspase-3), causing the death of nerve cells. Initiator Caspases, including Caspases-8 and -9, were released approximately 1 hour after rats were challenged with TBI *via* a fluid percussion brain insult, indicating apoptotic activity. Effector Caspases, such as Caspase-3, were elevated within 6 hours of TBI. Members of the IAP family, cleaved X-linked IAP (XIAP) and cellular IAPs (cIAP-1 and cIAP-2), were also reported to be elevated in TBI tissue. This indicates that Caspases and IAP cleavage may be involved in apoptosis in TBI [26]. Different types of Caspases and their functions are described in Table **1**.

2.1. Caspase Dependent Apoptosis

Elevated mRNA and protein levels of Caspase-8 are seen in adults who have experienced TBI; the Caspase-8 protein appears to be mainly expressed in the affected nerve cells. Active Caspase-3 and -9 are predominantly expressed in TUNEL-positive neurons. Caspase-3 and -9, on the other hand, are detectable in TUNEL-negative cells as well as TUNEL-positive cells that do not display activated Caspase. This association indicates that several Caspase-3 and -9 forms are implicated in TBI pathology [52]. Neuron-specific IAP (NIAP) also effectively inhibits the effector Caspase enzymatic activity [53]. As a result, Caspase inhibition techniques may be beneficial for reducing the apoptotic consequences of TBI [54].

Extrinsic or intrinsic mechanisms can trigger Caspase-dependent apoptosis. Cell surface receptors found across various cell types, especially neurons, are involved in extrinsic pathways. When cell surface tumour necrosis factor (TNF) interacts with extracellular TNF or Fas receptors interact with external Fas ligand, the receptors become

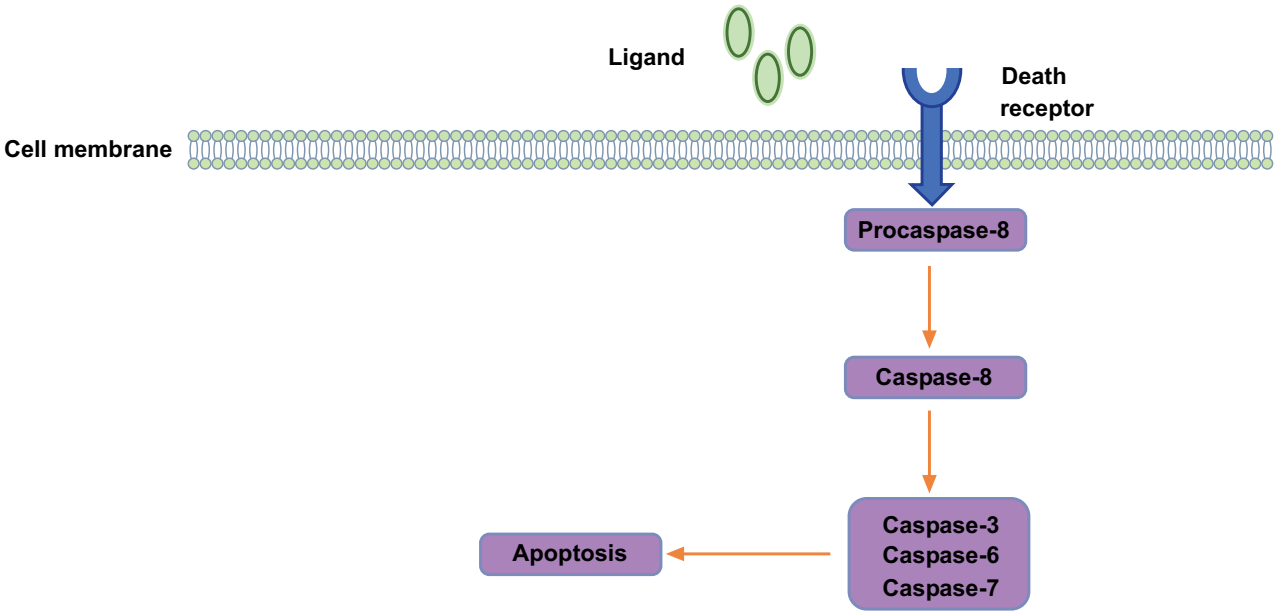

Fig. (2). Extrinsic apoptotic pathway. The death domain protein, which interacts with procaspase-8, is recruited when the Fas ligand adheres to death receptors. Death receptor, Fas ligand and procaspase-8 together form a death-inducing signaling complex. This complex activates caspase-8, which results in caspase-3-dependent cell death. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

Table 1. Different types of Caspases and their functions.

Caspase	Category	Organism	Function	References
Caspase-1	Pyroptosis-inflammation	Mouse and human	Take part in the inflammatory immune response	[27]
Caspase-2	Apoptosis initiator Caspase	Mouse and human	Participates in the cell apoptosis	[28]
Caspase-3	Apoptosis executioner Caspase	Mouse and human	Takes part in cell apoptosis	[29]
Caspase-4	Pyroptosis-inflammation	Human	Its function remains obscure, but it is believed to be an inflammatory Caspase	[30,31].
Caspase-5	Pyroptosis-inflammation	Human	It is an inflammatory Caspase and has a role in the immune system	[32,33]
Caspase-6	Apoptosis executioner Caspase	Mouse and human	Has a role in apoptosis, neurodegeneration, and immune response	[34,35,36]
Caspase-7	Apoptosis executioner Caspase	Mouse and human	Acts as an executioner of Caspase in apoptosis, and it has a role in inflammation	[37,38].
Caspase-8	Apoptosis initiator Caspase	Mouse and human	Acts as an initiator of Caspase in cell apoptosis and is involved in immune response	[39,40,41].
Caspase-9	Apoptosis initiator Caspase	Mouse and human	Its activation initiates the process of apoptosis	[42]
Caspase-10	Apoptosis initiator Caspase	Human	Has a role in cell apoptosis	[43,44]
Caspase-11	Pyroptosis-inflammation	Mouse	Is also called non-canonical inflammasome and takes part in the immune response.	[45,46,47]
Caspase-12	Pyroptosis-inflammation	Mouse and human	Participates in immune response and has a role in cytokine processing and activation.	[48,49,50]
Caspase-13	Pyroptosis-inflammation	Cattle	Unknown	-
Caspase-14	Other	Mouse and human	Has a role in keratinisation of the epithelium	[51]

trimerised, forming ensembles with intracellular signalling molecules: Fas-related protein with death domain and TNF receptor-linked death domain protein. This signalling complex then interacts with Caspase-8 [55] or Caspase-10 [56], causing their auto-cleavage and activation. These initiator Caspases eventually induce cleavage and activation of Caspase-3, causing the apoptosis process to become irreversible.

Stress impacting mitochondria and the endoplasmic reticulum (ER) trigger the intrinsic pathway. The mitochondrial generation and release of cytochrome-c, which occurs after depolarisation of the mitochondrial membrane and the development of mitochondrial permeability transition holes, can initiate Caspase-dependent apoptosis. The apoptosome is a complex formed when cytochrome-c combines with apoptotic protease activating factor-1 (Apaf-1), pro-Caspase-9, and ATP. Apaf-1 has a domain for Caspase recruitment that attaches with pro-Caspase-9. Numerous WD-40 repetitions in Apaf-1 allow Caspase-9 to self-oligomerise and auto-activate, cleaving and activating Caspase-3. Even though Caspase-9 can autocleave and be cleaved directly by active Caspases, this alteration is not required for its activation [57, 58].

2.2. Caspase-3

Caspase-3, the most significant of the effector Caspases, is activated by initiator Caspases, activating endonuclease Caspase-activated DNase (CAD). CAD occurs as a complex inhibitor, ICAD, in growing cells. On ICAD interaction with active Caspase-3, CAD is liberated in cells allowing it to cleave the genomic DNA into high-molecular-weight (HMW) DNA and the smaller fragments known as the internucleosomal DNA ladder, thus causing cell death [59]. CAD degradation of chromosomal DNA within nuclei causes the classical condensation evident in apoptosis.

Caspase-3 also causes the cytoskeleton to reorganise and the cell to disintegrate into apoptotic entities. Gelsolin, a protein that specifically binds to actin, has been recognised as one of the primary substrates for the activity of Caspase-3. The binding of gelsolin to phosphatidylinositol biphosphate results in the coupling of actin organisation and signal transduction. The gelsolin fragments, generated by caspase-3 mediated gelsolin cleavage, sequentially sever actin filaments in a calcium-independent way. Consequently, the cytoskeleton, cell division, signalling, and intracellular transport are all disrupted [60].

Caspase-3 has been shown to have a variety of effects. Many of these effects are necessary for the occurrence of cell death, but only in specific cell types that have been exposed to death inducers. Other Caspase-3 effects may be involved in ensuring that the apoptotic process is completed efficiently when the cell has already been committed to death [29].

2.3. Caspase-9

Caspases-9, which serves as the initiator Caspase subunit of the apoptosome, becomes activated after the release of cytochrome-c from mitochondria and hence has a significant role in the activation of effector Caspases consequently leading to various death stimuli. Caspase-9 is a crucial regulator at this stage in the apoptotic cascade, and apparently, it is one of the most well-characterised Caspases in terms of post-translational modifications [61].

Although Caspase-9 displays entire activity in its uncleaved state, dimerisation rather than cleavage stimulates its activity. This is likely due to the long loop which links the subunits. The linker loop is thought to migrate and reach the proximity of the active site without being cleaved because of its length and capacity of joining the large subunit with the small subunits and its dimerisation inside the apoptosome complex [57, 62]. Caspase-9 dimerization causes fast autocatalytic cleavage, resulting in Caspase-9 (p35/p12) [54,63]. Although caspase 9 and 2 activation have contrary effects, they play a role in neuronal differentiation [64], a process that may be relevant for the potential functional recovery after TBI.

2.4. Caspase 9 and Caspase 3 Dependent Apoptotic Pathway

Caspase-9, cytochrome-c, and Apaf-1 have previously been identified as members of a complex called 'apoptosome,' which is essential for Caspase-3 activity in *in vitro* experiments. Caspase-3 failed to activate *in vitro* when Caspase-9 was removed from cytosolic fractions. These data suggest that an apoptotic pathway depends on Caspase-9 and Caspase-3 [42]. Both Caspase-$9^{-/-}$ and Caspase-$3^{-/-}$ embryonic stem cells (ESCs) were resilient to many apoptotic stimuli, along with UV irradiation [65], indicating the existence of Caspase-9 and -3 dependent apoptotic pathways. Caspase-$9^{-/-}$ ESCs that died after UV irradiation had aberrant morphological characteristics similar to Caspase-3 deficiency. Moreover, the absence of Caspase-9 in UV-irradiated ES cells prevented Caspase-3 and other Caspases from being activated. Other Caspases, such as Caspase-8, appear to be unable to take over this function of Caspase-9 in ES cells, although Caspase-8 is produced in ES cells and can bind to Apaf1 [66].

Hence, UV irradiation preferentially activates an apoptotic pathway requiring the presence of Caspase-9 and -3 in ES cells. Caspase-9 and -3 mutations have similar actions on apoptosis and development of the brain, implying that the Caspase-9 and Caspase-3-dependent pathway is functionally needed for the process of programmed cell death (PCD) in the developing brain [67].

2.5. Caspase $9^{-/-}$ and Caspase $3^{-/-}$ Independent Apoptotic Pathway

Caspase-9 is required for apoptosis in specific cell types and in response to particular stimuli, indicating the presence of numerous apoptotic pathways. A deficiency of Caspase-9 failed to protect other cell types challenged using the same inducers for PCD, even though Caspase-$9^{-/-}$ ES cells were stable against a wide variety of apoptotic stimuli. Caspase-$9^{-/-}$ thymocytes and splenocytes, unlike ES cells, exhibited PCD due to UV irradiation. UV-mediated apoptosis affects Caspase-$3^{-/-}$ thymocytes and splenocytes as well. As a result, an apoptotic pathway that is independent of Caspase-9, as well as Caspase-3, is likely to exist *in vivo* [67].

2.6. Caspase $9^{-/-}$ Independent and Caspase $3^{-/-}$Dependent Apoptotic Pathway

The responsiveness of activated T lymphocytes to α-CD95 and α-CD3ε confirms that the apoptotic pathway, independent of Caspase-9 but dependent on Caspase-3, is

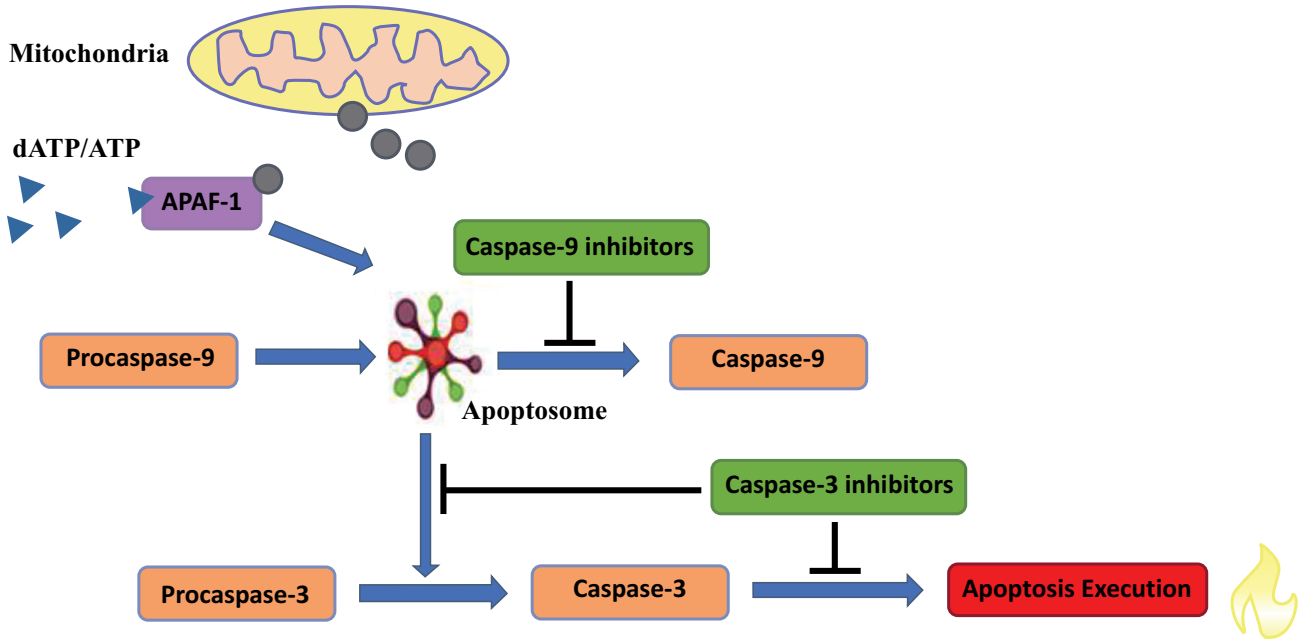

Fig. (3). Mechanism of Caspase 3 and Caspase 9 inhibitors. Following moderate TBI, caspases are activated in both the intrinsic and extrinsic apoptotic pathways, and caspase inhibitors play a defensive function inside the brain by altering the cleavage and activation of these apoptotic caspases. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

likely to exist. The deficiency of Caspase-3 has been reported to protect active splenocytes from apoptosis mediated by α-CD95 and α-CD3. The lack of Caspase-9, on the other hand, failed to safeguard activated T cells from death mediated by α-CD95 or α-CD3 *in vivo*, nor did it hinder the activation of Caspase-3 as a result of these stimuli. These findings strongly suggest that a Caspase-$9^{-/-}$ independent and a Caspase-$3^{-/-}$ dependent apoptotic pathway exists. Apaf-1 was found to interact with Caspases with extensive pro domains that contain CARDs (Caspase recruitment domains), such as Caspase-4 and -8 [66]. As a result, these Caspases could be involved in initiating this apoptotic pathway [67].

2.7. Caspase 9–Dependent and Caspase 3–Independent Apoptotic Pathway

All the apoptosis stimuli, including dexamethasone and γ irradiation, have been found to cause apoptosis in Caspase-$3^{-/-}$ thymocytes [68]. Caspase-$9^{-/-}$ thymocytes, on the other hand, were stable against apoptosis mediated by either dexamethasone or irradiation, demonstrating that these stimuli activate an apoptotic pathway in thymocytes that is Caspase-3 independent but Caspase-9 dependent. The discovery that γ irradiation selectively stimulates this apoptotic pathway is significant because Caspase-$9^{-/-}$, but not -$3^{-/-}$ thymocytes, ES cells, and splenocytes, was resilient to the apoptotic stimuli [69].

3. INHIBITION OF CASPASE-3 AND -9

Caspase inhibitors are a group of small peptide derivatives (usually 1-4 amino acids) and unique non-peptide therapeutic compounds [70]. These are competitive inhibitors, evaluated in TBI models, and are developed by particular amino acid target sequences at the cleavage site of the Caspase substrates [71, 72]. Independent studies have revealed that a specific Caspase-3 inhibitor (tetrapeptide) limits the Caspase enzyme activity, minimises loss of brain tissue, and improves neurological recovery following experimental TBI [73]. A tripeptide inhibitor has also been found to enhance physiological outcomes in rats following TBI [53,74] and minimise neurodegeneration due to apoptosis in developing rat brains following TBI. A negative aspect of these Caspase inhibitors is that they may not substantially cross the blood-brain barrier (BBB) unless administered soon after an injury, when the BBB has been damaged (generally within hours following an injury) [75]. Boc-aspartyl fluoro methyl ketone, a uni-peptide Caspase inhibitor, which can reasonably permeate the BBB, has been reported to prevent brain damage in a rat model of neonatal hypoxic-ischemic brain injury when administered either directly into the brain or systemically [76]. As yet, no results have been noted in TBI models. Treatment with these Caspase inhibitors carries special considerations, besides the possibility of delayed tumour formation. A) Inhibiting caspase-dependent apoptosis may cause cells to die by necrosis or Caspase-independent apoptosis [77], B) The activity of Caspase may be significant in cytoskeletal remodelling as well as other physiological activities [78] that could be inhibited, C) Caspase inhibitors may cause defective cells to survive, leading to survival but with little functional value. As an example, treatment with DEVD following TBI in rats minimised tissue damage without improving functional results [73]. The pattern of Caspase-3 and Caspase-9 inhibition is depicted in Fig. (**3**).

3.1. Inhibition of Caspase-3

Caspase 3 can be inhibited through various mechanisms, as described below (Table **2**).

Table 2. Caspase-3 inhibitors and their mode of action.

S. No.	Caspase 3 Inhibitors	Mechanism of Action	References
1.	PKCd and p38	p38 phosphorylation of Caspase-3 at Ser150	[79]
2.	IAPs	Ubiquitylation of a Caspase-3	[80,81]
3.	XIAP	Inhibition of Caspase-3 and Caspase-7	[82-87]
4.	SNAP and doxorubicin	nitrosylation on its active site cysteine	[88-90]
5.	Peroxynitrite	Reversible inhibition of Caspase-3	[91,92]
6.	2,2'-methylenebis (1,3-cyclohexanedione) (M50054)	Inhibition of Caspase-3	[93-95]
7.	Z-DEVD-fmk	Specific, irreversible inhibition of Caspase-3	[96]
8.	Mexiletine	Inhibition of Caspase-3	[97]
9.	Zinc	Inhibits PARP proteolysis induced Caspase-3	[98]

3.1.1. Phosphorylation

Caspase-3 has been reported to be phosphorylated by both PKCd and p38. It is directly inhibited by p38 phosphorylation at Ser150, which is localised within the main component of the protein. This has been demonstrated to hinder Fas-mediated apoptosis in neutrophils [79].

3.1.2. Ubiquitylation

Even though the exact mechanism via which IAPs can modulate Caspase-3 remains unknown, a study has demonstrated that cIAP2 can monoubiquitylate Caspase-3 *in vitro* [80]. The physiological significance of this mechanism has yet to be discovered. cIAP1-mediated ubiquitylation of a Caspase-3 intermediate has been identified in more recent research, resulting in proteasome-mediated destruction of Caspase-3 and more excellent protection against TRAIL-mediated apoptosis [81].

XIAP, an IAP, can bind to Caspases-3 and deactivate it directly [82]. Nevertheless, this action of XIAP is not necessary for its antiapoptotic activity, as a mutant of XIAP that cannot suppress the activity of Caspase-3 still has the complete ability to prevent UV-induced apoptosis. The XIAP BIR2 (Baculovirus IAP repeat domain) IBM- (IAP-binding motif) binding pocket binds the IBM of the large subunit of Caspase-3. The linker site ahead of BIR 2 impairs the substrate-binding site of Caspase-3, thereby inhibiting substrate accessibility [83-86]. Even though E3-ubiquitin ligase activity appears to be redundant, examination of cells of a transgenic mouse demonstrated that RING-dependent post-transduction alterations impacted XIAP-mediated Caspase-3 suppression [87].

3.1.3. Nitrosylation

Caspase-3 can undergo nitrosylation on its active site, cysteine. The same study discovered that when cells were treated with Fas ligand, Caspase-3 was denitrosylated, enhancing Caspase activity and cell death due to Fas treatment. Recent findings revealed that only a subgroup of Caspase-3 confined to mitochondria is nitrosylated [88]. Nitrosylation of Caspase-3 was found in some fibroblasts isolated from patients, and these cells have minimised apoptotic reactivity upon nitrosylation of Caspase-3 [89]. Other models, such as SNAP (S-nitroso-N-acetyl-penicillamine) and doxorubicin treatment of cardiomyocytes, have been utilised to confirm the importance of nitrosylation in the regulation of Caspase-3 [90].

3.1.4. Peroxynitrite

Peroxynitrite (PN) shows action either directly on Caspase-3 or on events that occur between the release of cytochrome-c and Caspase-3 activation. Cytochrome-c interacts with procaspase-9 and Apaf-1 to form an apoptosome once released. Caspase-9 is activated by apoptosome formation, resulting in Caspase-3 cleavage and activation. However, in previous experiments, PN treatment of cytochrome c did not affect apoptosome formation and subsequent Caspase-3 activation [91]. Although tyrosine nitration is a marker of PN activity, it does not always imply the inactivation of Caspase-3. An ex vivo system was employed to assess the effects of PN on both Caspase-3 and - 9 activations after demonstrating that PN causes the tyrosine-mediated nitration of proCaspase-3 [92].

3.1.5. 2,2'-methylenebis (1,3-cyclohexanedione) (M50054)

2,2'-methylenebis, commonly called M50054, a Caspase-3 selective inhibitor, was employed to modify apoptosis levels. Previous research studies have demonstrated that the M50054 molecule successfully blocks the activation of Caspase-3, thereby preventing apoptosis in both *in vitro* and *in vivo* models [93-95].

3.1.6. z-DEVD.fmk

z-DEVD.fmk is an inhibitor of Caspase-3, broadly employed across *in vitro* and *in vivo* models to inhibit the functions of Caspase 3 in apoptosis. It is a tetrapeptide inhibitor that selectively acts on Caspase-3. z-DEVD-fmk enhanced neurological performance and minimised damage in necrotic cells in which activated Caspase-3 was detected [96].

3.1.7. Mexiletine

After experimental spinal cord injury (SCI), mexiletine therapy suppressed the activation of Caspase-3 and

Table 3. Caspase 9 inhibitors and their mechanism of action.

S. No.	Caspase 3 Inhibitors	Mechanism of Action	References
1.	Phosphorylation	Reduction of Caspase-9 activation and/or cleavage	[99, 100]
2.	Thr125	Phosphorylation of Caspase-9	[100-105]
3.	Ser144	Phosphorylation of Caspase-9	[106-108]
4.	Nitrosylation	Nitrosylation of Caspase-9	[88, 109]
5.	XIAP	Ubiquitylation	[110-116]

maintained neuronal function more efficiently than methylprednisolone [97].

3.1.8. Zinc

A Caspase-3-containing extract of apoptotic tissue and purified recombinant Caspase-3 activate PARP proteolysis, although Zn^{2+} can potentially suppress it. Further research into the mechanism(s) through which suppression of Caspase-3 is mediated is needed. Nevertheless, ICE (IL-1β converting enzyme) crystal structure studies have revealed that His-237 and Cys-285 participate in this type of catalysis. Zn2+ may block Caspase-3 by forming a complex with one or both of these two sites [98].

3.2. Inhibition of Caspase-9

Caspase 9 can be inhibited through various mechanisms, as mentioned in Table **3**.

3.2.1. Phosphorylation

There are various phosphorylation sites for Caspase-9, which lead to a decline in its activation or activity [99].

Thr125, which is phosphorylated by numerous distinct kinases, tends to be the site of Caspase-9 modulation. Phosphorylated Thr125, located in between the CARD domain and Caspase-9 larger subunit, inhibits activation of Caspase-3 (thus proteolysis) by an unknown mechanism [100].

3.2.2. Thr125

Thr125 was the first identified site to be phosphorylated by Erk kinase; however, other kinases, such as DYRK1A, p38, and cdk1, have been found to cause phosphorylation at this site, lowering its activity and that of Caspase-3 [100-104]. In all circumstances, the kinases involved are thought to contribute to antiapoptotic signalling, avoiding full-fledged Caspase activation in the event of cytochrome-c release by mitochondria. The same kinases also prevent cytochrome c release implying that phosphorylation of Caspase-9 might effectively act as an additional layer of protection from apoptosis in the event of accidental cytochrome c generation/release. Protein phosphatase-1a (PP1a) can counteract Thr125 phosphorylation, which is a pro-apoptotic event [105].

3.2.3. Ser144

PKCz can phosphorylate Caspase-9 at another site, i.e., Ser144, in response to hyperosmotic stress, in addition to Thr125 phosphorylation. This alteration also reduces Caspase activity [106]. Additionally, three different sites for PKA phosphorylation of Caspase-9 have been identified: Ser99, Ser183, and Ser195 [107]. Nevertheless, the significance of these sites in inhibition of Caspase activation remains unknown because altering these sites did not stabilise the apoptosome against PKA signalling inhibition, implying that there may be additional significant sites or that PKA action is indirect. Caspases-9 can be phosphorylated at Ser196 and Ser348 by the Akt and CK2 kinases, respectively [108].

3.2.4. Nitrosylation

Caspase-9 can be nitrosylated in addition to its phosphorylation, which results when intracellular levels of nitric oxide (NO) are high [109]. Treatment with S-NitrosoN-acetyl-D, L-penicillamine (SNAP), an NO donor, lowers Caspase-9 activation but does not inhibit the release of cytochrome-c by mitochondria. SNAP was found capable of negatively inhibiting recombinant activity. Even though the vital site was not identified, human Caspase-9 *in vitro* [88,109] has also been found to cause Caspase-9 nitrosylation in various subcellular compartments, with a mitochondrial component of Caspase-9 being selectively nitrosylated.

3.2.5. Ubiquitylation

Caspase-9 can be modulated by ubiquitylation, in addition to alterations that affect its activity or activation. XIAP E3 ubiquitin ligase has been shown to ubiquitylate Caspase-9 [110]. The carboxy-terminal RING domain of XIAP is required for the protein's ubiquitin ligase function [111]. *In vitro*, XIAP can polyubiquitylate the Caspase-9 large subunit but does not affect the inactive proCaspase-9 [112]. When cells were treated with MG132, a proteasome antagonist, combined with XIAP, the aggregation of polyubiquitylated Caspase-9 was enhanced [112]. More research is needed to completely comprehend the mechanisms that regulate XIAP-mediated Caspase-9 ubiquitylation, along with the importance of this activity in controlling the progression of apoptosis.

It appears to have a two-site binding mechanism that directly attaches and blocks processed-Caspases-9 activity [113]. First, BIR3's surface groove binds the IBM, which is disclosed at the active small subunit's N-terminus after processing Caspase-9 [114]. Second, BIR3's C-terminal extremity attaches to Caspase-9's dimer interface, blocking the dimerisation of Caspase-9 and concealing the catalytic residue [115, 116]. Therefore, XIAP might limit apoptosomes by decreasing Caspase-9 activity and intervening in the Caspase-9 activation cycle.

CONCLUSION AND FUTURE PROSPECTS

Even though apoptosis, without doubt, actively participates in the cell death evident after TBI in animal models and humans, current research is insufficient to prove that apoptosis or selective caspase activation is purely deleterious after brain damage. Indeed, caspase 9 and 2 activations, though with opposite effects, appear to play a role in neuronal differentiation, a process that may be relevant for the potential functional recovery after TBI. As a result, attempts to minimise neuronal and glial apoptosis following TBI may have physiologic and technical constraints. However, it is logical to continue developing clinically relevant techniques for selective apoptosis reduction following TBI. More research into Caspase-dependent apoptosis is needed, as these pathways could provide potential targets for developing innovative treatment techniques.

LIST OF ABBREVIATIONS

AD	=	Alzheimer's Disease
Apaf-1	=	Apoptotic Protease Activating Factor-1
BBB	=	Blood-brain Barrier
BIR2	=	Baculovirus IAP Repeat Domain
CAD	=	Caspase-activated DNase
CARD	=	Caspase Recruitment Domain
CCCK18	=	Caspase-3-cleaved Cytokeratin 18
cIAP	=	Cellular Inhibitor of Apoptosis Protein
CSF	=	Cerebrospinal Fluid
CTE	=	Chronic Traumatic Encephalopathy
DIABLO	=	Direct IAP Binding Protein with Low PI (Propidium Iodide)
DNA	=	Deoxyribonucleic Acid
DYRK1A	=	Dual Specificity Tyrosine Phosphorylation-Regulated Kinase 1A
ER	=	Endoplasmic Reticulum
ES	=	Embryonic Stem Cells
IAP	=	Inhibitor of Apoptosis Proteins
IBM	=	IAP-binding Motif
ICAD	=	Inhibitor of CAD
LMICs	=	Low- and Middle-income Countries
mRNA	=	Messenger Ribonucleic Acid
MVP	=	Major Vault Protein
NIAP	=	Neuron-specific Inhibitor of Apoptosis Protein
NO	=	Nitric Oxide
PARP	=	Poly(ADP-ribose) Polymerase
PCD	=	Process of Programmed Cell Death
PD	=	Parkinson's Disease
PKA	=	Protein Kinase A
PKCd	=	Protein Kinase C delta
PN	=	Peroxynitrite
PP1a	=	Protein Phosphatase 1a
SBDP	=	αII-spectrin Breakdown Products
SCI	=	Spinal Cord Injury
SMAC	=	Second Mitochondrial Activator of Caspases
SNAP	=	S-nitroso-N-acetyl-penicillamine
TBI	=	Traumatic Brain Injury
TNF	=	Tumor Necrosis Factor
TRAIL	=	TNF-related Apoptosis-inducing Ligand
TUNEL	=	Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling
XIAP	=	X-linked Inhibitor of Apoptosis Protein
Zn^{2+}	=	Zinc

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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