Apoptotic Mechanisms for Neuronal Cells in Early Brain Injury After Subarachnoid Hemorrhage

Yu Hasegawa, Hidenori Suzuki, Takumi Sozen, Orhan Altay, and John H Zhang

Abstract *Objects*: The major causes of death and disability in subarachnoid hemorrhage (SAH) may be early brain injury (EBI) and cerebral vasospasm. Although cerebral vasospasm has been studied and treated by a lot of drugs, the outcome is not improved even if vasospasm is reversed. Based on these data, EBI is considered a primary target for future research, and apoptosis may be involved in EBI after experimental SAH.

Methods: We reviewed the published literature about the relationship between SAH induced EBI and apoptosis in PubMed.

Result: Most available information can be obtained from the endovascular filament perforation animal model. After onset of SAH, intracranial pressure is increased and then cerebral blood flow is reduced. Many factors are involved in the mechanism of apoptotic cell death in EBI after SAH. In the neuronal cells, both intrinsic and extrinsic pathways of apoptosis can occur. Some antiapoptotic drugs were studied and demonstrated a protective effect against EBI after SAH. However, apoptosis in EBI after SAH has been little studied and further studies will provide us more beneficial findings. *Conclusions*: The study of apoptosis in EBI after experimental SAH may give us new therapies for SAH.

Keywords Apoptosis · Cerebral blood flow · Early brain injury · Intracranial pressure · Subarachnoid hemorrhage

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Introduction

Subarachnoid hemorrhage (SAH) is associated with high mortality, and 12.4% of patients die suddenly before reaching the hospital [1]. These deaths were mostly due to the initial hemorrhage, and no effective treatment is available for brain injury after the hemorrhage [2]. For survivors, early brain injury (EBI) caused by the initial hemorrhage and delayed ischemic neurologic deficits due to cerebral vasospasm are major causes of the subsequent morbidity and mortality [3]. Although cerebral vasospasm has been studied and treated by a lot of drugs during the past several decades, the outcome is not improved by the reversal of vasospasm [4]. Based on these data, EBI is considered a primary target for future research and may be also an important factor in preventing symptomatic vasospasm because EBI may predispose the brain to ischemic injury due to vasospasm.

Recent studies showed that apoptosis is involved in the pathogenesis of EBI after experimental SAH or in a clinical setting [5, 6]. Therefore, it is thought that an antiapoptotic treatment can be one of the therapeutic candidates for EBI after SAH. In this review, we focus on the relationship between EBI after SAH and apoptotic mechanism in neuronal cells.

Pathophysiology of Early Brain Injury

Most available information about EBI after SAH comes from endovascular filament perforation animal models, which show a high mortality and acute metabolic changes similar to clinical settings [7–9]. Intracranial pressure (ICP) in this model was increased to 40 mmHg immediately after SAH and then decreased to plateau (15–25 mmHg), whereas cerebral perfusion pressure was decreased to 35–40 mmHg from 70 mmHg, cerebral blood flow (CBF) was 20–30% decreased from the baseline after SAH induction, and then

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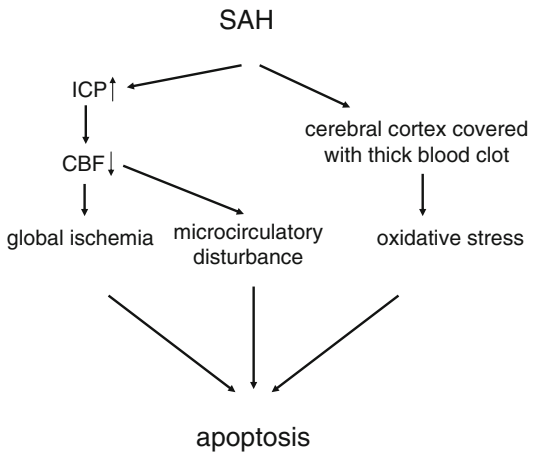

Fig. 1 Apoptotic cascade after subarachnoid hemorrhage. *SAH* subarachnoid hemorrhage, *ICP* intracranial pressure, *CBF* cerebral blood flow

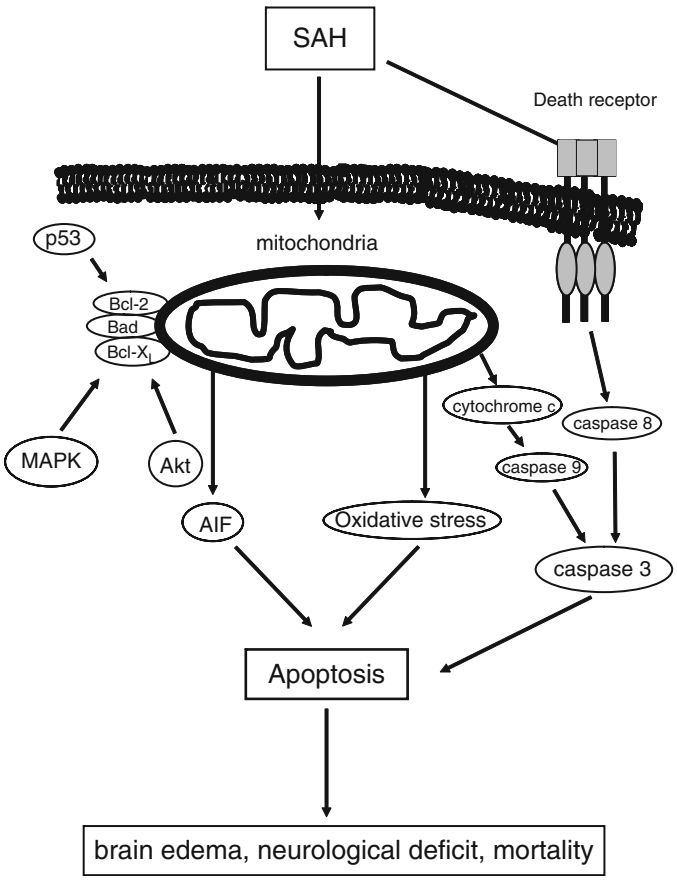

Fig. 2 Schematic representation of the pathway to neuronal apoptosis following subarachnoid hemorrhage. *MAPK* mitogen-activated protein kinase, *AIF* apoptosis-inducing factor

each of the values were gradually recovered [10]. Interestingly, the mortality rate was 100% when CBF was reduced to less than 40% of the baseline for 60 min after SAH, while less CBF reduction resulted in 19% mortality [11].

Many factors, such as global ischemia [12], microcirculatory disturbance [11], and subarachnoid blood toxicity [13] are involved in apoptosis-related mechanisms in EBI after SAH (Fig. 1), whereas distribution of apoptotic cell death is controversial [12, 14]. Although apoptotic cell death detected by TUNEL was seen in both the cortex and subcortex, neuronal cell death in the hippocampus, which is related to global ischemia, might depend on ICP [6, 14]. Blood immediately spreads in the subarachnoid space after SAH, and then the cerebral cortex is covered with a thick blood clot. Hemoglobin is metabolized by neurons and microglia [15], and the released iron induces apoptosis via lipid peroxidation. Thus, subarachnoid blood clotting, which has been linked to cell injury and oxidative stress [13], may cause greater apoptotic cell death in the cerebral cortex compared with the subcortex.

Apoptotic cell death has been reported to occur in neurons [13, 16, 17] and endothelial cells [18–20] in EBI after SAH, and both of them may be correlated with brain edema [21]. In this review, we focus on neuronal cell apoptosis, which consists of the intrinsic and extrinsic pathways [22] (Fig. 2).

Intrinsic Mechanisms of Apoptosis and SAH

Caspase-Dependent Pathway

The intrinsic pathway (mitochondrial pathway), which is mediated by the Bcl-2 family, starts with the increase of outer mitochondrial membrane permeability. The change of membrane leads to the leakage of mitochondrial proteins, including cytochrome c. Cytochrome c is translocated from mitochondria to the cytosolic compartment and interacts with apoptotic protease, activating factor-1, forming the apoptosome and leading to caspase-9 activation. Caspase-9, which is an initiator of the cytochrome c-dependent cascade, activates caspase-3, and results in DNA damage [23]. Caspase-3 is well known as one of the effectors of apoptosis, and cleaved caspase-3 was upregulated in the hippocampus and cortex after SAH [12, 24, 25].

It has been reported that some protein kinases might directly interact with mitochondrial proteins in cerebral ischemia, and their role mainly concentrates on the phosphorylation of pro- and anti-apoptotic proteins (Bad, Bax, Bcl-2, Bcl-xL) [26]. Akt (protein kinase B) and mitogen-activated protein kinase (MAPK) were the best studied of them in EBI after SAH. Akt, which is a serine/threonine kinase, is a key antiapoptotic signaling downstream of phosphoinositide 3-kinase (PI3K) in a growth factor mediated signaling cascade. Stimulation of receptor tyrosine

kinases or GTP-binding protein-coupled receptors activates Akt via PI3K, and activated Akt modulates many substrates, including Bax, Bad, glycogen synthase kinase-3, apoptosis signal-regulating kinase 1, and caspase-9, which inhibit apoptosis [27]. Moreover, Akt has also been shown to promote cyclic AMP response element-binding protein (CREB) phosphorylation and lead to Bcl-2 induction [28]. Decreased Akt activity is involved in ischemic neuronal cell death, and Akt activation is a principal factor in the prevention of apoptosis via the caspase-dependent pathway in cerebral ischemia [29–31].

Recent studies suggested that Akt might be involved in the mechanism for EBI after SAH, and this conclusion was drawn from using a PI3K inhibitor, which prevented phosphorylation of Akt and increased DNA damage [14, 32]. Moreover, Akt activation by overexpression of copper/zinc-superoxide dismutase (SOD1), which is one of the antioxidant enzymes, attenuated EBI caused by SAH [32]. Timing of Akt phosphorylation after SAH depended on brain regions; Akt were rapidly phosphorylated in the cortex, but it took 24 h to phosphorylate Akt in the hippocampus [14]. Since EBI after SAH may be the most severe in the cortex, it is suggested that Akt phosphorylation depends on the severity of brain injury [14].

The roles of MAPKs are very important in EBI after SAH [33]. MAPK, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38, is involved in the survival and apoptotic responses in certain cell death paradigms in cerebral ischemia [34]. These kinases are activated by various stimulants, including vascular endothelial growth factor (VEGF), oxidative stress, and inflammatory cytokines [35–38]. After SAH in a perforation model, these kinases were phosphorylated and induced brain edema, continuous high ICP, and high mortality [33, 39, 40]. Since ERK is activated in response to growth and differentiation factors and might be part of the survival pathway, whether activation of ERK is protective or detrimental to neurons in cerebral ischemia is controversial [41]. In contrast, JNK and p38 are activated in response to inflammatory cytokines and cellular stress, which were highly elevated in the cerebrospinal fluid and in cerebral arteries after SAH [42, 43]. JNK phosphorylates c-Jun, which upregulates apoptotic cascades by inducing expression of the proapoptotic member of Bcl-2 family Hrk/DP5, Bim, and Fas [44, 45]. Phosphorylated JNK and expression of c-Jun were increased after SAH induction and c-Jun mRNA were upregulated in the rat cerebral cortex and hippocampus after SAH [46, 47]. p38 activation by TNF-α and IL-1β was associated with neuronal death, and suppression of p38 activation by Bcl-2 suggested that p38 might be involved in apoptosis [48, 49].

Caspase-Independent Pathway

The caspase-independent component of the intrinsic pathway is carried out by the mitochondria-released apoptosis-inducing factor (AIF), endonuclease G and Bcl-2/adenovirus E1B 19kDa-interacting protein (BNIP3) [50]. AIF, which is the best studied among them, is normally in the mitochondrial intermembrane space and is translocated to the nucleus by some stimulations, inducing large-scale DNA fragmentation and cell apoptosis, which is independent of caspase activity [51]. Nuclear AIF upregulation was reported in cerebral ischemia [52], and the translocation might be triggered by poly (ADP-ribose) polymerase activity [53]. There has not been much reported about AIF expression in EBI after SAH and it is not clear which compartment of AIF expression increases [24].

Oxidative Stress and Early Brain Injury

It is important to hold the balance between reactive oxygen species (ROS) and antioxidants, which control oxidative stress. ROS such as superoxide anion (O^{-}_{2}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{-}) are generated at low levels and play important roles in signaling pathways [54]. Under normal conditions, they are regulated by endogenous antioxidants including SOD, glutathione peroxidase, glutathione, and catalase [55]. Overproduction of ROS and/or inactivation of antioxidants cause tissue injury from oxidative damage [54]. Oxidative stress can play important roles in the pathogenesis of EBI after SAH [56]. Mitochondria disruption, the production of hydroxyl radicals from extravasated hemoglobin, and disruption of the intrinsic antioxidant systems have all been reported in either experimental or human SAH [56–59]. O_2^{-} production was observed 1 h after SAH, and overexpression of SOD1 inhibited the production and reduced apoptotic cell injury after SAH [32]. The reduction in oxidative stress by SOD1 overexpression attenuated EBI after SAH via activation of Akt [32].

DNA Damage

p53 is a tumor suppressor gene involved in the regulation of apoptosis [60]. Responding to cell damage, p53 upregulates proapoptotic molecules including Bax, p53-upregulated modulator of apoptosis, and Bid, and downregulates antiapoptotic molecules Bcl-2 and Survivin [60]. p53 is upregulated after an

Table 1 Neuronal apoptosis related studies in early brain injury after SAH

Method/animal	Treatment	Neuronal apoptotic pathway	NS	Outcome	References
EP, rat	PP1	p-Erk, VEGF		BE↓	[33]
EP, rat	Z-VAD-FMK	caspase-3	+	BE↓	[12]
EP, rat	Hyperbaric oxygen	VEGF, BNIP3, TUNEL	+	BS↓, NS↑, MT↓	[69]
EP, rat	Hyperbaric oxygen	$gp91^{phox}$, NADPH oxidase, MDA	+	NS↑	[70]
EP, rat	Pifithrin	p53, cyto c, AIF, TUNEL, caspase-3, -8	+	BE↓, NS↑ MT↓	[62]
EP, rat	SOD1 overexpression	p-Akt, hydroethidine, Cell death assey		MT↓	[32]
EP, rat	SP600125	caspase-3	+	BE↓, NS↑	[40]
EP, rat	Tetramethylpyrazine	TUNEL, caspase-3	+	BE↓, NS↑	[63]
EP, rat	Atorvastatin	TUNEL, caspase-3, -8	+	BE↓, NS↑, MT↓	[24]
EP, rat	Argatroban	Cell death assey	+	BE↓, NS↑	[71]
BSI, rat	Meratonin	MPO, MDA, glutathione	+	BE↓, NS↑	[72]
BSI, rat	N-acetylcysteine	CuZn-SOD, GSH-Px, MDA	+	BE↓, NS↑	[73]
EP, mouse	Ac-YVAD-CMK	p-JNK	+	BE↓, NS↑	[39]
EP, rat	Hypothermia	–	+	NS↑, Weight↑	[10]

EP endovascular perforation, *BSI* blood single injection, *p-ERK* phosphorylated extracellular signal-related kinase, *VEGF* vascular endothelial growth factor, *BNIP3* BCL2/adenovirus E1B 19kDa-interacting protein 3, *TUNEL* terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick end-labeling, *NADPH* nicotinamide adenine dinucleotide phosphate, *MDA* malondialdehyde, *cyto c* cytochrome c, *p-Akt* phosphorylated Akt, *MPO* myeloperoxidase, *CuZu-SOD* copper/zinc-superoxide dismutase, *GSH-Px* glutathione peroxidase, *p-JNK* phosphorylated c-Jun N-terminal kinase, *NS* neurological score, *BE* brain edema, *MT* mortality, *BS* brain swelling

ischemia insult and induces mitochondrial damage and activation of caspases [61]. It was reported that in SAH, p53 is one of the key factors in neuronal cell death. p53 was upregulated both at 24 and 72 h after SAH, and p53 inhibitor decreased brain edema and neuronal cell death [24, 62, 63].

Extrinsic Pathway of Apoptosis

The death receptors, which are located on the cell surface, are involved in the extrinsic apoptosis pathway [31]. The receptor ligands expression, including Fas and tumor necrosis factor (TNF), are upregulated after cerebral ischemia [64, 65]. The death receptors can activate caspase-8 or -10, which then directly activate caspase-3 or cause Bid/Bax activation, inducing cytochrome c release [66]. Moreover, forkhead transcriptional factors were activated after cerebral ischemia and then expression of Fas ligand increased, resulting in neuronal cell death [67]. However, little is known regarding the relationship between EBI and death receptors or their ligands, whereas TNF-α were upregulated after SAH [68].

Treatments

Studies of neuronal apoptosis are summarized in Table 1. For evaluating neuronal apoptosis in EBI after SAH, neurological examination should be needed to examine the outcome of neuronal cell injury. These molecular apoptotic pathways in neurons may induce brain edema, neurological deficit, and higher mortality. Previous studies showed that apoptotic related pathway modulation by treatment could improve the outcome in EBI after SAH.

Conclusion

A lot of studies have demonstrated the apoptosis mechanism in cerebral ischemia, whereas relatively few have studied the relationship between apoptosis and SAH, especially in EBI. It would be helpful for us to study the relationship between SAH and another apoptotic mechanism, including autophagy and endoplasmic reticulum stress, which may lead to novel therapies in EBI. Studies regarding EBI after SAH are limited, and further studies are needed for clarifying the exact mechanism. For example, MAPKs, including ERK, JNK, and p38, were reported to induce apoptosis in the brain and cerebral artery after SAH [33], whereas it has reported that ERK phosphorylation induced a beneficial effect on cerebral vasospasm [74]. It is suggested that elevated ERK phosphorylation blocks apoptosis by enhancing the antiapoptotic protein Bcl-2 via CREB activation in cerebral ischemia [41]. The opposite effects may depend on the localization in the brain including neurons, glia, and endothelial cells.

In conclusion, apoptosis may play an important role in EBI after SAH. Further studies regarding apoptosis may lead to the development of new therapies and the improvement of outcome of SAH patients.

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Conflict of interest statement We declare that we have no conflict of interest.

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