

NEW AVENUES IN THE PATHOGENESIS OF SUBARACHNOID HEMORRHAGE.

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Abstract Background—Subarachnoid hemorrhage (SAH) remains a disease without any definitive treatment options. Research, to date, has concentrated on the pathophysiology of vasospasm. Recent evidence supports the concept of Early Brain Injury (EBI), a phenomena which may help to explain the complex pathophysiology seen in patients after a SAH. **Summary**—EBI aims to describe the pathophysiological events that occur in the brain within the first seventy two hours after a SAH, before the onset of vasospasm. A number of pathways have been identified which may play a role in the etiology of EBI. This review provides a brief synopsis of EBI and its implications for the future. **Conclusions**—EBI may represent a key event in the development of both vasospasm and Delayed Ischemic Neurological Deficit (DIND) after subarachnoid hemorrhage. Additional studies are required to determine the pathophysiology of EBI and to examine its role as a possible precursor to both vasospasm and DIND.

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Introduction

Subarachnoid hemorrhage (SAH) despite recent reviews indicating a decrease in the case fatality rate in patients with this disease continues to pose a significant threat to life and limb. The mortality and morbidity rates remain at unacceptably high levels, with an estimated mortality of 12% prior to hospital admission and a further 40% within a month of admission¹. This despite continued research and improvements in radiology, intensive care and the surgical management of patients with SAH. Since the first description of a SAH by Morgagni in 1761, it has largely been believed that vasospasm is the culprit for the mortality and morbidity rates observed. Perhaps with the exception of calcium channel blockers few studies have found a reliable method by which to alter the natural history of this disease. Indeed,

the Cochrane group have indicated through a meta-analyses of Nimodipine in patients with SAH, that the evidence is not over whelming in favor of SAH but that there is probably little harm in using it².

In recent years they have been a few groups that have tried to deviate away from established teaching with regard to vasospasm. It has been questioned as to whether vasospasm is indeed the culprit³. A new theory has emerged considering the possibility of Early Brain Injury (EBI) instead. This theory maintains that at the moment of a SAH, pathophysiological changes occur within the brain that may account for the disease process observed clinically. It has been suggested that these pathophysiological changes may be responsible for causing vasospasm as well as Delayed Ischaemic Neurological Deficit (DIND) together. In other words, vasospasm and DIND are two separate entities both caused by

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EBI and not necessarily related to each other⁴. There is evidence to support these statements which will be discussed in this review.

Early Brain Injury

EBI is a term that has only recently been adopted and describes the injury to the brain as a result of a SAH within the first seventy two hours of the ictus^{5,6}. The etiology of EBI lies within the complex pathophysiology encountered after SAH. It has well been established that the severity of a SAH is related to the blood load^{7,8}. The intracranial pressure (ICP) rises as blood enters the subarachnoid space, this has been demonstrated in both animal and in human models. The precise mechanism behind this rise in ICP remains unknown, although the increase in volume secondary to hemorrhage (Monroe-Kellie Hypothesis), vasoparalysis and cerebrospinal fluid (CSF) drainage obstruction have been implicated⁹. A rising ICP inevitably leads to a fall in the cerebral perfusion pressure (CPP), resulting in ischemia.

Given the fact that the rise in ICP is global it is not surprising that the resulting ischemia is also global. The mechanism behind this phenomenon, although incompletely understood, may be related to cortical spreading ischemia secondary to a reduction in cortical nitric oxide and a decrease in Na^+/K^+ ATPase activity^{10,11}. The fall in CBF and the concomitant rise in ICP may also be a protective mechanism, in an attempt to control blood loss from the aneurysm. CBF can fall to almost zero in experimental models^{5,12}. Blood pressure also falls, which again may be a protective mechanism to reduce blood loss. Finally, the cerebral blood volume (CBV) increases, perhaps as a result of vasodilatation. Ischemia has three possible outcomes: necrosis, apoptosis and recovery. Necrosis has been demonstrated in the brains of patients who have died following SAH and will not be discussed further here¹³.

Prolonged anoxia is not compatible with life and approximately 30% of these patients die at the time of the SAH. If the ischemic insult is brief, then cells within the brain may undergo apoptosis. The degree and extent of apoptosis is dependent on the amount of energy (i.e., ATP) available. With a fall in ATP levels, there is a concomitant rise in apoptosis. Not surprisingly, the most vulnerable part of the brain to ischemia, the hippocampus, is usually affected at low levels of hypoxia with additional brain parenchyma being affected as the duration of hypoxia increases. This may explain why many grade one patients demonstrate loss of hippocampal function in the long term^{14,15}.

Following the global ischemia seen with SAH, apoptosis has been shown to occur in the hippocampus, blood brain barrier, and vasculature with varying degrees of necrosis^{16,17}. Apoptosis has been implicated in the development of vasospasm and smooth muscle cell proliferation in spastic arteries¹⁷. Apoptosis has even been demonstrated in aneurysms and has been implicated in aneurysmal formation and rupture both in humans and in animal models¹⁸. However, when the injury is global, the degree of apoptosis can be more devastating than the injury itself.

Apoptosis

There are a number of apoptotic pathways that are believed to play a role in SAH: the death receptor pathway, cysteinyl aspartic acid-protease (caspase) dependent and independent pathways, as well as the mitochondrial pathway⁵. It is currently difficult to speculate which apoptotic pathway is important in SAH. To date, the death receptor/p53 pathway has been described as being particularly important^{5,19}. Of course, once initiated, all of the cascades come into play, as the relationships between these proteins are extensive and intricately interwoven. SAH can be considered to be an external stress event²⁰, which can possibly through changes in

the environment or physical structure of cells, lead to cellular suicide²¹. It has been shown in ischemic and hemorrhagic models that if severe enough, the injury can result in deoxyribonucleic acid (DNA) fragmentation²². In the case of SAH, the cell membrane death receptors, e.g., apoptosis stimulating fragment (Fas), tumor necrosis factor receptor 1 (TNFR1) and death receptors 3-5 (DR3-5), are believed to be responsible for the translation of the signal across the cell membrane by activating the TNFR family which appears to be the primary target in relation to SAH induced apoptosis²³. It has been shown in previous experimental models that TNF- α is up regulated after SAH²³.

One of the largest target groups of p53 is the Bcl-2 family, which contains a multitude of pro- and anti-apoptotic genes²⁴. Some of these include Bcl-2 associated x protein (Bax), Bid, Bcl-2 interacting killer (Bik), Bcl-2 antagonist/killer (Bak), Bcl-2 like 1 protein (Bcl-XL) and Bcl-2 like 11 protein (Bim) (pro-apoptotic) and Bcl-2, and myeloid cell leukemia 1 (Mcl-1) (anti-apoptotic). However, it has been shown that it is the overall ratio of pro- to anti-apoptotic signals which finally determines whether or not the cell dies²⁵. Therefore, one can hypothesize that it may be dependent on the strength of the signal or extent of the injury. That said, it is not known how exactly the Bcl-2 family influences apoptosis. One of the most widely accepted hypotheses suggests that it is the ability of Bcl-2 to inhibit caspases by binding to Apoptotic protease activating factor-1 (Apaf-1) and preventing cytochrome C release, thus preventing apoptosis²⁶. It is known that in neuronal cell death, it is the up regulation of Bax that initiates the apoptotic cascade. In fact, it has been shown that Bax is required for p53-induced caspase 3 activation in neuronal cell death²⁷. As a whole, the Bcl-2 family can either stimulate or inhibit cytochrome C release from the mitochondria depending on the dominant

signal, i.e. pro- or anti-apoptotic dominance²⁵.

It is important to note that apoptosis is not an all or none mechanism. In fact, in situations of sub lethal injury an apoptotic cell can recover and necrotic cells have been shown to possess the ability to switch to apoptosis in certain conditions. In addition, p53 cleaves procaspase 8 to form caspase 8, which in turn cleaves Bid to form truncated Bid (tBid). tBid then permits the release of cytochrome C from the mitochondria which is further regulated by Bcl-2 and Bcl-XL. As a result, cytochrome C is released into the cytosol where it binds to Apaf-1²⁸. The cytochrome C/Apaf-1 complex referred to as the apoptosome then recruits and cleaves procaspase 9 which activates the downstream caspase cascade³.

The critical step in this process is that of cytochrome C release, which is mediated by the Bcl-2 family. The Bcl-2 family is, in turn, controlled by p53²⁸. Caspase 9 is a prerequisite for the cleavage of procaspase 3 to caspase 3, which is known to be involved in p53 mediated apoptosis. Interestingly, the intrinsic pathway (mitochondrial pathway) is energy dependent and probably only occurs in areas, for example, in the penumbra, where ATP is available²⁹. In areas where ATP is not available, the extrinsic pathway occurs via the activation of caspases 8 and 3. Therefore, in SAH brains, either of these cascades can occur depending on the severity of the insult and the area of the brain being examined. For example, the hippocampal cells are far more prone to injury than other areas due to their sensitivity to ischemia and ATP requirements¹⁶.

As discussed above, p53 seems central to the apoptotic cascades in SAH. Recently, a new role for p53 has been found in the caspase independent cascade³⁰. In many experimental models of stroke and SAH, the inhibition of caspases has been shown to afford some protection, although apoptosis still occurs. Therefore, it seems clear

that another caspase independent cascade may be involved. Apoptosis inducing factor (AIF) is a mitochondrial intramembranous flavoprotein that has been shown to be released from the mitochondria and translocated to the nucleus in response to various death signals³⁰. p53 has been shown to trigger the release of AIF in the absence of Apaf-1, resulting in a caspase independent apoptotic cascade³⁰.

Brain Edema

One of the first complications related to both the pathophysiological aspects of SAH and the apoptotic cascades discussed above, is the disruption of the blood brain barrier (BBB)¹⁶. It is likely that the immediate pathophysiological upsets manifest themselves as early BBB disruption, while late BBB disruption is caused by the apoptotic phenomena³¹. The evidence for this is sparse as there is very limited information available from human studies regarding the time course of BBB disruption. Even in animal models, the time course is dependent on the animal model used³². Results from experimental models have found BBB changes ranging in time from one hour to six days. However, the overall pattern appears to be a biphasic response of the BBB to SAH in the short and long term³³. While one can tentatively suggest that a similar biphasic affect can be seen in humans, it is far from categoric.

Damage to the endothelial cells, as well as leading to BBB disruption, may also lead to a fall in the production of endothelial-dependent relaxing factors. This has been speculated to aggravate vasospasm locally, if not generally. This is further aggravated by denuding the vessels of endothelial cells through the process of apoptosis as a result of global ischemia which exposes the vessel to a host of vasoactive and toxic metabolites which can also aggravate vasospasm²³. Clinically, it is probably a combination of these factors and others that result in BBB disruption and

vasospasm. The destruction of the BBB and the subsequent edema has been implicated as one of the major predictors of cognitive dysfunction in the long run after SAH.

Brain edema is a major component of early brain injury as a direct consequence of the disruption to the BBB and not as a result of vasospasm³⁴. Although brain edema secondary to SAH has been largely ignored in the literature, Classen and colleagues showed that 8% of patients had global cerebral edema detected by computed tomography (CT) scan on admission and that an additional 12% developed appreciable edema over the first 6 days. The destruction of the BBB after a SAH is not well understood, although a number of different mechanisms have been proposed as outlined above. In patients with SAH, classical vasogenic edema has been described which is a direct result of BBB breakdown, which was also shown in experimental models³³.

Therefore, as mentioned above, the first arm of the biphasic response results in immediate brain edema. Through the mechanisms previously described, there is a resultant rise in the ICP which further reduces CBF and leads to further ischemia³⁵. As a result, there is more damage to the BBB and the apoptotic cascades are initiated, leading to a further breakdown in the BBB and suggesting a biphasic response. It is the disruption of the endothelial cells due to cell death that allows for the acute rise in both cerebral edema and ventricular volumes. Therefore, brain edema contributes to the rise in ICP seen immediately after a SAH³⁶. It is also believed to result in acute vasoconstriction which, when combined with the edema, leads to a further reduction in CBF and results in global ischaemic injury³⁷. The mechanism by which this occurs has not yet been fully elucidated. If unchecked, this cycle will repeat itself, leading to further edema and eventually death.

Vasospasm

There is considerable evidence demonstrating a close relationship between vasospasm and apoptosis/EBI. It has been shown that apoptosis occurs in vasospastic vessels^{38,39}, as well as aneurysms and has even been implicated in aneurysmal rupture^{40,41}. Furthermore, it has been shown that by reducing apoptosis in the basilar artery, severe vasospasm can be inhibited³⁸. A possible mechanism for this relates to the ability of the endothelial cells to prevent smooth muscle cell proliferation and vasoconstriction by producing inhibiting factors such as endothelial NO synthase (eNOS)⁴².

The time course for apoptosis in the rat monofilament model is approximately 24hrs, although up regulation of the apoptotic machinery can still be demonstrated at 72hrs³⁸. The time course in humans remains unknown. In the rat model the time course of apoptosis and the onset of vasospasm appear to validate the theory to some degree. This is reflected in measurements of outcome, BBB breakdown and brain edema, all of which have been shown to subside at 72hrs, in this model. This seems to suggest that the prevention of p53 orchestrated apoptosis in the endothelial layer preserves the integrity of the vasculature, thereby protecting the BBB and preventing edema. Finally, to date, most experimental data has concentrated on one aspect of the various apoptotic cascades. The role of inhibition at multiple levels may provide better protection from vasospasm and indeed EBI, for patients in the future and presents an exciting and unique therapeutic option deserving of further work.

Conclusions

Subarachnoid hemorrhage remains a life threatening disease which is poorly understood. While the surgical treatment for aneurysms has improved in recent years, comprehensive

treatment options for patients with SAH, is still lacking. Although vasospasm is believed by many to be the cause of ischaemic insults to the brain, there remains many unanswered questions. Most important of these was highlighted by the CONSCIOUS-1 trial in 2005, which was a double-blind, randomized clinical trial with clazosentan. The results of the trial indicated a significant dose-dependent 65% reduction in the relative risk of angiographic vasospasm. However, the trial failed to demonstrate an improvement in outcome. This has led to questions with regard to the clinical significance of angiographic vasospasm.

Therefore, it has been shown in both animal and human studies that despite treating vasospasm the outcomes do not appear to be dramatically different. As a result the search for alternate etiologies has begun. While EBI is currently a candidate and may explain the relationship between the pathophysiology seen after a SAH, vasospasm and DIND, it has not however been proven and there remains considerable work to be done to further elucidate the molecular mechanisms and their relationships to EBI, vasospasm and DIND.

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