The Pathology of Ischaemic Stroke: Development of Atherosclerotic Plaques and the Role of Nuclear Factor-κB and Notch Signalling in Cerebral Ischaemia

Background

Ischaemic stroke is a cerebrovascular disease that is one of the most prevalent causes of disability and death. It occurs due to an occlusion of a cerebral artery blocking blood flow to the brain, thereby reducing oxygen levels in the affected area of the brain. Characteristic symptoms of stroke are paralysis or weakness to one half of the body and incoherent speech. The impact of stroke can vary depending on age, sex, and environmental factors. Ischaemic strokes account for approximately 80% of strokes, often with poor prognosis.

Aim

The aim of this article is to describe the development of a cerebral arterial occlusion and the role of nuclear factor-κB (NF-κB) and the Notch signalling pathway in ischaemic stroke.

Pathology

The development of ischaemic stroke can be linked to the arterial plaques that are characteristic of atherosclerosis. In a cerebral artery, the ruptured plaque will occlude the vessel, thereby reducing oxygen to an area of the brain. Hypoxia activates NF-κB in ischaemic stroke which leads to neuroinflammation. Hypoxic conditions may stimulate the Notch signalling pathway which upregulates expression of NF-κB and the production of proinflammatory cytokines. Hypoxia-Inducible Factor-1 may also enhance this signalling pathway.

Conclusions

To conclude, the article described a handful of the factors contributing to the pathology of ischaemic stroke. Preventative measures against other vascular diseases should be taken to reduce the risk of stroke. NF-κB and components of the Notch pathway could be used in future research as potential therapeutic targets for treatment of ischaemic stroke.

Background

Ischemic stroke is a cerebrovascular disease that is caused by an occlusion of a cerebral artery or an artery leading to the cerebrum (1). Ischemic strokes account for approximately 80% of strokes worldwide, and according to the World Health Organization the occurrence of ischemic and haemorrhagic stroke in lower- and middle-class populations has doubled in the last four decades (1, 2). Within the 80%, it appears that older women have reduced risk of stroke compared to older men, however, the opposite is true for younger women compared to younger men (1, 3). This is possibly due to the use of hormonal contraceptives which are known to have coagulative effects from oestrogen (4). Additionally, populations of people living in polluted areas have been reported to have increased risk and prevalence of stroke (5-7). Ischemic stroke is also one of the most prevalent causes of disability and death worldwide and long-term prognosis is extremely poor due to neuronal death from lack of oxygen from the occluded artery. (3, 8). Because of the lack of blood flow to an area of the brain, the most common symptoms of ischemic stroke are weakness/paralysis on one side of the body and aphasia which is a condition that inhibits coherent

speech and/or comprehension of speech (9). In clinical settings, the acronym Face-Arms-Speech-Time is frequently used for the diagnosis of stokes which can be seen in Figure 1 (10). However, these symptoms also frequently present in haemorrhagic stroke, caused by a ruptured cerebral blood vessel, and therefore, computed tomography or magnetic resonance imaging are often used to determine the stroke type (9). The overall aim of this article is to describe the development of the arterial occlusion and investigate the activation of nuclear factor-κB (NF-κB) and the Notch signalling pathway in hypoxic conditions and their roles in ischaemic stroke.

Pathology

The Occlusion

Since ischaemic stroke is caused by an occlusion of a cerebral artery, it is heavily linked to vascular diseases such as atherosclerosis. The development of atherosclerosis begins with the formation of plaques under the tunica intima layer of endothelium due to build-up low-density-lipoproteins, immune cells, and lipid-laden macrophages (foam cells) (11, 12). Eventually a fibrous cap made of collagen and vascular smooth muscle cells will form to stabilize the plaque. If the cap ruptures, it will trigger a coagulation cascade leading to thrombosis and occlusion of the blood vessel. This plaque can form in any artery; if a plaque forms in a cerebral artery, its rupture can lead to ischaemic stroke, however, it can also lead to myocardial infarction or renal failure if the occlusion is in the heart or kidneys (12). The following sections will describe one of the responses to hypoxia and one of the hallmarks of ischaemic stroke that occurs after vascular occlusion.

Nuclear Factor-κB and the Notch Pathway

Necrosis and apoptosis of neurons that receive oxygen from the blood in the occluded artery occurs quite quickly. However, surrounding the dead cells is an ischaemic penumbra, an area of neurons with reduced oxygen consumption that can still be perfused by other arteries (13). Therefore, in the penumbra, the neurons continue to be salvageable for a short period of time; they respond to their hypoxia through activation of certain signalling pathways and transcription of important protein factors. One of the factors that are upregulated during hypoxic conditions is NF-κB. NF-κB is an important transcription factor for genes that code for proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β (14). Under homeostatic conditions, NF-κB is bound to inhibitory-κB (IκB) and is mainly inactive. However, during ischaemic stroke, IκB is phosphorylated by inhibitory-kappaB-kinase (IKK) causing it to dissociate from NF-κB (14). One study showed that IKK may be activated by hypoxic conditions due to a reduction in prolyl hydroxylase (PHD) activity (15). Under normal conditions, PHDs may inhibit IKK through hydroxylation, however, they require oxygen to be active. Due to their oxygen sensitivity, PHDs are also important regulators of hypoxia-inducible factor-1 (HIF-1), however, this molecular will be discussed later (15). A visual of the activation of NF-κB during hypoxia can be seen in Figure 2.

Another factor that is upregulated during hypoxic conditions is called Notch, a family of transmembrane receptors. Under homeostatic conditions Notch receptors produce a signalling pathway that is very important for neural embryonic development by keeping neural progenitor cells undifferentiated (13). Furthermore, Notch signalling is a regulator of neurogenesis, axonal

and dendritic growth, synaptic plasticity, and neuronal death over the course of a person's life (16). During and after an ischemic stroke, activation of the Notch signalling pathway has been shown to have neuroinflammatory effects. For example, one study found that Notch receptors are upregulated on the surfaces of microglial cells following cerebral ischemia (17). Microglia are the immunoinflammatory cells of the central nervous system. Microglia are known to cause apoptosis and necrosis of neurons and glial cells due to the production of proinflammatory cytokines such as TNF-α. The study found that it is likely that the Notch pathway indirectly regulates the production of cytokines from microglia by upregulating the expression of the transcription NF-κB (17). Interestingly, a transcription factor downstream of the Notch pathway called recombination signal binding protein-Jκ (RBP-Jκ) has been described as a transcriptional repressor of the NF-κB2 gene in another study (18). In the Notch pathway, the Notch intracellular domain (NICD) is translocated to the nucleus and binds to the RBP-Jκ on the DNA to form a complex. In the study, they investigated the binding of RBP-Jκ to DNA and the NICD and how it affected NF- κB expression. They found that the binding site for RBP-Jκ on DNA partially overlaps the promotor region of the NF-κB gene. Upon interactions with the NICD, transcription of NF-κB was upregulated. They inferred the possibility that RBP-Jκ and NICD may form a higher order complex which then promotes transcription of NF-κB, however, this mechanism is yet to be confirmed (18). A simplified visual of the Notch pathway in combination with the studies just described can be seen in Figure 3.

An additional study found that the transcription factor HIF-1 may be an important modulator of the Notch signalling pathway during ischemic stroke (19). They also provided evidence that NF-κB expression is likely upregulated by the Notch pathway which may be enhanced by HIF-1. In the study they found that the overexpression of both HIF-1 and the NICD individually increased the expression of NF-κB. Additionally, the inhibition of HIF-1 resulted in a decreased expression of NF-κB suggesting that HIF-1 may enhance Notch signalling. Finally, they discovered that overexpression of HIF-1 and NICD contributed to greater neuronal cell death together than individually.

Conclusions, Ethics, and Future Research

In conclusion, the article described the arterial occlusion, NF-κB, Notch signalling pathway, and HIF-1 as factors contributing to the pathology of ischaemic stroke. The occlusion of a cerebral artery was linked to the development of atherosclerosis. Therefore, preventative measures against atherosclerosis and other vascular diseases could help reduce the risk of stroke. However, due to the increased occurrence of stroke in lower- and middle-class populations, this could be indicative of poor access to adequate healthcare, and hence, it becomes more difficult to prevent strokes. NF-κB has been shown to be important for the progression of neuroinflammation during ischaemic stroke. Both NF-κB and the Notch pathway could be used in future stroke research as potential therapeutic targets to reduce neuroinflammation. Since the inhibition of HIF-1 also reduced NF-κB expression, it could also be an area of future research of therapeutic targets. However, while HIF-1 reduced the expression of NF-κB and was therefore connected to the modulation of the Notch pathway, it is still unknown how HIF-1 enhances this pathway.

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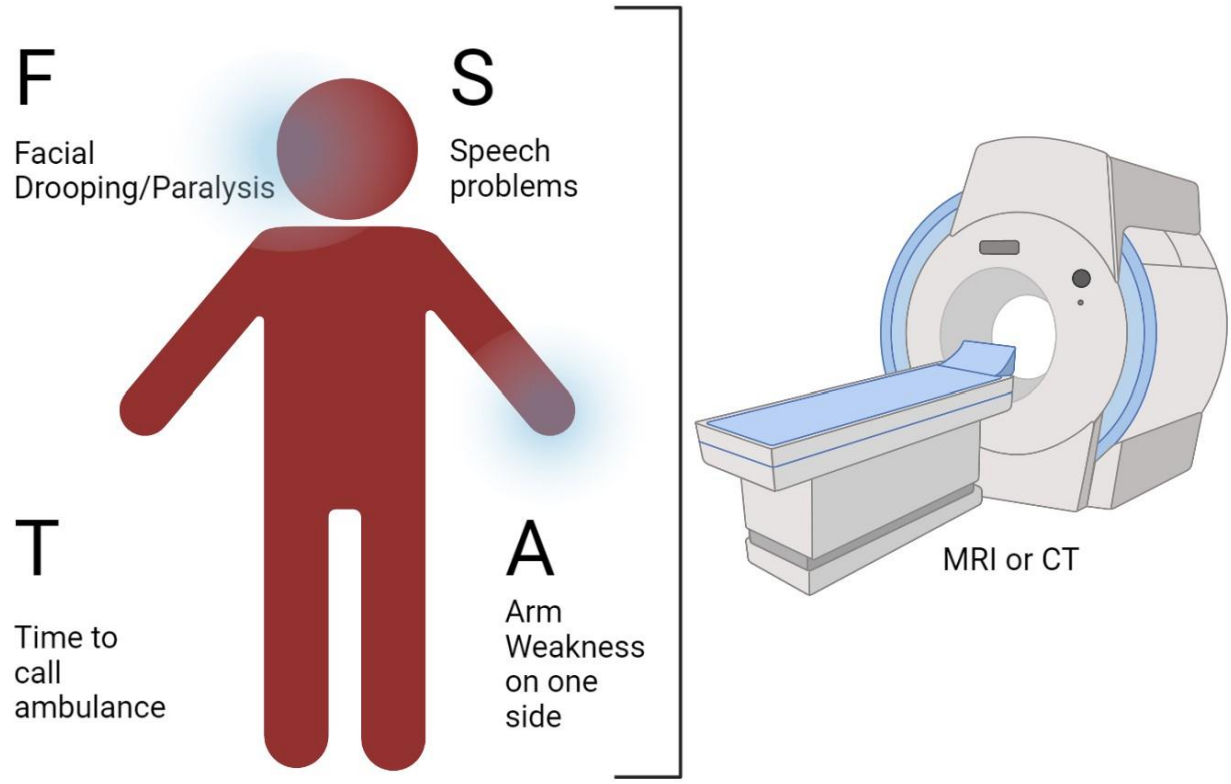

Figure 1: Early signs, symptoms, and diagnosis of stroke (9, 10). Figure 1 outlines the most prominent signs and symptoms of a stroke which are commonly found in both ischaemic and haemorrhagic stroke. The acronym frequently used in English speaking countries is F.A.S.T. which stands for facial drooping, arm weakness, speech problems, and time to call an ambulance. The acronym can be helpful for quick identification of these symptoms by the public and medical professionals. At the hospital magnetic resonance imaging (MRI) and/or computed tomography (CT) scans are frequently used to determine if the stroke is ischaemic or haemorrhagic. This figure was created using Biorender.com.

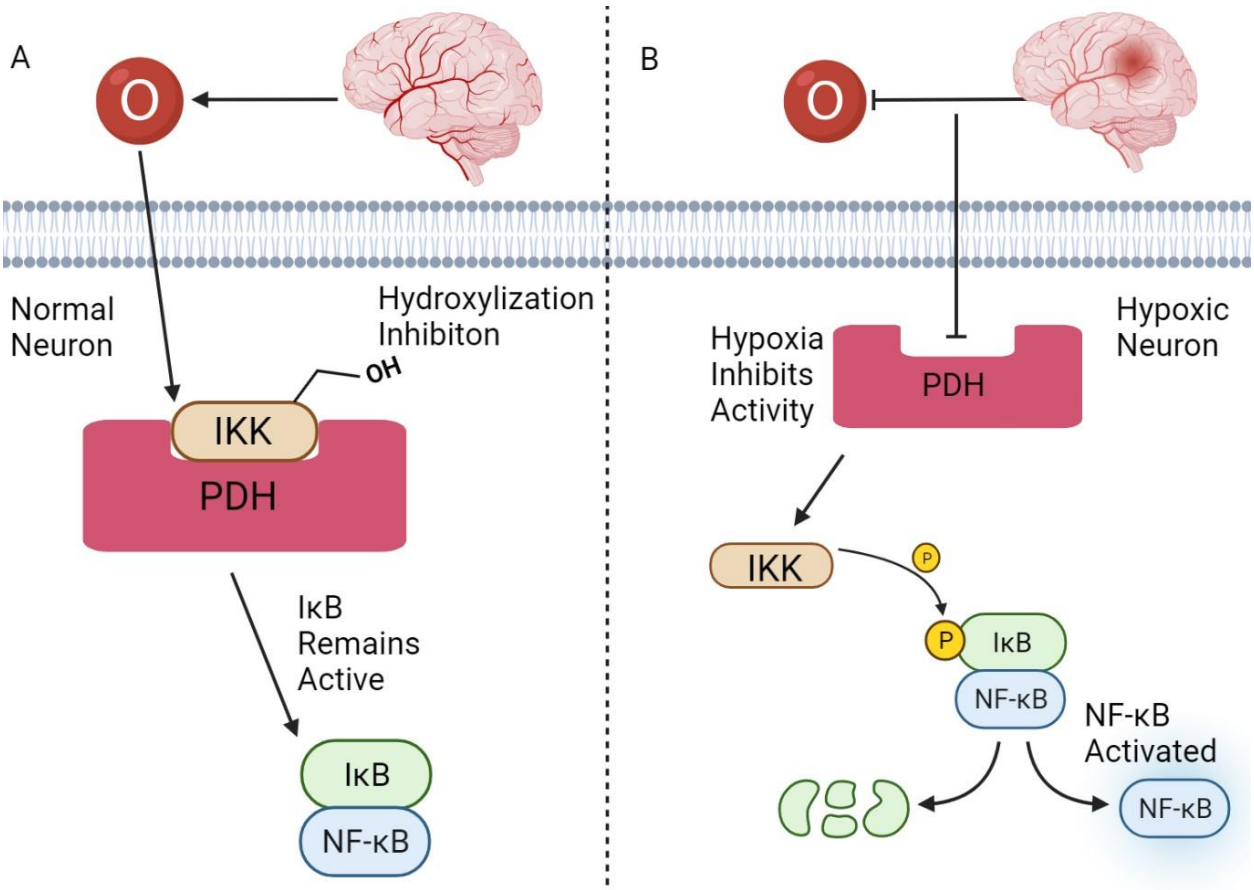

Figure 2: Comparison of the activity of nuclear factor-κB (NF-κB) in normal and hypoxic neurons (14, 15). A. Figure 2A shows how NF-κB stays inhibited under homeostatic conditions. Prolyl hydroxylase (PDH) requires oxygen to remain active. PDH may hydroxylate inhibitory-kappa-kinase (IKK) to inhibit it from phosphorylating inhibitory-κB (IκB). Since IKK cannot phosphorylate IκB, IκB remains active and inhibits NF-κB which prevents downstream transcription of proinflammatory cytokines. B. Figure 2B shows how NF-κB may be activated under hypoxic conditions. The lack of oxygen prevents PDH from functioning properly and it can no longer inhibit IKK through hydroxylation. IKK then phosphorylates IκB, marking it for degradation. Now NF-κB is active and can promote downstream transcription of proinflammatory cytokines. This figure was created using Biorender.com.

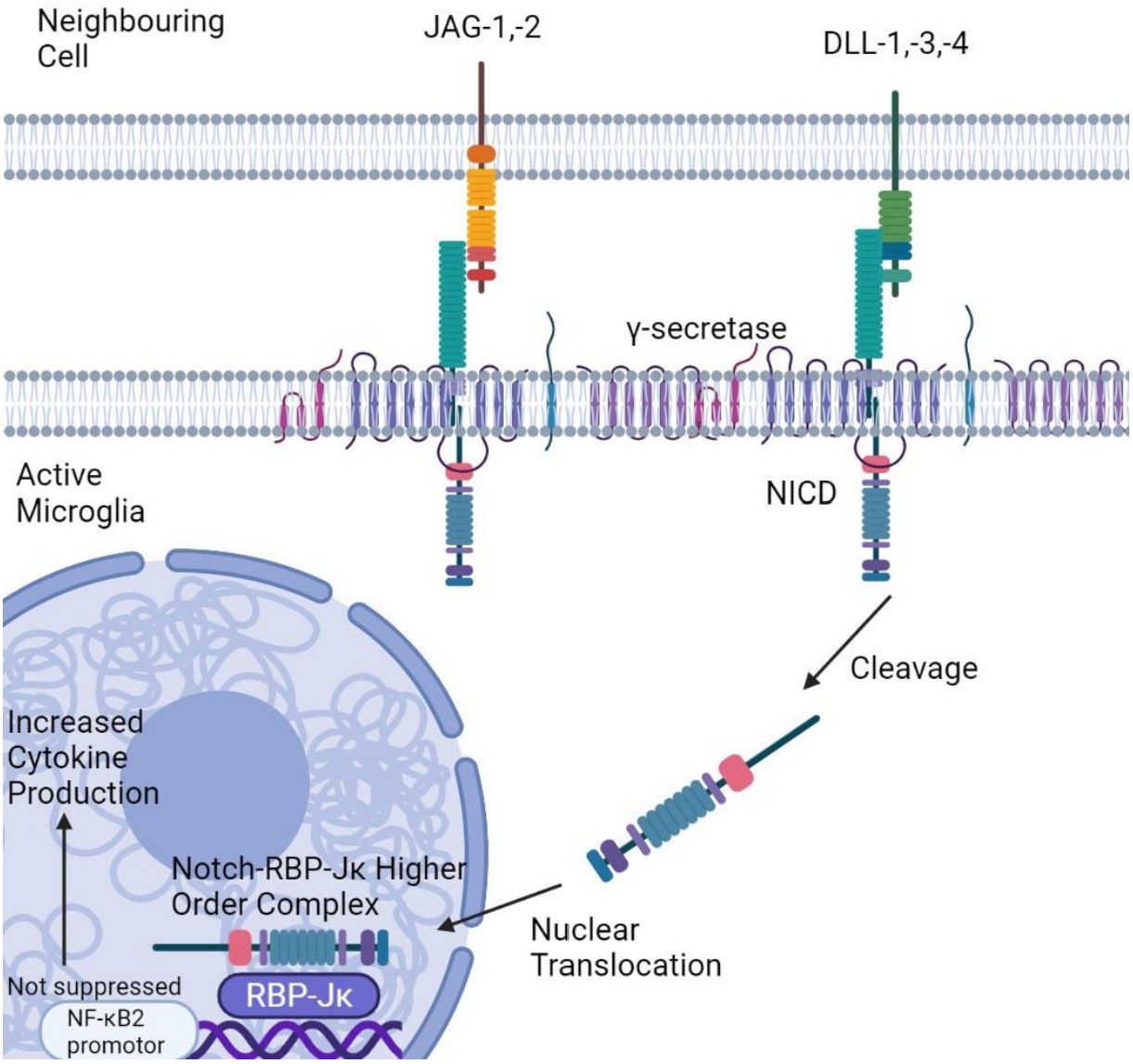

Figure 3: Simplified Notch Signalling Pathway (13, 17, 18). Notch receptors can be activated upon ligand bind with Jagged and Delta-like membrane proteins (JAG-1, -2 and DLL-1, -3, -4) resulting in ligand-receptor crosstalk between cells. Then an enzyme complex called γ-secretase will cleave the Notch intracellular domain (NICD). Following the cleavage via γ-secretase, the intracellular domain of Notch will then translocate to the nucleus to combine with the transcription factor recombination signal binding protein-Jκ (RBP-Jκ). Their association may lead to the formation of a higher order complex that no longer suppresses the nuclear factor-κB2 (NF-κB2) promotor region which promotes the production of proinflammatory cytokines from microglia. This figure was created using Biorender.com.