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A Review of the Paradoxical Effects of Microglia in Ischemic Stroke and the Future of Treatment

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Abstract

Deadly if attended to too late, ischemic strokes affect millions of individuals around the world yearly. Microglia are one of the first respondents to brain injury, acting quickly to repair and prevent damage in the event of a stroke. These cells have been long held as beneficial protectors and healers in the neuroimmune response as they are pro-inflammatory and remove toxic debris. Microglia have on the other hand also been found to mediate deleterious effects such as becoming neurotoxic when overactivated, which may compromise the damaged tissue instead of healing. Given its beneficial effects, researchers have attempted to target microglia in ischemic stroke intervention and treatments but due to its paradoxical role in inflammatory response, these attempts have encountered difficulties and many complications. The future of microglial-targeted treatment and intervention methods therefore is uncertain. This review summarizes the main findings of microglia following stroke to exemplify the need for continual research in this area. Currently, modulation of microglia rather than total blockage may be the best option, however, more research must be conducted using methods that can optimize the benefits while minimizing detriments as well as methods that are able to be easily translated to the people who experience strokes.

Keywords — Ischemic Stroke, Microglia, Treatment

1. Introduction

Microglia play an influential yet paradoxical role in the nervous system when damaged, providing both beneficial and harmful injury responses. As the brain's resident macrophages, microglia promote cellular differentiation by providing trophic support for astrocytes, oligodendrocytes, and vessels [1]. After injury, glial cells form containment zones around damaged brain cells and aid in accelerating the recovery process of damaged sites by removing harmful debris [2]. These are beneficial abilities, especially during the recovery process of strokes, however microglia have also been implicated in detrimental effects on the central nervous system post-stroke. In this review, by examining this seemingly paradoxical role of microglia in ischemic strokes, I hope to clarify the role of microglia in the intervention and treatment of stroke, as well as describe the current challenges researchers face with developing effective treatment methods.

Strokes are the third leading cause of death in Canada, with more than 400,000 Canadians living with long-term disability due to stroke [3, 4]. This number is predicted

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to double in the next 20 years by the Heart and Stroke Foundation of Canada [4]. Though there are multiple types of strokes, the focus of this review is on ischemic strokes as they are the most common type, caused by a blood clot in the brain [4]. If the stroke injury is not treated in time, the implications can be deadly as the blockage interrupts the regional blood supply, leading to neuronal dysfunction, and cell death (apoptosis) [5, 6]. The penumbra which surrounds the necrotic core, is an area of constrained blood flow comprised of potentially preserved energy metabolism which can progress to infarction due to the excitotoxicity that results from prolonged oxygen deprivation caused by the vascular blockage [6]. Alongside infarction, other secondary deleterious phenomena may also occur such as accumulation of excessive glutamate in extracellular space due to spreading depolarization, the production of toxic mediators activated by post-ischemic inflammation, and apoptosis of cells [6]. Excitotoxicity mechanisms can also cause inflammation and edema followed by acute cell death, or delayed apoptosis [6]. The severity of these effects however, affects the neuronal ability to recover from the stroke; the more severe, the worse the stroke outcomes [7]. After the site has been deprived of oxygen even for a brief moment, the brain is exposed to systemic responses that further the damage already done, potentially exacerbating the cognitive and physical deficits that occur from a stroke [7].

2. The Beneficial Role of Microglia

Microglia are an important component of the immune response, playing a beneficial role in response to injury. When the stroke injury is attended to in the brief time frame before extensive damage can be done, treatments can prevent further damage [8]. During this period of time, microglia elicit an immune response critical in post-ischemic injury recovery. Comprising 10% of the brain's cells, microglia lead the brain's immune system response following stroke and attend to the injured or damaged site [7]. Microglia are phagocytes which develop after the formation of the blood-brain barrier, making them unique, as they evolve in a specialized neural microenvironment devoid of other types of glial cells [7]. They continuously monitor the brain, responding to changes in brain homeostasis, even in their presumed resting state [9]. In order to search for these changes, microglia send out branching thin processes which survey the microenvironment and detect potential threats [9]. This is an important function of microglia, as it allows for them to remove toxic cellular debris, keeping sites of injury protected [9].

Alongside this function, studies on mice suggest that microglia may play a beneficial role in neuroprotection post-ischemic stroke [10, 11]. Lalancette-Herbert et al., [10] found that microglial proliferation may play a primary role in regulating or modulating both pro- and anti-inflammatory responses following ischemic injury in mice. As the inflammation process initiates, microglia are activated and differentiate into one of two phenotypes: M1 and M2 as a response to stress [12]. M1 microglia act as a pro-inflammatory mediator and defense against pathogens, typically being the first responder to the injury and infection [13]. This inflammation assists with stimulating myelin repair and removing toxic proteins from the central nervous system [14]. M1 is however also implicated in harmful effects which will be mentioned below [13]. M2

acts in opposition to M1 as an anti-inflammatory response and promotes repair gene expression following the initial microglial response [13]. M2 also produces neurotrophic factors, insulin-like growth factors 1 and 2 as well as brain-derived growth factors which aid the inflammation resolution process and promote neuron survival [13].

3. The Deleterious Role of Microglia

Though microglia have been implicated as the primary immune response to injury to the brain, there has been speculation regarding the neurotoxic effects resulting from microglial activation. As microglia carry out their neuroprotective role, they may produce reactive oxygen species and anti-inflammatory cytokines which can inhibit tissue repair when overactivated [15]. Mice studies have shown that when injecting the microglial activation inhibitor, minocycline, secondary oligodendrocytes, and axonal degeneration decrease following an injury to the central nervous system [11, 16]. Minocycline as well as other microglial inhibitors have also been found to provide some neuroprotection following stroke, producing better neurological outcomes with treatment [16]. Inhibition of microglia in these experiments contradicts the previous findings that microglia provide beneficial effects to sites of injury. Microglial overactivation may therefore turn beneficial effects into detrimental effects. Additional studies also indicate that this overactivation can be attributed to either direct stimulation via environmental toxins and endogenous proteins or reactive microgliosis after neuronal damage occurs [17, 18]. These seemingly opposing duties have been long questioned, as microglia play a large role following ischemic injuries.

Gomes-Leal [15] suggests that altered neurons, glia, and blood vessels among other sources, may release both beneficial and detrimental factors into the extracellular space where microglia reside. Other studies have found that in non-infectious diseases such as stroke, the destructive mechanisms used to eliminate pathogens may unintentionally also destroy healthy neurons [19]. Particularly in the case of ischemic strokes, disruption of the blood-brain barrier due to reactive oxygen species produced by overactive microglia can induce great damage within the barrier itself [20]. In doing so, neurotoxic agents may leak into the ischemic tissue, eliciting a cascade of post-ischemic inflammation events which can further damage the barrier [20]. Though these destructive effects appear detrimental, alongside the homeostatic role of microglia, they also play a key role in supporting the barrier and continue to attend the site of injury for hours after the initial damage occurs [21]. This relationship between microglia and the blood-brain barrier exemplifies the paradoxical role of microglia post-ischemic stroke.

4. Microglial-Targeted Treatment and Intervention

As microglia have been found to hold both beneficial and detrimental effects following neuronal injury, they are of great interest in the treatment of damaged tissue, especially in the recovery process of ischemic stroke. As summarized previously, the paradoxical effects of microglia appear to prevail mainly when overactivation of microglia or the release of neurotoxic agents occur [19, 20]. Though studies involving drugs and antibiotics such as minocycline have established that they are capable of fully inhibiting

microglia activation to alleviate the tissue damage resulting from ischemic stroke, many researchers oppose the use of abolishing microglia activation [15, 16]. Block et al., [17] suggest that early attenuation of microglial response to non-deleterious levels would be the ideal therapeutic approach in the treatment of strokes and neurodegenerative disorders. They hypothesize that given the progressive and cumulative process of microglial activation, intervening early on would allow for an opportunity to ensure that microglia are not overactivated at any point. Gomes-Leal [15] also believes that drug use should be avoided in addressing the microglia overactivation. He states that it is fundamental to identify which microglia receptors contribute to the beneficial or detrimental effects following strokes. By being able to identify these receptors, experimental manipulation or pharmacological applications may be able to enhance the beneficial effects of microglia while diminishing detrimental effects. Guruswamy et al., [7] agree with Gomes-Leal, elaborating further that fine-tuning immunomodulatory interventions while avoiding harmful immunosuppressive effects would be the most effective method in promoting the repair of damaged tissue post-stroke. A consensus among these researchers regarding the modulation of microglia activation levels rather than the total blockage of microglia activation is apparent. These methods of therapeutic remedy can and should be explored in the future through experimental design to analyze the effectiveness of modulating microglial activation post-ischemic stroke.

Recent studies have utilized these ideas to develop methods of intervention targeting microglia in ischemic stroke therapy [22, 23]. As suggested, modulation of microglial activation using pharmacological methods can improve the recovery process post-stroke [22]. Aside from minocycline, other treatments that modulate the effects of microglia rather than inhibit them such as noggin, which modulates microglial phenotypes, and tumour necrosis factor (TNF), which modulates microglial activation, can also be used to assist in post-stroke recovery in animal models [18]. Though these are promising steps toward creating the most optimal method of microglial intervention, nothing has been clinically approved for use in humans yet [22]. Other non-pharmaceutical methods utilizing cellular therapies in animal models that involve direct administration of microglia have also been found to yield improved axonal outgrowth and reduced tissue damage [23, 24]. Other animal studies using non-medical interventions such as exercise and environmental enrichment can also aid in stroke-recovery for older mice as the activity can modify age-related microglial dysfunction [25, 26].

5. Challenges of Treatment and Intervention

Despite these promising techniques, research on model systems is more extensive than humans as we do not perform these types of experiments on humans [22]. Human research comes with complications given the challenges of applying experimental and laboratory techniques from animal studies to human patients. This may be due to practical differences between lab studies and real stroke victims. Even if treatments are found to work in animals, they may only work in a brief window of time post-stroke when the individual may not even be aware that they are experiencing a stroke, leading to decreased efficacy if used [6]. These treatments may also work differently in animal models as for example, rats have three times the glucose, oxygen metabolism, and

blood flow as humans, and effects of treatment may be species dependent [6]. These studies are conducted in laboratories, using special equipment in 'ideal environments', while also often using young and healthy mice while stroke victims are often older human adults [6]. In addition, other reasons concern the approach of studying strokes, as every stroke is different and therefore not as heterogeneous and predictable as those studied in the lab [6]. Though it is useful studying animal models, the generalizability in some cases is questionable.

Treatments for ischemic stroke cannot be optimized using the findings of the current literature, likely due to a lack of understanding of the modulation of microglia [22]. The complexity that comes with attempting to selectively target the deleterious effects of microglia without impacting the beneficial effects also contributes to this difficulty in identifying treatment methods [22, 27]. Animal models may further complicate this problem as drug concentrations and limits of tolerated dosages vary between, for example, rodent models and humans which may exacerbate side effects 6. Even if methods targeting microglia are found to be effective in improving post-stroke symptoms, due to its paradoxical effects, side effects could potentially further decrease the quality of life in these individuals who already face many daily struggles as a result of their injury. Drug side effects can be monitored in animal models, but side effects may be missed or more complicated than can be observed with laboratory tools. Not only do researchers have to keep in mind the paradoxical effects but also the psychosocial impacts on the individuals that they are trying to help since inevitably these humans are more complex than animal models.

6. Conclusion

Researchers hold consensus that microglia play a large role in the neuroimmune system, possessing healthy inflammatory, and debris clearing properties as well as destructive neurotoxic, and disruptive properties. Microglia are vital in the immune response following stroke, however the paradoxical effects make these cells complex targets for treatment and intervention. Due to this reason, the progress research has made toward developing clinically safe therapeutic methods of intervention targeting microglia has been overall met with limited success. In attempting to yield the most beneficial effects of microglia, deleterious effects may result instead. Though it has been established that the selective modulation of microglia yields the most optimal improvements in stroke symptoms, research from animal models has not yet been able to be replicated in humans, resulting in this stand still in the literature. This finding prompts the need to explore the modulation of this activation and experimentally test if it is a feasible intervention method while being aware of its potentially harmful effects. Microglia hold great potential as a treatment target for ischemic stroke, therefore if selective modulation of microglia is able to be applied to humans, while yielding minimal deleterious or harmful effects, perhaps science may someday significantly change the outcome for stroke victims.

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