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Interventions in Ischemic Stroke Targeting Microglial Activation

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Abstract. Ischemic stroke is one of the biggest health concerns nowadays, leading to nonnegligible disability and mortality in its patients. During its pathological development, resident microglia in the central nervous system play a significant role to repair the damage, but may also expand the lesion because of the excessive microglial activation. As a result, microglial activation is considered a potential therapeutic target for ischemic stroke. Since microglia polarized into different phenotypes ranging from M1 to M2 display proinflammatory and neuroprotective functions, regulation of the morpho-functional change of microglia became a further way of intervention in ischemic stroke. This review qualitatively analyses drugs for ischemic stroke that were studied by credible research in the recent ten years in terms of experimental methods, applied animal models, and potential signaling pathways, so as to provide clues for future studies on the fine regulation of microglial activation to treat ischemic stroke.

Keywords: ischemic stroke, microglial activation, microglial polarization, treatment

1. Introduction

Stroke is one of the leading health concerns worldwide, affecting as many as 11.71 million people annually while accounting for 10% of deaths globally [1-2]. Moreover, stroke is responsible for dysfunction in up to 75% of its patients and disability in up to 45% of its survivors [2-3]. These mortalities and disabilities resulting from stroke cast a huge economic and mental burden on families as well as societies.

Among two broad categories of stroke including hemorrhagic stroke and ischemic stroke, the latter is the majority which constitutes 85% of the total number of stroke cases [4]. Recent evidence has reiterated the critical role of inflammatory responses in the development of ischemic stroke, during which glial cells such as microglia are actively involved in restoring the damaged brain tissue as well as expanding the lesion [5].

Microglia are resident macrophages that account for 10% of cells in the central nervous system, with a special origin from precursors in the embryonic yolk sac [6]. Highly ramified resting microglia swing their fine processes around to monitor the microenvironment of a healthy brain. But it becomes amoeboid-like after being activated by injury signals including ischemic stroke, participating in phagocytosis, cytokine release, and degradation of extracellular matrix proteins by MMPs [4,6]. These activities of activated microglia, as part of the neuroinflammation process, contribute to the

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remodeling of the lesioned brain tissue after ischemic stroke, thus empowering potential therapies of ischemic stroke to focus on microglial activation.

The review provides a qualitative analysis of drugs and cytokines reported by previous research aimed at decreasing the activation of microglia and altering the phenotypic transformation of microglia as potential therapeutic interventions for ischemic stroke. The related experimental methods, animal models, stages that drugs take effect, and the regulated signaling pathways are discussed in detail. A few other possible microglial targets of ischemic stroke treatment are further mentioned in brief. The listing of drugs herein offers hints for the possible interventional targets on microglia in the treatment of ischemic stroke.

2. Microglial activation and polarization in the context of ischemic stroke

Activated microglia experience morphological and functional polarization into M1 (classical) or M2 (alternative) phenotypes [6]. In general, M1 microglia excrete proinflammatory cytokines and cytotoxic substances such as tumor necrosis factor-α (TNF-α) and nitric oxide (NO), while M2 microglia are anti-inflammatory and release IL-10, arginase-I, as well as growth factor VEGF [6-7].

Specifically, microglia activation is actively involved in the pathology of ischemic stroke through blood-brain-barrier disruption, interruption of neurogenesis, and secondary neurodegeneration. Key pro-inflammatory cytokine TNF-α expressed by M1 microglia induces endothelium necroptosis in the oxygen-glucose deprivation/reperfusion (OGDR) model and middle cerebral artery occlusion (MCAO) model, thus contributing to the disruption of BBB which further expands the lesion [8]. Furthermore, the reduction of microglial activation shows a significant correlation with the increase in the number of neuroblasts in focal ischemia rat models, suggesting a potential role that microglial activation plays in the inhibition of neurogenesis [9]. Together with microglial morphological analyses, confocal imaging of GFP-Cx3CR1 expressed on activated microglia and NeuN in Neurons in photothrombotic occlusions in mice also suggested a significant correlation between neuron loss and classical microglial activation in secondary neurodegeneration, though the basic molecular machinery remains to be investigated [10].

Although not easy to explicitly quantify in human clinical cases in a real-time manner, both categories of activated microglia exhibit a distinct spatiotemporal localization in the ischemic brain in experimental settings. During the acute stage of ischemic stroke in the permanent MCAO model in rats, activated microglia can be found in the boundary of the lesion after thirty minutes, but appear in the core of ischemia 24 hours after the ischemic onset and become more in 72 hours [11]. Other MCAO studies in mice that applied RT-PCR and immunofluorescence staining to detect M1 and M2 markers suggested an increase in M2 gene expression since the first day of MCAO treatment, peaking in 7 d, while the expression of M1 microglia increased 3 d after MCAO and peaked at 14 d [12-13]. These pieces of evidence suggest that M2 microglia dominate the lesion in an early stage and transform into the M1 phenotype later in the sub-acute phase and possibly chronic phase of ischemic stroke. Moreover, the photothrombotic stroke model in rats also revealed that microglial reaction declined whereas ameboid cells can be hardly detected 28 d and 60 d after the lesion, suggesting a gradual decrease of microglia activation in the chronic stage of microglia. [14]. Immunofluorescence staining coupled with confocal imaging of slices in another photothrombotic stroke experiment proved the existence of a dense layer of microglia surrounding the infract developing region in 3 d, followed by its reduction and the decrease in microglial activation after a month [10]. Overall, microglia present rapid responses to the ischemia by activation and migration to the infarct region and create a physical barrier between the lesion and surrounding tissue. They experience a phenotypic transition from anti-inflammatory to pro-inflammatory in the subsequent phases after stroke onset and gradually deactivate along the chronic stage.

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3. Inhibiting microglial activation as intervention

Since excessive microglial activation can be deleterious in terms of its role in interrupting neurogenesis, disrupting BBB, and inducing neurodegeneration, it is reasonable to consider microglial activation as an interventional target for ischemic stroke.

Various strategies have been brought up to inhibit the activation of microglia in animal models as potential therapies (Table 1). As an example, the intraperitoneal injection of HAMI 3379, an antagonist for Cysteinyl leukotriene receptor 2 (CysLT¬2R), reduced the number of Iba-1 positive microglia and ED-1 positive activated microglia in the ischemic core and boundary zone 72 hours after MCAO, which is coupled with a decrease in pro-inflammatory cytokine concentration and improved neuron survival [11]. After MCAO and ischemia-reperfusion (IR), atorvastatin, a statin used for the treatment of high blood cholesterol, also lowered the number of ED-1 positive cells in 72 hours and reduced the lesion volume [15]. Unlike the previous two drugs taking effects in a relatively short period, the anti-inflammatory drug Indomethacin significantly reduced the microglial activation 14 d after injuries [9]. Except for conventional chemicals, overexpression of long non-coding RNA such as LncRNA-1810034E14Rik also inhibited overall microglial activation 24 hours after oxygen-glucose deprivation and reperfusion (OGD/R) and decreased infarct volume in MCAO, implying the potentiality of LncRNA therapies for the acute stage of ischemic stroke [16]. Zinc chelators (CaEDTA, TPEN) were reported to inhibit the expression of M1 specific markers (CD16/32, iNOS) in a study on the effect of extracellular Zinc on microglial polarization, suggesting reducing Zinc as a possible therapy [17]. Similarly, Genistein-3'-sodium, a polyphenol, aims at suppressing M1 activation and the expression of pro-inflammatory IL-1β [18]. Luteolin, another polyphenol, reduced the absence of microglia in the hippocampus 72 hours after ischemia [19]. Interestingly, long-term treatment of Argon, a noble gas, was also reported to attenuate the activation of microglia 30 days after the injury, preserving neurons throughout the chronic phase of ischemic stroke [20].

Table 1. List of drugs that regulate microglial activation.

Drug	Microglial activation	Animal	Experimental model	Target stages of ischemic stroke	References
HAMI 3379	decrease	Rats	MCAO/R	Sub-acute (72 h)	Shi et al. (2015) [11]
Atorvastatin	decrease	Mice	MCAO/R	Sub-acute (72 h)	Potey et al. (2015) [15]
Indomethacin	decrease	Rats	Focal striatal ischemia	Sub-acute/chronic (14 d)	Lopes et al. (2016) [9]
Zinc chelator (CaEDTA, TPEN)	decrease M1	Mice	LPS-stimulated BV2, IR	Sub-acute (72h)	Higashi et al. (2017) [17]
LncRNA-1810034E14Rik	decrease	Mice	MCAO, OGD/R	Acute (24 h)	Zhang et al. (2019) [16]
Luteolin	decrease	Rats	MCAO	Sub-acute (72 h)	Liu et al. (2020) [19]
Genistein-3'-sodium	decrease M1	Rats	tMCAO	Acute (24h)	Liu et al. (2021) [18]
Argon	decrease	Rats	tMCAO	Chronic (30 d)	Liu et al. (2022) [20]

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4. Regulating the M2:M1 ratio as intervention

The drugs above that generally block microglial activation or reduce M1 microglia do present neuroprotective characteristics to some extent. However, broad inhibition of activation of microglia as a whole without considering their critical roles in remodeling the damaged brain tissue can be problematic, while failing to identify the condition of anti-inflammatory M2 polarized microglia simultaneously is also risky. A better approach is to finely modulate microglial polarization so as to promote M1 to M2 transition or increase the M2:M1 ratio [21].

In fact, many studies do show advances in modulating the M2:M1 ratio via different signaling pathways (Table 2). Although not originally designed for stroke, metformin, an adenosine 5'monophosphate-activated protein kinase (AMPK) activator, raised M2 but suppressed M1 polarization in the long-term treatment after MACO, improving angiogenesis and neurogenesis potentially through the promotion of M1 to M2 transition by AMPK activation [21]. In the bilateral carotid artery stenosis (BCAS) induced chronic hypoperfusion experiments, a considerable decrease in CD16/32 positive and Iba-1 positive cells was found after fingolimod (FTY720) treatment but the expression level of CD206 remained stable [22]. Further investigation indicated that FTY720, this approved drug for multiple sclerosis, weakened M1 activation and strengthened M2 polarization after the stimulation of LPS plus IFN-γ in primary microglia via upregulation of STAT3 [22]. Comparably, Minocycline mediated similar M1 to M2 transition in vitro and improved neuronal survival and functional recovery in vivo, accompanied by the mounting concentration of phosphorylated STAT1 and STAT6 [23]. A plant-derived chemical named baicalein also inhibited neuroinflammation partially attributed to the phenotypic shift of microglia from M1 to M2 which is possibly mediated by inhibited phosphorylation of MAPK and NF-κB signaling pathways [24]. Moreover, endogenous substances are the potential sources of drugs for ischemic stroke. Noggin, for instance, is an endogenous antagonist against bone morphogenetic proteins that not only increased microglial activation in general and facilitated the M1 to M2 transition in special but also decreased the glial scar made of astrocytes [25]. Another endogenous glycoprotein cytokine, erythropoietin, was demonstrated to show a similar effect on decreasing glial scar, but regulated microglial polarization differently via blocking M2 to M1 shift and maintaining M2 polarization [26]. It is worth mentioning that other than conventional chemicals and proteins, well-designed drug complexes that take an integrated drug delivery system into consideration can be great candidates. Ma@(MnO2 +FTY), as an example, is a macrophage-disguised honeycomb manganese dioxide (MnO2) nanosphere with fingolimod (FTY) loaded on this nanoparticle [27]. Such a drug complex wrapped by macrophage cell membrane managed to obtain a prolonged half-life through a protective coating, target ischemic lesion specifically through retained macrophage surface proteins, reduce reactive oxygen species through the catalytic MnO2, and augment the M2/M1 ratio through the loaded fingolimod [27]. This reveals novel possibilities of multi-target complex treatment of ischemic stroke.

Despite showing the promising effect of drugs on neurofunctional improvement, these studies on microglial polarization are almost completely dependent on the co-label of Iba-1 and M1/M2 specific markers, which might be biased. Recent evidence has revealed the possibility of concurrent expression of both M1 and M2 markers on the same microglia, implying the urgent need for a more careful investigation into distinguishing microglial phenotypes [28]. Brand-new subtypes of microglia with varied gene expression profiles also remind us of the limitations of using an oversimplified M1/M2 model to analyze therapeutic effects [29].

Table 2. List of drugs that modulate microglial polarization (continue).

Drug	M2: M1	Animal	Experimental model	Signaling pathway	References
Metformin	increase	Mice	MCAO/R, LPS-stimulated BV2	AMPK	Jin et al. (2014) [21]
Noggin	increase	Mice	tMCAO	Not indicated	Shin et al. (2014) [25]

Fingolimod (FTY720)	increase	Mice	BCAS, stimulated microglia	LPS/IFN-γ primary	STAT3	Qin et al. (2017) [22]
Erythropoietin	increase	Mice	tMCAO		Not indicated	Wang et al. (2017) [26]
Baicalein	increase	Rats	MCAO/R		NF-κB, MAPKs	Yang et al. (2019) [24]
Minocycline	increase	Mice	MCAO/R, stimulated microglia	LPS/IFN-γ primary	STAT1/STAT6	Lu et al. (2021) [23]
Ma@(MnO_2 +FTY)	increase	Rats	tMCAO		Not indicated	Li et al. (2021) [27]

5. Discussion

Due to their critical and complicated roles in the CNS, microglia are a highly potential treatment target for ischemic stroke. Apart from regulating microglial activation and polarization as intervention, some other aspects of microglia are still considerable and are to be researched further. These alternative directions include but are not limited to microglial depletion, repopulation, migration, and their interaction with other cells such as neurons and astrocytes [30-32].

It is worth noticing that although the disruption of BBB along with ischemic stroke allows drugs to permeate into the brain tissue, the measure of BBB opening is still dependent on the extent of the lesion [33]. Therefore, in order to determine the practicability and implement further biochemical modifications of the interventions, the permeabilities of therapeutic agents are to be examined. Corresponding experiments on human microglia might also provide a more solid basis for further clinical applications.

6. Conclusion

Microglia are important macrophages in the CNS that are involved in the pathogenesis and development of ischemic stroke. Upon activation, microglia polarize into different phenotypes interlinked with distinct and even opposing functions to regulate the surrounding brain environment. Therefore, instead of suppressing microglial activation as a whole to inhibit inflammation-induced damage in ischemic stroke, the fine modification of phenotypic shift of microglia to induce its neuroprotective ability and impair the inflammation progress seems to be a more promising therapeutic method. Interestingly, most drugs reviewed were tested in rodent models or mice microglia cell lines. Future studies should also notice the possible difference between rodents and humans, and carefully examine the clinical practicability of drugs in humans.

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