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Ischemic Stroke Cell and Gene Therapy; Using Type 2 Microglia and Gene profile of IL-35 and HGF

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Abstract

Ischemic Stroke (IS) is the second cause of death and the third cause of disability in the world. The main mechanisms which have a role in IS immune-pathogenesis are oxidative stress, apoptosis, and inflammation. It is noteworthy that pro and anti-inflammatory cytokines and chemokines implicated in the all named mechanisms. There are limited approved treatments in the American Food and Drug Administration (FDA) for ischemic stroke, which include endovascular recanalization and tissue plasminogen activating factor (t-PA). Also, other proposition therapeutic methods for IS are gene therapy, cell therapy, and combined gene-cell therapy. For these types of treatments, various genes and cells have been utilized, possessing different advantages and disadvantages. The main requirement in this type of therapy is the anti-inflammatory microenvironment to reduce the destruction of remained host cells and increase the function of the transplanted cells. The selected cell in our hypothesis is Type 2 Microglia cell within the anti-inflammatory effect in the IS microenvironment. In addition to selecting appropriate cells, there is a need to use anti-inflammatory factors. Our suggested anti-inflammatory factors are IL-35 (an anti-inflammatory cytokine) and Hepatic Growth Factor (HGF) (an anti-apoptotic and immuno-suppressor factor). AAV has been suggested as a potential vector for utilizing in this transferring gene approach. The current study aimed to propose a gene-cell therapy strategy to improve repairing, anti-apoptotic, and immuno-suppressive mechanisms in the IS. This procedure could be a reliance for neurologists to tackle their obstacles in treatment and for patients to qualify their lifestyle.

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Introduction

Is the second cause of death and the third cause of disability in the world [1-3]. According to the underlying pathophysiology, there are two types of strokes; ischemic and hemorrhagic. Ischemia is the dominant pathophysiology of the stroke due to permanent or transient damp down of cerebral blood flow which per se can diminish brain cellular function through ATP deprivation [3-5]. The vulnerability of the brain in ischemic stroke is due to the different mechanisms, comprising oxidative stress, intracellular poisoning with calcium, cytokine-induced necrosis, apoptosis, and inflammation [6,7]. In addition to these molecular processes, there is a multiplicity of cell-mediated pathways in this deleterious state, containing platelets and leukocytes (exactly monocytes and macrophages) cross talk. Secondary to this interaction, lesion progression, plaque rupture, thrombus formation, and embolization are implicated. Platelet function is a link between hemostasis, innate immunity, atherosclerosis, and inflammation that exerted through its granules content (pro-inflammatory cytokines and chemokines). Furthermore, macrophages aggravate ischemic events via the expression of tissue factors, C-Reactive Protein (CRP), and Tumor Necrosis Factor-Alpha (TNF-α). Altogether, these complex mechanisms contribute to thrombosis formation through inflammation [8,9]. According to irreversible abnormalities, high prevalence, and reduced quality of life, there is a critical need for a fundamental treatment. However, the limited FDA approved treatments, including endovascular recanalization, decompressive surgery, and tissue plasminogen activating factor (t-PA) are not efficient and IS remains as an incurable disease [10].

Pathogenesis

The intricate process of atherosclerosis is characterized by an essential group of cellular and molecular mechanisms. Each component constitutively exerts a beneficial role in devastating atherosclerotic events.

Molecular mechanism of atherosclerosis

Molecular mechanisms are crucial parts of the inflammatory process involved in the initiation and persistence of atherosclerosis. The intensity of ischemic stroke extensively depends on the nervous system inflammation, microglia-mediated inflammatory factors, and nuclear factor kappa B (NF-kB) signaling [1,5,7,11]. The initiation of atherosclerosis is depending on the sub-endothelial Apo lipoprotein (APO) B-100 accumulation following dyslipidemia. After APO B-100 entering, oxidation and glycation mechanisms lead to the sub-endothelial retention of this lipoprotein. Placement of this deformed molecule can induce the inflammatory response in atherosclerosis. Intensifying the expression of Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Protein-1 (VCAM-1) on the endothelial cell surface is an essential implication of inflammatory-associated response that facilitates the entry of immune cells like monocytes into the target tissue. Monocyte-derived macrophages in the injured area can induce the monocyte and neutrophil attraction through the secretion of CCL3, CCL5, and Monocyte Chemoattractant Protein-1 (MCP1). Also, tissue-resident macrophages attempt to devour the entrapped molecule. Secondary to structural changes in APO B 100 and macrophages (reduction of CCR7), swallowing is impaired, which causes the foam cell formation, a source of CCL3 excretion [12]. CCL3 is exerted as a neutrophil chemo-attractor by binding to its receptor. Concerning these series of mechanisms, the inflammatory state is mediated by the TLR4/MyD88/NF-kB pathway. By the means of this cascade, divers' inflammatory mediators (IL-1B, IL-18, and TNF-α) and multi-protein components, named inflammasomes are secreted as critical factors for systemic and local inflammation. Inflammasome activation induces the exosome (heterogeneous membrane containing biomolecules) secretion by neurons, astrocytes, microglia, and oligodendrocytes in CNS. The exosomes contain various kinds of bioactive mediators, such as tubulin, clathrin, heat-shock proteins (Hsp 70 and 90), actin, flotillin-1, metabolic enzymes of glucose metabolism, signal transduction proteins (kinases, heterotrimeric G proteins), proteins involved in transport and fusion (annexins, Rab proteins), Major Histocompatibility (MHC) molecules, translation elongation factors, and IL-1β [13]. The beneficial member of the inflammasome's family in atherosclerosis and ischemic stroke is NLR Family Pyrin Domain Containing 3 (NLRP3), which is associated with thrombosis formation. Ultimate products of this cascade, comprising IL-1β, IL-18, and TNF-α increase in primary hypoxia. Inflammasomes enhance transcription of pro-inflammatory cytokines and also induce converting the procaspase-1 to the active form. Upon the activation, the functional inflammatory phase is amplified [4,10,14-17].

Cellular mechanisms of atherosclerosis

The effective cells in IS immune-pathogenesis include Dendritic Cells (DC), CD4+ T cells, gamma delta (γδ) T cells, microglial cells, and astrocytes. However, the microglial cells are the most crucial component in the brain tissue. Two polarities of these cells, M1 and M2 phenotypes have been deciphered in response to brain injury. The M1 phenotype is characterized as a pro-inflammatory cell, which can cause neuroinflammation and neurodegeneration by inducing the secretion of pro-inflammatory factors (Reactive Oxygen Species (ROS), TNF-α, Interleukin-1β (IL-1β), Nitric Oxide (NO), superoxide, and proteases). The M2 phenotype switches the immune system to T helper 2 (Th2)-dominated response through the STAT6 signaling pathway and anti-inflammatory cytokines production include IL-10, IL-13, and TGF-β. The M1/M2 ratio depends on the severity and stage of destruction, in which a higher M1/M2 ratio is associated with brain injury and neurodegeneration enhancement [4,11,16-22]. Also, M2 cell regulates the revascularization and repairing process by inducing the Resistin like molecule a (RELMa), Arginase-1 (Arg-1), and Chitinase 3 like-3 (Chil3, also called Ym1) genes [23]. Hence, the M2 cell can reduce the brain tissue degeneration, inflammation, and increase the post IS repairing. Furthermore, there are types of factors that can affect on repairing process and immune-regulation in the IS microenvironment, such as IL-35 and HGF [24-26]. Since IL-35 and HGF are immunosuppressive factors, they may be utilized as immune-regulator components to control the inflammatory and repair processes in IS immuno-pathogenesis. According to the above-mentioned categories, it can be considered that the combined immune cell therapy with a genetic profile in IS using the HGF, IL-35, and a viral vector (to gene transduction into M2 cell), may emerge as an effective therapeutic method in the context of IS treatment [27,28].

IL-35 signaling pathway

IL-35 is a heterodimer member of the IL-12 cytokine family with two subunits of P53 and Epstein-Barr virus-induced gene3 (EBI3). IL-35 activates the STAT4 and STAT1 pathway through binding to the protein Interleukin-12 Receptor Beta 2 (IL-12Rβ2) and Interleukine-27 Receptor Alpha (IL-27Rα). Substantially, the downstream signaling pathway is established through suppressing the TH1 and TH17, increasing the Treg cells activity,

anti-inflammatory and immunosuppressive mediators, and production of IL-10 as well as the IL-17 downregulation. Moreover, IL-35 suppresses T cells and converts them to IL-35-producing induced regulatory T cells. These mechanisms are the imperative pathways of ischemic stroke pathogenesis. IL-35 has also been characterized as an appropriate cytokine in targeted therapy in inflammatory, autoimmune, and infectious diseases. Also, IL-35 regulates the function of T cells, B cells, macrophages, and dendritic cells [25,29-32] (Figure 1).

HGF signaling pathway

Hepatic Growth Factor (HGF) is a pleiotropic cytokine that known as "scatter factor" in the gene map 7q21.11, which is produced by damaged heart and brain cells. The HGF has mitogenic, mutagenic, morphogenic, anti-fibrotic, and anti-apoptotic effects on different tissues. The biological response to this factor is via binding to the tyrosine kinase receptor MET that has different domains: Sema, PSI, IPT, Juxtamembrane, Kinase, and multifunctional docking site. Following the HGF- tyrosine kinase receptor MET binding, the Signal transducer and activator of transcription 3 (STAT3), Phosphoinositide 3-Kinase (PI3K), and Rat Sarcoma (RAS) signaling pathways are activated. HGF induces anti-inflammatory cytokines production, cell migration, inflammatory and immune response, cytokine production, maturation, antigen presentation, and T cell function. HGF also plays a critical role in protecting the tissues from inflammatory damages [24,26,33-35]. Furthermore, HGF can reduce the production of IL-6, TNF-α, ICAM-1, and High Mobility Group Protein B1 (HMGB1) via the NF- kB pathway inhibition [36] (Figure 1).

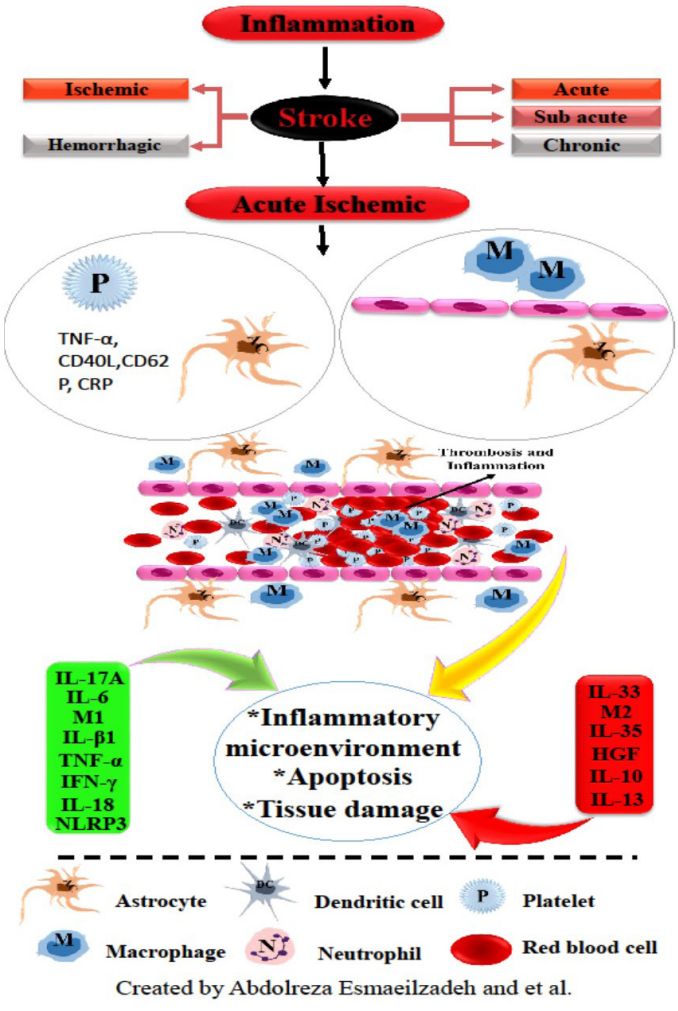

Figure 1

The hypothesis

Stroke is one of the most debilitating chronic diseases with no effective treatment. Appropriate treatment for strokes should be able to cover three important goals, which include reducing the inflammation and apoptosis and also improving the repairing process. None of the existing therapies are comprehensively enough for covering all these goals. Besides, the present treatments cannot be generalized to all patient populations. Hence, a combination therapeutic strategy is required for IS, which can contain a complex of the cell, gene, and vector. The recommended cell in this hypothesis is the M2 phenotype of the microglial cells, which has an anti-inflammatory effect with the dominant late-onset function. The sources of these cells are the fetal yolk sac and blood monocytes i.e. The valuable features of the M2 phenotype are healing the central nervous system and synapses, producing anti-inflammatory cytokines and mediators such as IL-10 and TGF-β, and regenerating the neurons and myelin. According to the mentioned characteristics, M2 microglia has been considered as a suitable candidate for target therapy in IS in this hypothesis [37-40]. The IL-35 cytokine and HGF are other selected components, which covert the anti-inflammatory condition in the IS microenvironment. Also, HGF has anti-apoptotic and restorative effects [25,29-32,36]. Which another existed gap in IS combined therapy is a vector, which transforms the selected genes into target cells. There are various types of viral and non-viral vectors. The viral types are herpes simplex virus, Adenovirus, lentivirus, and Adeno-Associated Virus (AAV). AAV is an effectively recognized vector in IS animal model gene therapy. Also, the notable potentials of AAV include reducing the inflammatory mediators, increasing glucose uptake and angiogenesis, inhibiting the apoptosis and inflammation, and reducing the calcium cytosol [25,27,36]. In general, according to these points, AAV can be an appropriate vector for gene transfer in ischemic stroke [27]. In this hypothesis, it is hoped that the immune cell-gene therapy of IS, which is performed by utilizing M2 cells transfected with the recombinant AAV-GFP vector with the HGF and IL-35 gene profile will play the appropriate role in tissue repairing and reducing the inflammatory factors.

Evaluation of the hypothesis

According to cases pointed earlier, it is proposed the following levels to test and assess the hypothesis. In each group, there are ten rats as syngeneic animal models (Figure 2).

Summary of procedure:

1. Type 2 microglia (M2) cells are taken from rats, isolated by MACS (Magnetic-activated cell sorting), and cultured under a biological hood.
2. The new AAV-Green Fluorescent Protein (GFP) viral vector is created using the HGF and IL-35 genes profile.
3. The isolated type 2 microglia cells from rats are transplanted into the culture and transfected with the recombinant AAV-GFP vector.
4. The transfected Type 2 microglia cells with a recombinant AAV-GFP vector are intrathecally injected into the rat, which is no rejection of the transplantation due to autologous injection.

Control groups:

Group 1: The target rats receive saline.

Group 2: The target rats receive type 2 microglia cells infected by an AAV-GFP vector (free of the foreign gene).

Group 3: The target rats receiving the AAV-GFP will contain the HGF and IL-35 genes.

Methods for evaluation of study:

1. Using GFP to identify and confirm transfection [28].
2. Evaluation of anti-inflammatory cytokines and restorative factor proteins (IL-10, IL-35, TGF-β, and HGF), by Enzyme-Linked Immunosorbent Assay (ELISA).
3. Evaluation of anti-inflammatory cytokines and restorative factor genes (IL-10, IL-35, TGF-β, and HGF) by Real-time Polymerase Chain Reaction (RT-PCR).
4. Evaluation of apoptosis by Terminal deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay.
5. Measurement of the lesion extending created with Magnetic Resonance Imaging (MRI).

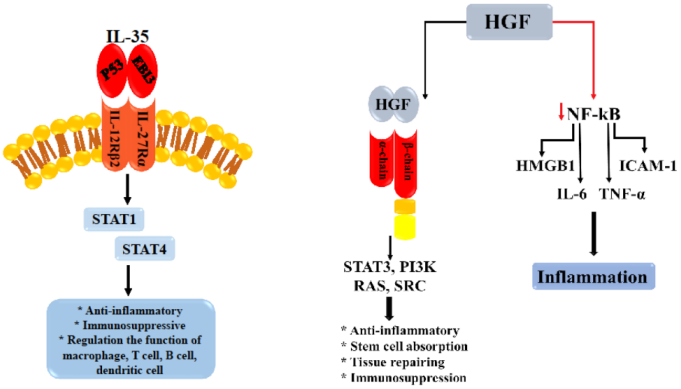

Figure 2

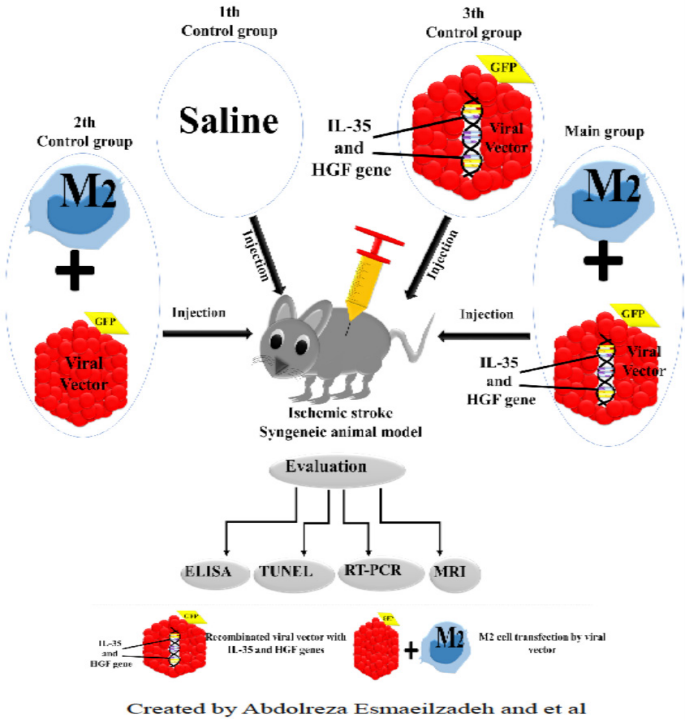

Figure 3

Discussion and conclusion

In this hypothesis, given the importance of ischemic stroke, the authors attempted to propose better and comprehensive treatment with the least complications. Previous existing treatments such as t-PA and endovascular recanalization did not have enough constructive effects for all patients [10]. According to previous studies, inflammation and apoptosis have deleterious roles in ischemic stroke pathogenesis, so targeted-therapy by preventing the inflammation and apoptosis could be beneficial. Understanding the immunological and molecular mechanisms of ischemic stroke microenvironment could be efficient for finding the applicable treating assay [7]. Gene- and cell-based immunotherapy could be a novel hopeful suggestive method in ischemic stroke, which may decrease the IS-mediated disability. In short, a complex of the appropriate gene, applicable cells, and vectors are required for efficacious therapy in IS. IL-35 is an anti-inflammatory (via inhibiting the NF-kB pathway) and immunosuppressive mediator, which activates the STAT4 and STAT1 pathways through binding to the IL-12Rβ2 and IL-27Rα receptors. Also, IL-35 suppresses the TH1 and TH17 cells increase the Treg cells and IL-10 activity, anti-inflammatory, and immunosuppressive mediators, and down-regulates the IL-17 cytokine [25,29-32]. HGF can repair damaged tissue and suppresses apoptotic and inflammatory mechanisms. HGF modifies cytokine profile inducing anti-inflammatory cytokines production and has a role in neuroprotection in the inflammatory microenvironment. This factor can regulate the immune response by suppressing the pro-inflammatory agents affecting the NF-kB signaling pathway. NF-kB inhibition prevents transcriptional actions, so as a result, proteomics suppression is observed. HGF absorbs stem cells to injured tissue and protects the neurons from apoptosis, damages, and inflammatory compensations. Generally, focusing this kind of mechanisms, HGF has an anti-inflammatory and immunosuppressive function in ischemic stroke inflammatory microenvironment [24,26,33-36]. Another part of effective combined treatment is regarding target cell. In this hypothesis, the selected cell is microglia which regulates scar formation and has a phagocytic function that can remove any pathogens and debris from the damaged tissue. Microglia plays an important role in the Central Nervous System (CNS) homeostasis by removing damaged neurons [41]. There are two types of microglia (M1and M2) [42]. Type 2 phenotype is the potent cell to heal nervous system damage through its late onset anti-inflammatory effects in IS and also can regulate the immune response by shifting TH1 immunity to TH2 and innate immune response with suppressing the pro-inflammatory agents [20-22,38]. M2 macrophages have three subtypes that are known M2a, M2b, and M2c. Each subtype produces different types of chemokines and cytokines, separately. M2a produces IL-10, TGF-β, and IL-1Rα cytokines and CCL17, CCL22, and CCL24 chemokines. M2b produces IL-4, IL-6, IL-10, and TNF-α cytokines also CCL1 chemokine. M2c produces IL-10 and TGF-β as cytokine and CCR2 as a chemokine [21]. There is a need for an appropriate vector to form combined therapy. Our suggested vector is AAV, which has a high transduction efficacy and low immunogenicity [27]. Each of these factors and cells has disadvantages lonely, that can cover each other when they are used together. Considering the above, it is hoped that using the M2 cells to repair and prevent scaring, HGF for tissue repairing, and regulating the immune response as well as using the IL-35 as an immune regulatory and anti-inflammatory factor would enhance the therapeutic successes in IS and improve the quality of life of patients.

Author Statements

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Conflict of interest statement

The authors have no conflicts of interest to declare.

Author's contribution

This hypothetic study couldn't be completed unless unsparing efforts and technical guides of *Abdolreza Esmaeilzadeh* for conceptualization qualifying, project administration, study design, scientific writing, theoretical and academic peer reviewing, definitive approval of the final manuscript, sincere manner and also *Azita Mohammadzadeh* and *Maryam Zarerafie* for collecting data, conclusive literature review, images designation, normative writing (last original drafts preparation). All authors have approved the final version of the article.

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