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# Tissue plasminogen activator followed by antioxidant-loaded nanoparticle delivery promotes activation/mobilization of progenitor cells in infarcted rat brain

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ABSTRACT

Inherent neuronal and circulating progenitor cells play important roles in facilitating neuronal and functional recovery post stroke. However, this endogenous repair process is rather limited, primarily due to unfavorable conditions in the infarcted brain involving reactive oxygen species (ROS)-mediated oxidative stress and inflammation following ischemia/reperfusion injury. We hypothesized that during reperfusion, effective delivery of antioxidants to ischemic brain would create an environment without such oxidative stress and inflammation, thus promoting activation and mobilization of progenitor cells in the infarcted brain. We administered recombinant human tissue-type plasminogen activator (tPA) via carotid artery at 3 h post stroke in a thromboembolic rat model, followed by sequential administration of the antioxidants catalase (CAT) and superoxide dismutase (SOD), encapsulated in biodegradable nanoparticles (nano-CAT/SOD). Brains were harvested at 48 h post stroke for immunohistochemical analysis. Ipsilateral brain slices from animals that had received tPA + nano-CAT/SOD showed a widespread distribution of glial fibrillary acidic protein-positive cells (with morphology resembling radial glia-like neural precursor cells) and nestin-positive cells (indicating the presence of immature neurons); such cells were considerably fewer in untreated animals or those treated with tPA alone. Brain sections from animals receiving tPA + nano-CAT/SOD also showed much greater numbers of SOX2- and nestin-positive progenitor cells migrating from subventricular zone of the lateral ventricle and entering the rostral migratory stream than in t-PA alone treated group or untreated control. Further, animals treated with tPA + nano-CAT/SOD showed far fewer caspase-positive cells and fewer neutrophils than did other groups, as well as an inhibition of hippocampal swelling. These results suggest that the antioxidants mitigated the inflammatory response, protected neuronal cells from undergoing apoptosis, and inhibited edema formation by protecting the blood–brain barrier from ROS-mediated reperfusion injury. A longer-term study would enable us to determine if our approach would assist progenitor cells to undergo neurogenesis and to facilitate neurological and functional recovery following stroke and reperfusion injury.



## 1. Introduction

Stroke is the number one cause of long-term disability and the third leading cause of death in the US. Of the two major types of stroke, ischemic and hemorrhagic, ischemic stroke is the more common [1]. It is caused by thrombotic occlusion in a cerebral or carotid artery, resulting in hypoxia that leads to rapid neuronal cell death of parenchyma, creating a stroke epicenter composed of dead tissue (infarct). The poorly perfused tissue surrounding the infarct, the so-called penumbra, is affected by ischemia in a time- and blood flow-dependent manner, thus eventually becoming part of an expanding infarct [2].

Timely intervention is critical to saving the penumbra and minimizing the progression of the infarct [3]; penumbral injury is reversible during the first few hours of ischemia, but only if blood

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**List of abbreviations**

| | |
|---|---|
| BBB | Blood-brain barrier |
| CAT | Catalase |
| ECA | External carotid artery |
| GFAP | Glial fibrillary acidic protein |
| HRP | Horseradish peroxidase |
| ICA | Internal carotid artery |
| MCAO | Middle cerebral artery occlusion |
| MPO | Myeloperoxidase |
| NANO-CAT/SOD | Nanoparticles loaded with catalase or superoxide dismutase |
| NPs | Nanoparticles |
| NPCs | Neural precursor cells (stem and progenitor cells) |
| PBS | Phosphate-buffered saline |
| PLGA | Poly (D,L-lactide *co*-glycolide) |
| PVA | Polyvinyl alcohol |
| RGL | Radial glia-like |
| RMS | Rostral migratory stream |
| ROS | Reactive oxygen species |
| RSA | Rat serum albumin |
| RT | Room temperature |
| SOD | Superoxide dismutase |
| SVZ | Subventricular zone |
| tPA | (recombinant human) Tissue-type plasminogen activator |
| VZ | Ventricular zone |
| IHC | Immuno Histochemistry |
| IF | Immuno Fluorescence |

flow is promptly restored [2]. Clinically, reperfusion is achieved by administering the only US FDA-approved treatment for ischemic stroke, recombinant human tissue-type plasminogen activator (the fibrinolytic agent commonly termed tPA). However, treatment with tPA is not safe beyond ~4.5 h post stroke, due to increased risk of hemorrhagic complications, breakdown of the blood–brain barrier (BBB) [4], and neurotoxic effects of tPA [5]; thus only about 5% of patients with ischemic stroke can be treated with this agent today [6]. Although reperfusion after tPA treatment brings oxygen and nutrients to the ischemic tissue, it simultaneously causes increased production of reactive oxygen species (ROS) by ischemic neural and glial cells, infiltrating neutrophils, and vascular cells constituting the BBB [2]. Under normal circumstances, neuronal cells contain high levels of endogenous antioxidants to counteract ROS [7]; these antioxidants are continuously produced during metabolism of excitatory amino acids and neurotransmitters [8]. However, endogenous antioxidants in the ischemic brain cannot attain high enough levels to neutralize the excessive levels of ROS formed during ischemia/reperfusion, resulting in loss of the redox balance and causing oxidative stress [9], which then initiates a cascade of degenerative events such as inflammation, excitotoxicity, hyperglycemia, and apoptosis. This stress condition in turn expands the injury response and infarct volume over time, causing irreversible damage to the brain, especially in the penumbral areas [10].

It was long thought that neurogenesis occurs only in the developing brain; however, some decades ago, *in vitro* studies of brain cells from the subventricular zone (SVZ) showed that these cells have stem-cell properties [11], and more recent studies have given *in vivo* evidence of neurogenesis in adult mammalian brain [12]. It has been shown that endogenous neural precursor cells (NPCs; i.e., stem cells and progenitor cells) become activated after stroke, then maturate and migrate to the site of injury, where they differentiate into neuronal cells (Fig. 1) [13]. However, this process of neurogenesis is limited, some postulate, because of excessive production of ROS following stroke and reperfusion [14], creating unfavorable conditions for progenitor cells to activate, mobilize, survive, and eventually differentiate into functional neuronal tissue [15]. In fact, Huang et al. [16] have shown a marked reduction in neurogenesis in superoxide dismutase (SOD)-deficient mice with radiation-induced brain damage, which also caused a large increase in ROS levels in the injured brain for a sustained period of time.

In this study, we hypothesized that targeting the initial excess of ROS formation with the antioxidant enzymes catalase (CAT) and SOD loaded into nanoparticles (NPs; termed nano-CAT/SOD) at the time of reperfusion (i.e., immediately after tPA delivery) would prevent the ROS-mediated cascade of inflammatory and degenerative events, creating conditions favorable for the activation and mobilization of progenitor cells that could eventually promote the endogenous mechanisms of neurogenesis in the infarcted area.

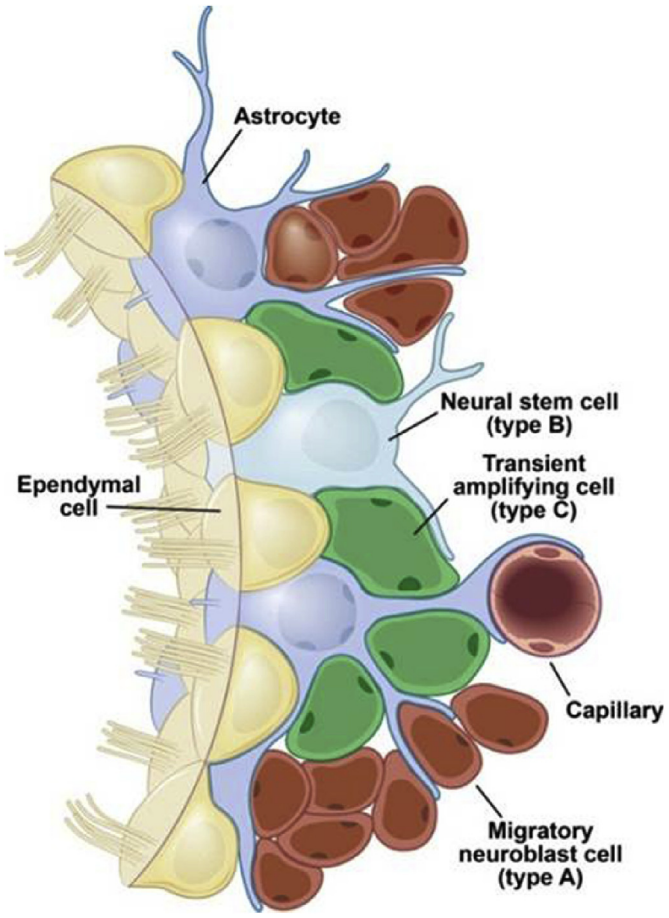


**Fig. 1. Schematic representing various stem and glial cells associated with the inherent process of neurogenesis in post-stroke conditions**. The figure illustrates the "inherent neurogenesis" concept, achieved by various stem cell types and glial cells, typically associated with spontaneous self-recovery after stroke.