Novel Anti-Oxydative Stress Nanoparticle for Ischemia Reperfusion Injury

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Introduction: Ischemic cardiovascular disease is a second cause of death in the world due to a disturbance of blood supply to the vital organs. It is known that arterial re-canalizations achieved by thrombolysis and intravascular intervention are main treatment strategies to restore blood supply in ischemic stroke and heart attack. However, reperfusion has a dilemma of ischemia-reperfusion injury caused by reactive oxygen species (ROS), which is produced after a long ischemic period and can extend a damaged area. Therefore, protection of organs affected by ROS has been investigated to suppress the damaged area than that associated with the arterial occlusion. The prior articles revealed that nitroxides and spin trapping compounds, such as 2,2,6,6-tetramethylpiperidin 1-oxyls (TEMPO) and •-phenyl-N-tert butylnitrone (PBN) could improve the functional outcome of ischemic brain and heart by scavenging free radicals on ischemia-reperfusion injury. However, these compounds have not been applied clinically because they were inactivated due to the acute reduction by anti-oxidant systems such as catalase, glutathione peroxidase in vivo. To overcome poor bioavailability and biocompatibility, we developed a novel core-shell type nanoparticle containing nitroxyl radicals in the core and named radical-containing-nanoparticle (RNP)[1].

RNP showed the prolonged blood circulation time by the compartmentalization of TEMPO into the micelle and pH-sensitivity to help TEMPO radicals act as antioxidant due to the collapse of nanoparticle in low pH condition such as ischemic-tissue in vivo[2]. The objective of this study is to evaluate the ability of RNP to deliver nitroxyl radicals to the ischemic brain, and neuroprotective effects by scavenging free radicals using transient middle cerebral artery occlusion (MCAO) model in rats.

Experiment

Poly(ethyleneglycol)-*block*-poly(chloromethylstyrene) (acetal-PEG-*b*-PCMS) (**2**) was synthesized by the free-radical telomerization of chloromethylstyrene (CMS) using acetal-PEG-SH as a telogen. The chloromethyl groups of **2** were quantitatively converted to 2,2,6,6-tetramethylpiperidinyloxys (TEMPOs) via the amination of **2** with 4-amino-TEMPO to obtain acetal-PEG-*b*-PCMS containing TEMPO moieties (**1**). The obtained **1** formed core-shell-type nanoparticles in aqueous media when

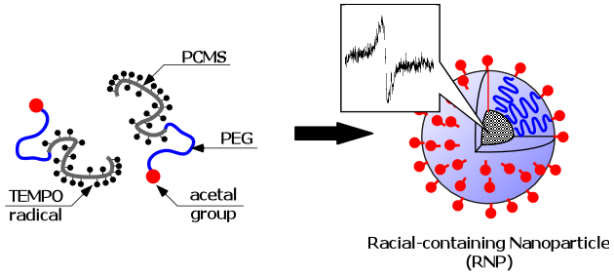

Figure 1. Schematic illustration of nanoparticle with pH responsive EPR signals

subjected to the dialysis method: the cumulant average diameter of the nanoparticles was ca. 40 nm and the nanoparticles emitted intense electron paramagnetic resonance (EPR) signals. Reduction resistance, blood circulation and effect of cerebral infarction damage of RNP were investigated in detail.

Results and discussions

The TEMPO radicals in the core of the nanoparticles showed reduction resistance even in the presence of 3.5 mM ascorbic acid. The EPR signal in blood stream of mice was observed even 2h after tail vein injection. It is sharp contrast against low molecular weight TEMPOL, which was reduced within 2 min in blood stream (Figure 2). As can be seen in Figure 3, the infarction volume in RNP group was significantly smaller than that in saline. These data revealed that RNP could limit the extension of cerebral infarction caused by the ischemia-reperfusion injury, and improve neurological deficits. The RNP thus prepared was found to be effective for brain injury by cerebral ischemia via anti-oxidative stress damage. The RNP is thus promising as high-performance bionanoparticle drug, which can be used *in vivo*.

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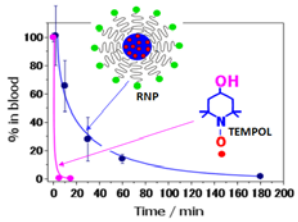

Figure 2. Blood Circulation of RNP

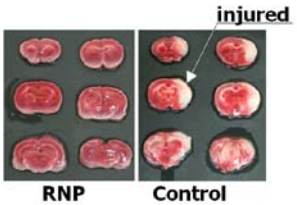

Figure 3. RNP Effect of Cerebral Infarction of Ischemia Reperfusion

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[2] Yoshitomi Toru, Suzuki Rie, Mamiya Takashi, Hirofumi Matsui, Hirayama Aki Nagasaki Yukio pH-Sensitive Radical-Containing-Nanoparticle (RNP) for the L-Band-EPR Imaging of Low pH Circumstances. Bioconjugate Chemistry 20 1792-1798(2009).