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TRANSIENT ISCHEMIC ATTACK INDUCED BY MELTED SOLID LIPID MICROPARTICLES PROTECTS RAT BRAINS FROM PERMANENT FOCAL ISCHEMIA

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Abstract—This study aims to develop a transient ischemic attack (TIA) model in conscious animals and uses this model to investigate the effect of TIA on subsequent permanent ischemia. TIA was induced by injecting designed temperature-sensitive melted solid lipid microparticles with a melting point around body temperature into male Wistar rats via arterial cannulation. Neurologic deficit was monitored immediately after the injection without anesthesia. According to the clinical definition of TIA, rats were divided into neurologic symptom durations <24-h, 24–48-h and ⩾48-h groups. The lipid microparticle-induced infarct volumes were small in the <24-h and 24–48-h groups, while the volumes were five times larger in the ⩾48-h group. Permanent ischemic stroke was induced 3 d after the induction of TIA by injecting a different kind of embolic particle manufactured by blending chitin and PLGA. The <24-h group had less severe neurologic deficits and smaller infarct volumes than that of 24–48-h and control (without prior lipid microparticle treatment) rats. Taken together, we successfully develop a TIA animal model which allows us to monitor the neurologic deficit in real-time. By adopting this model, we validate that TIA (<24 h) preconditioning protects the brain from subsequent permanent ischemic stroke.

Key words: transient ischemic attack, solid lipid microparticles, ischemic preconditioning, ischemic stroke, chitin/PLGA-blended microparticles.

INTRODUCTION

Transient ischemic attack (TIA) is the sudden onset of transient neurologic deficit with full recovery within 24 h (Easton et al., 2009). TIA has been recognized as a risk factor for permanent ischemic stroke (Easton et al., 2009). However, in the animal model that TIA is induced by transient occlusion of ipsilateral cerebral arteries, ischemic preconditioning has been recognized as a beneficial factor for ischemic stroke (Perez-Pinzon et al., 1997; Kitagawa et al., 2005; Zhang et al., 2008). Several clinical studies also support the premise that prodromal TIA may reduce the severity of permanent ischemic stroke and decrease the infarct size as detected in the perfusion scan of magnetic resonance imaging (Weih et al., 1999; Moncayo et al., 2000). However, it has been argued that the latter studies were retrospective in nature, with most of the information (relationship between TIA and a following permanent ischemic stroke) being culled from medical histories or descriptions from family members of patients. A more recent study investigating a much larger cohort failed to find the protective effects of preceded TIA on permanent ischemic stroke (Johnston, 2004). The effect of preceded TIA on ischemic stroke deserves further characterization.

A major drawback of previous studies in characterizing the effect of TIA preconditioning on permanent stroke severity is that they were unable to evaluate the exact temporal relationship between TIA and neurologic deficit. In clinical studies, it has been demonstrated that the duration of TIA-induced neurologic deficit is closely related to the incidence of stroke. For example, TIA symptoms lasting more than one h increase the incidence of stroke or stroke recurrence (Giles et al., 2006; Ois et al., 2008; Sciolla and Melis, 2008). However, few animal studies focused on the duration of neurologic deficit induced by TIA, because in most animal studies TIA was induced by transient occlusion of brain circulation under anesthesia. It is impossible to evaluate the onset time of neurologic deficit secondary to TIA until hours after recovering from anesthesia. Therefore, an animal model that permits inspection of the behavior immediately after the induction of

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Abbreviations: ANOVA, analysis of variance; CITS, cerebral infarction with transient signs; ECA, external carotid artery; PLGA, poly(D,L-lactide-co-glycolide; TIA, transient ischemic attack; TTC, Triphenyl Tetrazolium Chloride.

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TIA is essential to examine the association of preceded TIA and the severity of the following ischemic stroke.

The objectives of this study are to develop a TIA animal model that allows us to evaluate the TIA-induced neurologic deficit in real-time and to evaluate the effect of TIA on the neurologic outcomes of subsequent permanent ischemic stroke. We develop the TIA animal model by injecting artificial emboli which were designed to melt *in vivo* after injection into the brain circulation. Lipid microparticles, especially solid lipid microparticles, have been used for drug delivery carriers because of their ideal characteristics of homogenous sphere appearance and rapid and consistent dissolvability (Jannin et al., 2008). By taking these advantages, we designed solid lipid microparticles that melt at body temperature to produce artificial emboli. The injection of melted solid lipid microparticles was performed without anesthesia, so the TIA-induced neurologic deficit could be evaluated immediately from the onset of neurologic symptoms. After completely recovering from neurologic deficit, a different type of artificial particle emboli, manufactured by chitin and poly(D,L-Lactide-co-glycolide) (chitin/PLGA)-blended microparticles, were injected to induce permanent ischemic stroke (Tsai et al., 2011). Neurologic deficit was evaluated immediately after the injection of chitin/PLGA microparticles. Regional cerebral blood flow was determined before, during, and after the injection of microparticles. Brain infarction was assessed by Triphenyl Tetrazolium Chloride (TTC) stain.

EXPERIMENTAL PROCEDURES

Materials

Gelucire 33/01 and Gelucire 43/01 were purchased from Gattefosse Co. (St-Priest, France). PLGA 50/50 (MW: 40 dDa, Lactide/glicolide ratio: 50/50) was obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Chitin was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Tetrazolium Red (2,3,5-triphenyl-tetrazolium Chloride) was obtained from Alfa Aesar Co. (Ward Hill, MA). All other chemicals and solvents were of analytical grade and purchased from Sigma–Aldrich Co.

Preparation of melted solid lipid microparticles for TIA

The solid lipid microparticles were made by adding the oil phase into the water phase containing hydrophilic surfactant. The preparations were manufactured with the rationale that the used lipids and surfactant would be biocompatible and biodegradable. Gelucires have been used as surfactants, co-surfactants and lipid matrixes in drug delivery systems and are generally recognized as safe (Shimpi et al., 2005). The oil phase was prepared by mixing Gelucire 43/01 (melting point of 43 °C/hydrophile–lipophile balances of 1) and Gelucire 33/01 (melting point of 33 °C/hydrophile-lipophile balances of 1). Gelucires are solid lipid materials composed of mono-, di-, and triglycerides and mono- and di-fatty acid esters of polyethylene glycol (Jannin et al., 2008). A ratio of 1:1 between Gelucire 43/01 and Gelucire 33/01 was used to produce solid lipid microparticles that have a melting point of 38 °C, which was verified using a melting point apparatus (Mel-Temp II, Laboratory Devices, Inc., Tarzana, CA, USA). The blended oil phase was homogenously mixed by heating the Gelucire lipids above their melting point. The water phase was prepared by adding sodium dodecyl sulfate in purified water to get a final concentration of 1%. Both the oil phase and water phase were kept at 70 °C and were mixed by slowly dripping the oil phase directly into the water phase while stirring at the speed of 300 rpm for 5 min. The temperature was then allowed to slowly cool down to room temperature. The gelled lipid microparticles were allowed to harden in the cool water phase while stirring at the same speed for another 24 h. After hardening, the microparticles were filtered and rinsed with deionized water and the particles were sieved by standard U.S. size meshes from 20 to 355 mesh (Analytical Test, Retsch, Germany) before drying overnight. The finished microparticles were kept at 4 °C in the refrigerator. The particle size of 75–90 μm was used as emboli for inducing transient brain ischemia.

Differential scanning calorimetry of melted solid lipid microparticles

The thermal transitions of the lipid microparticles were analyzed by differential scanning calorimetry. The melted solid lipid nanoparticles were freeze-dried before the measurement. The differential scanning calorimetry was performed by a PerkinElmer DSC calorimeter (DSC7, Waltham, MA, USA). Solid lipid nanoparticles (5 mg) were put in aluminum pans and the thermal profiles were obtained as the temperature increased from 10 to 55 °C at a rate of 5 °C/min under nitrogen.

Scanning electronic microscopy of melted solid lipid microparticles

This method has been described previously (Tsai et al., 2012). The melted solid lipid microparticles were attached onto double-sided adhesive tape and fixed to an aluminum stage. The microparticles were sputter-coated with gold using a Hitachi coating unit and the surface of the microparticles were examined using a Hitachi S-2300 Scanning electronic microscopy (Japan).

Preparation of chitin/PLGA microparticles for ischemic stroke

The detailed procedure to prepare chitin/PLGA microparticles has been described elsewhere (Tsai et al., 2011). Briefly, chitin powder was dissolved in dimethylacetamide solution containing 5% LiCl to make 1% chitin solution. Chitin/PLGA 50/50 mixed solution was formulated by directly suspending the PLGA 50/50 powder in the prepared chitin solution in 1:1 ratio. To produce microparticles, chitin/PLGA 50/50 mixed solution maintained at 70 °C was dropped through a syringe (27 gauge) into 1% sodium lauryl sulfate solution bath maintained at 25 °C, and then allowed to harden the blended microparticles for 12 h. Finished microparticles were