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Research Report

Effect of internal carotid artery reperfusion in combination with Tenecteplase on clinical scores and hemorrhage in a rabbit embolic stroke model

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ABSTRACT

In the present study, we used a modification of the rabbit small clot embolic stroke model (RSCEM), a multiple infarct ischemia model to achieve reperfusion (REP) through the internal carotid artery (ICA) following small clot embolization. We determined if increasing regional cortical blood flow (RCBF) following an embolic stroke is beneficial to neurological outcome. We compared this to cerebral reperfusion induced by the administration of the thrombolytic Tenecteplase (TNK, 1.5 mg/kg, IV bolus) in the presence or absence of REP. In this study, we also measured the incidence of ICH following REP and thrombolytic treatment. Following embolization, RCBF was reduced to 48–55% of baseline. When REP was induced by removal of a CCA ligature, RCBF initially increased to 185% of baseline. REP (P_{50}=1.18±0.43 mg) had no effect on embolization-induced behavior measured 24 h following embolization compared to control (P_{50}=1.01±0.48 mg). However, TNK treatment (2-hours post-embolization) in the absence or presence of REP (initiated 2 h following embolization) significantly ($p<0.05$) increased the group P_{50} to 2.92±0.55 mg and 2.42±0.40 mg, respectively. In addition, ICH was increased in the REP (42%, $p<0.05$) and REP-TNK (35%, $p>0.05$) group compared to either the control group (5.5%) or TNK group (10%). This study show that reperfusion of ICA can increase RCBF following embolization, but this is not associated with improved neurological outcome measured using quantal analysis. However, TNK administration significantly increased behavioral outcome when given 2 h following embolization; an increase that is not affected by combining TNK with REP.

1. Introduction

The rabbit small clot embolic stroke model (RSCEM) is a well-developed cerebral ischemia model that allows for effective identification of treatments with neuroprotective properties (Lapchak et al., 2004a,b,d, 2007b). The RSCEM has been used successfully to develop various preclinical treatments for acute ischemic stroke (Lapchak et al., 2004a,b,d, 2007b), such as the only FDA-approved treatment Alteplase (tPA) and transcranial near infrared laser therapy (Lampl et al., 2007; Lapchak, 2002; Lyden et al., 2006; Zivin et al., 2009) that has been shown to be positive in the NEST-1 and NEST-2 clinical trials. In the classical RSCEM, the common carotid artery (CCA) is permanently ligated after a catheter is inserted retrogradely into the CCA (Lapchak et al., 2004a, 2007b,c; Zivin et al., 1988) Unilateral occlusion of the CCA does not cause acute symptomatic strokes or behavioral deficits in rabbits, but it is possible that the hemodynamic status of the brain may be compromised or

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neurological outcome would be further affected following embolization in this model.

In humans, stenosis (Bertges et al., 2003; Muluk et al., 1999) or chronic occlusion of internal carotid artery (ICA) (Blaser et al., 2002) in usually associated with measurable changes of cerebral hemodynamics and patients are at risk for strokes (Flaherty et al., 2004; Maldonado et al., 2008; Parthenis et al., 2008) Emergency endovascular recanalization via a carotid endarterectomy may lead to resolution of severe clinical symptoms probably due to restoration of blood flow to critically hypoperfused and dysfunctional, but salvageable brain tissue (penumbra) (Du Mesnil De Rochemont et al., 2004) However, cerebral hyperperfusion syndrome can be a devastating complication of endarterectomy-induced reperfusion (Fukuda et al., 2007; Ogasawara et al., 2007). Moreover, following reperfusion of ICA there is an increased incidence of hemorrhagic transformation (HT), which is associated with a 67% mortality (McCabe et al., 1999; Morrish et al., 2000; Ogasawara et al., 2007; Russell and Gough, 2004).

Even though the changes that occur in humans are the result of chronic ICA occlusion due to carotid artery plaque formation (Maltezos et al., 2007), we modeled stenosis or ICA occlusion in the rabbit, in order to study the effects of acute reperfusion (REP) or opening of the ICA on stroke-induced behavioral function using the RSCEM (Lapchak et al., 2004d, 2007a,b; Lapchak and Zivin, 2009). For this, we modified the ligation method used in the RSCEM to allow for reperfusion of the common carotid artery following embolization, in order to increase RCBF through the common and internal carotid arteries. We measured the physiological effects of ligature removal on RCBF using laser Doppler analysis and we used quantal analysis to measure the behavioral effects of ligature removal following an embolic stroke. Quantal analysis is a statistical analysis method using iterative curve fitting to determine if a treatment condition produces a statistically significant increase in the P_{50} value or the amount of microclots in the brain that produce neurologic dysfunction in 50% of a group of animals. If the difference between the P_{50} for a treatment group and the control group is significantly different, the data is indicative of a behavioral improvement. Quantal analysis, our primary clinical endpoint is based upon the motor function components of the National Institute of Health Stroke Scale (NIHSS) for stroke in humans (Broderick et al., 2000; Clark et al., 2000). Lastly, since recanalization or reperfusion in humans can lead to a greater incidence of ICH, we determined if REP in the presence or absence of TNK had an effect on ICH rate in embolized rabbits. The overall goal of this study was to determine if REP of the ICA in the presence or absence of thrombolytic recanalization of cerebral vasculature can promote enhanced neurological outcome following small clot embolization.

2. Results

2.1. *RSECM surgical modification to produce REP via the ICA*

Fig. 1 shows the anatomy of the carotid artery and the ICA. For REP, 2 h after embolization, rabbits were anesthetized with halothane and the PE-50 tube ligature around the CCA was removed to restore blood flow through ICA. This is then referred to reperfusion or REP.

2.2. *Effect of REP on RCBF following embolization*

Fig. 2 shows the effects of embolization on RCBF measured in New Zealand white rabbits. After embolization, RCBF was reduced to 48–55% of pre-embolization baseline, which is maintained throughout the measurement period. When reperfusion was induced by removal of a CCA ligature, RCBF initially increased to 185% of baseline that declined to a 110% increase by 90 min following ligature removal.

2.3. *Effect of REP on clinical rating scores following embolization*

For these studies, we used quantal analysis to measure the effects of REP on behavior 24 h following embolization. Fig. 3 shows the result of the quantal analysis studies. Even though REP initiated 2 h following embolization produced a significant increase of RCBF, there were no significant effects of REP on P_{50} values measured using quantal analysis. The P_{50} value in the REP group was 1.18 ± 0.43 mg compared to 1.01 ± 0.48 mg in the control group that was run in parallel.

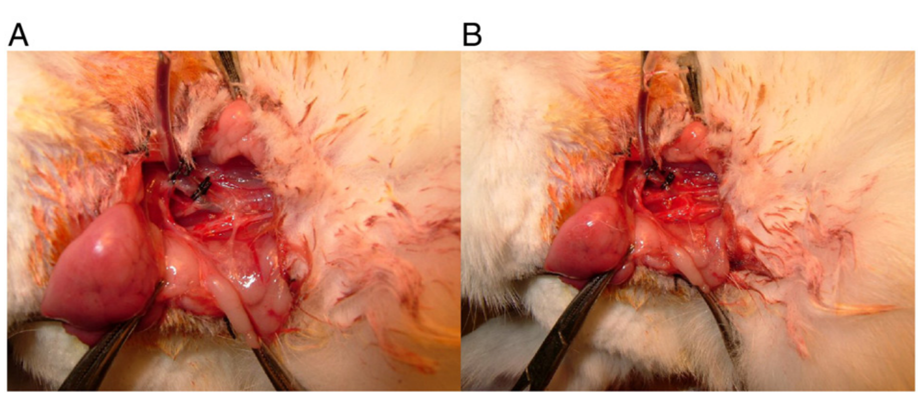

Fig. 1 – Effect of reperfusion on ICA and RCBF in rabbits. A (Left) View the CCA and the ICA. Before removal of the ligature, the ICA is hypofused. B (Right) After removal of the ligature, blood flow to the ICA is restored.