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Combination treatment with dipyridamole, aspirin, and tPA in an embolic model of stroke in rats

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Abstract

Antithrombotic therapy has been shown to be effective in preventing secondary strokes. Inhibition of platelet function may reduce formation of thrombi thereby reducing the incidence of stroke. However, stronger inhibition of platelets is correlated with increased risk of bleeding events. The purpose of this study was to test the protective effects of combination therapy with dipyridamole and acetylsalicylic acid (ASA) in comparison to ASA alone, and whether such combination treatment may produce any added benefits when tissue plasminogen activator (tPA) treatment is also used. The study was divided into three parts. In part A, effect of antiplatelets on infarct volume was assessed. In part B, perfusion deficits were measured. In part C, efficacy of antiplatelet therapy in combination with tPA was assessed. In part A, dipyridamole and aspirin treatment significantly reduced infarct volume ($P<0.05$). In part B, treatment with dipyridamole significantly reduced the perfusion deficits as compared to control ($P<0.05$). In part C, dipyridamole plus tPA or dipyridamole and aspirin plus tPA significantly decreased infarct volume as compared to tPA alone ($P<0.05$). The present study suggests that there is significant protection with dipyridamole as both infarct volume and perfusion deficits are significantly reduced. Dipyridamole with tPA also significantly reduced infarct volume as compared to tPA alone. Our data suggests that higher doses of antithrombotic therapy with dipyridamole offer best neuroprotection.

Keywords: Antiplatelets; Antithrombotic; Brain injury; Cerebral ischemia

Introduction

Thrombosis and thromboembolic occlusions of major and minor blood vessels are involved in various illnesses. If such an occlusion happens within the arterial tree of the vasculature, the onset of clinical symptoms in most cases is rapid and with devastating consequences (Eisert, 2001a). The observation by pathologists that the occluding thrombi in the arteries are platelet-rich in content promoted the strategy of preventing the untimely occlusion by inhibiting platelets from forming aggregates. Aspirin is a member of the antiplatelet class of drugs and it inhibits platelet aggregation by irreversibly blocking cyclooxygenase in all cells. Dipyridamole, previously only known for its antithrombotic activity, also inhibits platelet aggregation via a number of mechanisms including inhibition of cellular uptake and metabolism of adenosine leading to increased adenosine concentration at the platelet/vessel wall interface resulting in reduced platelet aggregation and adhesion. Dipyridamole also increases intracellular cGMP through inhibition of cGMP phosphodiesterase leading to potentiation of effects of nitric oxide (NO) on platelets, resulting in inhibition of platelet aggregation and adhesion (Eisert, 2002; Bult et al., 1991a; Sakuma et al., 1991).

Many studies have demonstrated that dipyridamole is not purely an antiplatelet agent like acetylsalicylic acid (ASA) (Eisert, 2001b, 2002; Uddin et al., 2003), but it also enhances the antithrombotic mechanisms of the local vasculature by a variety of mechanisms which include, direct stimulation of the vascular endothelium to cause PGI_2 (Eisert, 2001b, 2002; Uddin et al., 2003; Neri Serneri et al., 1981) and tPA (Kim et al., 2005) release. Dipyridamole is a scavenger for oxy as well as peroxy radicals, which is known to inhibit damage to tissue under oxidative and metabolic stress (Chakrabarti et al., 2005). Dipyridamole also inhibits smooth muscle cell proliferation resulting in inhibition of restenosis (Eisert, 2002). Very recent

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data show a marked suppression of monocyte chemoattractant protein-1 (MCP-1) production by dipyridamole in activated monocytes. MCP-1, a proinflammatory cytokine, is known to play a major role in the adhesion and migration of white blood cells to the vessel wall and subsequent blockade of microvessels under oxidative and metabolic stress (Weyrich et al., 2005).

Antiplatelet agents have been widely studied in preventing cerebrovascular disease (Diener et al., 1996). The effects of antiplatelet agents in neuroprotection are under debate. Shuaib et al. (Shuaib et al., 2001; Yang et al., 1998) have shown a glycoprotein IIb/IIIa antagonist in conjunction with fibrinolytic therapy to be effective in significant attenuation of neuronal damage following focal cerebral ischemia in rats. In the present study, we studied the effects of dipyridamole and ASA at different doses in the focal cerebral ischemia using an embolic model of stroke in rats. We examined the effects of these antiplatelets on infarction volume following cerebral ischemia. We also studied their effects on perfusion deficits following ischemia to determine whether they play any role in reestablishment of reperfusion following ischemic injury. As majority of ischemic strokes are caused by thrombotic or thromboembolic arterial occlusions, therapeutic strategies designed to restore cerebral perfusion hold great promise for these patients (Bult et al., 1991a). Indeed, intravenous infusion of tissue plasminogen activator (tPA) is the only scientifically proven effective therapy for acute ischemic stroke (Albers et al., 1998). Therefore, we also studied whether treatment with dipyridamole and ASA in combination with tPA provides any added benefits to treatment with tPA alone.

Materials and methods

Male Sprague-Dawley rats, weighing 250–300 g, were purchased from Charles River (St. Constant, Canada). The rats were housed in a 12-h light/dark cycle and had free access to water and food. The Animal Ethics Committee of the University of Alberta approved animal care and the general protocols for animal use. Animal model of embolic focal cerebral ischemia was induced by embolizing a preformed clot into the middle cerebral artery (MCA) (Noor et al., 2003; Wang et al., 2001b). Briefly, the animal was anesthetized initially with 3.0% halothane and then maintained with 1.5% halothane in a mixture of 30:70 oxygen/nitrous oxide with a face mask during surgery. A longitudinal incision of 1.5 cm in length was made in the midline of the ventral cervical skin. The right common carotid artery (CCA), right internal carotid artery (ICA) and right external carotid artery (ECA) were exposed. A modified PE-10 catheter connected with a PE-50 tubing (40 mm in length for 10 μl thrombus and 20 mm for 5 μl), filled with bovine thrombin 10 NIH U/μl and attached to a 100-μl Hamilton syringe (Fisher), was introduced into the lumen of the right ECA. After the blood was withdrawn, the catheter was advanced 17 mm up in the ICA until its tip was 1–2 mm away from the origin of the MCA. The catheter was retained there for 15 min to allow formation of a clot. Once the clot formed, it was then gently injected into the MCA. The catheter in the right ICA was removed 5 min after the clot injection and the ECA was ligated. After the wound was closed, the animal was allowed to recover from anesthesia and returned to its cage. The rectal temperature in the animals was kept at 37 °C throughout the surgery procedure with a feedback-controlled heating system.

Measurement of infarct size

The procedures for assessment of infarct volume have been detailed previously (Noor et al., 2003). Briefly, at the end of each experiment (48 h after embolization), the anesthetized animal was sacrificed by decapitation and the brain removed. For morphometric study, 2-mm-thick coronal sections were cut using a rat brain matrix. A total of 8 coronal sections were collected and the sections were stained using a 2% 2,3,5-triphenyltetrazolium chloride solution. The stained brain sections were scanned with a color flatbed scanner. The images were analyzed with Adobe PhotoShop. The total volume of infarction was determined by integration of the areas from the sections. The infarct volume was expressed as a percentage of the total volume from the ipsilateral hemisphere.

Measurement of dipyridamole concentration

Dipyridamole concentration was measured at 2 h and 12 h in rats receiving 200 mg/kg dipyridamole orally (gavage) twice daily ($n=20$). Briefly, 1 ml of blood was collected from the tail vein in a tube containing 0.1 ml of heparin. The blood was then centrifuged and the plasma collected was to be analyzed using the phosphorylation method.

Detection of perfusion deficits

Perfusion deficits were analyzed using an Evans blue staining procedure, as described previously (Uddin et al., 2003; Wang et al., 2001a). Briefly, 2% Evans blue solution was injected i.v. in the anesthetized rats and allowed to circulate for 10 s. The animals were then decapitated and their brains removed. The brains were frozen in 2-methylbutane kept in dry ice, embedded in optimal cutting temperature (OCT) medium, and stored at −70 °C until analyses. The brains were sectioned at 10 μm in thickness with a cryomicrotome commencing 3.7 mm anterior to bregma. A total of 9 brain sections were collected serially with an interval of 1 mm. The perfused microvessels in the brain were visualized under fluorescent microscopy and images were captured with Qimaging camera. Areas of perfusion deficits in the brain were traced, calculated with Openlab system and expressed in mm^2.

Experimental design

The present study consisted of three parts. In part A, we examined the effects of antiplatelet agents, dipyridamole and aspirin, on infarct volume following cerebral ischemia. Animals were randomly assigned and treated orally with Simple syrup ($n=14$), dipyridamole 80 mg/kg ($n=10$), dipyridamole 200 mg/kg ($n=10$), aspirin 0.625 mg/kg ($n=10$) and