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Research Report

Neuroprotection by pyrroloquinoline quinone (PQQ) in reversible middle cerebral artery occlusion in the adult rat

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ABSTRACT

Pyrroloquinoline quinone (PQQ) is a naturally occurring redox cofactor that acts as an essential nutrient, antioxidant, and redox modulator. It has previously been reported to reduce infarct size in 7-day-old rat pups with an in vivo cerebral hypoxia/ischemia model (Jensen et al., 1994). In this study, we tested whether improvement is found in both behavioral measures of protection and by histological measures of infarcted tissue at 72 h after reversible middle cerebral artery occlusion (rMCAo) in adult rats. Two-hour rMCAo was induced in adult rats using the intraluminal suture technique. PQQ (10, 3, and 1 mg/kg) was given once by intravenous injection at the initiation, or 3 h after the initiation, of 2 h rMCAo. Neurobehavioral deficits were evaluated daily for 3 days followed by infarct volumes measurements by 2,3,5-triphenyltetrazolium chloride (TTC) staining. PQQ at 10 mg/kg infused at the initiation, or 3 h after the initiation, of rMCAo was effective in reducing cerebral infarct volumes measured 72 h later. At 3 h after ischemia, a dose of 3 mg/kg significantly reduced infarct volume compared to vehicle-treated animals, but 1 mg/kg was ineffective. Neurobehavioral scores were also significantly better in the PQQ-treated group compared to the vehicle controls when PQQ was given at 10 and 3 mg/kg, but not at 1 mg/kg. Thus, PQQ is neuroprotective when given as a single administration at least 3 h after initiation of rMCAo. These data indicate that PQQ may be a useful neuroprotectant in stroke therapy.

1. Introduction

Pyrroloquinoline quinone (PQQ; Fig. 1) is a free, water soluble, anionic compound that is a redox cycling planar orthoquinone, which also has free radical scavenging properties (Gallop et al., 1989a,b, 1993; Paz et al., 1989). PQQ-dependent enzymes such as methyl alcohol and alcohol dehydrogenases bind PQQ as a prosthetic group and also contain cytochrome c that accepts electrons and donates them to ubiquinone, which functions as an electron carrier in the mitochondrial respiratory chain (Bishop et al., 1998; Davidson, 2004; Gallop et al., 1989b). PQQ has been demonstrated to depress *N*-methyl-D-asparate (NMDA)-induced electrical responses and is neuroprotective in vitro against NMDA-mediated neurotoxic injury (Aizenman et al., 1992; Alexandrova and Bochev, 2005). Jensen et al. (1994) first showed that PQQ given intraperitoneally at 30 min prior to hypoxia reduces infarct size without causing measurable neurobehavioral side effects in an in vivo cerebral hypoxia/ischemia (bilateral carotid ligation in combination with hypoxia) model in 7-day-old rat pups. We know of no

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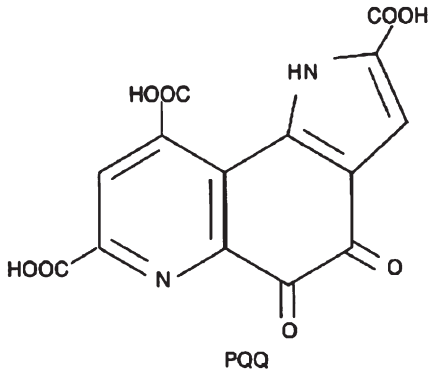

Fig. 1 – Structure of pyrroloquinoline quinone (PQQ).

study that examined whether PQQ given systemically can improve neurobehavioral outcome or salvage infarcted brain resulting from focal cerebral ischemia in adult animals. Therefore, we evaluated whether PQQ was effective in producing neuroprotection as assessed by neurobehavioral measures and infarct size measurement following 2 h of reversible middle cerebral artery occlusion (rMCAo) in adult rats. We also characterized the dose–response curve for PQQ on infarct volume.

2. Results

2.1. *Neuroprotection by PQQ at 10 mg/kg*

We first studied PQQ at a dose of 10 mg/kg, a dose based on the previous report by Jensen et al. (1994). Infarct volume was 319 mm^3 (SD = 96.2; n = 7) in vehicle-treated animals and was significantly reduced by 85% to 50 mm^3 (SD = 39; n = 8) in the animals given 10 mg/kg PQQ at the initiation of ischemia ($P < 0.01$; Mann–Whitney test). Infarct volume was 362 mm^3 (SD = 110; n = 5) in vehicle-treated animals and was also significantly less at 67 mm^3 (SD = 53; n = 8; 81% reduction) in the animals given 10 mg/kg PQQ 3 h after the onset of ischemia ($P < 0.05$; Mann–Whitney test). These data are shown in Fig. 2A. Examples of TTC staining are shown in Fig. 3.

Behavioral scores were also better in the PQQ-treated groups compared to the corresponding vehicle-treated controls when PQQ at 10 mg/kg was given at the initiation or 3 h after the initiation of ischemia, as shown in Figs. 4A and B.

2.2. *Neuroprotection by PQQ at 3 mg/kg and lack of protection at 1 mg/kg*

PQQ at 10 mg/kg given 3 h postinitiation of ischemia appeared to be as effective as when it was administered simultaneously with ischemia. Therefore, we tested the effect of lower doses at 3 h after initiation of ischemia because 3 h postinitiation of ischemia provides a clinically utilizable therapeutic window for treatment (Stroke Therapy Academic Industry Roundtable 1999). When PQQ was given at 3 mg/kg at 3 h after the onset of ischemia, infarct volume was 406 mm^3 (SD = 114; n = 10) in the vehicle-treated animals and was significantly less at 120 mm^3 (SD = 47; n = 6; 70% reduction) in the PQQ-treated animals ($P < 0.01$; Mann–Whitney test; Figs. 2A and 3). At this dose, behavioral scores were also better in the PQQ group compared to the vehicle groups (Fig. 4C).

When PQQ was given at 1 mg/kg 3 h after ischemia, infarct volume was 361 mm^3 (SD = 132; n = 6) in vehicle-treated animals and there was no significant difference at 328 mm^3 (SD = 112; n = 6) in PQQ-treated animals (P = 0.42; Mann–Whitney test; Fig. 2A). Behavioral scores were also not significantly different in the PQQ-treated animals compared to the vehicle-treated animals (Fig. 4D). The full dose–response curve for decreased infarct size is shown in Fig. 2B.

3. Discussion

PQQ is a water-soluble, anionic, quinonoid substance that has been shown to be an essential nutrient in mice (Killgore et al., 1989; Steinberg et al., 1994; Stites et al., 2000). It was initially isolated from cultures of methylotropic bacteria as a crystalline acetone adduct and was proposed to be a cofactor of many bacterial primary alcohol dehydrogenases (Salisbury et al., 1979). Thus far, PQQ has been shown to be an antioxidant and redox modulator in a variety of systems (He et al., 2003). Such agents are well known to be protective in experimental stroke models (Alexandrova and Bochev, 2005; Stroke Therapy Academic Industry Roundtable (STAIR), 1999; Zhang and Rosenberg, 2002). Only one prior report by Jensen et al. (1994) investigated the neuroprotective effects of PQQ in a global ischemia/hypoxia model in postnatal day 7 rat pups. PQQ was also reported to be effective in an animal model of epilepsy (Sanchez et al., 2000). In addition, cardioprotection by PQQ has also been recently reported by Zhu et al. (2004) in an ex vivo model of myocardial ischemia/reperfusion in rat. PQQ decreased myocardial infarct size and improved cardiac function in their study. Thus, the present study appears to be the first that examines neuroprotection, assessed by both infarct volume and neurobehavioral outcome, in a widely used model of focal reversible middle cerebral ischemia/reperfusion and in adult rats. Our data demonstrate that PQQ is effective in producing behavioral and infarct volume neuroprotection when given either at the onset of ischemia or at 3 h after the onset of ischemia. The neuroprotection provided by PQQ was dose related with 85% and 81% reduction in infarct size at doses of 10 and 3 mg/kg, respectively, but no effect at 1 mg/kg. Because many neuroprotective effects can disappear weeks and months after the injury (Corbett et al., 2000), long-term investigation on neuroprotection by PQQ after 3 days will be needed in future studies.

Several properties of PQQ could be involved in neuroprotection. First, PQQ may suppress peroxynitrite formation. The neurotoxicity of nitric oxide in ischemic stroke has been suggested to depend upon its conversion to peroxynitrite (Eliasson et al., 1999). PQQ may suppress peroxynitrite formation (Zhang and Rosenberg, 2002) because it is a free radical scavenger and a cofactor for quinoprotein enzymes (He et al., 2003; Urakami et al., 1997). Secondly, PQQ may oxidize the NMDA receptor redox site. Pathological activation of NMDA receptors has been implicated in various CNS disorders including ischemia (Choi and Rothman, 1990; Meldrum and