A dose-response study of dextrorphan in permanent focal ischemia

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Excitatory amino acids (EAA) are important mediators of ischemic injury in stroke [1]. The noncompetitive N-methyl-D-aspartate (NMDA) antagonist dextrorphan is effective in attenuating stroke injury in a number of in vivo and in vitro systems [2-4]. The current study is designed to document the dose-response curve and the time window of effectiveness of dextrorphan in a rodent model of permanent middle cerebral artery (MCA) occlusion leading to infarction.

Focal cerebral ischemia was induced in isoflurane-anesthetized rats (275-300 g) by occlusion of the MCA using the technique of Shiraishi and Simon [5]. The MCA was approached through the base of the skull; the foramen ovale was enlarged to expose the origin of the MCA which was coagulated under direct vision from its origin in the olfactory tract. The femoral artery and vein were cannulated. Normothermia was maintained by a heating pad and a heating lamp; contralateral temporalis muscle temperature, rectal temperature, arterial blood gases and blood pressure were monitored.

Dextrorphan or an equivalent volume of saline was administered over 1 minute in doses of 10, 20, or 30 mg/kg i.v. 5 min prior to MCA occlusion (n = 6 per group). Infarct volume in these experiments was determined by TTC (2,3,5-triphenyltetrazolium-HCl) staining [6]. Two additional groups (n = 8 per group) comparing saline and the maximum effective dose of dextrorphan (20 mg/kg i.v. administered 5 min prior to MCA occlusion) were prepared for histologic evaluation with hematoxylin and eosin staining (H&E).

Animals were sacrificed after 24 h following MCA occlusion with an overdose of chloral hydrate. Brains were rapidly removed and coronally sectioned at 2 mm intervals for TTC staining [6], or cut on a cryostat (20 μm sections at 400 μm intervals) for H&E staining. Eight sections were photographed for analysis of infarct size using an image analysis system. The infarct area in each section was determined by subtracting the area of normal staining brain in the hemisphere ipsilateral to the MCA occlusion from the total area of the contralateral hemisphere to minimize the effect of edema of infarcted brain [7]. The infarct volume was determined by summing the areas and multiplying by the distance between the sections. The results from TTC staining were compared to H&E stained 20 μm thick coronal sections obtained every 0.5 mm from anterior cingulate to posterior hippocampus. Areas of infarction were subdivided by brain region (caudate versus cortex) on a single section through midcaudate.

Differences between groups were determined by analysis of variance and post hoc testing by the Newman-Keuls test. P < 0.05 was used as the accepted confidence level.

Arterial blood pressure, contralateral temporalis muscle temperature and arterial blood gas values 5 min prior to and 30 min following MCA occlusion in saline and dextrorphan animals were examined; no significant dif-
Fig. 1. Infarct volume from TTC stained sections following MCA occlusion. Dextrophan doses i.v. 5 min prior to MCA occlusion (A) and dextrophan 20 mg/kg i.v. administered at designated times following MCA occlusion (B). Infarct volume, from H&E-stained sections, following MCA occlusion. Dextrophan dose 20 mg/kg i.v. 5 mins prior to MCA occlusion (C) and subdivided by brain region (D).
ference in the measured variables were found between groups or time points, except transient hypotension lasting less than 5 min occurred with the maximum dose in the dextrophan animals.

The volume of brain infarction resulting from permanent MCA occlusion was reduced in a dose dependent manner by dextrophan pretreatment. The reduction reached statistical significance at 20 mg/kg and was unchanged at 30 mg/kg (Fig. 1A). These brain volume calculations were performed on TTC stained sections. A similar statistically significant reduction in infarct size was confirmed in serially sectioned H&E stained material from animals pretreated with dextrophan 20 mg/kg (Fig. 1C). Delayed treatment with dextrophan (20 mg/kg) continued to significantly attenuate infarct size when administered at 15, 30, and 45 min following MCA occlusion (Fig. 1B). Protection was most prominent in cortex but also occurred in basal ganglia (Fig. 1D).

The results reported here expand those previously reported with dextrophan in vivo, to demonstrate the compound’s dose–response characteristics, and its time window of usefulness in a model of permanent focal ischemia in the rat which produces a large cortical and basal ganglia lesion evolving to frank infarction over 4 h [3]. The cortex forms the penumbral region and the basal ganglia represents the ischemic core. The maximum effective dose in this model is 20 mg/kg, which is similar to the result of Steinberg in transient focal ischemia [3, 4]. This dose produces brief, mild attenuation in blood pressure when administered in a bolus fashion. The majority of the cerebral protection occurred in the cortical penumbra, but some sparing was also seen in ischemic core of the basal ganglia (Fig. 1D).

As pretreatment paradigms have limited counterparts in clinical situations, the time window of efficacy of the maximally effective dose was studied in this model. A significant reduction in infarct volume was found when the compound was withheld as long as 45 min after permanent MCA occlusion (Fig. 1B). Although the extrapolability of this time window in rodents to that in humans is uncertain, the results demonstrated here suggest an adequate potency and time window to support the potential of effective treatment of human focal ischemia.

Dextrophan is a potent, non-competitive antagonist of NMDA channel function which also blocks voltage gated Ca$^{2+}$ channels [8]. It is lipophilic and therefore crosses the blood-brain barrier readily. Brain concentrations are twice those of plasma [3]. In culture, dextrophan attenuates neuronal injury induced by NMDA, glutamate, and hypoxia [2, 9]. In an in vivo system, attenuation of ischemic neuronal injury induced by transient focal ischemia has been reported [3, 4]. These demonstrated neuroprotective properties, and the fact that the compound may lack toxicity in humans [10] make the drug an appealing candidate for a neuroprotectant in human ischemic injury.

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