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Reduction of the prenatal hypoxic-ischemic brain edema with noscapine

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Cytotoxic free radicals and release of several neurotransmitters such as bradykinin contribute to the pathogenesis of hypoxic-ischemic brain damage. We have studied the efficacy of noscapine, an opium alkaloid and a bradykinin antagonist, in reducing post-hypoxic-ischemic damage in developing brain of 7-d-old rat pups. Hypoxic-ischemic injury to the right cerebral hemisphere was produced by legation of the right common carotid artery followed by 3 h of hypoxia with 8% oxygen. Thirty to 45 min before hypoxia the rat pups received noscapine (dose = 0.5–2 mg/kg) or saline. Pups were scarified at 24 h post recovery for the assessment of cerebral damage by histological methods.

Our results showed that noscapine was an effective agent in reducing the extent of brain injury after hypoxic-ischemic insult to neonatal rats. Therefore, it is concluded that noscapine may be a useful drug in the managements of patients after stroke.

Keywords: noscapine, bradykinin, rat pups, hypoxic-ischemic insult, brain edema

Noscapine is an isoquinoline alkaloid found in opium (1). Unlike most other alkaloids obtained from the opium latex, this drug is devoid of any significant analgesic, sedative or euphoric effects generally associated with this group of drugs. Although recently, this drug was shown to be an inducer of apoptosis in some cell lines (5), the only important clinical effect associated with noscapine is its antitussive activity (1, 5).

Opioid antitussive drugs can suppress cough (2) by acting on either the opioid receptors on the airway sensory nerves or the opioid receptors located in the CNS. Agonists of the μ, σ and δ opioid receptors are believed to have central antitussive effects (3, 4). However recent work in our laboratory (10) has shown noscapine to be an effective antitussive agent especially when cough is caused by ACEIs and bradykinin agonist FR190997 (11).

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It has been reported that bradykinin and other kinins play a role in various types of neuronal injury (4). Therefore, this suggests that bradykinin antagonists may be clinically useful in the therapeutic management of neurosurgical patients (5, 6). Recent work in our laboratory on the contractile effects of bradykinin on the guinea pig ileum showed noscapine to be a non-competitive inhibitor of this peptide (9). Therefore, the present work was carried out to study the effect of noscapine in reduction of brain injury after hypoxic-ischemic insult using neonatal rats as a model of brain edema.

Materials and methods

Hypoxic-ischemic insult to the right cerebral hemisphere of 7-d-old rat pumps was introduced as described by Palmer et al. (6), briefly: 7-d-old rat pups of either sex, weighing 12–18 g were anaesthetized using ether. The right common artery of each pup was ligated with 4–0 surgical silk thread. The wound was then sutured and the animal allowed to recover for 5 min. Pups from mixed litters were then randomly divided into four groups. Animals in normal (control) group did not have any surgery or hypoxic insult. Group I received an injection of only normal saline (0.1 ml) given i.p. while groups II–IV received 0.5, 1.0, and 2.0 mg/kg of noscapine in normal saline (0.1 ml). The pups in groups I–IV underwent hypoxic-ischemic insult. Pups were return to their dams for 2 1/2 h rest and then they were placed in jars in which a mixture of 8% oxygen and 92% nitrogen was continuously circulated. The jars were partially submerged in a 37 °C water bath to maintain a constant thermal environment. Air temperature in the jar was measured to be 35 °C. After 3 h of hypoxia, the jars were opened to room air and the surviving pups were returned to their dams for 24 h. Animals were killed and the amount of injury was assessed histologically. Rats in the control group did not have any surgical treatment.

Histological assessment

For assessment of the extent of edema or ischemia, the whole brain was removed and put into formalin for 3 days, molded in paraffin and 10 μm slices were prepared by a fine microtome. Slides were stained by H&E and viewed under light microscope by a trained pathologist and the degree of edema was assessed according to Rice et al. (12) as follows:

Grade 0 = no visible swelling, grade 1 = slight swelling, grade 2 = moderate swelling, grade 3= marked swelling.

Similarly the degree of ischemic neural damage was graded (12) as follows:

Grade 0 = normal, grade 1 = a few neurons damaged, grade 2 = a moderate number of neurons damaged, grade 3 = the majority of neurons damaged, grade 3* = infarction.

Noscapine was a gift from Temad-DP Pharmaco-chemical Company, Tehran, Iran. Other drugs were of analytical grade or higher.

SPSS package was used to carry out student *t*-test on the grades of edema and ischemia.

Results

It was found that hypoxic-ischemic insult could produce some degree of edema or ischemic injury in neonatal rats which could be prevented by an injection of 0.5–2 mg/kg of noscapine (Figs 1 and 2) The relationship between the extent of injury and dose is shown in Figs 1 and 2. It was found that the effect of noscapine on prevention of such insult was more pronounced in the cortex (Figs 1 and 2). Representative histograms of normal brain of neonatal rats, brain of rats after hypoxic-ischemic insult and the brain of rats treated with noscapine (1 mg/kg) after hypoxic-ischemic insult are shown in Fig. 3 (A to C, respectively).

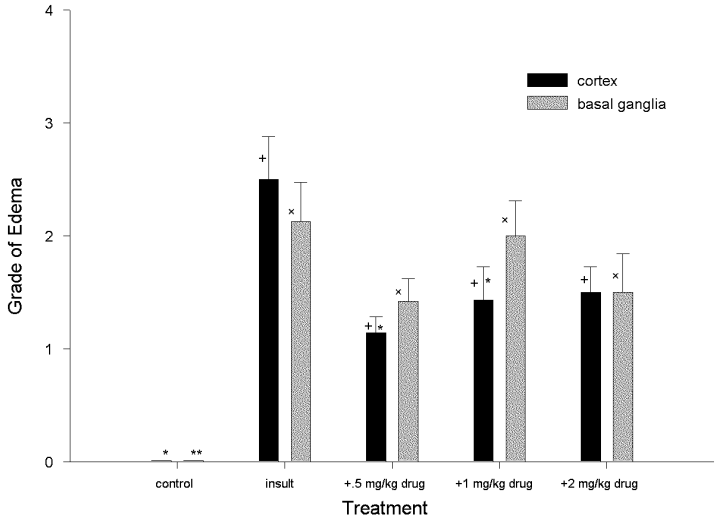


Fig. 1. Effect of noscapine treatment on edema. The injury was evaluated by histological method and graded as described in the text. Each region was compared with the similar region (cortex: black column, basal ganglia: shaded column) of the control group. + Significant difference ($p>0.05$) with the cortex of control group. * Significant difference ($p>0.05$) with the cortex of insulted group. x Significant difference ($p>0.05$) with the basal ganglia of the control group. ** Significant difference ($p>0.05$) with the basal ganglia of the insulted group

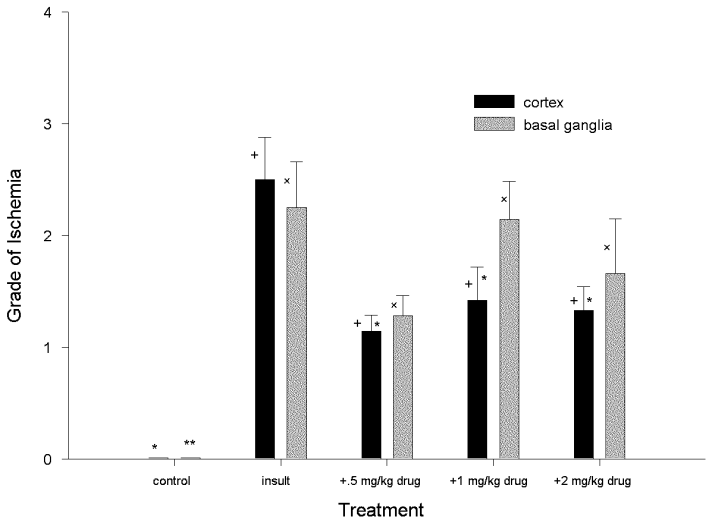
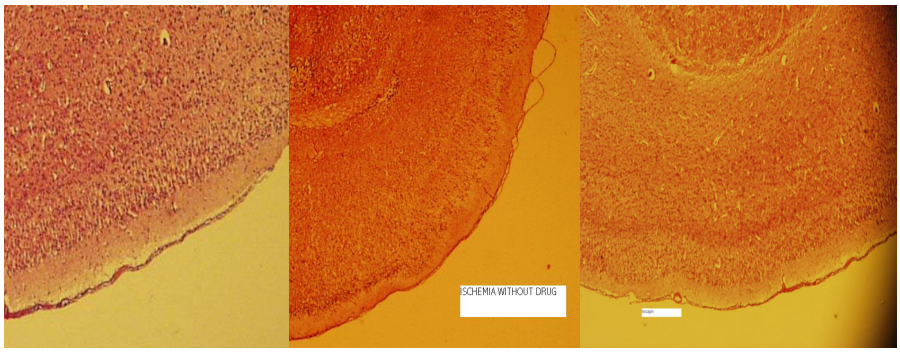


Fig. 2. Effect of noscapine treatment on ischemia. The injury was evaluated by histological method and graded as described in the text. Each region was compared with the similar region (cortex: black column, basal ganglia: shaded column) of the control group. + Significant difference ($p>0.05$) with the cortex of control group. * Significant difference ($p>0.05$) with the cortex of insulted group. x Significant difference ($p>0.05$) with the basal ganglia of the control group. ** Significant difference ($p>0.05$) with the basal ganglia of the insulted group



A **B** **C**

Fig. 3. Representative histograms of normal rat brain **(A),** of rat brain after ischemic-hypoxic insult **(B)**, and of rat brain treated with noscapine (1 mg/kg) after ischemic-hypoxic insult **(C)**

Discussion

The hypoxic-ischemic model used in the present study was developed by Rice et al. (12). The model has the advantages of inducing highly reproducible brain damage in spontaneously breathing animals, with low mortality rate, and in the absence of convulsions or cardiopulmonary complications for at least the first 50 h of survival. The model is also inexpensive and relatively simple to prepare, so that large numbers of predictably brain-damaged animals can be produced for study of drugs.

It was found that noscapine which is used clinically as an antitussive drug can decrease the extent of edema after hypoxic-ischemic insult in neonatal rats. Work carried by Mahmoudian et al. (9), suggested that in the guinea pig ileum, noscapine acted as a noncompetitive antagonist of bradykinin. Also our previous work in guinea pigs showed that noscapine could effectively prevent cough reflexes induced by enalapril and captopril, two ACEIs. It is thought that bradykinin is involved in the production of brain edema and bradykinin antagonists are reported to be able to prevent it. It has been shown that drugs with B2 antagonistic activity could reduce the extent of brain edema (2, 11). Considering the tussive and contractile effects of bradykinin, it is expected that noscapine can decrease edema and brain damage after ischemic-hypoxic enjury. Our results clearly demonstrate that noscapine, at doses which inhibited the cough reflex in guinea pig (4), could effectively prevent the extent of brain edema after hypoxic-ischemic insult. However, it remains to be seen if noscapine is also effective in other types and models of edema. It is interesting to note that effect of noscapine was reduced at higher doses. A finding which was reported by Zausinger et al. (13) also for LF 16-0687 Ms, another bradykinin B2 receptor antagonist.

The present results provide evidence for a role of kinins in secondary brain damage evolving from ischemic-hypoxic insult in neonatal rats and effectiveness of bradykinin antagonists such as noscapine to enhance the neurological recovery.

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