International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology 3 (3): 248-253, 2007
ISSN 1811-7775

Effect of Calcium Channel Blocker Nicardipine on Brain Edema in Rats

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Abstract: The aim of the present study was to examine the therapeutic effect of nicardipine on diffuse brain injury. Male Wistar adult rats were subjected to a diffuse brain injury. Nicardipine, as a calcium channel blocker was injected intravenously 15 min following induction of injury at doses of 10 or 20 μg kg^{-1}. Quantitative measurements of water content, as index of brain edema and Evans Blue (EB) content as index of blood brain barrier permeability were determined using standard procedures. The histological examination was done by hematoxylin-eosin staining. The results indicated that there were no significant differences in the percent of water content between nicardipine treated, trauma alone and trauma + vehicle groups. The content of EB was significantly lower in nicardipine-treated groups in comparison with trauma and trauma + vehicle groups. There was no difference between nicardipine and other groups in histological verification. The present data indicated that nicardipine could have a protective effect on vascular permeability after brain injury.

Key words: Brain edema, calcium channel blocker, nicardipine, rats

INTRODUCTION

The brain edema is recognized as one of the crucial factors in mortality and morbidity after head injury. Overall mortality in trauma patients is about 14-30 per 100,000 of the population per year. In developed countries approximately 100-300 per 100,000 of the population are at risk of suffering a head injury per year with a slightly higher incidence in less developed countries (Wasserberg *et al*., 2004; Jeevaratnam *et al*., 1996). Several mechanisms are associated with the development of functional deficits following Traumatic Brain Injury (TBI). At the time of the traumatic event, mechanical disruption of tissue is known as primary injury(Graham *et al*., 1993). After the primary injury, a cascade of biochemical and physiological changes take place as a secondary injury that cause significantly more neuronal injury than the primary event (Graham *et al*., 1993; McIntosh *et al*., 1992, 1996).

Intracellular Ca^{2+} signals have been implicated in the regulation of cellular functions such as production and regulation of many vasoactive factors, excitatory amino acids, proteases, reactive oxygen intermediates ions, complement proteins under physiological and pathological conditions. Calcium signals may be triggered via Ca^{2+} channels such as receptor-activated, store-operated, capacitative Ca^{2+} entry or Voltage-operated channels (Nilius *et al*., 2001; Beatman *et al*., 1988). Increasing body of knowledge indicates that neuronal calcium overload is a main cause of ischemia and brain edema after trauma. By stimulating release of inflammatory and ischemic agents such as endothelin, prostaglandins, leukotrienes following traumatic injury to brain (Colwell and Levine, 1999; Miyazaki *et al*., 1999; Bootman *et al*., 2001; Chan and Fishman, 1980), calcium plays an critical role in the extent of injury following trauma. In fact, excessive entry of calcium into cells has been proposed as a final common pathway of cell death (Schanne *et al*., 1979). Reduction of calcium entry has been proposed as a therapeutic approach for protection of excessive brain damage following trauma (Grotta *et al*., 1986; Wieloch *et al*., 1982).

Nicardipine is a dihydropyridine calcium channel blocker. Recent studies have reported that nicardipine has protective effects in ischemic brain injury (Miyazaki *et al*., 1999; Chew *et al*., 1991; Kittaka *et al*., 1997), prevents or

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reverses vasogenic brain edema (Lin *et al*., 1994) and counters brain damage due to hypertension (Amenta *et al*., 1996). In a clinical study, it was shown that nicardipine reduces the symptoms of vasospasm and improves neurological outcome in patients with subarachnoid hemorrhage (Flamm *et al*., 1988). Finally, it was demonstrated that nicardipine reduces changes in tissue ion concentrations and water content in rat brains after regional cerebral ischemia (Hadani *et al*., 1998). The benefit effects on ischemia have been attributed to diminished entry of calcium into ischemic cells (Wieloch *et al*., 1982).

As far as the literature is concerned, almost there is no data about the effects of nicardipine on brain edema following a diffuse brain injury in rat. Therefore, current study was planned to investigate the effects of post-traumatic treatment of nicardipine on brain edema in rat model of traumatic diffuse brain injury.

For this propose different groups of rats were injected with nicardipine shortly following an induction of brain diffuse injury. The rats sacrificed 24 h and 4 h after trauma and their brains taken out. Quantitative measurements of water content, as index of brain edema and Evans Blue (EB) content as index of blood brain barrier permeability were determined using standard procedures, respectively.

MATERIALS AND METHODS

Animals: Adult male Wistar rats (300-350 g) were obtained from breeding colony of Kerman University of Medical Sciences. Animals were housed five per cage in a room with natural light cycle and constant temperature (24±2°C). Food and water were available *ad libitum*. All procedures were conducted in agreement with Guidelines of the Kerman University of Medical Sciences for care and use of laboratory animals.

Drugs: Nicardipine (NIC) (Sigma, England) (10 or 20 μg kg^{-1}) was injected intravenously at a volume of 10 μL g^{-1} 15 min following induction of brain injury. The drug doses were mainly derived from pilot studies and a survey of reports on these drugs (Chew *et al*., 1991; Lin *et al*., 1994).

Induction of brain trauma: Brain trauma was induced according to procedures were described by others (Marmarou *et al*., 1994). Briefly, a trauma device was used to produce a diffuse brain injury, it was delivered by dropping the weight from a 2 m height.

Experimental groups: One hundred two male rats were divided into 5 groups (n = 20-21 in each group): sham, trauma, trauma + vehicle, trauma plus nicardipine (10 or 20 μg kg^{-1}). Each group divided into three subgroups of 6-7 rat for each: one for measuring water content, one for Evans blue and the last one for histological verification as explained.

The sham group undertook the surgical procedure but was not injured. In trauma group, an induction of injury was done without any treatment. At 15 min after induction of injury in treatment groups, animals were administered an intravenous dose of nicardipine (10 or 20 μg kg^{-1}) or equal volume of vehicle.

Measurement of brain edema

Water content: The rats sacrificed 24 h after trauma and the brains taken out. The wet weight of each brain was measured in a pre-weighed plate. The brains were then dried at 60°C for 72 h and dry weight was measured. Water content of the brain (%) was expressed as (wet weight-dry weight)/wet weight×100.

Evans blue (EB): Evans blue (EB, 2 mL kg^{-1}, 2%) was injected 3 h following brain trauma to each rat through the tail vein. One hour later, the amount of EB in the brain was determined. Duration of 1 h was sufficient for the tracer to accumulate in the brain tissue with a high reproducibility (Esen *et al*., 2005). The tracer remaining in brain vessels were removed by perfusion of saline. The brain was removed and homogenized, then serum was extracted and the content of EB in the extract was determined using a spectrophotometer at 620 nm. Extravasation of EB expressed as a μg EB g^{-1} tissue.

Histological observation: Rats were decapitated under halothane anesthesia 24 h after the trauma and brain were removed immediately. Rat's brains were collected in buffered formaldehyde for histology and coronal brain sections (10 μm) were made on a brain slicer with a razor blade. Slices then stained by Hematoxylin-Eosin (HE).

Statistical analysis: The data were analyzed using one-way ANOVA followed by Tukey's HSD test to determine the source of detected significances. Values of $p<0.05$ were considered as significant.

RESULTS

Effect of nicardipine on brain water content: One-way ANOVA revealed a significant differences among groups ($F_{(4,30-)} = 7.49$, $p = 0.001$). Post-hoc comparisons indicated there is a significant difference between sham and trauma groups ($p<0.05$), but no significant difference were found between trauma with nicardipine-treated groups (Fig. 1).

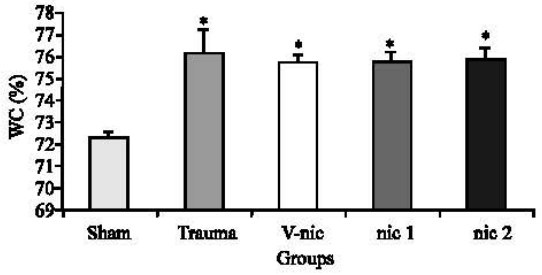

Fig. 1: Mean±SEM Water Content (WC) in different groups of animals. *: p<0. 01, significant different from the sham group, respectively. V-nic = nicardipine vehicle, nic1 = 10 μg kg^{-1} nicardipine, nic2 = 20 μg kg^{-1} nicardipine

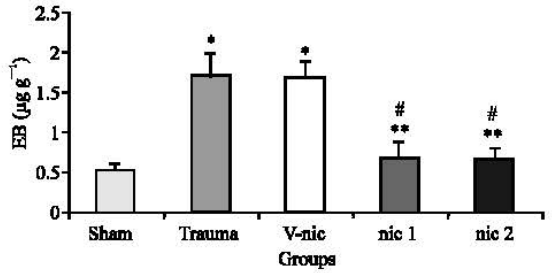

Fig. 2: Mean±SEM Evans Blue (EB) content in different groups. V-nic = nicardipine vehicle, nic1 = 10 μg kg^{-1} nicardipine, nic2 = 20 μg kg^{-1} nicardipine, *:p<0.0001 in comparison with sham group, **:p<0.005, in comparison with vehicle treated (V-nic) group. #: p<0.001 significant different from the trauma group

Effect of nicardipine on EB content: One-way ANOVA indicated a significant differences among the groups [$F_{(4, 32)} = 11.17$, $p = 0.001$). Post-hoc comparisons indicated that nicardipine at both 10 and 20 μg kg^{-1} doses significantly reduced the content of EB in brain in comparison with trauma alone ($p<0.002$, $p<0.001$, respectively), or trauma + vehicle ($p<0.005$, in both doses). There was no significant deference between high and low dose of nicardipine on EB content (Fig. 2).

Histological findings: Sub-arachnoid Hemorrhage (SAH) could be observed in the majority of rats except in sham groups (Fig. 3). Engorged vessels were seen in the cortical area. Abnormality in the injured brains includes neuronal vaqualization and irregularity was seen. Edema was evident in brain tissue and peri-capillary. In rats of nicardipine treatment groups, the abnormalities and edema were the same as trauma group.

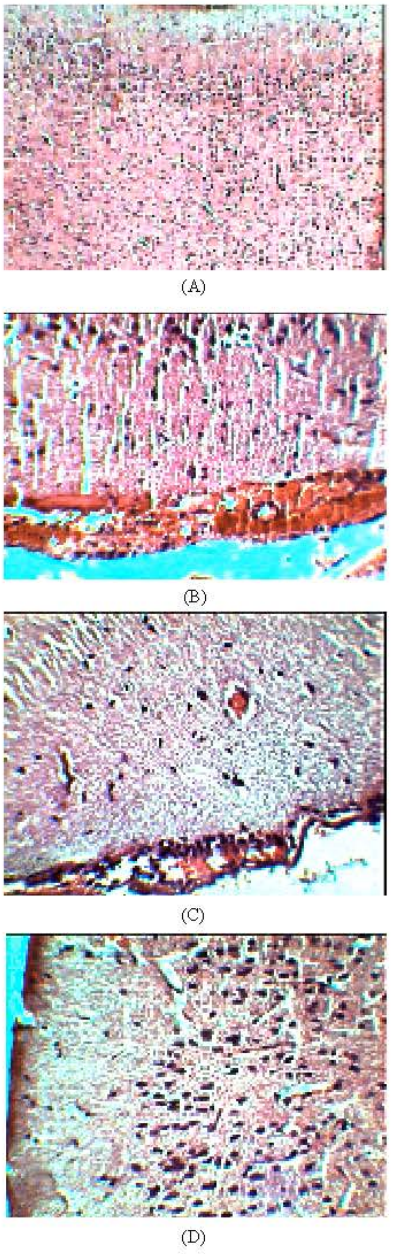

Fig. 3: (A) Histology section of the sham operated brain, absence of injury in the brain tissue can be observed. (B) Engorged vessels sub-arachnoid hemorrhage (SAH), neutrophil infiltration and edema can be observed in brain tissue of the trauma group. (C and D) Nicardipine treated groups in doses of 10 and 20 μg kg^{-1}, respectively. At both doses of nicardipine treated rats, tissue injury is similar to the trauma group. All sections were obtained 24 h post trauma. Magnification power of A is 40X and that of B, C and D is 400x

DISCUSSION

The aim of the present study was to examine whether nicardipine as a calcium channel blocker has any therapeutic effect on diffuse brain injury. The data indicated that nicardipine reduces blood brain barrier permeability as assessed by extravasation of evans blue, but has no any significant effects on water content in a diffuse brain injury. The present data indicated that nicardipine could have a protective effect on vascular permeability after brain injury.

As mentioned in introduction, calcium is a vital component of intracellular signaling system that regulates the activity of various cells. The accumulation of intracellular Ca^{++} is considered a major trigger for the development of tissue damage after brain injury (Miyazaki *et al*., 1999; Colwell and Levine, 1999). Calcium voltage-operated channels are designated as the most important in the regulation of cellular function, especially in the synthesis and release of vasoactive factors (Bootman *et al*., 2001; Nilius and Droogmans, 2001). Excessive entry of calcium into cells following brain trauma triggers a cascade of biochemical pathways which results in cell death (Schanne *et al*., 1979). Thus, calcium channels blockers are potential drugs for protection of excessive brain damage following trauma.

There are some studies suggesting that nicardipine has neuroprotective in the CNS. In brain injury models, nicardipine has been shown to be benefit in stroke and some trauma models (Chew *et al*., 1991; Lin *et al*., 1994; Amenta *et al*., 1996; Kittaka *et al*., 1997). The most of these studies have assessed mechanisms of the proposed benefit of nicardipine in ischemic models or in combination with other drugs in vasogenic brain edema (Lin *et al*., 1994).

To maintain the restricted intercellular transport systems responsible for blood brain barrier permeability, brain capillary endothelial cells require the support of astrocytes (Abbott, 2002). Previous studies have shown that selective destruction of astrocytes resulted in an increase in BBB permeability (Guerin *et al*., 2001; Nishino *et al*., 1997). Cerebral microvascular endothelial cells also express L-type voltage-dependent Ca^{++} channels. These channels have several important roles including production of vasoactive factors, endothelia proliferation and angiogenesis (Momoh *et al*., 2002). Entry of calcium into cells through these channels may be involved in ischemia-induced alteration of cerebral microvascular reactivities and development of vasospasm. The findings of the present study support this assumption. We have found that nicardipine has a inhibitory effects on blood brain permeability on rat diffuse brain injury as assessed by extravasation of evans blue in the brain (Fig. 2). This finding confirms the results of others showing a benefit effect of nicardipine on brain vascular permeability in a cold induced brain injury (Lin *et al*., 1994).

To evaluate brain edema, we measured the brain water content 24 h after diffuse brain injury. It seems that sodium instead of calcium ions play the critical role in the brain edema formation during pathological conditions (Gotoh *et al*., 1985). An increase in activity of sodium-potassium pump (Shigeno *et al*., 1989) and production of nitric oxide (Nagafuji *et al*., 1992) may contribute to brain edema during ischemia. We have found that nicardipine did not affect increased brain water content induced by injury. Present finding confirmed by study of others indicating that nicardipine did not protected against delayed neuronal death after ischemia and increased the brain water content on rat ischemic brain injury (Sauter and Rudin, 1991; Miyazaki *et al*., 1999). Although the last studies used different models of injury and different doses of nicardipine, but their findings are similar to present data).

In conclusion, present findings indicated that nicarcipine may not be effective in reducing water content (brain edema) in diffuse brain injury. This may suggest involvement of other pathways rather than the L-type Ca^{++} channel such as NMDA receptor-operated ion channels, nonspecific membrane channels, Na-Ca exchangers. On the other hand, nicarcipine has a benefit effect on blood brain barrier via blocking function of L-type Ca^{++} channel more likely located in endothelial cells and astrocytes. Thus, pharmacological manipulations may have beneficial and protective effects in brain trauma.

ACKNOWLEDGMENT

This study was supported by Grant from Neuroscience Research Center and Afzalipour Faculty of Medicine in Kerman-Iran and it was a part of Ph.D Thesis of Bahram Bibak.

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