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Original Article

Neuroprotective effects of potassium channel openers on cerebral ischemia–reperfusion injury in diabetic rats

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ABSTRACT

Objectives: This study was done to estimate the potential neuroprotective role of potassium channel openers in cerebral ischemia–reperfusion (IR) injury in streptozotocin (STZ) induced type-I diabetic rats (T1DR).

Methods: Potassium channel openers – cromakalim, cinnarizine and nicorandil; potassium channel blocker –glibenclamide, insulin (as an antidiabetic standard), telmisartan (as an anti-hypertensive standard agent) and vitamin E (as an antioxidant and antiapoptotic standard agent) were given for 3 days in streptozotocin (45 mg/kg i.v.) induced type I diabetic rats along with middle cerebral artery occlusion. After 24 h of surgery, plasma glucose, neurobehavioral score, cerebral infarct volume, blood pressure and caspase-3 levels were measured to evaluate the mechanism of potassium channel openers (KCOs) for neuroprotection.

Results: Following STZ administration and ischemia–reperfusion, blood sugar, neurobehavioral score, cerebral infarct volume and caspase-3 levels were significantly high in diabetic-IR groups. Treatment with cromakalim, cinnarizine, nicorandil, insulin and vitamin E significantly reduce neurobehavioral score while nicorandil and vitamin E significantly reduced cerebral infarct volume. Caspase-3 levels were significantly reduced by cromakalim and nicorandil treated animals. Except insulin and glibenclamide, none of the agents significantly reduce plasma glucose levels.

Conclusion: Treatment of ischemic stroke with potassium channel openers in T1DR is neuroprotective. Inhibition of apoptosis may contribute to their neuroprotective effects after stroke in T1DR.

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1. Introduction

Cerebral ischemic stroke is caused due to obstruction of blood supplied to the brain. It is classified either as ischemic stroke (IS) or hemorrhagic stroke. There are around 83% cases of strokes with IS while the remaining have hemorrhagic brain stroke which results in leakage of blood into the brain. Important etiological factors for pathogenesis include hypercholesterolemia, hypertension and hyperglycemia. It is reported that diabetes mellitus (DM) increases the risk of brain stroke 2 to 3 times more. DM increases the risk of macrovascular and microvascular complications [1,2].

Current treatments options for brain stroke include the use of anti-platelet agents and tissue plasminogen activators (tPA) for their thrombolytic effects. Anti-oxidants such as vitamin C, E and growth factors are found to be neuroprotective in IS. Furthermore, anti-hypertensives, anti-hyperlipidemics as well as oral hypoglycemic agents are beneficial for prevention of IS [3].

However, tPA treatment of stroke after 3 h in patients with DM increases the risk of death and intracerebral hemorrhage [4,5]. Even, reports have found that tPA treatment within 2 h after stroke in type-I diabetic rats significantly increases brain hemorrhage, and increases neurobehavioral score after stroke [6,7]. Thus, there is a need to identify new treatment agents with neuroprotective action in IS and its related disorders like diabetes.

K⁺ ion channels of CNS (central nervous system) play an important role for providing neuroprotection in animal models of ischemic brain stroke [8]. ATP sensitive potassium channel openers such as nicorandil and cromakalim showed free radical scavenging effect and anti-apoptotic effect in streptozotocin-induced diabetic

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rats and in cultured myocytes [9–11]. They produce neuroprotective action IR injury through anti-apoptotic, anti-oxidant and anti-inflammatory actions in various experimental animal models [12]. Thus they are newer therapeutic treatment targets for neuroprotection in ischemic brain stroke. However, their mechanism of neuroprotection in stroke and its related disorders like DM remains unknown. Present acute type of study assumed that potassium channel openers might treat IR injury in STZ induced type-I diabetes mellitus by inhibiting apoptosis pathways. Animal model of IR was induced by cerebral artery occlusion in STZ induced type - 1 diabetes mellitus (T1DM). The neuroprotective actions of potassium channel openers cromakalim, nicorandil and cinnarizine were determined to find out the mechanism of action in diabetic rats.

2. Materials and method

2.1. Drugs and chemicals

All the drugs and chemicals for study were of laboratory grade. Except vitamin E, all other drug solutions were freshly prepared in distilled water and given intraperitoneally (i.p.) in appropriate doses as written in the experimental design section. STZ solution was dissolved in phosphate buffer and given 45 mg/kg intravenously (i.v.). Vitamin E suspension was prepared by dissolving it in 4% tween 80 and given orally. Here, cromakalim, cinnarizine and nicorandil were taken as potassium channel opener agents. Glibenclamide was taken as potassium channel blocker. Insulin, telmisartan and vitamin E were taken as antidiabetic, anti-hypertensive and antioxidant-antiapoptotic standard agents respectively.

2.2. Animals

Adult male Wistar Albino rats weighing between 180 and 210 g were procured from the Animal House of Parul Institute of Pharmacy, Vadodara. The animal experimental protocols, including all use, care, and operative procedures, were approved by the Institutional Animal Ethics Committee (IAEC). Every effort was made to minimize the number of animals used and their suffering. Animals were maintained at 18 ± 2 °C in polypropylene cages with food and water *ad libitum*. Animals were divided into sixteen groups.

2.3. Experimental design

Group 1 (normal control, $n = 6$) animals were administered with tween 80 (4%). Group 2 (Diabetic control, $n = 6$) animals were administered with STZ (45 mg/kg i.v) on 1st day. On day 3, diabetic glucosuria was confirmed using Diachex urine strip. Group 3 (Diabetic Sham surgery operated, $n = 6$) animals were given with tween 80 (4%) for 3 days along with STZ on 1st day. Sham surgery was done on 2nd day. Group 4 (IR control, $n = 12$) animals received tween 80 (4%) for 3 days. IR was done on 2nd day from initiation of experiment. Group 5 ($n = 12$), 6 ($n = 12$), 7($n = 12$) and 8 ($n = 12$) were induced with IR and treated with cromakalim (10 mg/kg i.p.) [13], cinnarizine (5 mg/kg ip) [14], nicorandil (5 mg/kg ip) [15] and vitamin E (150 mg/kg orally) [16] respectively for 3 days. Group 9 (Diabetic-IR control, $n = 12$) animals were administered with tween 80 (4%) for 3 days after STZ (45 mg/kg i. v.) induced diabetes. Cerebral IR was done on 2nd day. Group 10 animals ($n = 12$) were administered with cromakalim (10 mg/kg i. p. for 3 days) along with STZ (45 mg/kg i.v. on 1st day) along with IR injury on 2nd day. Similarly group 11 ($n = 12$), 12 ($n = 12$), 13 ($n = 12$), 14 ($n = 12$), 15 ($n = 12$), and 16 ($n = 12$), were treated with cinnarizine (5 mg/kg ip), nicorandil (5 mg/kg ip), glibenclamide

(5 mg/kg i.p), [17] insulin (5 IU/day) [18], telmisartan (10 mg/kg i.p) [19] and vitamin E (150 mg/kg orally for 3 days) respectively.

2.4. Induction of cerebral ischemia–reperfusion injury

Cerebral IR was induced as per transient middle cerebral artery occlusion (tMCAO) method of Wang et al. [13]. Rats were anesthetized with an i.p. injection of 100 mg/kg ketamine. A 2–3 cm incision was made in the middle of the neck line, separating the left carotid artery, the superior thyroid artery, and the occipital artery, as well as the internal and external carotid communicating arteries. The occipital artery branches of external carotid artery (ECA) were isolated and tied with a cotton thread. Cotton thread was tied loosely around the ECA stump near the bifurcation. Then internal carotid artery (ICA) and common carotid artery (CCA) were temporarily occluded by a fine vessel clip. Through a small incision to the ECA stump, blunt Poly-L-lysine coated 4-0 monofilament was inserted from the left external carotid artery into the left internal carotid artery to a depth of 18.0 mm, vessel clip from ICA removed. After a variable length of suture had been inserted into the ECA stump, resistance was felt and slight curving of suture was observed, indicating that the suture had passed the middle cerebral artery (MCA) origin and reached to proximal segment of anterior cerebral artery (ACA-it has small diameter). Hence the suture had blocked all sources of blood from ICA, ACA and posterior cerebral artery. Finally the vessel clip from CCA was removed to restore the blood flow. The midline incision was closed, leaving the suture protruding so it could be withdrawn to allow reperfusion. The thread was maintained for 2 h and subsequently removed to restore blood flow to the common carotid and internal carotid arteries. Here 18 mm of suture was pulled back until resistance was felt, indicating that the tip cleared the ACA-ICA lumen and was in the ECA stump, then trimmed. The animals were transferred to a fresh cage with free access to food and water.

2.5. Tissue homogenate preparation

Brain samples were washed with isotonic saline and homogenized using ice-cold 10% w/v 0.1 M phosphate buffer of pH 7.4. Supernatant was obtained by centrifuging the homogenate at 12000 rpm (20 min). This supernatant was used to estimate caspase-3 [20].

2.6. Tissue total protein level

It was estimated as per the method of Lowry et al. [21] using bovine serum albumin, alkaline copper reagent Solution A (2% sodium carbonate in 0.1 N NaOH solution in distilled water), solution B (0.5% copper sulfate in 1.0% sodium potassium tartarate) and Folin's phenol reagent.

2.7. Plasma glucose levels

Glucometer (One Touch Ultra 2, Lifescan Inc, USA) was used to estimate glucose levels.

2.8. Neurobehavioral score

Neurobehavioral score was obtained for the group after 24 h of IR injury [22]. This score was monitored for group 4 to 16. Score 0: no behavioral deficit; score 1: forelimb flexion and positive tail suspension test; Score 2: Reduced hold of the forelimb when tail pulled; Score 3: Spontaneous circling or contralateral circling movement when tail pulled; Score 4: Spontaneous circling; Score 5: Death.

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