Remote ischaemic preconditioning and prevention of cerebral injury

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Bilateral carotid artery occlusion of 10 min followed by reperfusion for 24 hr was employed in present study to produce ischaemia and reperfusion induced cerebral injury in mice. Cerebral infarct size was measured using triphenyltetrazolium chloride staining. Short-term memory was evaluated using elevated plus maze. Inclined beam walking test was employed to assess motor incoordination. Bilateral carotid artery occlusion followed by reperfusion produced cerebral infarction and impaired short-term memory, motor co-ordination and lateral push response. A preceding episode of mesenteric artery occlusion for 15 min and reperfusion of 15 min (remote mesenteric ischaemic preconditioning) prevented markedly ischaemia-reperfusion-induced cerebral injury measured in terms of infarct size, loss of short-term memory, motor coordination and lateral push response. Glisbenclamide (5 mg/kg, iv) a KATP channel blocker and caffeine (7 mg/kg, iv) an adenosine receptor blocker attenuated the neuroprotective effect of remote mesenteric ischaemic preconditioning. It may be concluded that neuroprotective effect of remote mesenteric ischaemic preconditioning may be due to activation of adenosine receptors and consequent activation of KATP channels in mice.

Keywords: Cerebral injury, Ischaemic preconditioning, Reperfusion

Ischaemic preconditioning has been reported to protect various organs including heart1, brain2, kidney3, small intestine4, skeletal muscle5 and liver6. Remote ischaemic preconditioning refers to protective effect of brief episodes of ischaemia in one region, which provides protection to severe ischaemia in other distant organ7. Such a protective effect of remote ischaemic preconditioning has been produced in myocardium by prior occlusion and reperfusion of infrarenal aorta8, mesenteric9, renal10 or femoral artery11. The mesenteric ischaemic preconditioning has been observed to increase the release of insulin12,13. The activation of adenosine receptors and ATP-sensitive potassium channels has been implicated in cardioprotective effect of remote ischaemic preconditioning14. The remote ischaemic preconditioning induced cardioprotective effect is mediated through activation of bradykinin and opioid receptors8,9,15. Therefore, the present study has been designed to investigate the effect of remote mesenteric ischaemic preconditioning on global ischaemia and reperfusion induced cerebral injury.

Materials and Methods

Male inbred BALB/C mice weighing 25±2g, maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water were employed in the present study. They were housed in the departmental animal house and were exposed to 12 hr cycle of light and dark. All the animals used in study were naïve to elevated plus-maze test. The experiments were conducted in a semi-sound proof laboratory. The animal experiments were carried out as per the guidelines of institutional ethical committee.

Drugs and chemicals—Glibenclamide (Aventis Pharma Limited, Goa, India), caffeine (Central Drugs House (P) Ltd., New Delhi, India) and chloral hydrate (Riedel-deHaen, Germany) were dissolved in normal saline. All other chemicals used in the present study were of analar quality. All drug solutions were freshly prepared before use.

Remote mesenteric ischaemic preconditioning—Mice were anaesthetized with chloral hydrate (400 mg/kg, ip). Body temperature of mice was maintained at 37°C using heated surgical platform. A median incision was made on the abdomen. Superior mesenteric artery was located, freed from surrounding tissue and a suture (numbered 5/0) was passed below it. The mesenteric artery was occluded for 15 min by tying a shoelace knot and was untied for reperfusion of 15 min. The abdomen was sutured in layers after the completion of one episode of remote mesenteric ischaemic preconditioning.
Ischaemia-reperfusion induced cerebral injury—Mice were anaesthetized using chloral hydrate (400 mg/kg, ip). A midline ventral incision was made in the neck to expose right and left common carotid arteries, which were isolated from surrounding tissue and vagus nerve. A cotton thread was passed below each of the carotid artery. Global cerebral ischaemia was induced by occluding the carotid arteries. After 10 min of global cerebral ischaemia, reperfusion was allowed for 24 hr. The incision was sutured back in layers. The sutured area was cleaned with 70% ethanol and was sprayed with antiseptic dusting powder. The animals were shifted individually to their home cage and were allowed to recover.

Assessment of cerebral infarct size—At the end of 24 hr of reperfusion after global cerebral ischaemia, animals were sacrificed by spinal dislocation and the brain was removed. The brain was kept overnight at -4°C. Frozen brain was sliced into uniform coronal sections of about 1 mm thickness. The slices were incubated in 1% triphenyltetrazolium chloride (TTC) at 37°C in 0.2 M tris buffer (pH 7.4) for 20 min. TTC is converted to red formazone pigment by NAD and lactate dehydrogenase and thus stained the viable cells deep red. The infarcted cells have lost the enzyme and cofactor and thus remained unstained dull yellow. The brain slices were placed over glass plate. A transparent plastic grid with 100 squares in 1 cm² was placed over it. Average area of each brain slice was calculated by counting the number of squares on either side. Similarly, number of squares falling over non-stained dull yellow area was also counted. Infarcted area was expressed as a percentage of total brain volume. Whole brain slices were weighed. Infarcted dull yellow part was dissected out and weighed. Infarct size was expressed as percentage of total wet weight of brain. Investigator estimating the infarct size was not aware about the type of treatment received by the animal.

Evaluation of short term memory using elevated plus maze—Plus maze consisted of two open (16 × 5 cm) and two enclosed (16 × 5 × 12 cm) arms, connected by a central platform (5 × 5 cm). The apparatus was elevated to a height of 25 cm above the floor. A fine line was drawn in the middle of the floor of each enclosed arm. All the animals were given a single trial on plus maze. Each mouse was individually placed at the end of open arm facing away from central platform of the maze. The time taken by the mouse to enter from open arm with all the four legs into the enclosed arm was taken as transfer latency time (TLT). In case the animal did not enter the enclosed arm within 90 sec, it was gently pushed into the enclosed arm and TLT of 90 sec was assigned to it. The animal was allowed to explore the maze for an additional 10 sec after the measurement of TLT. The animal was put to elevated plus maze test for three consecutive days. TLT recorded on the third day served as an index of short-term memory. Animal was then subjected to global cerebral ischaemia for 10 min followed by reperfusion for 24 hr and was again put to elevated plus maze test. Utmost care was taken not to change the relative location of plus maze with respect to any object serving as visual clue in laboratory.

Inclined beam-walking test—Inclined beam-walking test was employed to evaluate fore and hind limb motor coordination. Each animal was individually placed on a metallic bar 55 cm long and 1.5 cm wide, inclined at an angle of 60° from ground. The motor performance of mouse was on a scale ranging from 0 to 4. A grade of 0 was assigned to animal that could readily traverse the beam, grade 1 was given to animal demonstrating mild impairment, grade 2 was assigned to animal demonstrating moderate impairment, grade 3 was given to animal demonstrating severe impairment and grade 4 was assigned to animal completely unable to walk on the beam. Inclined beam-walking test was performed before global cerebral ischaemia and 12 and 24 hr after global cerebral ischaemia and reperfusion.

Lateral push test—Mouse was placed on a rough surface for firm grip and evaluated for resistance to lateral push from either side of shoulder. The test was performed before global cerebral ischaemia and 12 and 24 hr after global cerebral ischaemia and reperfusion. Mice with increased or decreased resistance to lateral push after global ischaemia were assigned + or - score respectively.

Experimental protocol—In total 7 groups were employed and each group comprised of 5 animals. Sham group (Group I): Mouse was subjected to surgical procedure and suture was passed below the superior mesenteric artery but the artery was not occluded. After 30 min of surgery carotid arteries were isolated and thread was passed below it but the arteries were not occluded. After 10 min, thread was removed and the animal was sutured back and allowed to recover for 24 hr. Control group
(Group II): Mouse was subjected to surgical procedure and suture was passed below the superior mesenteric artery but the artery was not occluded. After 30 min of surgery the mouse was subjected to 10 min global cerebral ischaemia followed by reperfusion for 24 hr. Mesenteric preconditioning group (Group III): Mouse was subjected to 15 min of mesenteric artery occlusion followed by a reperfusion period of 15 min. This was immediately followed by 10 min of global cerebral ischaemia and 24 hr reperfusion. Glibenclamide control group (Group IV): Mouse was administered glibenclamide (5 mg/kg, iv) 10 min prior to mesenteric artery isolation. Rest of procedure was same as described for group-II. Glibenclamide treated preconditioning group (Group V): Mouse was administrated glibenclamide (5 mg kg^{-1}, iv) 10 min prior to mesenteric artery occlusion. Rest of procedure was same as described for group-III. Caffeine Control Group (Group VI): Mouse was administrated caffeine (7 mg kg^{-1}, iv) 10 min prior to mesenteric artery isolation. Rest of procedure was same as described for group-II. Caffeine treated preconditioning group (Group VII): Mouse was administrated caffeine (7 mg/kg, iv) 10 min prior mesenteric artery occlusion. Rest of the procedure was same as described for group-III (Fig. 1).

Statistical analysis—Statistical analysis for infarct size and TLT was done using one-way ANOVA followed by Dunnet’s test and Tukey’s multiple range test as post-hoc analysis. Statistical significance for lateral push and beam walking were calculated using Chi square and Wilcoxon Rank sum test respectively. A value of $P<0.05$ was considered to be statistically significant.

Results

Effect of mesenteric preconditioning on ischaemia and reperfusion induced cerebral infarct size—Global cerebral ischaemia followed by reperfusion produced cerebral infarction measured by volume and weight method. Mesenteric preconditioning markedly attenuated ischaemia and reperfusion induced increase in cerebral infarct size (Fig. 2).

Glibenclamide (5 mg/kg, iv) per se did not affect ischaemia-reperfusion induced cerebral infarction. However, its 5 mg/kg, iv dose significantly attenuated mesenteric preconditioning induced decrease in cerebral infarct size occurred as a result of ischaemia and reperfusion. Caffeine (7 mg/kg, iv) per se...
markedly reduced ischaemia and reperfusion induced increase in cerebral infarct size. Moreover, it (7 mg/kg, iv) significantly prevented mesenteric preconditioning induced decrease in cerebral infarct size (Fig. 2). The effect of glibenclamide and caffeine were identical on cerebral infarct size measured by volume and weight method.

Effect of mesenteric preconditioning on ischaemia and reperfusion induced impairment of short-term memory and motor performance—Global cerebral ischaemia followed by reperfusion produced significant percentage change in TLT. Mesenteric preconditioning significantly attenuated ischaemia-reperfusion induced increase in percentage change of TLT. Glibenclamide (5 mg/kg, iv) and caffeine (7 mg/kg, iv) per se did not modulate ischaemia-reperfusion induced increase in percentage TLT. However they significantly attenuated mesenteric preconditioning induced decrease in percentage TLT (Fig. 3).

Global cerebral ischaemia followed by reperfusion produced significant motor incoordination in mice noted after 12 and 24 hr of reperfusion. Mesenteric preconditioning markedly prevented ischaemia-reperfusion induced motor incoordination. Glibenclamide (5 mg/kg, iv) and caffeine (7 mg/kg, iv) per se did not affect ischaemia-reperfusion induced motor incoordination. However, they significantly attenuated mesenteric preconditioning induced decrease in motor incoordination (Fig. 4).

Global cerebral ischaemia followed by reperfusion produced a significant decrease in percentage of mice demonstrating resistance to lateral push noted after 12 and 24 hr of reperfusion. Mesenteric preconditioning significantly prevented ischaemia-reperfusion induced decrease in percentage of mice demonstrating resistance to lateral push. Glibenclamide (5 mg/kg, iv) and caffeine (7 mg/kg, iv) per se did not modify ischaemia-reperfusion induced decrease in percentage of mice demonstrating resistance to lateral push. However, they significantly attenuated mesenteric preconditioning induced increase in percentage of mice demonstrating resistance to lateral push (Fig. 5).

![Figure 1](image1.png)

**Fig. 1**—Effect of mesenteric preconditioning, glibenclamide and caffeine on ischaemia and reperfusion induced impairment of short-term memory and motor performance. [Values are mean ± SE. Wilcoxon Rank sum test was used to test the statistical significance of difference between various groups. Other details are same as in Fig. 2.]

![Figure 2](image2.png)

**Fig. 2**—Effect of mesenteric preconditioning, glibenclamide and caffeine on ischaemia and reperfusion induced impairment of short-term memory and motor performance. [Values are mean ± SE. Wilcoxon Rank sum test was used to test the statistical significance of difference between various groups. Other details are same as in Fig. 2.]

![Figure 3](image3.png)

**Fig. 3**—Effect of mesenteric preconditioning, glibenclamide and caffeine on ischaemia and reperfusion induced impairment of short-term memory in mice. [Values are mean ± SE. Statistical analysis for TLT was done using one-way ANOVA followed by Dunett’s test and Tukey’s multiple range test as post-hoc analysis. Other details are same as in Fig. 2.]

![Figure 4](image4.png)

**Fig. 4**—Effect of mesenteric preconditioning, glibenclamide and caffeine on ischaemia and reperfusion induced impairment of motor coordination noted after 12 and 24 hr of reperfusion in mice. [Values are mean ± SE. Wilcoxon Rank sum test was used to test the statistical significance of difference between various groups. Other details are same as in Fig. 2.]

![Figure 5](image5.png)

**Fig. 5**—Effect of mesenteric preconditioning, glibenclamide and caffeine on ischaemia and reperfusion induced impairment of resistance to lateral push noted after 12 and 24 hr of reperfusion in mice. [Values are percentage of mice showing resistance to lateral push. Chi square test was used to test the statistical significance of difference between various groups. Other details are same as in Fig. 2.]
Discussion

The remote mesenteric ischaemic preconditioning has been observed to prevent ischaemia and reperfusion induced cerebral infarct size and impairment of short-term memory, motor incoordination and decrease in resistance to lateral push. This neuroprotective effect of remote mesenteric ischaemic preconditioning has been attenuated by glibenclamide treatment. Glibenclamide is reported to selectively block ATP-sensitive potassium channels ($K_{\text{ATP}}$) which are documented to be present in brain and blood vessels of brain. Therefore, it may be suggested that neuroprotective effect of remote mesenteric ischaemic preconditioning may be due to the activation of $K_{\text{ATP}}$ channels. Similarly, cardioprotective effect of remote ischaemic preconditioning has been documented to be mediated through activation of $K_{\text{ATP}}$ channels. Moreover, caffeine treatment has been observed to attenuate the ameliorative effect of remote mesenteric ischaemic preconditioning. Caffeine is an adenosine receptor antagonist which may be responsible to prevent mesenteric ischaemic preconditioning induced neuroprotection. Activation of adenosine A1 receptors is known to stimulate $K_{\text{ATP}}$ channels. Thus, it may be possible that remote ischaemic mesenteric preconditioning may release adenosine to activate adenosine receptors, which may consequently activate $K_{\text{ATP}}$ channels and it may be responsible to provide neuroprotection. Caffeine per se has been observed to decrease markedly ischaemia and reperfusion induced cerebral infarct size. This effect of caffeine may not be ascribed to blockade of adenosine A1 receptors because selective blockade of adenosine A1 receptors have been noted to increase the ischaemia induced cerebral injury, moreover other effects of caffeine such as adenosine A2A receptor antagonism, antioxidant activity and inhibition of phosphodiesterase may be responsible to reduce cerebral infarct size.

On the basis of above discussion, it may be concluded that remote mesenteric ischaemic preconditioning exerts neuroprotective effect possibly mediated through adenosine receptors and consequent activation of $K_{\text{ATP}}$ channels.

References


