Endothelin B agonist, IRL-1620, reduces neurological damage following cerebral ischemia in Rats

Mary Leonard[1,2], Seema Briyal[1], Anil Gulati[1,2]. [1]Midwestern University, Chicago College of Pharmacy, 60515, United States, [2]University of Illinois at Chicago, Department of Bioengineering, 60607, United States.

Background: Despite recent studies indicating that stimulation of ET_B receptors may enhance cerebral blood flow and provide neuroprotection, no one has yet determined the involvement of these receptors in cerebral ischemia. The purpose of this study was to determine the effect of IRL-1620, a selective ET_B receptor agonist, on cerebral ischemia produced following middle cerebral artery occlusion in rats.

Methods: Cerebral ischemia was produced in ketamine (100mg/kg, i.p.) and xylazine (10mg/kg, i.p.) anesthetized male Sprague-Dawley rats weighing 250-350g by permanently occluding the middle cerebral artery. Animals (n=6/group) were treated with either vehicle or ET_B agonist IRL-1620 (5µg/kg, i.v.) at 2, 4 and 6 hours after occlusion, or with ET_B antagonist BQ788 (1mg/kg, i.v.) followed by either vehicle or IRL-1620 to determine the role of ET_B receptors in cerebral ischemic conditions. At 24 h after the occlusion, rats were assessed for neurological deficit and motor function using the grip test, foot fault error and rota rod. Animals were then euthanized and the brains were removed for assessment of infarct volume, ET receptor estimation, and oxidative stress parameters. One-way analysis of variance followed by Bonferroni's post-hoc test was used for intergroup comparison with $P<0.05$ considered significant.

Results: Rats treated with vehicle showed significant cerebral ischemia with an infarct volume of 153±32 mm^3 along with marked neurological and motor function deficit following middle cerebral artery occlusion. There was a significant reduction in infarct volume (24.5±4.4 mm^3; $P<0.01$) as well as neurological and motor deficit in animals treated with IRL-1620, when compared with vehicle treated group. Pretreatment with BQ788 completely blocked the effect of IRL-1620 on infarct volume (139±15 mm^3) and neurological symptoms. Cerebral ischemia caused an increase in lipid peroxidation as measured by malondialdehyde (MDA) (574±35 nmol/g wet tissue), an effect which was reversed with IRL-1620 treatment (179±27 nmol/g wet tissue). Conversely, antioxidants superoxide dismutase (SOD) and reduced glutathione (GSH) decreased following occlusion (8.26±0.82 u/mg protein and 97±7 µg/g wet tissue, respectively). IRL-1620-treatment reversed these effects, increasing SOD and GSH in the occluded rat brain (13.17±0.69 u/mg protein and 188±13 µg/g wet tissue, respectively). Pretreatment with BQ788 blocked the effects of IRL-1620 on oxidative stress, increasing MDA (602±17 nmol/g wet tissue) and decreasing SOD and GSH (4.66±0.17 u/mg protein and 86±7 µg/g wet tissue, respectively). Expression of ET_A receptors was elevated in the ischemic rat brain while ET_B receptor expression remained unchanged. These results were not altered by IRL-1620 treatment.

Conclusions: Activation of ET_B receptors with specific agonist IRL-1620 reduced neurological damage, infarct volume, and oxidative stress following cerebral ischemia in rats. These effects were reversed by ET_B antagonist BQ788. The present study demonstrates that selective ET_B receptor activation may be a novel neuroprotective therapy in the treatment of cerebral ischemia.