Rapamycin treatment ameliorates brain metabolite levels after transient focal ischemia in rats

Anjali Chauhan1, Uma R Sharma2, Naranamangalam R Jagannathan2, and Y K Gupta1

1Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, Delhi, India, 2Department of NMR & MRI Facility, All India Institute of Medical Sciences, New Delhi, Delhi, India

Introduction: Worldwide, stroke is one of the major causes of mortality and morbidity in humans. Recombinant tissue plasminogen activator (rtPA) is the only approved drug for management of ischemic stroke. However, rtPA is associated with limitations like narrow therapeutic window and increased risk of intracranial hemorrhage. Therefore exploring other drugs as neuroprotective agents becomes essential. In experimental models of central nervous system disorder, rapamycin treatment has been shown to have neuroprotection [1-3]. Using MRI, we have earlier demonstrated that rapamycin afforded neuroprotection in the middle cerebral artery occlusion (MCAo) model of transient ischemic stroke in rats by decreasing the infarct volume and area as well as improving the motor deficits [4]. The objectives of present study are to evaluate the comprehensive metabolic profile of rat brain after induction of cerebral ischemia, to investigate the effect of rapamycin treatment on the brain metabolite profile in MCAo and to understand the mechanism of neuroprotection.

Materials and Methods: Male Wistar rats (180-230g) were anesthetized and focal cerebral ischemia was induced as described previously [4]. Briefly, there were three investigational groups namely; Group 1- sham (n = 6), where no ischemia was induced and no treatment was given, Group 2-vehicle group (n = 6) in which dimethyl sulfoxide was administered after 1 hour of ischemia and in Group 3- rapamycin (n = 6) was administered in dose of 250 μg/kg i.p. 1 hour after MCAo. In Group 2 and 3, reperfusion was done after 2 hours of ischemia. After 22 hours of reperfusion, the brain was removed and snap freeze in liquid nitrogen. For performing the in vitro NMRS, the water-soluble metabolites were extracted from the rat brain using perchloric acid extraction as described previously [5].

Results: The various metabolites were assigned in all the 3 groups using 1D and 2D NMR (Figure 1). The level of lactate (Lac) was increased while the levels of glutamine/glutamate (Gln/Glu), creatine/phosphocreatine (Cr/PCr), glycerophosphocholine/phosphoryl choline (GPC/PC), myo-inositol (mi), N- acetyl aspartate (NAA), taurine (Tau) and γ-amino butyric acid (GABA) were decreased in vehicle group as compared to sham group (P<0.05; Figure 2). Treatment with rapamycin decreased the level of Lac and increased the levels of Gln/Glu, Cr/PCr, GPC/PC, mi, NAA, Tau and GABA in the drug treated group as compared to vehicle group (P<0.05; Figure 2).

Discussion: The results of the present study demonstrated an increase in Lac levels and decrease in the levels of Gln/Glu, Cr/PCr, GPC/PC, mi, NAA, Tau and GABA in the vehicle group as compared to sham control. The increase in Lac levels in vehicle group might be attributed to ischemia induced depletion of oxygen and glucose leading to lactic acidosis. On the other hand, we observed decrease in Gln, Glu and GABA in vehicle group which might be ascribed to ischemia induced deficiency of glucose and shift in these metabolites as alternative substrates to glucose in the tricarboxylic acid cycle. Furthermore, decrease in levels of Cho, PC and GPC along with mi was observed in the vehicle treated rats, this decrease might reflect to injury to cell membrane as these metabolites are integral components of membrane phospholipids and hence are associated with membrane metabolisms. Likewise, the Cr/PCr are important entities involved in the energy metabolism in mitochondria and regulate the energy homeostasis in the brain and their decrease in vehicle group reflect mitochondrial injury in this group. On the other hand, decrease in NAA and tau which was observed in vehicle group might reflect to neuronal damage and dysfunction. These changes corroborated well with others [7]. In the rats treated with rapamycin, there was decrease in Lac and increase in the Glu/Glu, Cr/PCr, GPC/PC, mi, NAA, Tau and GABA levels. Protection by rapamycin might be attributed to its antioxidant and anti-inflammatory activity [4] hence suggesting action of rapamycin on ischemia induced injury including; metabolic changes, disruption in cell membrane metabolism and changes in the neurotransmitters.

Conclusion: The results of this study indicated the neuroprotective effect of rapamycin on the brain biochemistry after MCAo induced stroke. This protection of rapamycin might be ascribed to its effect on changes on levels of brain metabolites and neurotransmitters that are affected in focal ischemia.