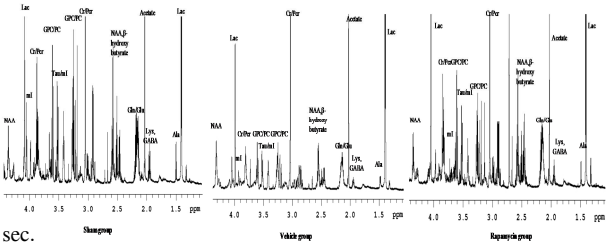
Rapamycin treatment ameliorates brain metabolite levels after transient focal ischemia in rats

Anjali Chauhan[1], Uma R Sharma[2], Naranamangalam R Jagannathan[2], and Y K Gupta[1]

[1]*Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, Delhi, India,* [2]*Department 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].



3-Trimethyl silyl propionic acid (TSP) was added to the sample that served both as a chemical shift reference and concentration standard. Proton NMR spectroscopy was carried out on a 700 MHz NMR spectrometer (Varian). The data was acquired using a standard 5 mm dedicated multinuclear broadband inverse probe at 25°C. The 1D spectra with water suppression were acquired using a single 90° pulse over a spectral width of 7716 Hz using 32 K data points, 64 scans and a relaxation delay of 14 seconds. 2D COSY and the TOCSY were also carried out. Typical parameters used for TOCSY experiments were: data points 2 K in F2 dimension, spectral width 7716 Hz and a relaxation delay of 2 sec. The number of t_1 increments were 256 and 64 free induction decays per increment was acquired. The concentrations of the metabolites were determined by comparing the integrated intensity of the isolated resonances of the compounds of interest with that of the TSP signal [6].

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/phosphorlyl 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).

Figure 1: various metabolites assigned in all the 3 groups; 1D of sham, vehicle and rapamycin group

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 Glu, Gln 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, decease 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 Gln/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.

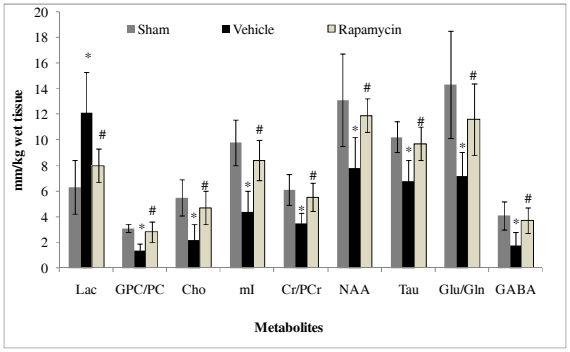


Figure 1: The concentration of metabolites in sham, vehicle and rapamycin treated groups (mm/kg wet weight). *P<0.05 as compared to normal; #p<0.05 as compared to vehicle.

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.

References: (1) Erlich S et al., Neurobiol Dis 2007, 26, 86–93; (2) Caccamo A et al., J Biol Chem 2010, 285, 13107–20; (3) Ehninger D et al., Nat Med 2008, 14,843–8;(4) Chauhan A et al., Behavioural Brain Research 2011, 225,603– 609; (5) Payen JF et al, Anaesthesiology 1996, 84, 1077-82; (6) Sharma U et al., Magn Reson Imaging 2003, 21,145-5; (7) Yang M et al., Talanta 2012, 88,136-44.