Effects of 2-deoxy-D-glucose and 3-O-methyl-glucose on focal cerebral ischemia in hyperglycemic rats

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Synopsis

The effect of 3-O-methylglucose (3OMG) and 2-deoxy-D-glucose (2DG) on neurological outcome was evaluated on a hyperglycemic rat middle cerebral artery occlusion/reperfusion (MCAO/R) model by using proton MRI/MRS. The results showed that daily injection of 2DG (300mg/kg, ip) combined with single dosage of 3OMG (500mg/kg, iv) 10 min prior to MCAO significantly reduced DWI measured lesion volume by 48% and lactate/NAA ratio by 56% at 4 h after MCAO/R. The results indicated that the competitive 3OMG and 2DG inhibition of glucose uptake (glucose transporter) and initial metabolism (hexokinase) has a beneficial effect in reduing brain damage in hyperglycemic rats exposed to MCAO/R.

Introduction

Pre-existing hyperglycemia exacerbates neurological deficits in a rat MCAO/R model. Glucose availability appears to be associated with tissue lactate accumulation during ischemia/reperfusion. The degree and extent of tissue damage in this model correlates with the intracellular concentration of lactic acid[1,2] and a worse outcome during hyperglycemia has been attributed to excessive lactate production and the resulting acidosis.[3] We hypothesize that agents such as 2DG inhibit hyper-metabolic neuronal necrosis, (which involves lactate accumulation, glutamate excitotoxicity, nitric oxide production, free radical formation and ATP depletion[4]) and limit irreversible brain damage by suppressing the energy dependent pathological vicious cycle. In the present investigation we have extended our previous studies[5] to examine the protective effect of daily treatment with 2DG, combined with a single dosage of 3OMG prior to MCAO/R on hyperglycemic rats. By performing sequential diffusion weighted imaging (DWI) and localized, water-suppressed proton MRS, we examined the time course of the early changes of the lesion size and lactate/NAA peak ratio in hyperglycemic rats undergoing a 90 min MCAO followed by 24h reperfusion.

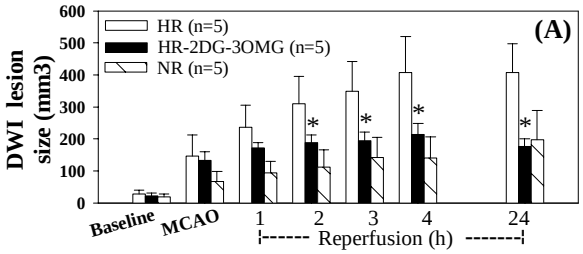
Materials and Methods

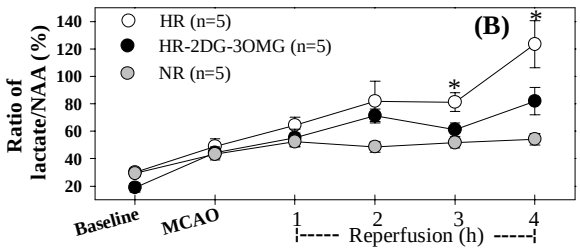
Male Sprague-Dawley rats (300-330g) were anesthetized with 3% isoflurane in 30%O_2/70%N_2, and then intubated and mechanically ventilated with isoflurane maintained at 1.0-1.5% during the surgery and MRI procedures. The femoral artery was cannulated for monitoring blood gasses and mean arterial blood pressure (MABP). A cannula was inserted in the femoral vein to deliver 3OMG or contrast agents. Hyperglycemia was induced by ip injection of streptozotocin 60 mg/kg. MCAO was induced using the intraluminal suture insertion method. Animals were divided into two groups: **1**.hyperglycemic rats (HR-2DG-3OMG, n=6) treated with 2DG (ip 300 mg /kg) daily up to 7 days, and intravenously injected 500 mg/kg of 3OMG 10 min before MCAO. **2**.hyperglycemic rats (HR, n=5) treated with saline. **3**. normoglycemic rats treated with saline (NR, n=5).

Proton MRI was performed using a 4.7 Tesla, 33-cm horizontal bore system (Varian, USA). Excitation and signal detection were achieved with a 5-cm surface coil. MRI and MRS were acquired before and 30 min after MCAO and repeated every 60 min up to 4.5h. The protocol consisted of: **1**) Multislice spin echo and DWI; **2**) water-suppressed proton MRS (PRESS technique) positioned in the ischemic territory. The voxel dimension (x, y, z) was 3.5× 3.5 × 3.5 mm^3, TR=2500ms, TE=25ms. Ischemic lesion volumes, estimated from DWI, were calculated in mm^3 from the number of hyperintense pixels in the ischemic hemisphere. Peak integrals of baseline-corrected localized proton spectra were used to measure the changes in lactate, creatine and NAA. The peak at 2.02 ppm was assigned to NAA, 1.33 ppm to lactate and 3.02 ppm to creatine. Lactate levels were expressed as the ratio of the lactate:NAA and lactate:creatine.

Results

Figure A summarizes the ischemic lesion growth. Animals treated with 2DG and 3OMG showed a significantly reduced DWI-measured lesion volume. By 4 h into reperfusion, lesion volume in the 2DG-3OMG treated HR group was 213 mm^3, which was significantly lower than that in the saline treated group (408 mm3, * p< 0.05). The ratio of lactate/NAA (Fig. B) followed a similar time course with the DWI-detected lesion volume.





Discussion

These results indicate that daily administration of 2DG combined with a single dose of 3OMG 10min prior to MCAO is neuro-protective in strepotozotocin induced hyperglycemic rats. Nonmetabolizable glucose analogs 2DG and 3OMG inhibit cellular metabolism by competitively inhibiting glucose uptake and anaerobic glycolytic flux. Inhibiting glucose metabolism may exert cytoprotection in postischemic brain tissue by decreasing hyper-metabolic necrosis. By using *in vivo* ^{1}H MRS and MRI, this study shows that 2DG and 3OMG reduced lactate levels and minimized the neuropathological consequences of ischemic injury.

References

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