SUMMARY

EFFICIENCY OF PENTOXYPHILLINE AND MELATONIN TREATMENT ON NEWBORN RAT MODEL OF HYPOXIC ISCHEMIC ENCEPHALOPATHY

Perinatal asphyxia is an important cause of neonatal mortality and subsequent serious sequelae such as motor and cognitive deficits and seizures. The principal mechanisms leading to neuronal death after hypoxia-ischemia are initiated by energy depletion, accumulation of extracellular glutamate, and activation of glutamate receptors. The cascade of events that follows involves accumulation of cytosolic calcium and activation of a variety of calcium-mediated deleterious events, such as activation of lipases, proteases and phospholipases, and formation of oxygen free radicals as by-products of xantine and prostaglandin synthesis. The intracellular calcium induces the production of nitric oxide. The combined effects of cellular energy failure, acidosis, glutamate release, intracellular calcium accumulation, lipid peroxidation, and NO neurotoxicity disrupt essential components of the cell, resulting in death by mechanisms of necrosis and apoptosis. Melatonin, the chief secretory product of the pineal gland, is an effective antioxidant which scavenges free radicals and up-regulates several antioxidant enzymes. It also has a strong anti-apoptotic signaling function. Pentoxyphilline is a xanthine derivative, potent inhibitor of TNF-α production and apoptosis.

The aim of this study is to investigate the effects of the melatonin and pentoxyphilline on neurodegeneration and cerebral nitric oxide production in a neonatal rat model of hypoxic-ischemic brain injury.

Seven-day-old Wistar Albino rat pups have been used in the study (n=60). Experimental groups in the study were; sham operated group, melatonin treated hypoxia-ischemia group, pentoxyphilline treated hypoxia-ischemia group, melatonin+pentoxyphilline treated hypoxia-ischemia group, etanole (melatonin's dissolver) treated hypoxia-ischemia group and vehicle-treated hypoxia-ischemia group. In hypoxia-ischemia groups, left common carotid artery was ligated permanently on the seventh postnatal day. Two hours after the procedure, hypoxia (%92 nitrogen and %8 oxygen) was applied for 2.5 hour. Melatonin (10 mg/kg), pentoxyphilline (40 mg/kg) and melatonin+pentoxyphilline were injected (intraperitoneally; ip) as a single dose immediately after the hypoxia period. Brain

nitrite levels, neuronal cell death, and apoptosis were evaluated 72 hours after the hypoxic-ischemic insult.

Histopathological evaluation demonstrated that melatonin and pentoxyphilline significantly diminished number of “apoptotic cells” in the hippocampus. Melatonin, when administered separately, significantly preserved the number of neurons only in the CA2 regions of hippocampus. When compared with vehicle-treated group, combination treatment with melatonin and pentoxyphilline significantly reduced “apoptotic cell death” and preserved the number of neurons CA1, CA2, CA3, and dentate gyrus regions of hippocampus. Brain nitrite levels were evaluated by Griess reagent and showed that hypoxic-ischemic injury caused a significant increase in NO production. Melatonin+pentoxyphilline treatment significantly decreased NO overproduction in the hypoxic-ischemic hemisphere, whereas no significant change appeared in hypoxia alone and also in the sham-operated group.

These results suggest the beneficial neuroprotective effect of melatonin and pentoxyphilline combination treatment in this model of neonatal hypoxic-ischemic brain injury. To our knowledge, this is the first study that demonstrates a protective effect of melatonin and pentoxyphilline combination treatment against hypoxia-ischemia in the developing brain.

Key Words: Hypoxia, ischemia, brain, newborn, melatonin, pentoxyphilline, apoptosis, nitric oxide.