[S1-2] [4/17/2003(Thur) 14:30-15:00/Grand Hall]

# Neuroprotective Effects of Antioxidatives of Constituents Isolated from Plants

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Cerebral ischemia, the most prevalent form of clinical stroke, is a medical problem of the first magnitude. Substantial efforts are being made to develop drugs which will protect the brain from the neurodegeneration that follows ischemic stroke. However, no medical treatment is currently approved for the treatment of stroke to reduce brain infarction or neurological disability beyond tissue plasminogen activator. It is now convincing that free radical generation is involved in the pathophysiological mechanisms of ischemic stroke, particularly in ischemia-reperfusion injury. The seminar, therefore, will discuss our efforts to develop antioxidative constituents isolated from several plants as neuroprotective agents using *in vitro* neuronal cell culture systems and *in vivo* rat models of focal cerebral ischemia.

The first series of experiments examined neuroprotective effect of aloesin isolated from Aloe vera, which was known to have antioxidative activity, in a rat model of transient focal cerebral ischemia. Transient focal cerebral ischemia was induced by occlusion of middle cerebral artery for 120 min with a silicone-coated 4-0 nylon monofilament in male Sprague-Dawley rats under isoflurane anesthesia. Aloesin (1, 3, 10, 30 and 50 mg/kg, i.v.) was administered 3 times at 0.5, 2 and 4 hr after onset of ischemia. Neurological score and infarct volume were measured 24 hr after onset of ischemia. Treatments with the doses of 1 or 50 mg/kg did not significantly reduce infarct volume compared with the saline vehicle-treated control group. However, treatments with the doses of 3 and 10 mg/kg significantly reduced both infarct volume and edema by approximately 47% compared with the control group, producing remarkable behavioral recovery effect. Treatment with the dose of 30 mg/kg also significantly reduced infarct volume to a lesser extent by approximately 33% compared with

the control group, but produced similar degree of behavioral recovery effect. In addition, general pharmacological studies showed that aloesin was a quite safe compound.

The second series of experiments examined neuroprotective effects of antioxidative flavonoids isolated from *Opuntia ficus-indica* var. *saboten* Makino. The flavonoids, quercetin (Q), (+)-dihydroquercetin (DHQ), and quercetin 3-methyl ether (QME), were isolated from the ethyl acetate fractions of the fruits and stems of the plant. The *in vitro* study evaluated their protective effects against the oxidative injuries induced in primary cultured rat cortical cells and their antioxidative activities using three different cell-free bioassays. Quercetin inhibited the $H_2O_2$- or xanthine (X)/xanthine oxidase (XO)-induced oxidative neuronal injury, with the estimated $IC_{50}$ values of 4-5 μg/ml. (+)-Dihydroquercetin exhibited concentration-dependent inhibitions of the oxidative neuronal injuries, but it was less potent than Q. Quercetin 3-methyl ether, on the other hand, exhibited potent and dramatic inhibitions of the $H_2O_2$- and X/XO-induced neuronal injuries. The $IC_{50}$ values were 0.6 and 0.7 μg/ml, respectively. All three principles markedly inhibited lipid peroxidation and scavenged 1,1-diphenyl-2-picrylhydrazyl free radicals. These results indicate that Q, DHQ, and QME are the active antioxidative principles in the plant exhibiting neuroprotective actions against the oxidative injuries induced in cortical cell cultures, and that QME appears to be the most potent neuroprotectant among the three flavonoids. In addition, *in vivo* treatment with 10 mg/kg of QME (i.v.) 30 min after induction of 120-min transient focal cerebral ischemia significantly reduced both infarct volume and edema by approximately 50% compared with the vehicle-treated control group, producing behavioral recovery effect.

The results suggest that several antioxidative constituents isolated from the plants can serve as lead chemicals for the development of neuroprotective agents by providing neuroprotection against oxidative and focal ischemic neuronal injuries.