of a superoxide radical and oxidized lipids in vessel-wall abnormalities has also been hypothesized in atherogenesis.[3]

SOD is essential for the normal functioning and survival of aerobic cells.[6] It is one of the natural defense mechanisms that catalytically scavenge superoxide radicals produced intracellularly during respiration. In eukaryotic cells, 2 forms of SOD have been found, copper-zinc SOD (CuZn-SOD) in the cytosol and manganese SOD (Mn-SOD) in mitochondria. During the past decade, various investigators using different models have found that endogenous SOD activity appears to decline during ischemia and reperfusion.[7,8] This proposed change in SOD activity may result in a lowering of the defense of the myocardium against free-radical-mediated damage. Based on these observations, antioxidant enzymes have been added to reperfusion therapies after thrombolysis or revascularization, although the issue of whether SOD reduces the degree of myocardical damage remains unresolved.[9]

Catechins are a group of polyphenolic compounds contained abundantly in green tea (*Cmellia sinensis*). The main polyphenolic components in green tea are (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. Catechins are considered to exert protective effects against cancer and inflammatory and cardiovascular disease.[10,11] These protective effects have been mainly attributed to their antioxidative activities by scavenging free radicals.

Trilinolein is a potent antioxidant recently purified in our laboratory from the traditional Chinese medicinal plant *Panax pseudoginseng*.[12,13] It has a triacylglycerol with linoleic acid as the only fatty acid residue in all 3 esterified positions of glycerol.[13] It has myocardial protective effects during ischemia and reperfusion both in vivo and in vitro.[5,14] Our previous study showed that this natural lipophilic trilinolein has a beneficial effect on SOD,[14] i.e., it can potentially affect the expression of SOD. Since catechin is also a natural hydrophilic antioxidant, it should also possess beneficial effects as does SOD.This study was undertaken to evaluate the short-term (2 days) and long-term (7 days) effect of catechin on SOD activity, and SOD-mRNA and SOD protein amounts in rat aortic smooth muscle cells (A7r5).

MATERIALS AND METHODS

Preparation of Aortic Smooth Muscle Cells

A7r5 cells (embryonic thoracic aorta, smooth muscle, DBIX rat), obtained from the Food Industry Institute, Hsinchu, Taiwan, were incubated in Dulbecco's modified Eagle's medium that contained 10% fetal bovine serum. A7r5 cells were washed in phosphate-buffered saline (PBS) containing 0.1% EDTA and 0.5% trypsin and were centrifuged at 1000 r.p.m. for 5 min. The supernatant was discarded, and a cell suspension was obtained after adding culture medium. A 100 l sample of the cell suspension was mixed thoroughly with 100 l of PBS containing 0.04% trypan blue. The number of surviving cells was counted using a light microscope and a Coulter counter. Surviving cells were divided into 4 groups: a control group incubated with culture medium containing 0.5% ethanol (without catechin) and 3 treatment groups incubated with culture medium in addition to 1, 10, or 100 M catechin in 0.5% ethanol, respectively, for 2 or 7 days.

Assay of SOD Activity

Superoxide dismutase activity was measured using a commercial assay kit (Wako, Osaka, Japan). Cells from 1 dish were harvested and homogenized in 1 ml of 0.9% NaCl.[15] The crude homogenate was centrifuged at 10,000 g for 1 h to get the supernatant (cytosolic) and pellet (particulate). For assay of Mn-SOD activity in the pellet, 1 mM potassium cyanide was added to the incubation mixture to inhibit CuZn-SOD activity. The activity of CuZn-SOD was derived by subtracting Mn-SOD activity from total SOD activity in the pellet. Superoxide dismutase activity values were derived from a standard curve constructed using purified SOD from bovine erythrocytes (S-2515; Sigma Chemical. St. Louis, MO, USA). Results are expressed as units per milligram of protein, which was determined by the protien dye-binding method.[16]