have been attributed to oxygen-derived free radicals.[3, 4] Furthermore, oxygen free radical species produce deleterious effects in various diseases such as cancer, AIDS, chemical-induced liver damage, aging, rheumatoid arthritis, and several autoimmune diseases.[5, 6] Oxygen free radicals are also thought to be involved in neurodegenerative diseases such as Parkinson's and Alzheimer's disease.[7, 8] Specific cellular enzymatic defense mechanisms mediated by superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) have been suggested to offer protection against oxygen free radical species.[9, 10]

It has been suggested that sulfhydryl containing certain agents participates in protecting against radical-induced damage.[11] Recently, growing interest has been focused on the role of metallothionein as a radical scavenger because of its high thiol content which is involved exclusively in the formation of diamagnetic metal-thiolate clusters.[12] Zinc-metallothionein has been shown to scavenge hydroxyl radicals in vitro and to be more effective than glutathione in preventing hydroxyl radical-induced DNA degradation.[13] Furthermore, the role of metallothionein in cardiac protection against oxidative injury has been demonstrated with adriamycin, an important anticancer drug which causes heart damage. Preinduction of metallothionein in mouse hearts by bismuth subnitrate significantly inhibited adriamycin-induced lipid peroxidation.[14]

In the present study, we examined the effectiveness of metallothionein in free radical-scavenging activity and cytoprotective activities in in vitro models. We subsequently utilized these findings to characterize the detailed effects of metallothionein in these reactions.

MATERIALS AND METHODS

Materials

Cytochrome c (type III, from bovine liver), probucol, butylated hydroxytoluene (BHT), DMSO, DMPO, malondialdehyde, desferrioxamine mesylate, 2-thiobarbituric acid, tetramethoxypropane, diphenyl-p-picrylhydrazyl, bovine serum albumin (BSA), Hepes, hydrogen peroxide (30% solution), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), superoxide dismutase (SOD, type I, from bovine liver), trypan blue, diphenylenieiodinium (DPI), dextran (T500), cytochalasin B, and formyl-Met-Leu-Phe (FMLP) were purchased from Sigma Chemical Co. (USA). Metallothionein was obtained from Pharmaceutical Research Co. (PRC; USA). Ficoll-Paque was obtained from Pharmacia Biotech. Penicillin, streptomycin, M199 medium, Hanks' balanced salt solution (HBSS), glutamine and fetal calf serum (FCS) were obtained from Gibco-BRL. All other chemicals used in this study were of reagent grade.

Antioxidant Activity in Rat Brain Homogenate

Rat brain homogenates were prepared from brains of freshly killed Wistar rats, and their peroxidation induced by different methods was measured using the thiobarbituric acid (TBA) method, as described by Hsiao et al.[15] Tetramethoxypropane was used as a standard, and the results were expressed as nanomoles of malondialdehyde equivalents per milligram of protein of both preparations. Protein contents of brain homogenates were determined by the Bio-Rad method,[16] using bovine serum albumin as a standard.

Stable Free Radical-scavenging Action

Stable radical-scavenging activity was measured according to the method of Mellors and Tappel.[17] An ethanolic solution of the stable nitrogen-centered free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH, 100 μM), was incubated with normal saline or metallothionein, and the absorbance was monitored spectrophotometrically at 517 nm. The change in absorbance of DPPH by the antioxidant during 30 min was taken as the potency of its free radical-scavenging activity.

Preparation of Human Neutrophils and Measurement of Superoxide Anion Production

Citrated blood samples were obtained from healthy individuals by venipuncture after informed consent had been given. Neutrophils were isolated by sedimentation through dextran (6% w/v), centrifugation through Ficoll/Hypaque gradient medium (Pharmacia),