μM), metallothionein increased the MTT reduction to approximately 90% as compared with the normal control.

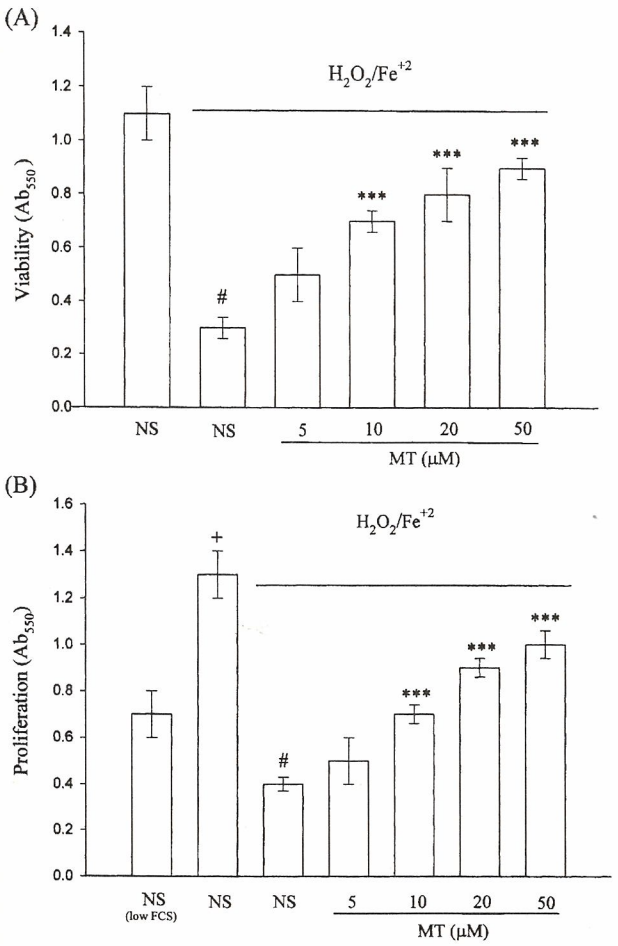

Fig. 5. (**A**) Cytoprotective effect and (**B**) cellular proliferation of metallothionein on oxidative insult in HUVECs. Oxidative damage to HUVECs was caused by the addition of H_2O_2 (10 mM)/Fe^{+2} (50 μM) (H_2O_2/Fe^{+2}) in the presence of normal saline or various concentrations of metallothionein (5-50 μM) in humidified air at 37 °C for 24 h. Cell viability and proliferation were measured using a colorimetric assay based on MTT reduction in viable cells. The normal condition is represented as treatment with no oxidative challenge. Data are presented as the means ± S.E.M. (n = 5). $p < 0.001$ as compared with the normal saline (NS; low FCS); $^{\#}p < 0.001$ as compared with the normal saline (NS) group; $^{***}p < 0.001$ as compared with the normal saline (NS) group challenged with H_2O_2/Fe^{+2}.

DISCUSSION

The results obtained from this study demonstrate that metallothionein exhibits free radical-scavenging and cytoprotective activities. The phospholipid bilayers of cellular and subcellular membranes are undoubtedly major targets for these radicals. Any compound that inhibits membrane phospholipid peroxidation seems to exert a pharmacological effect in the prevention of radical-induced oxidative pathological events.[22] In this study, 4 in vitro models were used to assess the activities of metallothionein in free radical-scavenging activity. Among cell-free systems, brain homogenates are usually chosen to evaluate antioxidant effects on lipid peroxidation.[23] Rat brain homogenates exposed to ferrous ion exhibit lipid peroxidation in air by a mechanism whose induction step may primarily involve site-bound iron-mediated decomposition of lipid hydroperoxides to yield alkoxy or peroxyl radicals, leading to the chain reaction of lipid peroxidation.[24] In this system, metallothionein dose-dependently inhibited lipid peroxidation. This is probably due to the sulfhydryl group of metallothionein which more readily associates with the homogenized brain tissue membrane. The 1,2-diphenyl-2-picrylhydrazyl tests provided direct information about the reactivity of metallothionein with stable free radicals. In DPPH tests, metallothionein acts as a direct free radical scavenger.

In recent years, reactive oxygen species have been thought to play a critical role in many diseases such as cancer and sepsis.[25] It has reported that the reduction of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) spin adducts in ESR was applied to determine free radical-scavenging activities, but it is difficult to apply this method to water-soluble substances. The H_2O_2/NaOH/DMSO system was developed to evaluate the antioxidative ability of both water- and oil-soluble antioxidants.[20] Mechanisms of free radical formation in the H_2O_2/NaOH/DMSO system assume that the superoxide anion and hydroxyl radical are generated from degradation of hydrogen peroxide, and that the methyl radical is generated from degradation of DMSO by the hydroxyl radical. The superoxide anion changes into the hydroxyl radical by catalytic action of contaminated trace iron, so that the amount of the hydroxyl