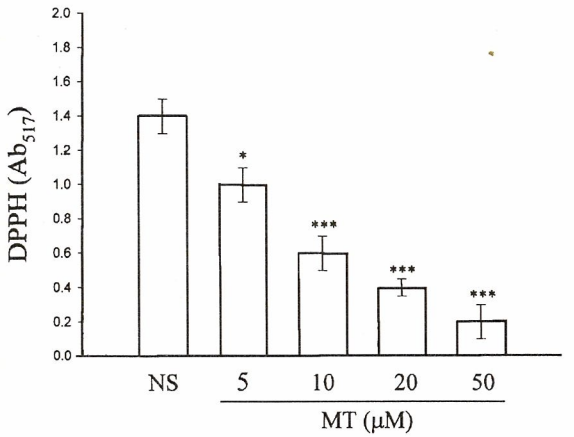

Fig. 2. Effect of metallothionein in the 1,2-diphenyl--2-picrylhydrazyl (DPPH) test. An ethanolic solution of the stable nitrogen-centered free radical, DPPH (100 μM), was incubated with normal saline or various concentrations of metallothionein (5-50 μM). The absorbance was monitored spectrophotometrically at 517 nm. Data are presented as the means ± S.E.M. (n = 5). *$p < 0.05$ and ***$p < 0.001$ as compared with the normal saline (NS) group.

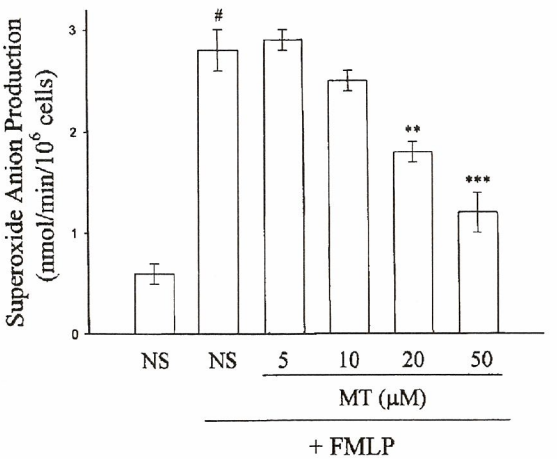

Fig. 3. Effect of metallothionein on superoxide anion production in human neutrophils. Neutrophils were preincubated with normal saline or various concentrations of metallothionein (5-50 μM) for 3 min, and stimulated with formyl-Met-Leu-Phe (FMLP, 100 nM). The initial rate of superoxide anion generation was determined using a SOD-sensitive cytochrome c reduction assay. Data are presented as the means ± S.E.M. (n = 5). #$p < 0.001$ as compared with the normal saline (NS) group. **$p < 0.01$ and ***$p < 0.001$ as compared with the normal saline (NS) group challenged with FMLP.

production. On the other hand, metallothionein also inhibited the enhanced production of superoxide anion by cytochalasin B (1 μg/ml)-primed neutrophils by 90% (data not shown).

Free Radical-scavenging Activity of Metallothionein

The rate of free radical-scavenging activity is defined by the following equation: inhibition rate = 1 [signal height (metallothionein)/signal height (control)].[20] In this study, typical ESR signals of the superoxide anion, hydroxyl radical, and methyl radical were observed as in Fig. 4A. Metallothionein (10 μM) markedly suppressed superoxide anion, hydroxyl radical, and methyl radical formation by about 28.5 ± 2.1, 23.5 ± 2.7, and 26.6 ± 1.7 %, respectively (n = 4). At a higher concentration (20 μM), metallothionein further inhibited superoxide anion, hydroxyl radical, and methyl radical formation by about 67.4 ± 5.8, 79.2 ± 6.7, and 55.3 ± 4.6 %, respectively (n = 4). However, the suppression rate of metallothionein against the methyl radical was smaller than those against the superoxide anion and hydroxyl radical. This observation may provide in vitro evidence suggesting the usefulness of metallothionein for its free radical-scavenging activity.

Effect of Metallothionein on Cell Viability and Proliferation in HUVECS

The reduction in MTT absorbance by confluent cells was 1.1 ± 0.1. When H_2O_2/Fe^{+2} was used to challenge HUVECs, cell viability significantly decreased as compared with the normal control. The decrement in cell viability was concentration-dependently restored by metallothionein (Fig. 5A). At a concentration of 50 μM, metallothionein almost completely restored cell viability reduced by H_2O_2/Fe^{+2}.

On the other hand, reduction in MTT absorbance in quiescent cells (with 5% FCS) was about 1/2 that of proliferative cells (normal FCS, 20%) (0.7 ± 0.1 vs. 1.3 ± 0.2, n = 5). When H_2O_2/Fe^{+2} was used to challenge HUVECs, it was clearly shown that MTT reduction in cells was markedly lower than that of quiescent cells (with 5% FCS). Metallothionein increased cell proliferation in a concentration-dependent manner (5-50 μM) (Fig. 5B). At a higher concentration (50