and 20-s hypotonic lysis of erythrocytes. Washed neutrophils were finally resuspended in a modified Hanks' balanced salt solution consisting of 145 mM NaCl, 10 mM K_2HPO_4, 10 mM Hepes, 4.2 mM $NaHCO_3$, 5.5 mM glucose, and 200 μg/ml human serum albumin, pH 7.4 (HBSS), and the concentration of neutrophils was measured using a cell counter (Coulter, AcT) and adjusted to 2×10^7 neutrophils/ml with the same medium. Final preparations contained 97% neutrophils, and viability was above 98% as assessed by Riu stain[18] and trypan blue (0.4%, w/v) exclusion, respectively.

Superoxide anion production generated by human neutrophils was determined by measurement of the reduction of cytochrome c in the presence or absence of superoxide dismutase as previously described.[19] Briefly, human neutrophils (2×10^6/ml) in HBSS containing Ca^{+2} (1 mM)/Mg^{+2} (0.5 mM) and cytochrome c (40 μM) were combined in a thermostat-controlled stirred cuvette (at 37 °C). After cells were incubated with normal saline or metallothionein for 2 min, the reaction was started by addition of FMLP (100 nM). The change in absorbance at 550 nm was continuously recorded for 10 min. Results were calculated and are expressed as the initial rate of superoxide anion production (nmol/min/10^6 neutrophils) as previously described.

Electron Spin Resonance Spectrometry

Electron spin resonance (ESR) spectra were recorded at room temperature on a Bruker EMX ESR spectrometer using a quartz flat cell designed for aqueous solutions. Conditions of ESR spectrometry were as follows: 3456 ± 50 G; power, 0.635 mW; modulation frequency, 100 kHz; frequency, 9.663 GHz; modulation amplitude, 1 G; receiver gain, 6.3×10^{-4} ; time constant, 81.92 ms; and conversion time, 327.68 ms. The ESR spectrum was obtained in a H_2O_2/NaOH/DMSO system as described previously.[20] Briefly, 100 μl of DMSO and the same volumes of 25 mM NaOH and a metallothionein solution were mixed in a test tube, followed by the addition of 10 μl of DMPO and 100 μl of 30% hydrogen peroxide. The reaction mixture was sucked into a quartz flat cell and placed in the ESR apparatus; scanning was begun 10 min after mixing all reagents.

Cell Cultivation

Human umbilical vein endothelial cells (HUVECs) were isolated from fresh umbilical cords obtained at normal deliveries. The umbilical vein was cannulated with 50 ml of cord buffer to remove any blood, after which the vein was filled with 20 ml of 0.1% collagenase I dissolved in cord buffer and incubated for 10 min at 37 °C. The collagenase solution was drained from the cord and collected, and the cord was gently flushed with 20 ml M199 medium. Cells in these pooled solutions were recovered by centrifugation at 150 × *g* for 7 min and transferred to 100 mm culture dishes in M199 medium (M199, Gibco-BRL) supplemented with 20 mM Hepes, 20% heat-deactivated fetal calf serum (FCS), 1% (w/v) penicillin/streptomycin, 25 μg/ml endothelial cell growth supplement, 50 μg/ml heparin, and 2 mM glutamine at 37 °C in a humidified atmosphere with 5% CO_2. For subculturing, confluent dishes were detached by 0.05% trypsin in a 0.53 mM EDTA solution (Gibco-BRL), then neutrilized with FCS at a split ratio of 1:3 every 4 days. Trypsinized HUVEC cells were seeded at a density of 8.5×10^3 /cm^2 in Corning 100 × 20-mm flat-bottomed tissue culture petri dishes. Throughout the experiments, cells were used between passages 3 and 8 from the origin of preparation. Before the experiments, the confluent monolayers of HUVEC cells were seeded onto 24-well plates.

Cell Viability

After 22 h of continuous exposure to oxidative stress as H_2O_2 (10 mM)/Fe^{+2} (50 μM) and metallothionein, HUVEC viability was measured using a colorimetric assay based on the ability of mitochondria in viable cells to reduce the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) as previously described.[21] Treated cells in 24-well plates were incubated with MTT (0.5 mg/ml) for 3 h. Culture medium was removed by aspiration, and cells were solubilized in DMSO (0.5 ml). The extent of reduction of MTT to formazan within cells was quantified by the measurement of absorbance at 550 nm (OD_{550}) with a microplate reader (ZL