A 211

Prevention of H2O2 mediated DNA damage by over expression of Glutaredoxin in bovine endothelial cells

Glutaaredoxin over expressing cells were grown on four 10 cm dishes labeled 1,2,3 and 4 induced with ITPG for 12 hrs at 37C^{o} Dishes 1,2 and 3 were incubated with 0.25 mM H_2O_2, 0.5 mM H_2O_2 and 1 mM H_2O_2 for 30 min. at 37C^{o} respectively. Dish number 4 was incubated with serum free medium for 30 min. at 37C^{o}. Normal endothelial cells were also grown on 10cm dishes labeled 5,6,7 and 8 and treated with ITPG for 12 hrs at 37C^{o}. Dishes 6,7 and 8 were treated with 0.25mM H_2O_2, 0.5mM H_2O_2 and 1mM H_2O_2 for min. at 37C^{o} respectively. Dish number 5 was treated with serum free medium for 30 min. at 37C^{o} respectively. Dish number 5 was treated with serum free medium for 30 min. at 37C^{o}. After 30 min. incubation period cells were removed from dishes and total DNA was extracted. Extracted DNA was electrophoresed on agarose gel and stained with ethidium bromide and observed under UV light. Normal endothelial cells incubated with serum free medium gave a single high molecular weight DNA band. Normal endothelial cells treated with three different concentrations of H_2O_2 insult. Glutaredoxin over expressing cells treated only with serum free medium also gave a single

highmolecular weight DNA band. Glutaredoxin over expressing cells treated with H_2O_2 also gave single high molecular weight bands indicating that DNA of those cells are resistant to damage caused by H_2O_2 as compared to normal endothelial cells treated with H_2O_2.