

radical is consequently relatively greater than that of the superoxide anion. Using this system, free radical-scavenging activities of the superoxide anion, hydroxyl radical, and methyl radicals can be simultaneously evaluated. In this study, we found that metallothionein effectively inhibited hydroxyl radical, superoxide anion, and methyl radical formation in vitro (Fig. 4).

On the other hand, it has been reported that metallothionein acts as a neuroprotective agent by scavenging superoxide radicals.²⁷ Metallothionein also provides protection against ischemia reperfusion-induced heart injury.²⁸ These studies seem to imply that metallothionein has cytoprotective activity via its free radical-scavenging activity. In the present study, we found that metallothionein concentration-dependently inhibited cell damage induced by H₂O₂/ Fe⁺² challenge in HUVECs, indicating the free radical-scavenging properties displayed by metallothionein may have cytoprotective implications.

In conclusion, the data presented in this report show that metallothionein is a potent free radical scavenger and cytoprotectant in in vitro tests. It will be of interest to further study the cytoprotective activity of metallothionein in various radical-mediated pathological events in vivo in order to evaluate its possible therapeutic use.

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REFERENCES

- Hamer, D. H. Metallothionein. *Annu. Rev. Biochem.* 1986; **55**: 913-951.
- Kagi, J. H. R. Overview of metallothionein. *Meth. Enzymol.* 1991; **205**: 613-626.
- Brown, R. K., Mcburney, A., Lunec, J., Kelley, F. J. Oxidative damage to DNA in patients with cystic fibrosis. *Free Rad. Biol. Med.* 1995; **18**: 801-806.
- Vile, G. F., Tyrrell, R. M. UVA radiation-induced oxidative damage to lipids and proteins in vitro and in human skin fibroblasts is dependent on iron and singlet oxygen. *Free Rad. Biol. Med.* 1995; **18**: 721-730.
- Halliwell, B., Cross, C. E. Reactive oxygen species, antioxidants and acquired immunodeficiency syndrome. *Arch. Intern. Med.* 1991; **157**: 29-31.
- Jaeschke, H. Mechanisms of oxidant stress-induced acute tissue injury. *Proc. Soc. Exp. Biol. Med.* 1995; **209**: 104-111.
- Schapira, A. H. V. Oxidative stress in Parkinson's disease. *Neuropathol. Appl. Neurobiol.* 1995; **21**: 3-9.
- Gotz, M. E., Kunig, G., Riederer, P., Youdim, M. B. H. Oxidative stress: free radical production in neural degeneration. *Pharmac. Ther.* 1994; **63**: 37-122.
- Michiels, C., Raes, M., Toussaint, O., Remacle, J. Importance of se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. *Free Rad. Biol. Med.* 1994; **17**: 235-248.
- Hussain, S., Slikker, W. Jr., Ali, S. F. Age related changes in antioxidant enzymes, superoxide dismutase glutathione peroxidase and glutathione in different regions of mouse brain. *Int. J. Dev. Neurosci.* 1995; **13**: 1-8.
- Rhee, S. G., Kim, K. H., Chae, H. Z., Yim, M. B., Uchida, K., Netto, L. E., Stadtman, E. R. Antioxidant defense mechanisms: a new thiol-specific antioxidant enzyme. *Ann. N.Y. Acad. Sci.* 1994; **738**: 86-92.
- Sato, M., Bremner, I. Oxygen free radicals and metallothionein. *Free Rad. Biol. Med.* 1993; **14**: 325-337.
- Abel, J., de Ruiter. Inhibition of hydroxyl-radical generated DNA degradation by metallothionein. *Toxicol. Lett.* 1989; **47**: 191-196.
- Satoh, M., Naganuma, A., Imura, N. Metallothionein induction prevents toxic side effects of cisplatin and adriamycin used in combination. *Cancer Chemother. Pharmacol.* 1998; **21**: 176-178.
- Hsiao, G., Teng, C. M., Wu, C. L., Ko, F. N. Marchantin H as a natural antioxidant and free radical scavenger. *Arch. Biochem. Biophys.* 1996; **334**: 19-26.
- Marion, M. B. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976; **2**: 248-254.
- Mellors, A., Tappel, A. L. The inhibition of mitochon-