Pharmacology of enantiomers of higenamine and related tetrahydroisoquinolines

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**Abstract**

Oxidative stress is a constant threat to all living organisms and an immense repertoire of cellular defense systems is being employed by most pro- and eukaryotic systems to eliminate or to attenuate oxidative stress. Ischemia and reperfusion is characterized by both a significant oxidative stress and characteristic changes in the antioxidant defense. Heme oxigenase-1 (HO-1) is up-regulated by various stimuli including oxidative stress so that it is thought to participate in general cellular defense mechanisms against ischemic injury in mammalian cells. Higenamine, an active ingredient of Aconite tuber, has been shown to have antioxidant activity along with inhibitory action of inducible nitric oxide synthase (iNOS) expression in various cells. In the present study, we investigated whether higenamine and related analogs protect cells from oxidative cellular injuries by modulating antioxidant enzymes, such as HO-1, MnSOD etc. R-form of YS-51 was the most potent inducer of HO-1 in bovine endothelial cells, which inhibited apoptotic cell death by $H_2O_2$. HO-1 induction by YS 51 was mediated by $PI_3$ kinase activation in which PKA- as well as PKG pathway is considered as important regulators. YS-51 also induced Mn-SOD mRNA expression by activating c-jun N-

terminal kinase in endothelial cells and Hela cells. In ROS17/2.1 cells, higenamine and enetiomers of related compounds inhibited iNOS expression by cytokine mixtures. Taken together, higenamine and related compounds can be developed as possible protective agents from oxidative cell injury or death.

Key words: tetrahydroisoquinolines, oxidative stress, heme oxygenase, Mn-SOD

**Introduction**

Oxidative stress is the result of excessive production of oxidant species and/or depletion of intracellular antioxidant defenses, leading to an imbalance in the redox status of the cell. In recent years, the inducible isoform of heme oxygenase (HO-1) has been suggested to function as an effective system to counteract the oxidative threat. HO is the rate-limiting enzyme for the degradation of proheme IX to biliverdin, which is a precursor of bililubin, the terminal heme-degradading product. Three isoforms of HO have been identified. HO-1 is inducible, whereas HO-2 and HO-3 are constitutively expressed. Carbon monoxide (CO) is another HO product of the HO reaction, which has well been recognized as a physiologically important vasoactive substance rather than a toxic waste product. This gaseous monoxide is necessary to maintain microvascular patency in the liver under both unstimulated and stress conditions. The exact functional role of HO-1 induction in response to oxidative stress is not fully understood. However, as HO-1 provides cytoprotection in various cell culture and animal models, HO-1 gene activation is considered to be an adaptive cellular defense mechanism. Overexpression of the HO-1 gene has been shown to attenuate the toxic effects of heme and

hemoproteins in transfected coronary endothelial cells and to protect pulmonary epithelial cells against hyperoxia. The important physiological function of HO-1 has been confirmed by observation in HO-1 knockout mice. Cultured fibroblast cells from these animals are highly susceptible to heme- or hydrogen peroxide-mediated toxicity. In addition, exposure of HO-1 deficient mice to endotoxin results in increased hepetocellular necrosis and in higher mortality from endotoxic shock as compared to control animals. The findings in HO-1 deficient mice were essentially confirmed in human HO-1 deficiency. Since the specific induction of heat shock proteins by pharmacological stimuli has significant clinical implications, targeted induction of HO-gene expression by 'non-stressful' stimuli may serve as a novel approach to therapeutic intervention. The proposed antioxidant role for HO-1 is based on some crucial experimental observations: (i) HO-1 gene expression is extremely sensitive to up-regulation by oxidative stress in a variety of mammalian tissues; (ii) induction of HO-1 protein or transfection of cells with the HO-1 gene protect tissues against oxidant-mediated injury; and (iii) HO-1 knock out mice exhibit reduced stress defenses when exposed to oxidative challenge. Since biliverdin and bililubin have been shown to possess potent antioxidant properties and up-regulation of HO-1 is usually accompanied by increased levels of ferritin, a protein which sequesters intracellular catalytic iron, HO-1 appears to be an excellent candidate for cytoprotection. In previous studies we have reported that higenamine and related compounds showed anti-inflammatory actions. Thus, in the present study, we investigated further in detail for the protective mechanism of higenamine and its analogs in relation with anti-oxidant enzyme expression such as HO-1 and Mn-SOD.

**Results and Discussion**

Although we will discuss on cytoprotective action of higenamine and related compounds in regard to HO-1 expression in oral presentation, we cordially ask readers' understanding not to present HO-1 related data as written form, because it is now in preparation. Instead, we will present the recent data, which will be published in coming May 2004 in Pharmacology journal.

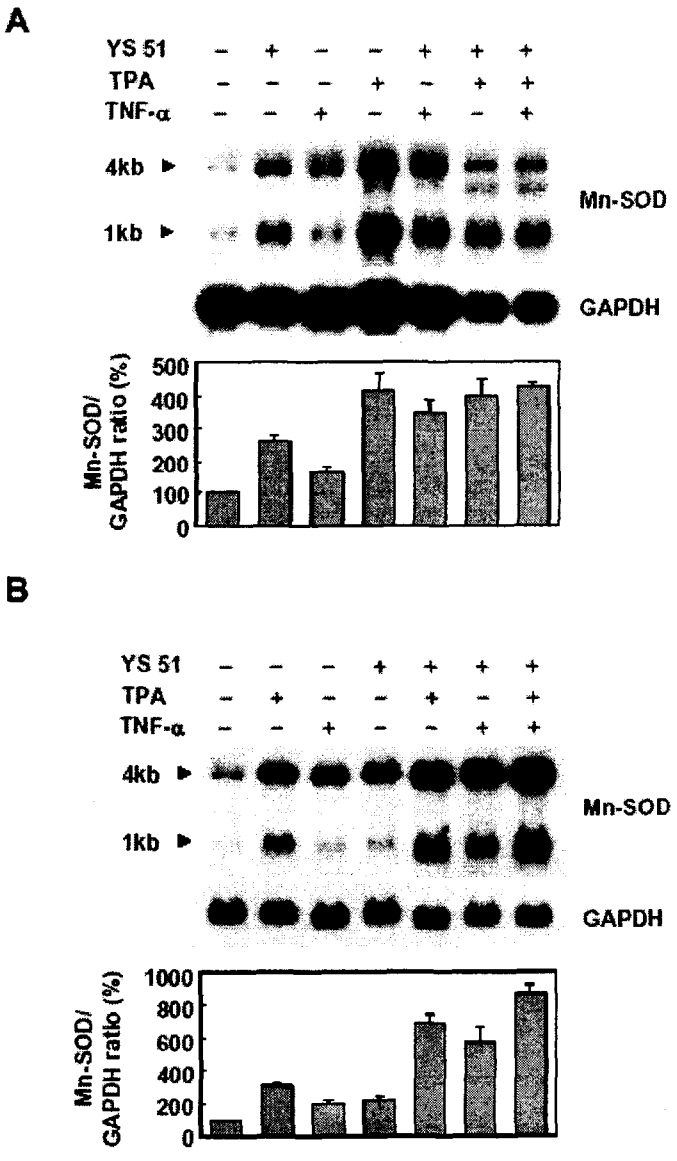

Fig.1. Induction of Mn-SOD mRNA in SPAEC and Hela cells. Cells were incubated with TPA and TNF-a for 9 h with or without YS 51.

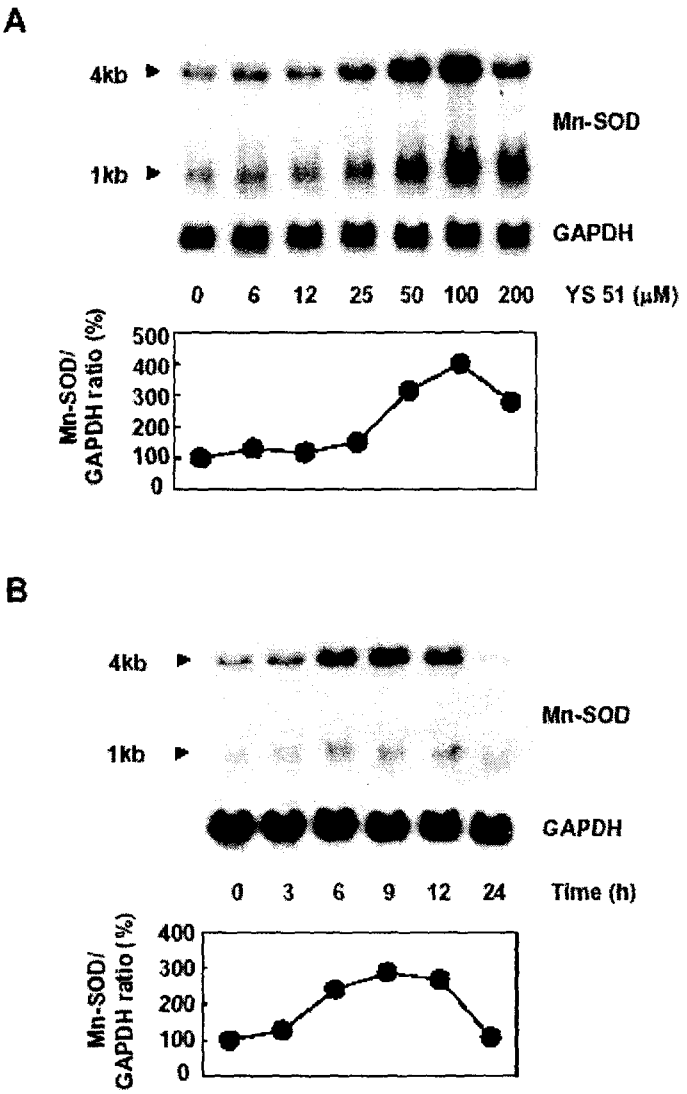


Fig.2. Dose- and time-denpendent up-regulation of MN-SOD mRNA by YS 51 in Hela cells.

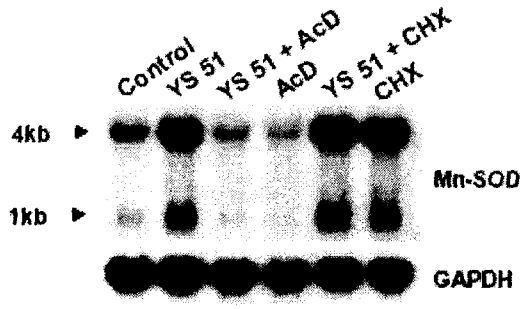


Fig.3. Effects of actinomycin D and cyclohexamide on YS 51-induced Mn-SOD mRNA expression.

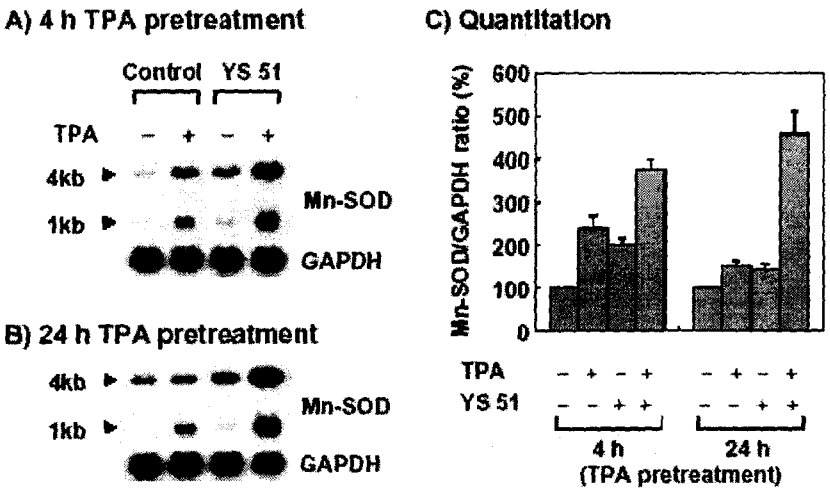


Fig.4. Effects of TPA on Mn-SOD mRNA induction by YS 51 in Hela cells.

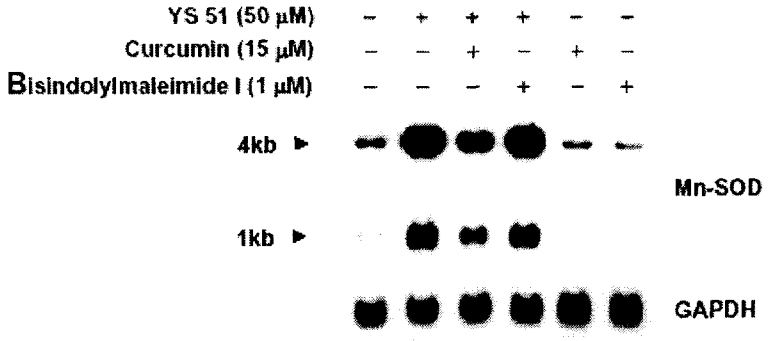


Fig.5. Effects of curcumin and bisndolylmaleimide I on Mn-SOD mRNA expression induced by YS 51 in Hela cells.

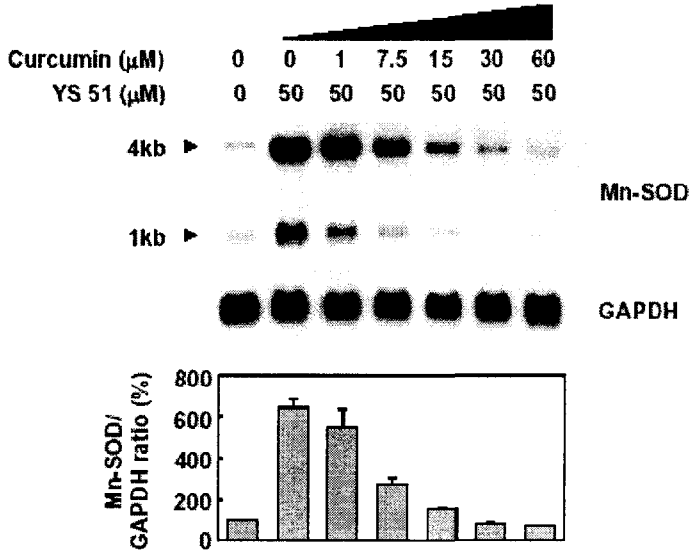


Fig.6. Dose-dependent inhibition of YS 51-induced Mn-SOD mRNA by curcumin in Hela cells.

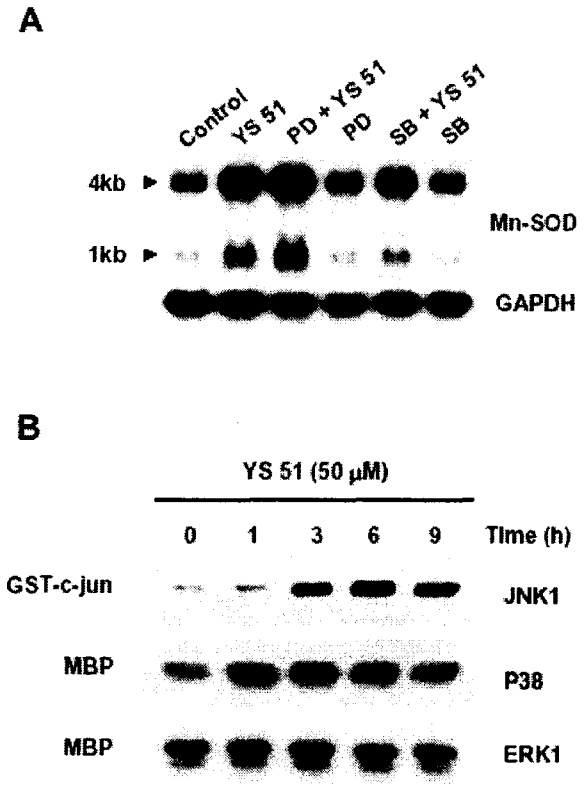


Fig. 7. Activity of MAP kinases on the expression of YS-51 induced MnSOD mRNA in Hela cells.