환경독성

Heme Oxygenase-1(HO-1) induction by UVB(290-320nm) radiation in ICR mice

Wookhee Choi* · Taehwan Kim** · Ryoungme Ahn*

*Department of Health science, Dongduk women's University, Seoul, 136-714 Korea

**College of Veterinary medicine, Kyungbook national University, Daegu, 702-701 Korea

〈Abstract〉

The induction of heme oxygenase-1(HO-1) by ultraviolet(UV) radiation provides a protective defense against oxidative stress, and has been well demonstrated in UVA-irradiated skin, but not UVB. In this study in mice, we show that the UVB(290-320nm) radiation can be attributed to the induction of cutaneous heme oxygenase-1. The expression of HO-1 mRNA was assessed in vivo by the reverse transcription-polymerase chain reaction (RT-PCR) analysis, and HO-1 enzyme activity was measured in microsomal preparation from irradiated mice.

The mRNA level of HO-1 increases in liver and skin from 24h to 72h after UVB(3KJ/㎡) radiation. The results of gene expression were same pattern of HO enzyme activity in skin, but not in liver. HO-1 mRNA in liver resulted in a progressive increase to 96h after UVB radiation, but HO activity in liver increased to 48h. This finding indicates that UVB radiation is an important inducer of HO-1 and increases in HO activity may protect tissues directly or indirectly from oxidative stress.

1. Introduction

Ultraviolet(UV) radiaiton regulates several stress response enzyme in mammalian tissue. The major stress protein induced by UV radiation is identified as heme oxygenase(HO). HO is the rate-limiting microsomal enzyme that catalyses the catabolism of heme to biliverdin, releasing Fe and carbon monoxide, and in most tissue the cytosolic NADPH dependent enzyme biliverdin

reductase rapidly converts biliverdin to the more stable bilirubin. HO-1 is strongly induced by oxidant stress and increases in HO activity appear to protect tissue from oxidative stress. This study was carried that the UVB(290-320nm) radiation can be attributed to the induction of cutaneous heme oxygenase-1 in mice.

2. Materials & Methods

Mice and UVB irradiation Female inbred ICR mice were purchased from the Samtako Co. and housed in treatment groups of four. The mice were 7-8 weeks old at the start of the study. The dorsal skin of mice was shaved with clipper one day before UVB irradiation. The shaved skin was exposed to 3KJ/㎡ UVB radiaiton, and the time periods of irradiation were about 2-3min.

Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) Total RNA was extracted from mice skin and liver and treated with the test substancesusing TRI-zol solution according to the manufacturer's instructions. RNA concentrations were determined spectrophotometrically from absorbance at 260nm. One microgram of total RNA was reversibly transcribed into first strand cDNA, which was then amplified using PCR. The PCR primers were purchased or synthesized from Bioneer Co.(Seoul, Korea). RT reactions were performed at 48℃, for 1 hour and subsequently then PCR was performed in same tube using RT-PCR pre-mix according to the manufacturer's manual. The reaction was cycled 30times through 30sec at 94℃, 30sec at 62℃ and 30sec at 72℃. The products were analyzed by electrophoresis on 1% agarose gels and stained by with ethidium bromide.

HO enzyme activity Enzyme activity was assayed by a modification of the method of Lincoln et al in microsomal preparations from dorsal skin and liver. Mice were killed at 24, 48, 72, 96h post-UVB exposure. The HO activity was measured by the rate of bilirubin formation at 37℃, indicated by the increase in absorbance at 470nm versus 540nm. Protein was measured by the method of Lowry et al with BSA (Bovine serum aluumin) as standard.

3. Results & Discussion

In irradiated skin, HO-1 mRNA expression resulted in a strong and time-dependent increase. A significant increase in HO-1 expression was first measured at 24h post irradiation and peaked at 96h. Similar effects of HO-1 mRNA level were found in liver of UVB-irradiated mice. HO-1 mRNA in liver was significantly induced from 24h to 96h post irradiation. Dorsal skin and liver microsomal HO activity was first assayed at 24h post irradiation. The HO activity of increased steadily to a 4-fold increase of control by 96h. In contrast, a significant increase of HO activity in liver was peaked at 24h , and then decreased to 96h.

These results show that UVB radiation induced HO-1 on the mRNA level and enzyme activity. Also, exposure to UVB radiaiton can cause biological damage in both UV-irradiated direct tissue and unirradiated indirect tissue.

4. Conclusion

UVB irradiaiton was induced the HO-1 mRNA and increased HO activity in dorsal skin and liver. In conclusion, we have identified cutaneous HO-1 induction by UVB radiation. Also, HO activity in UV-irradiated mice may play a role to understand protective mechanism against oxidative damage.

〈References〉

Allanson M, Reeve VE. (2004) Immunoprotective UVA(320-400nm) irradiated upregulated heme oxygenase-1 in the dermis and epidermis of hairless mouse skin. *J Invest Dermatol.* 122(4), 1030-1036.

Obermuller-Jevic UC, Schlegel B, Flaccus A, Biesalski HK. (2001) The effect β-carotene on the expression of interleukin-6 and heme oxygenase-1 in UV-irradiated human skin fibroblasts in vitro. *FEBS Letters.* 509, 186-190.

Reeve VE, Tyrell RM. (1999) Heme oxygenase induction mediates the photoimmunoprotective activity of UVA radiation in the mouse. *Immunology.* 96, 9317-9321

Soriani M, Rice-Evans C, Tyrell RM. (1998) Modulation of the UVA activation of haem oxygenase, collagenase and cyclooxygenase gene expression by the epigallocatechin in human skin cells. *FEBS Letters.* 439, 253-257.

Trekli M, Riss G, Goralczyk R, Tyrell RM. (2003) Beta-carotene suppresses UVA-induced HO-1 gene expression in cultured FEK4. *Free radical biology & medicine.* 34(4), 456-464.