Relationship between nitric oxide and heme-oxygenase in human pulp cells

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I. Objectives

Heme oxygenase(HO) is a microsomal enzyme, widely distributed in mammalian tissues, which has a major role in heme metabolism. The role of HO in different tissues has not, as yet, been fully characterized, but it is becoming evident that it is involved in a variety of cellular regulatory and protective mechanism.

Therefore, in this report, we confirmed the idea of whether the presence of HO in human pulpal cell, and HO can be a principal mechanism of nitric oxide(NO) mediated pulpal cell damage, by adding a deprivation of NO and to gain clinical relationship. We also accessed the effects of HO in pulpal cells treated with hydrogen.

II. Materials and Methods

Human dental pulp cell were cultured by using an explant technique. To determine the effect of hemin and S-nitroso-N-acetyl-penicillamine(SANP) on the expression and activity of heme oxygenase in human pulp cells, the complete medium was replaced with incubation medium.

Four sets of experiments were performed. The first was to determine the time and dose dependence of heme oxygenase protein expression by hemin in human pulp cells. The second set of experiments was to determine the time and concentration dependence of HO-1 expression by SNAP(0-1 mM) in human pulp cell. A third set of experiments was performed to investigate the mechanism of induction of HO-1 by SNAP. In addition to NO, SNAP is known to release iron, cyanide, and oxygen free radicals, all of which may potentially induce HO-1. hydroxocobalamin(HCB) was used as a scavenger of NO to determine the contribution of NO to SNAP-mediated HO-1 induction. The fourth set of experiments was to determine the effect of HO-1 on hydrogen peroxide-induced cell damage.

III. Results

1. Effect of dose and duration for hemin and SNAP in pulp cell viability.

   There was a gradual concentration-dependent decrease in viability, compared with control cells, which was more marked in the hemin- than the SNAP-treated group.

   The decline in HO protein seen with both higher concentrations of inducing agent and longer times of incubation coincided with the decrease in cell viability.

2. Induction of HO protein by hemin and SNAP.

   Expression of HO protein in pulp cells following incubation with hemin and SNAP were analyzed by Western blot analysis. Hemin-treated cells showed a marked concentration-dependent increase in HO-1 protein levels above control. After incubation of pulp cells with SNAP, a marked increase in HO-1 protein was seen. A time-dependent response was also seen after incubation of cells with hemin and SNAP.

HCB on SNAP-stimulated heme oxygenase protein expression.

ine whether expression of HO is stimulated by the release of NO from SNAP, cells were incubated with HCB, an NO scavenger. Compared with control, a marked increase in HO-1 protein expression was seen in response to SNAP and, this was greatly attenuated by coincubation of cells with 0.5 mM HCB.

4. Effect of hemin-mediated cytoprotection against oxidative stress.

   Zinc protoporphyrin Ⅸ(ZnPP Ⅸ), an inhibitor of HO protein, itself did not cause any evident cytotoxic effect at the concentrations tested. Injury following exposure to increasing concentrations of hydrogen peroxide(20-100 mM) was significantly diminished in cells pre-treated with 100 μM hemin.

IV. Conclusions

It was first time to demonstrate that expression of HO pretein is significantly increased in human pulp cells following exposure to hemin and NO donor, SNAP.

This study was revealed that increased HO protein as a consequence of hemin contributes to cytoprotection of pulp cells against oxidant-mediated damage. The participation of additional defence mechanisms cannot be excluded since ZnPP Ⅸ did not completely reverse the protective effect mediated by hemin. Data suggesting that pulp cells responds to oxidative stress at the molecular level and the apparent clinical safety of nightguard vital bleaching procedure may, in part, be due to this response by odontoblasts with increased HO-1 production.