MASTER'S THESIS

Effects of indirubin on the expression of RANTES in influenza virus infected human bronchial epithelial cells

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Abstract

Human bronchial epithelial cells are the primary sites of influenza virus infection. In the present study, the effects of indirubin on the expression of chemokine RANTES (regulated on activation, normal T cell expressed and secreted) by the influenza A/WSN/33 or B/Lee virus infected H292 human epithelial cell line were examined. The expression of RANTES mRNA was analyzed using RT-PCR (reverse transcription polymerase chain reaction) and the concentrations of RANTES production were determined by the ELISA method (enzyme-linked immunosorbent assay). At the non-cytotoxic concentrations, indirubin was found to reduce both of the expression and production of RANTES in influenza A/NWS/33 infected H292 cells. The inhibitory effect was also observed in influenza virus B/Lee infected cells. Significant reduction of the expression and production of IL-8 was not observed after infection. Indirubin-3'-oxime, a recently developed derivative with kinase inhibitory activity, also mediates a potent inhibitory effect on the expression of RANTES. The influenza virus infection-induced phosphorylation of the nuclear transcription NF-κB regulatory molecule IκBα, p38 MAP kinase and JNK were inhibited by indirubin-3'-oxime. This finding suggests that indirubin may be one of the components in the Chinese medicinal herbs *Isatis indigotica* and *Strobilanthes cusia* with immunomodulatory activity on the expression of RANTES. DNA macroarray technology was also used to examine the effect of indirubin-3'-oxime on the cytokine genes expression profile in the influenza infected cells. Preliminary results from DNA array analysis showed that indirubin-3'-oxime up-regulates the expression of certain cytokine gene (e.g. IL-9, IL-15, IL-21 and macrophage migration inhibitory factor (MIF)). Further analysis on the expression of these

cytokines may enhance our understanding on the immuno-modulatory activities of indirubin.

In conclusion, results presented in this thesis support the recent findings that indirubin is one of the immuno-modulators in *Isatis indigotica* and *Strobilanthes cusia.* The inhibition of chemokine RANTES expression and production is through the regulation of phosphorylation of IκBα, p38 MAP kinase and JNK.

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