

Indirubin-3'-oxime as an antiviral and immunomodulatory agent in treatment of severe human influenza virus infection

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KEY MESSAGES

1. Two indirubin derivatives (indirubin-3'-oxime and E804) demonstrate strong antiviral and immunomodulatory effects on human macrophages and type-I alveolar epithelial cells after influenza H5N1 virus infection.
2. In mice infected with H5N1 virus, the use of E804 does not improve survival or weight loss but significantly reduces cytokine and chemokine expression and secretion, compared with controls.
3. Cyclin-dependent kinases pathway is involved in

the antiviral and immunomodulatory effects of indirubin upon influenza H5N1 virus infection.

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Introduction

Patients with influenza H5N1 virus infection have a fulminant viral pneumonia with rapid progression to adult respiratory distress syndrome and multiple organ dysfunctions. Although virus replication and tissue tropism were important drivers of pathogenesis.¹ Clinical and *in vivo* studies showed that pro-inflammatory cytokines were highly induced during H5N1 infection, suggestive of pathogenesis.² We have shown that primary human peripheral blood-derived macrophages (macrophages) and type-I alveolar epithelial cells (ATI) were target cells of H5N1 virus and produced higher levels of cytokines.³ These results highlighted the need to modulate cytokine responses during H5N1 infection as an adjunct to antiviral therapy.

Indirubin, a 3,2'-bisindole isomer of indigo, is an active ingredient of a traditional Chinese medicine preparation Danggui Longhui Wan for various chronic diseases.⁴ This chemical compound exhibits strong anti-inflammatory and anti-leukaemic activities. One of the derivatives, indirubin-3'-oxime (IDO) has been found to have anti-inflammatory and anti-viral effects.⁵ The therapeutic role of IDO and E804 in H5N1 virus infection has yet been investigated. This study aimed to demonstrate that IDO and E804 can be a beneficial adjunctive therapy for human H5N1 infection through its antiviral and immunomodulatory effects.

Methods

Antiviral activity of IDO and E804 was examined by evaluating the infectious virus titres in human

macrophages and ATI using plaque assay. We then infected human macrophages and ATI with H5N1 and H1N1 viruses with or without IDO and E804 treatment, and determined the mRNA and protein expression of cytokine and chemokine by real-time qPCR and ELISA. cDNA microarray was performed to identify the gene expression profile that involved in the antiviral and immunomodulation of cytokines by E804. Mice infected with H5N1 (A/Hong Kong/486/97) and H1N1 (A/HK/54/98) viruses were used to study the effect of E804 on survival, weight loss, infectious viral titre, and cytokine and chemokine expression.

Results

Compared with cells without treatment, indirubin derivatives (IDO and E804) inhibited viral replication by about 10-fold in H5N1 virus-infected macrophages and ATIs and in H1N1 virus-infected macrophages at 24 hours post-infection, as well as in H1N1 virus-infected ATIs at 48 hours post-infection (Figs 1a and 1b). IDO effectively suppressed the viral matrix 1 protein expression in H5N1 virus-infected macrophages (Fig 1c).

In infected macrophages, E804 (5 µM) suppressed the induction of IP-10, MIG, IL-1β, and RANTES (Figs 2a to 2d), as well as IL-8, MIP-1α, MIP-1β, and MCP-1 (data not shown). In infected ATIs, E804 significantly reduced the secretion of IP-10 and RANTES (Figs 2e and 2f). E804 exhibited a stronger and more potent immunomodulatory effect than IDO.

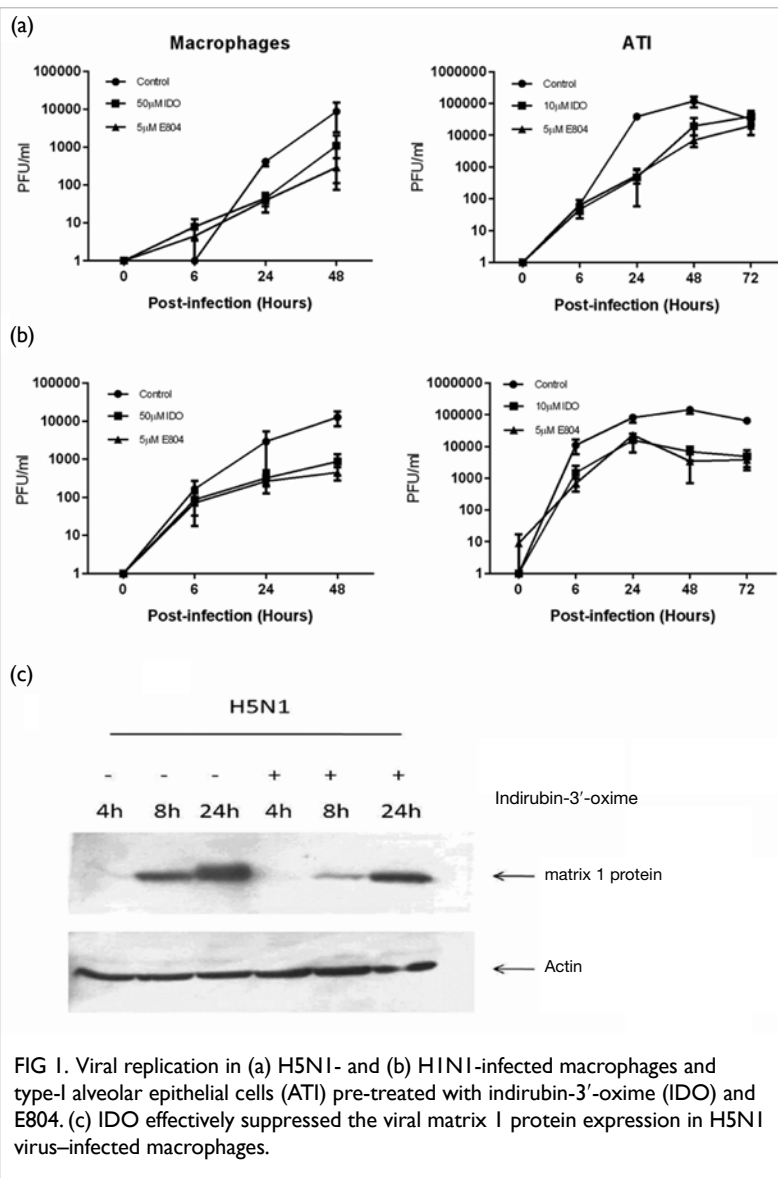


FIG 1. Viral replication in (a) H5N1- and (b) H1N1-infected macrophages and type-I alveolar epithelial cells (ATI) pre-treated with indirubin-3'-oxime (IDO) and E804. (c) IDO effectively suppressed the viral matrix I protein expression in H5N1 virus-infected macrophages.

Discussion

Indirubin derivatives, IDO and E804, exhibited strong cyclin-dependent kinase inhibition and suppressed induction of pro-inflammatory cytokines together with inhibition of viral replication in human macrophages and ATIs, the two major cellular targets of H5N1 virus. The antiviral and immunomodulatory effects of indirubin derivatives are partly due to cyclin-dependent kinase inhibition. The underlying mechanism needs further investigation.

Mice treated with E804 did not have any survival advantage. Mice treated with both peramivir and E804 had better lung pathology with less inflammation and infiltration of immune cells at day 6 post-infection, compared with other treatment groups. Mice treated with E804 alone or peramivir and E804 showed a

significant decrease in the expression and secretion of pro-inflammatory cytokine and chemokine in the lung lysate. This suggests that direct injection of E804 can improve the immunopathology, and recovery and regeneration of injured lung may be more robust after E804 treatment. Nonetheless, mice infected with H5N1 cannot be used to evaluate the E804 treatment. A better animal model (eg, ferrets) is needed to evaluate the potential therapeutic effects. In addition, we were unable to use a higher concentration of E804 to treat the H5N1-infected mice, owing to the poor solubility of E804. Developing novel indirubin derivatives with higher solubility is needed to achieve therapeutic effect. Furthermore, the infection dosage (1-2 LD₅₀) of the H5N1 virus in the in vivo model was too high; it is the marginal infectious dosage to develop lethal outcome for the mice. In future studies, sub-lethal dosage may be used to determine the therapeutic effect of E804 on weight loss, virus titre, and cytokine level.

Pandemic influenza is a public health concern. The long vaccine production process can hardly prevent the first wave of the pandemic, and high morbidity and mortality may be unavoidable. Antivirals may lead to amelioration of disease but are not effective in preventing severe complications. Novel therapeutic strategies against severe influenza infection are needed. Additional therapeutic strategies such as modulating the immune response may be key to successful treatment. Indirubin derivatives, IDO and E804 have antiviral and immunomodulatory effects and could be used in treatment of H5N1 infection.

Indirubin has been used to treat leukaemia. This naturally occurring compound has low toxicity and may be used to treat H5N1 infection and other respiratory infections such as novel influenza H7N9, SARS-CoV, MERS-CoV, for which the 'cytokine storm' may be involved in the pathogenesis.

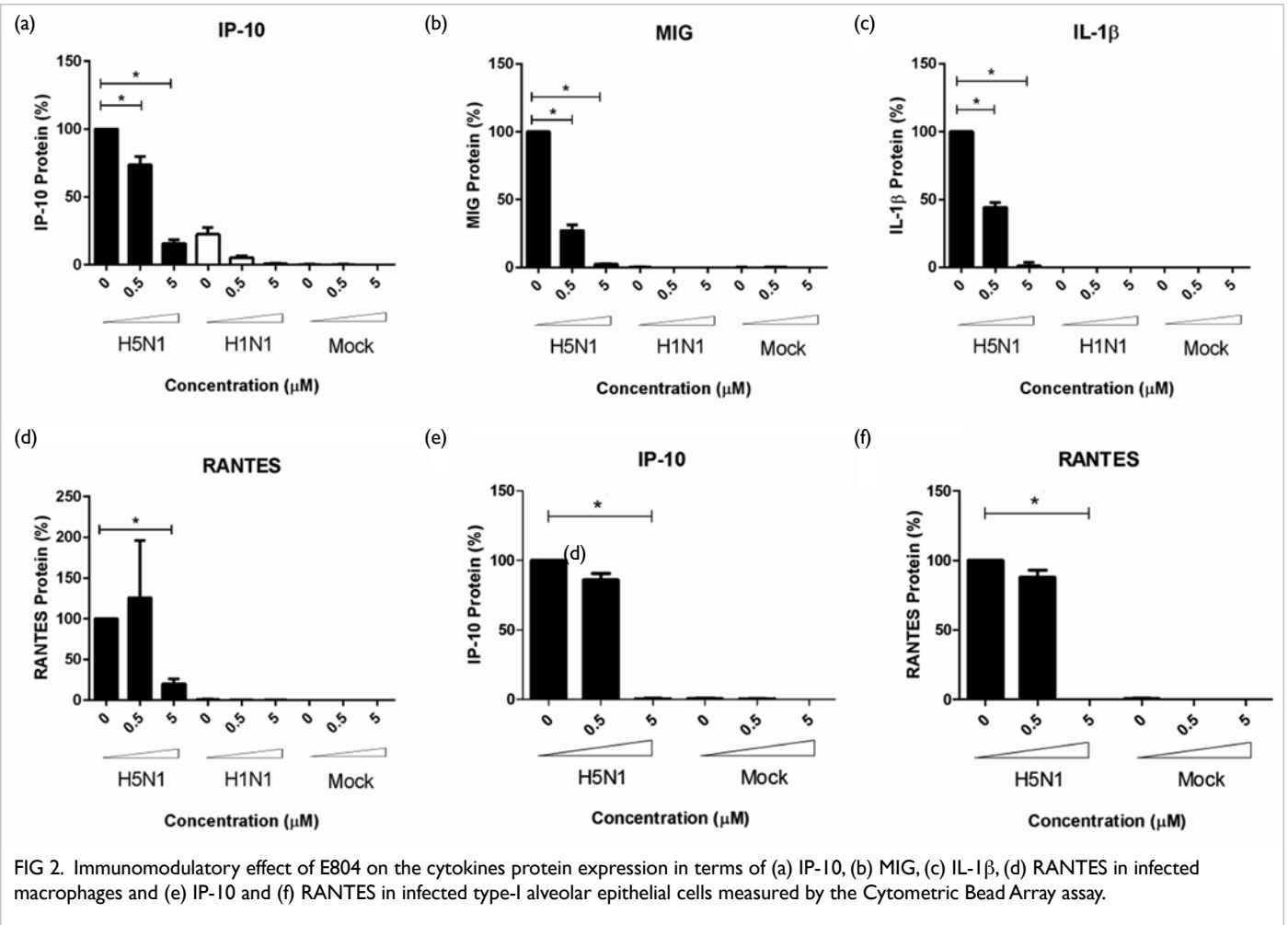
Conclusion

The two indirubin derivatives (IDO and E804) demonstrate strong antiviral and immunomodulatory effects on human macrophages and ATIs after influenza H5N1 virus infection. In mice infected with H5N1 virus, the use of E804 does not improve survival or weight loss but significantly reduces cytokine and chemokine expression and secretion, compared with controls.

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influenza A (H5N1) virus infected primary human peripheral blood-derived macrophages and alveolar epithelial cells. *Antiviral Res* 2014;106:95-104.

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