Antiviral Role of Toll-Like Receptor-3 Agonists Against Seasonal and Avian Influenza Viruses

J.P. Wong[1,*], M.E. Christopher[1], S. Viswanathan[1], X. Dai[1], A.M. Salazar[2], L.-Q. Sun[3] and M. Wang[3]

[1]*Molecular Biology Group, Biotechnology Section, Defence R&D Canada – Suffield, Ralston, Alberta, Canada;* [2]*Oncovir Inc. Washington, DC, USA and* [3]*College of Veterinary Medicine, China Agriculture University, Beijing, China*

Abstract: The divergence and antigenic shifts in influenza viruses represent significant challenges for the development of effective vaccines and antiviral drugs against influenza viruses. In view of current challenges and/or deficiencies in the influenza pandemic influenza preparedness, novel antiviral strategies which are robust and can respond to constant viral mutations, are particularly needed to combat future pandemic threats.

Toll-like receptor-3 (TLR-3) is an integral part of the host's innate immune system and serves as an important signaling pathway for the recognition of dsRNA for the triggering of antiviral and inflammatory responses to combat viral infections. This review examines dsRNA including Poly ICLC and liposome-encapsulated Poly ICLC (LE Poly ICLC) as TLR-3 agonists for their antiviral activity against seasonal and highly pathogenic avian influenza (HPAI) viruses. Furthermore, their roles in attenuating the antiviral and inflammatory cytokines in the host will also be explored. Preclinical studies in experimental animals suggest Poly ICLC and liposome-encapsulated Poly ICLC are safe and offer broad-spectrum protection against both seasonal and HPAI viruses, as well as other respiratory viruses including respiratory syncytial virus and SARS. Preliminary results from recent studies suggest these drugs up-regulate the production of interferons (-α, -β, and -γ), and tumor necrosis factor (TNF-α) but downregulate some proinflammatory cytokines including IL-2 and IL-4.

Taken together, these results suggest these TLR-3 agonists have a promising role to play as safe, effective and broad-spectrum anti-influenza drugs that could complement other antiviral drugs to combat seasonal, zoonotic and pandemic influenza viruses. The clinical safety of these drugs and their efficacy in pre-clinical studies may provide sufficient justification for regulatory agencies to consider their fast track development for use in future outbreaks of pandemic influenza or of other emerging respiratory pathogens.

INTRODUCTION

Viruses which constantly mutate and develop drug-resistance, including influenza and human immunodeficient viruses, represent formidable targets for pharmaceutical drug design and development. In light of pandemic threat from the current highly pathogenic avian influenza H5N1 virus (HPAI), the race to develop new and more effective antiviral drugs and vaccines against influenza has never been more intense.

All existing marketed antiviral drugs against influenza viruses are targeting the various virus protein components, examples are adamantanamines (amantadine and rimatadine) which target the M2 ion channel protein, and the neuraminidase inhibitors (zanamivir and oseltamivir) which prevent the progeny virions from being released from infected cells [for review see 1]. The major drawback of targeting influenza virus particles is that the viral genes that encode for these proteins undergo constant mutations, and in so doing, render these drugs ineffective against the virus variants. The widespread drug-resistance of seasonal and H5N1 influenza viruses to both amantadine and rimantadine [2, 3], and the rapid emergence of oseltamivir-resistant clinical isolates of H5N1 virus [4], raises concerns about efficacy of these drugs in a global pandemic caused by HPAI. Thus, there are compelling reasons to fast-track development of novel antiviral agents which are effective, robust and broad-spectrum, and are less likely to give rise to drug resistance resulting from viral mutation.

During infection, influenza virus inhibits the alpha/beta interferon (IFN-α/β) cascade in the host immune responses [5], thereby suppressing the antiviral defence mechanism in the host. Thus, the use of interferons and interferon-inducers is a rationale part of the antiviral strategy to combat the virus. In recent years, many significant advances have been made in discovery of distinct families of pathogen-associated molecular pattern (PAMP)-recognition receptors that initiate intracellular signaling pathways, which when activated, lead to complex immune responses that function to eliminate invading microbial pathogens. They thus function as 'alarm' or 'danger' signals for the host. Among these groups of specialized recognition receptors are the toll-like receptors (TLRs), which are transmembrane signaling proteins that are found expressed by cells of the innate immune system. There are more than 11 TLRs identified in mammalian cells that are designed for high specificity recognition of various protein, lipid, nucleic acid components of invading microorganisms,

*Address correspondence to this author at the Defence R&D Canada – Suffield, Box 4000 Main Station, Medicine Hat, Alberta, Canada T1A 8K6; E-mail:jonathan.wong@drdc-rddc.gc.ca

1381-6128/09 $55.00+.00

and activate signaling cascade in immune defence cells and trigger immune, inflammatory responses to combat the infectious agents [6]. Of particular relevance are the TLRs recognizing nucleic acids, which include TLR-3 that recognizes dsRNA, TLR-7 (ssRNA), TLR-8 (ssRNA) and TLR-9 (unmethylated CpG motifs sequences) [6]. Nucleic acid-recognized TLRs are located in the endosomal membrane while non-nucleic acid recognized TLRs are located on the plasma membrane.

Activation of these TLRs plays a pivotal role in the host's innate and adaptive immune systems and their ability to recognize pathogen derived nucleic acids. TLRs and TLRs agonists have become hot area of anti-infective drug designs in recent years. In addition to binding TLR-3, dsRNAs also activate key enzymes which play a central role in the host antiviral state, including the interferon-inducible protein kinase R (PKR), the 2'-5' oligoadenylate synthetase (2'-5'OAS) [7], RIG-I Helicase, and the MDA5 [8, 9]. It follows that dsRNA may play a critical role in arming the innate immune and inflammatory pathways to fight viral infections.

This paper will outline the current understanding of immunological signaling events induced by TLR-3 agonists, specifically, pharmaceutically formulated dsRNA, and highlight their potential antiviral applications against influenza virus infections. In addition, we provide an overview on their safety and efficacy in preclinical studies. The development and applications of agonists for other TLRs against infectious and malignant diseases are subjects covered elsewhere and are therefore not discussed in this review.

ROLE OF TLR-3 IN INFLUENZA VIRUS INFECTIONS

Influenza virus infects primarily the pulmonary epithelial cells, causing airway respiratory distress, pulmonary exacerbations, and in the elderly and immunocompromised patients, causes secondary bacterial pneumonia which may lead to mortality. Influenza infection is characterized by the *in vivo* release of cytokines TNF-αl, IFN-γ, IL-6, various chemokines (MCP-1, -α, -β) [10]. Infected pulmonary epithelial cells attract migration of neutrophils, macrophages/monocytes, followed by natural killer cells to the infected respiratory tract. Infected epithelial cells undergo necrosis, whereas infected macrophages/monocytes respond by programmed cell death. The activation of this pathway also induces release of inflammatory cytokines and chemokines. The difference between avian or a pandemic influenza and seasonal influenza viruses is found in the magnitude of the inflammation in the respiratory tract of the infected individuals. The inflammation of the respiratory tract caused by avian and pandemic influenza viruses is estimated to be 10 times higher than the level of that caused by normal seasonal influenza [11]. The hyperinduction of these pro-inflammatory and apoptotic cytokines, also known as "cytokine storm", may lead to lung injury and multiple organ failures, and is a contributing factor to the increased fatality seen in both bird flu and pandemic flu victims [11, 12], as well as for other emerging viruses such as SARS. Unfortunately, young people, with healthy and normal immune functions, are particularly susceptible to HPAI-induced "cytokine storm".

Maintaining a delicate balance between inducing protective antiviral immunity without the overwhelming inflammatory effects is a challenging yet very important strategy in the antiviral prophylaxis and therapy of influenza infection, particularly against the deadly HPAI virus. Important mediators of the antiviral immune responses and the inflammatory and apoptosis pathways are various TLRs expressed in dendritic cells, macrophages, natural killer cells and B cells, as well as in respiratory epithelium.

Influenza viruses are negative-stranded RNA viruses belonging to the family *Orthomyxoviridae*. The viral genomes of influenza viruses consist of eight segmented ssRNAs, encoding for 10 viral proteins and other peptides. The viral segmented RNA is encased by the viral lipid envelope which is derived from the host cells it infects. Viruses are obligate intracellular pathogens, which replicate intracellularly by interfering with the normal gene functions of infected cells to reproduce their own genetic and protein components. During the influenza viral replication cycle, both single and double stranded RNAs of viral origin accumulate intracellularly in infected cells. The presence of these viral RNAs is detected by nucleic acid-recognizing signaling pathways (including TLR-3, -7, -8 and -9), and triggers host defence mechanisms.

The primary ligand for TLR-3 activation is double stranded RNA of either viral or non-viral origin [6, 8]. Intense efforts have been made to study TLR-3 and dsRNA interactions and to explore the antiviral potential of this ligand-receptor relationship. In a very recent study, the crystal structure of the complex between dsRNA and TLR-3 ectodomain was determined [8]. This study revealed that one single dsRNA binds to two TLR-3 ectodomains which are located at each terminal end of the horseshoe structure. It is postulated that it is the formation of this TLR-3:dsRNA 2:1 complex that is the signaling factor that recruits the adaptor protein (TRIF) to its cytoplasmic toll interleukin-1 receptor (TIR) domain, and results in the expression and secretion of type I interferons and other mediator cytokines and chemokines [8].

The induction of type I interferons by TLR-3: dsRNA signaling is unique in that it uses the adaptor protein TRIF and the transcription factor IRF-3, and it is independent of the adaptor protein myD88. This cascade pathway is unique because other nucleic acid-recognized TLRs, including TLR-7, -8, -9, use myD88 as the mediating protein for its interactions with transcription factor, IRF-7, TRAF6 and IRAK4 [6].

THE ANTIVIRAL ROLE OF TLR-3 AGONISTS

Partly because of the ability of TLR-3 ligands to stimulate the hosts's innate and adaptive immunity, they have been explored for their potential role in the prevention and treatment of viral diseases, including influenza infections. A number of pre-clinical studies in animals using the synthetic stabilized dsRNA, Poly ICLC, have provided evidence that it is an effective broad-spectrum immunomodulator that protects against a number of viral diseases, including influenza virus in mice.

Poly ICLC, is a synthetic double-stranded RNA consisted of complementary strands of polyriboinosinic-polyribocy-

tidylic acid (poly IC) stabilized with poly-L-lysine:carboxymethyl cellulose (LC) [13]. Poly IC is a well established TLR-3 agonist, and has been shown in crystal structure study to bind to TLR-3 to form a complex [8]. For poly IC to be an effective signal transductor of the TLR-3 pathway, the minimal length is at least 40-50 base pair [8]. Poly IC, when complexed with LC, was found to be more nuclease resistant and was more potent inducer of inteferons in monkeys and man compared to plain Poly IC [13, 14]. Poly ICLC has been shown in animal studies to be effective in providing protection against a range of infectious viruses including those causing yellow fever, Rift Valley fever, rabies and Venezuelan equine encephalomyelitis [14, 15]. The antiviral activity of this complex is believed to be mediated by the ability of dsRNA to stimulate the production of α, β, and γ-interferons *in vivo* and to stimulate specific components of the cellular and humoral immune systems, including the activation of natural killer cells [13, 16], TLR-3, PKR, OAS, RIG-I, MDA5 and others.

THE ANTIVIRAL ACTIVITY AGAINST SEASONAL INFLUENZA VIRUS

The antiviral efficacy of poly ICLC was determined using a lethal respiratory influenza virus model in mice. Intranasal (IN) pretreatment with Poly ICLC (1 mg/kg body weight) provided complete protection against both influenza A/Aichi/2 (H3N2) and influenza A/PR/8 (H1N1) [17]. A liposome formulation for the encapsulation of Poly ICLC was developed [18]. When the antiviral activity and toxicological profiles of Poly ICLC and liposomal Poly ICLC were compared in mice, a number of therapeutic characteristics were observed. Liposome encapsulation of Poly ICLC prolonged the window of protection from 14 days for Poly ICLC to 21 days [18]. When mice were pretreated with liposomal Poly ICLC within 21 days prior to virus challenge, they were completely protected from virus challenge, while untreated mice succumbed to the lethal influenza virus challenge [18]. When the toxicity profiles of the free and liposomal Poly ICLC were compared in mice, it was found that hypothermia and body weight loss induced by Poly ICLC were either completely mitigated or significantly reduced in mice given equivalent and therapeutically active doses of Poly ICLC in the liposomal form [18].

ANTIVIRAL ACTIVITY AGAINST HPAI H5N1 VIRUS

The antiviral efficacy against the HPAI virus in mice was evaluated to determine whether the protection provided by Poly ICLC and LE Poly ICLC was broad-spectrum and effective against the highly virulent influenza viruses. Additionally, this study could provide insights as to whether pretreatment with Poly ICLC and the consequential activation of TLR-3 signaling pathway would be beneficial or harmful for antiviral therapy against H5N1 virus infection, in view of the "cytokine storm" induced by the HPAI virus. An immunotherapeutic approach which adds "cytokine burden" to the respiratory tract in bird flu infected patients could be counterproductive in increasing mortality. This study would therefore be important in ensuring the safety and beneficial effects of TLR-3 agonists in such antiviral application.

To achieve these goals, a lethal challenge model using an influenza A/H5N1/chicken/Henan (clade 2) virus was established in BALB/c mice [19]. This model was then used to evaluate the immunoprophylactic efficacy of Poly ICLC and LE Poly ICLC to protect against a low and a high virus challenge with the HPAI virus. Control mice infected with either the low (1 LD_{50}) or high (4 LD_{50}) virus doses succumbed to the influenza infection starting at day 5-6 days post infection. Half of the control mice infected with the low virus chal-

Table 1. Summary of Results from qRT-PCR Analysis of the Effects of Nasally Administered Poly ICLC and LE Poly ICLC on the Expressions of Various Antiviral and Inflammatory Cytokine in Lungs of Mice

Cytokine	Produced by	Down-Regulation (-) Up-Regulation (+)	Functions
IL-1	Macrophages	+	Activation of B-and T-cells, induction of cytokines by macrophages, induction of fever
IL-2	T-lymphocytes	-	T-cell proliferation, natural killer cell activation
IL-4	Mast cells, CD4 lymphocytes	--	Proliferation of activated T- and B-cells, promotion of IgG1 and IgE synthesis
IL-5	Mast cells, CD4 T-helper cells	--	Proliferation and activation of B-cells
IL-10	Macrophages, CD4 T-cells	++	Inhibits mononuclear cell inflammation, inhibition of IFNγ secretion
IL-12b	Monocytes and macrophages	+	Induction of Th1 cells
IL-15	Mononuclear phagocytes	++	Activation of natural killer cells which promotes killing of virus infected cells
IFN-γ	T-lymphocytes	++	Intracellular killing of virus, activation of 2'-5' OAS
IFN-β	Dendritic cells Macrophages Natural killer cells	+++	Antiviral Anti-inflammatory Modulation of adaptive immunity
TNF-α	Macrophages, T-lymphocytes	++	Same as IFN-γ Mediation of inflammation and apoptosis

lenge dose died from the infection by day 10 post virus infection [18]. In the high virus challenge dose, all mice died from the infection by day 13 post infection (median = 8 days). Liposome-encapsulated Poly ICLC provided complete protection (100% survival) against the low virus challenge, and 63-75% protection against the high virus challenge. These levels of increased survival provided by liposomal Poly ICLC were statistical significant ($p < 0.0402$ vs control, $p < 0.0014$ vs control , respectively, for the low and high virus doses) The mean survival times of mice pretreated with liposomal Poly ICLC were found to be increased by 2 days from 8 days (control) to 10 days [19].

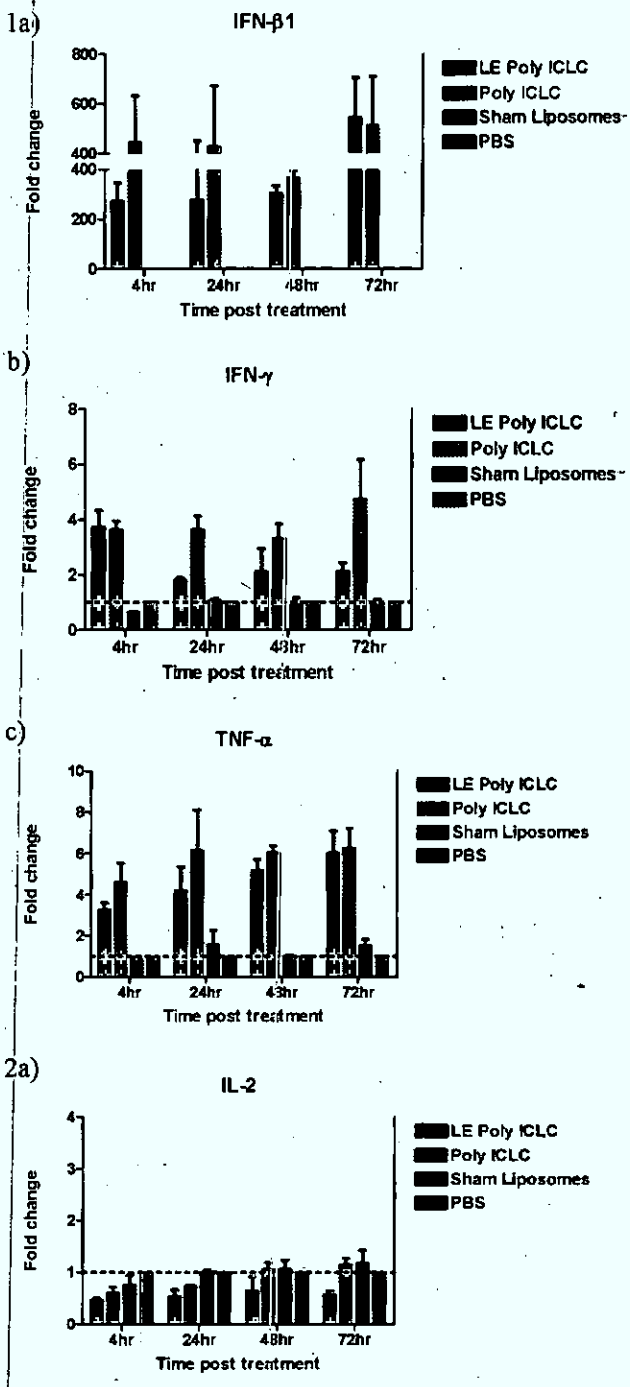
This study showed that pre-treatment with LE Poly ICLC enhanced survival and did not cause any significant adverse effects thus providing evidence that TLR-3 activation by dsRNA such as Poly ICLC did not cause "cytokine burden" to the mice. The protection provided by LE Poly ICLC suggests that the antiviral immunity elicited by LE Poly ICLC was indeed broad-spectrum and effective against multiple strains of seasonal and avian influenza viruses.

MODULATION OF ANTIVIRAL AND INFLAMMATORY CYTOKINES

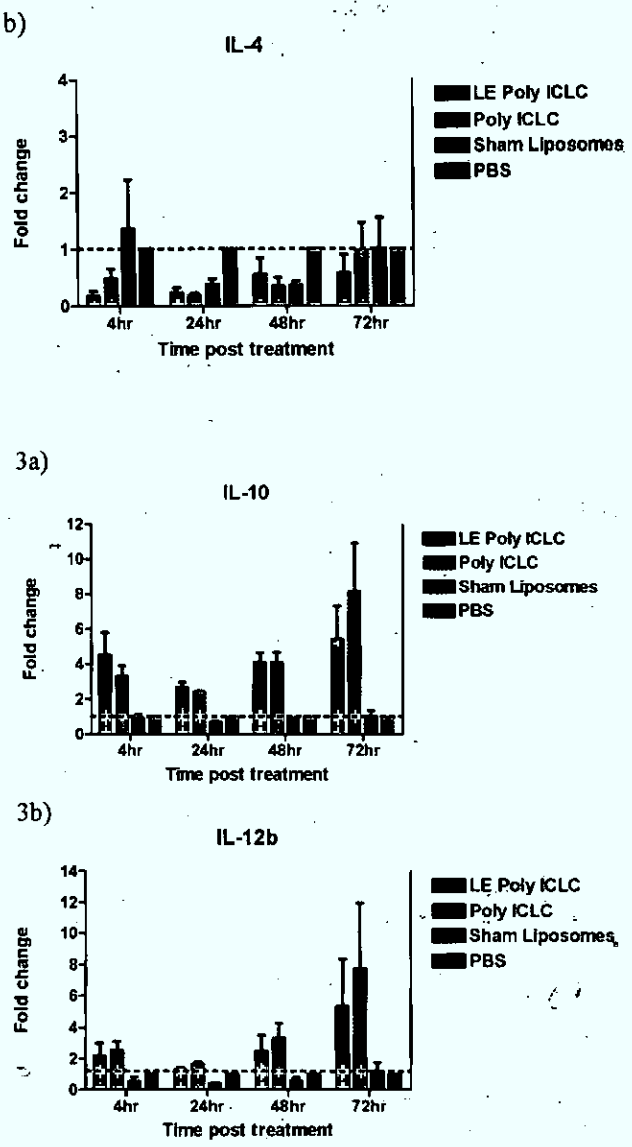
The effects of nasally administered TLR-3 agonists on the induction of antiviral and inflammatory cytokines in the respiratory tract may provide valuable insights on the nature of the protective antiviral immunity elicited by these drugs, as well as further clarifying any potential for exacerbation of a viral induced cytokine storm. To study the modulating effects on the cytokine gene expressions in mice, a quantitative real time polymerase chain reaction assay (qRT-TCR) was established and used to assay for various antiviral and inflammatory cytokine mRNAs in the lungs of mice which received the standard two prophylactic doses of nasal Poly ICLC and LE Poly ICLC [18]. The results of the qRT-PCR analysis are summarized in Table 1.

The effects on Poly ICLC and LE Poly ICLC on the expression of antiviral cytokines at various time points post drug treatment are also highlighted in Fig. (1). Both Poly ICLC and LE Poly ICLC significantly up-regulated the production of key antiviral cytokines such as IFN-β (Fig. 1a), IFN-γ (Fig. 1b), and TNF-α (Fig. 1c). IFN-γ is produced by T-lymphocytes, is responsible for the induction of anti-viral state and intracellular killing of pathogens in host cells, and is important in the regulation of immune responses. TNF-α is produced by macrophages, natural killer cells, and has antiviral, antiparasitic and anti-tumor activities, and is responsible for the activation of phagocytic cells and mediation of inflammatory responses. Both IFN-γ and TNF-α induce 2'-5' oligoadenylate synthetase (2'-5' OAS), a protein present in host cells which plays an important role in viral killing. In contrast, pre-treatment of mice with LE Poly ICLC and Poly ICLC resulted in the slight downregulation of IL-2 (Fig. 2a), IL-4 (Fig. 2b) and IL-5 (results not shown). IL-2 is a key mediator of the T-cells proliferation and production of T-cell lymphokines. The T-cell lymphokines are responsible for the generation of antigen-specific chronic inflammatory reactions.

The production of anti-inflammatory cytokines such as interleukins IL-10 and IL-12b upregulated (Fig. 3). IL-10 and IL-12 are anti-inflammatory cytokines, and both cause profound inhibition and suppression of the production of inflammatory cytokines produced by macrophages. IL-10 is produced by Th2 cells while IL-12 is produced by macrophages and B-cells.



(Figures) contd....



Figs. (1-3). The modulation of cytokine gene expressions in the mouse lung following pre-treatment with Poly ICLC and LE Poly ICLC as measured by qRT-PCR.

As expected, both Poly ICLC and LE Poly ICLC upregulate the expression of TLR-3 receptor in mouse lungs (Fig. **4**). Even at 72 hours post drug treatment, TLR-3 expression remained elevated. In contrast, sham liposomes have little or no effect on TLR-3 expression.

CONCLUSIONS

The pre-clinical studies described in this review affirm the potential of TLR-3 agonists including Poly ICLC and LE Poly ICLC for prophylaxis against influenza virus infections. Since these promising drug candidates work by targeting the host's innate and adaptive immune responses, rather than virus particles, they may represent a more robust and broad-spectrum strategy to combat pathogenic viruses. Recent studies have suggested that TLR-3 agonists such as Poly ICLC are very effective against other respiratory viruses including the respiratory syncytial virus (RSV) [20], and deadly SARS virus [21]. Against highly variant influenza viruses, the unique mechanism of action of these drugs offers strategic advantage in that they are less susceptible to the emergence of drug-resistance, and they protect against seasonal, zoonotic and pandemic strains. The demonstrated clinical safety of both systemically and intramuscularly administered (IM) Poly ICLC in phase I trials conducted in the US further supports its use for this indication (results not shown).

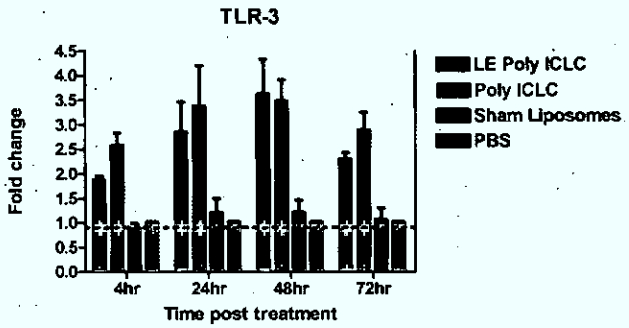


Fig. (4). The modulation of TLR-3 expression in mouse lung following pre-treatment with Poly ICLC and LE Poly ICLC as measured by qRT-PCR.

In view of possible global influenza pandemic caused by H5N1 bird flu or by other avian influenza viruses, and in view of the rapid increase drug-resistance of the bird flu virus to conventional ant-influenza drugs, there is justification for the regulatory agencies to consider fast track development of these drugs for the prophylaxis and/or adjunct treatment of H5N1 infection in humans.

ACKNOWLEDGEMENT

This work was partly funded by CRTI-06-0301 TO.

REFERENCES

[1] De Clercq E. Antiviral agents active against influenza viruses. Nat Rev Drug Discov 2006; 5: 1015-25.

[2] Bright RA, Medina MJ, Xu X, Perez-Oronoz G, Wallis TR, Davis XM, *et al.* Incidence of adamantine resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. Lancet 2005; 366: 1139-40.

[3] He G, Dong C, He C, Zhao L, Tian Y. Amantadine-resistance among H5N1 influenza viruses isolated in N China. Antiviral Res 2008; 77: 72-6.

[4] de Jong MD, Tran TT, Truong HK, Vo MH, Smith GJ, Nguyen VC, *et al.* Oseltamivir resistance during treatment of influenza A (H5N1) infection. New Engl J Med 2005; 353: 2667-72.

[5] Talon J, Horvath CM, Polley R, Basler CF, Muster T, Palese P, *et al.* Activation of interferon regulatory factor 3 is inhibited by the influenza A virus NS1 protein. J Virol 2000; 74: 7989-96.

[6] Sen GC, Sarkar SN. Transcriptional signaling by double-stranded RNA: role of TLR3. Cytokine Growth Factor Rev 2005; 16: 1-14.

[7] Havanessian AG. On the discovery of interferon-inducible, double-stranded RNA activated enzymes: the 2'-5' oligoadenylate synthetases and the protein kinase PKR. Cytokine Growth Factor Rev 2007; 18: 351-61.

[8] Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, Segal DM, Davies DR. Structural basis of toll-like receptor 3 signaling with double-stranded RNA. Science 2008; 320: 379-81.

[9] Sirén J, Imaizumi T, Sarkar D, Pietilä T, Noah DL, Lin R, *et al.* Retinoic acid inducible gene-I and mda-5 are involved in influenza A virus-induced expression of antiviral cytokines. Microbes Infect 2006; 8(8): 2013-20.

[10] Julkunen I, Melen K, Nyqvist M, Pirhonen J, Sareneva T, Matikainen S. Inflammatory responses in influenza virus infection. Vaccine 2000; 19: 32-7.

[11] de Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, *et al.* Fatal outcome of human influenza (H5N1) is associated with high virus load and hypercytokinemia. Nat Med 2006; 12: 1203-7.

[12] Osterholm, MT. Preparing for the next pandemic. N Engl J Med 2005; 352: 1839-42.

[13] Levy HB, Baer G, Baron S, Buckler CE, Gibbs CJ, Iadorola MJ, *et al.* A modified polyriboinosinic-polyribocyticylic acid complex that induces interferon in monkeys. J Infect Dis 1975; 132: 434-9.

[14] Stephen EL, Sammons ML, Pannier WL, Baron S, Spertzel RO. Effect of a nuclease-resistant derivative of a polyriboinosinic-polyribocytidylic acid complex on yellow fever in rhesus monkeys (*Macaca mulatta*). J Infect Dis 1977; 136: 122-6.

[15] Kende M. Prophylactic and therapeutic efficacy of poly (I,C)-LC against Rift Valley fever virus infection in mice. J Biol Response Mod 1985; 4: 503-11.

[16] Wiltrout RH, Salup RR, Twilley TA, Talmadge JE. Immumomodulation of natural killer activity by polyribonucleotides. J Biol Response Mod 1985; 4: 512-7.

[17] Wong JP, Saravolac EG, Sabuda D, Levy HB, Kende M. Prophylactic and therapeutic efficacies of poly(IC.LC) against respiratory influenza A virus infection in mice. Antimicrob Agents Chemother 1995; 39: 2574-6.

[18] Wong JP, Yang H, Nagata L, Kende M, Levy H, Schnell G, *et al.* Liposome-mediated immunotherapy against respiratory influenza virus infection using double stranded RNA poly ICLC. Vaccine 1999; 17: 1788-95.

[19] Wong JP, Christopher ME, Salazar AM, Dale RMK, Sun L-Q, Wang M. Nucleic acid-based antiviral drugs against seasonal and avian influenza viruses. Vaccine 2007; 25: 3175-8.

[20] Guerrero-Plata A, Baron S, Poast JS, Adegboyega PA, Casola A, Garofalo RP. Activity and regulation of alpha interferon in respitaory syncytial virus and human metapneumovirus infections. J Virol 2005; 79: 10190-9.

[21] Barnard DL, Day CW, Wandersee M, Kumaki Y, Salazar AM. Evaluation of interferon inducers, ribavirin and mouse hyperimmune serum in a pathogenesis/lethal mouse model infected using a mouse-adapted SARS-CoV. International Conference for Antiviral Research (ICAR): Montreal 2008.