ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Effects of respiratory syncytial virus infection on the levels of host translational initiation factors

Azimah Abdul-Wahab*[1], Rusydatul Nabila Mahmad-Rusli[1], Manraj Singh Cheema[2]

[1]Universiti Kuala Lumpur, Institute of Medical Science Technology (UNIKL MESTECH), 43000 Kajang, Selangor, Malaysia
[2]Universiti Putra Malaysia, Faculty of Medicine and Health Science, 43400, Seri Kembangan, Selangor, Malaysia

Article History:

Received on: 02.10.2018
Revised on: 12.12.2018
Accepted on: 14.12.2018

Keywords:

eIF4A,
eIF4E,
eIF4F,
eIF4G,
Respiratory syncytial Virus,
Translational initiation factors

ABSTRACT

Respiratory Syncytial Virus (RSV) is a leading cause of respiratory infection primarily in children. Presently, there are no effective vaccines available whereas current treatments are only limited to relieve the signs and symptoms. Currently, the development of RSV antivirals and vaccines are much more focused on viral attachment and RNA transcription and few information are produced on the effect of RSV infection on host translation initiation factors. Therefore, this study aimed to analyse the level of translational initiation factors (eIF4A, eIF4E, eIF4G) in RSV-infected cells, in which they play a major role as the translation control target. Time course infection study was carried out at 0, 24, 48, 72, 96, 120 hours at Multiplicity of Infection 1 (MOI 1) to determine virus growth curve and level of host proteins. Findings of the study demonstrated that the virus particles were detected in the supernatant as early as 12 hours post infection (hpi) and the titre was found to increase drastically after 48 hpi before reaching the highest concentration at 72 hpi. However, RSV titre started to reduce both out-cellular and in-cellular between 96 hpi to 108 hpi, due to the latent period of RSV infection. In addition, eIF4G and eIF4E levels were found to be maintained throughout the infection. However, eIF4A levels decreased during early infection before increased at a later stage of infection. This finding shows that the eIF4G and eIF4E are probably required in synthesizing the viral proteins. The reduction of eIF4A level, however, primarily suggests that this helicase factor might be very crucial in the early stage of the RSV replication.

* Corresponding Author

Name: Dr. Azimah Abdul Wahab
Phone: +603 87395894
Email: azimahaw@unikl.edu.my

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v9iSPL2.1729

Production and Hosted by
IJRPS | https://ijrps.com

INTRODUCTION

Respiratory syncytial virus (RSV) was discovered in 1956 and classified as a non-segmented negative-sense single-stranded RNA virus (Collins and Melero, 2011). The *Paramyxoviridae* family members have been reported to cause problems to the human respiratory system such as bronchiolitis and pneumonia (Müller-Pebody *et al.,* 2002; Psarras *et al.,* 2004). It was reported that RSV mostly affects infants, young children, elderly person and immunodeficient individual (Nair *et al.,* 2010). People infected with RSV develop symptoms such as a headache, low-grade fever, runny nose, cough and sore throat. However, in healthy adults, these symptoms disappear after a certain period, although serious illness may implicate in those with

low immune systems (Openshaw and Tregoning., 2005).

Extensive attempts have been conducted to identify the best treatment against RSV infection (Hu and Robinson, 2010; Huang *et al.,* 2010). Currently, there are no vaccines available to combat RSV infection effectively, whereby treatments are also limited in relieving signs and symptoms only (Collins and Melero, 2011). At present, the USA Food and Drug Administration (FDA) had approved the aerosolized ribavirin as an antiviral agent to treat infants and young children with severe lower respiratory tract disease caused by RSV (Marcelin *et al.,* 2014). Ribavirin is known as a synthetic nucleoside analogue that incorporates with viral RNA which later suppresses RSV replication in host cells. However, a study has shown that the treatment was associated with a diminished antibody response in treated children by impairing the development of the specific mucosal antibody response, IgE (Rosner *et al.,* 1987). Palivizumab, the humanized monoclonal antibody for RSV is able to inhibit RSV replication, but the therapy should be restrained in children with severe immunodeficiency or those at risk of nosocomial RSV infection (Olchanski *et al.,* 2018).

Generally, replication of a virus involves attachment, penetration, replication, assembly and release stages. The success of viruses in replicating themselves is mainly focused on the mechanisms used in hijacking the host system, for example, RNA translation process. Synthesis of virus proteins occurs in many ways including cap-dependent and cap-independent processes. Shut-off of the host protein synthesis mechanism may vary even within the same viral family. Some viruses act effectively on blocking the host's mRNA translation process by hijacking the host cell translation machinery in synthesizing their own protein (Walsh and Mohr, 2011). During Picornavirus infection, recruitment of cellular translation proteins to the virus RNA was found to occur via the secondary structure of the viral RNA known as Internal Ribosomal Entry Site (IRES) (Willcocks *et al.,* 2011). Meanwhile, translation of Calicivirus RNA is initiated by interaction between the viral protein, VPg and host ribosomal subunits (Goodfellow, 2011). Removal of VPg from the viral RNA by proteinase K dramatically reduces translation and mutation of a conserved amino acid, tyrosine (Tyr)-24 in VPg which has been shown to prevent viral replication indicating that VPg is essential for calicivirus infection (Brierley *et al.,* 1997; Mitra *et al.,* 2004).

As for RSV scope of the study, there is little information regarding the effect of RSV infection on host translation initiation factor. Likewise, the development of RSV antivirals and vaccines has been focusing on viral attachment (Lambert *et al.,* 1996; Jordan *et al.,* 2015) and RNA transcription (Mason *et al.,* 2004; Liuzzi *et al.,* 2005) rather than viral RNA translation. Therefore, this study was experimentally performed to investigate the mechanism used by RSV to take over their host's biological activities in promoting their replication, thus facilitating the future research in finding new approaches for future treatment toward RSV infection. Study on the requirement of the translation initiation factors and other molecular activities during viral infections would be able to provide great information in developing antivirals, vaccines or other treatments.

MATERIALS AND METHODS

Cell Culture

Human Epithelial type 2 (HEp-2) cells were obtained from Universiti Kebangsaan Malaysia Medical Centre (UKMMC) and cultured in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% foetal bovine serum (FBS), 1% non-essential amino acids and 1% penicillin/streptomycin (Life Technologies). The cells were then incubated at 37°C with 5% carbon dioxide.

Production of Respiratory Syncytial Virus (RSV) Stock

RSV stock was also obtained from UKMMC. The HEp-2 cells were infected with RSV incubated at 37°C with 5% carbon dioxide overnight. The cells were then harvested and centrifuged at 2000 rpm for 5 minutes. The supernatant was collected and virus titre was determined using the Tissue Culture 50% Infectious Dose ($TCID_{50}$) Assay (Ramakrishnan, 2016).

Preparation of Protein Lysate

Approximately 3×10^5 of HEp-2 cells was infected with RSV at MOI 1 and uninfected cells were used as a control experiment. The cells were incubated at 37°C and harvested at the following time-points post infection; 0, 24, 48, 72, 96 and 120 hpi. The cells were lysed in nuclear lysis buffer (NLB) [50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7.4, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA), 2 mM Sodium Orthovanadate (Na3VO4), 25 mM glycerophosphate, Complete Mini protease inhibitor cocktail (Roche), 0.5% NP4O] and centrifuged at 14000 rpm for 5 minutes at 4ºC. Cell lysates were stored at -20°C.

Protein Analysis

The concentration of protein samples was determined using Bicinchoninic Acid (BCA) Protein Assay Kit (PIERCE-Thermo Scientific). The proteins

were separated using SDS-PAGE and immunoprecipitated (Western Blotting technique) using specific antibodies; eIF4A, eIF4G and eIF4E (Cell Signalling Technology). GAPDH expression was monitored as a loading control and showed an even loading for each sample. The protein blots were treated with Super Signal West Pico Chemiluminescent substrate (Pierce Biotechnology) and visualized using Fusion FX Chemiluminescence and Fluorescence Imaging (VilberLourmat).

RSV Growth Curve

Time course infection was conducted and cells were harvested at 0, 12, 24, 36, 48, 60, 72, 96, 108 and 120 hours post infection. Supernatants were collected, and the remaining cell lysates were added with DMEM and homogenized using cell homogenizer (OMNI-TH) for 2 minutes on ice to break the cell membrane. The cell materials were centrifuged at 2000 rpm for 5 minutes immediately after the supernatant containing virus material inside the cells was collected. Titre of virus in the supernatants was determined using $TCID_{50}$ assay. The virus titre was plotted against time post-infection for determination of the virus growth curve.

RESULTS AND DISCUSSION

RSV Growth Curve

Figure 1 shows the infected cells started to release virus particles after 36 hours of infection and concentration of the out-cellular virus was found to increase significantly at 60 hours post-infection. The highest concentration of virus was detected at 80 hours post-infection before decreasing immediately after. Reduction of the virus concentration might be due to the unavailability of live host cells that could be utilized by the viruses to replicate.

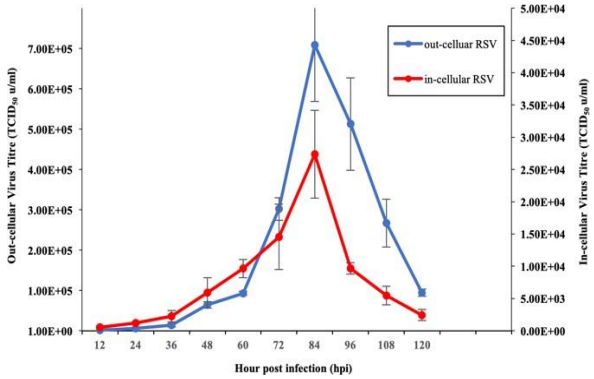

Figure 1: Growth curve of respiratory syncytial virus (RSV)

Effects of RSV on eIF4E and eIF4G Levels

This study focused on the levels of cap-binding (eIF4E), scaffold (eIF4G) and helicase (eIF4A) proteins. As shown in Figure 2, there were no changes on the levels of eIF4E and eIF4G throughout the virus infection which suggested that these two proteins are probably required during viral replication which warrants further investigation. The confirmation study is important as a previous study conducted by a group of researchers on Murine norovirus-infected cells found that the eIF4E level maintained throughout the virus infection. However, inhibition of the interaction between eIF4E and eIF4G using specific inhibitor, 4E2RCat led to the reduction of virus titre that confirmed the requirement of eIF4E and eIF4G for the virus protein synthesis (Royall *et al.,* 2015).

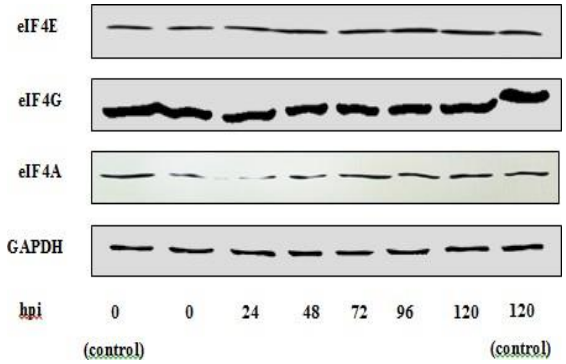

Figure 2: Effect of RSV infection on levels of eIF4E, eIF4G and eIF4A

Effects of RSV on eIF4A Levels

The level of eIF4A was observed to reduce at early of infection (24 hpi) and increased again at a later stage of infection (72 hpi). To confirm whether reduction of eIF4A level was influenced by RSV replication, the level of the protein in cells infected with inactivated RSV was analysed. Several studies have reported that virus inactivation using UV irradiation at wavelength 254 nm were associated with irreparable damage of RNA resulting in the loss of viral RNA replication (Nuanualsuwan and Cliver, 2003; Pfaender *et al.,* 2015). In this study, the RSV stock was exposed to UV light at a wavelength of 254 nm for 10 minutes using UV Transilluminator before infecting Hep-2 cells at MOI 1. Protein and virus samples were collected after a time course infection experiment was conducted. Our result showed that the level of eIF4A was maintained during viral infection (Figure 3). Meanwhile, growth curve plotted for the inactivated-RSV study also demonstrated that there was no replication observed as compared to normal RSV (Figure 4) which confirmed that the eIF4A level was influenced by the viral replication. A study conducted on Foot-and-Mouth Disease virus (FMDV) also showed the same result as RSV whereby the level of eIF4A in FMDV-infected cells reduced at later infection due to the presence of the viral 3C protease (Belsham *et al.,* 2000).

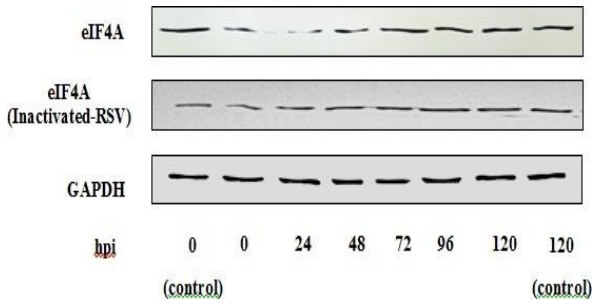

Figure 3: Effect of inactivated-RSV infection on the level of eIF4A

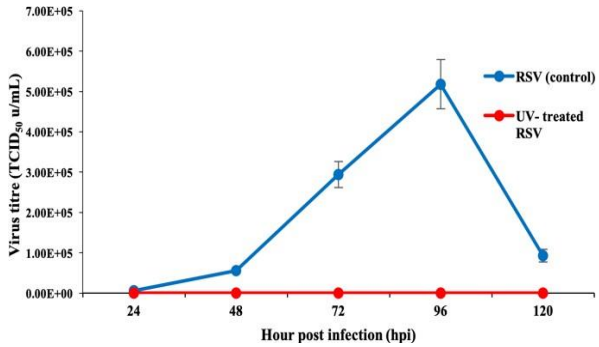

Figure 4: Growth curve of RSV (control) and UV-treated RSV

CONCLUSION

Investigation of the effect of RSV infection on host translation initiation factors is significant in understanding the mechanism used by the virus to synthesis their proteins. In this study, it was found that the RSV infection may cause different effects on the levels of cap-binding (eIF4E), scaffold (eIF4G) and helicase (eIF4A) proteins. Time-course infection conducted following RSV infection demonstrated that the virus caused no changes on levels of eIF4E and eIF4G proteins. However, level of eIF4A was observed to reduce at early infection and raised again at later infection. The study using UV inactivated-RSV proved that the changes on the eIF4A level was influenced by viral replication. Therefore, more investigations must be conducted including performing RNA silencing or using specific inhibitors to understand the functional requirement of the eIF4E, eIF4G and eIF4E.

Acknowledgement

We would like to acknowledge the Ministry of Education for funding the project under the Fundamental Research Grant Scheme (FRGS). We also thank UKMMC for their generosity in donating the HEp-2 cells and RSV for this project.

REFERENCES

Belsham, G. J., McInerney, G. M. and Ross-Smith, N. 2000. Foot-and-mouth disease virus 3C protease induces cleavage of translation initiation factors eIF4A and eIF4G within infected cells. *Journal of Virology*, 74(1), 272–80.

Brierley, I., Herbert, T. P. and Brown, T. D. 1997. Identification of a protein linked to the genomic and subgenomic mRNAs of feline calicivirus and its role in translation. *Journal of General Virology*, 78(5), 1033–1040.

Collins, P. L. and Melero, J. A. 2011. Progress in understanding and controlling respiratory syncytial virus: Still crazy after all these years. *Virus Research*, 162(1–2), 80–99.

Goodfellow, I. 2011. The genome-linked protein VPg of vertebrate viruses — a multifaceted protein. *Current Opinion in Virology*, 1(5), 355–362.

Hu, J. and Robinson, J. L. 2010. Treatment of respiratory syncytial virus with palivizumab: a systematic review. *World Journal of Pediatrics*, 6(4), 296–300.

Huang, K., Incognito, L., Cheng, X., Ulbrandt, N.D., Wu, H. 2010. Respiratory syncytial virus-neutralising monoclonal antibodies motavizumab and palivizumab inhibit fusion. *Journal of Virology*, 84(16), 8132–40.

Jordan, R., Show, M., Mackman, R.L., Perron, M., Cihlar, T., Lewis, S.A., Eisenberg, E.J., Carey, A., Strickley, R.G., Chien, J.W., McEligot, H.A., Anderson, M., L., Behrens, N.E., and Gershwin, L.J. 2015. Antiviral Efficacy of a Respiratory Syncytial Virus (RSV) Fusion Inhibitor in a Bovine Model of RSV Infection. *Antimicrobial Agents and Chemotherapy*, 59(8), 4889–4900.

Lambert, D. M., Barney, S., Lambert, A.L., Guthrie, K., Medinas, R., Davis, D.E., Bucy, T., Erickson, J., Merutka, G., Petterway, S.R. 1996. Peptides from conserved regions of paramyxovirus fusion (F) proteins are potent inhibitors of viral fusion. *National Academy of Sciences*, 93(5), 2186–2191.

Liuzzi, M., Mason, S.W., Cartier, M., Lawetz, C., McCollum, R.S., Dansereau, N., Bolger, G., Lapeyre, N., Gaudette, Y., Lagacé, L., Massariol, M.J., Dô, F., Whitehead, P., Lamarre, L., Scouten, E., Bordeleau, J., Landry, S., Rancourt, J., Fazal, G., Simoneau, B. 2005. Inhibitors of Respiratory Syncytial Virus Replication Target Cotranscriptional mRNA Guanylylation by Viral RNA-Dependent RNA Polymerase. *Journal of Virology*, 79(20), 13105–13115.

Marcelin, J.R., Wilson, J.W., Razonable, R.R. 2014. Oral ribavirin therapy for respiratory syncytial virus infections in moderately to severely immunocompromised patients. *Transplant Infectious Disease*, 16(2), 242–250.

Mason, S.W., Lawetz, C., Gaudette, Y., Dô, F., Scouten, E., Lagacé, L., Simoneau, B., Liuzzi, M.

2004. Polyadenylation-dependent screening assay for respiratory syncytial virus RNA transcriptase activity and identification of an inhibitor. *Nucleic Acids Research*, 32(16), 4758–4767.

Mitra, T., Sosnovtsev, S. V and Green, K. Y. 2004. Mutagenesis of tyrosine 24 in the VPg protein is lethal for feline calicivirus. *Journal of Virology*. 78(9), 4931–5.

Müller-Peabody, B., Edmunds, W.J., Zambon, M.C., Gay, N.J., Crowcroft, N.S. 2002. The contribution of RSV to bronchiolitis and pneumonia-associated hospitalizations in English children, April 1995-March 1998. *Epidemiology and Infection*, 129(1), 99–106.

Nair, H., Nokes, D.J., Gessner, B.D., Dherani M., Madhi, S.A., Singleton, R.J., O'Brien, K.L., Roca, A., Wright, P.F., Bruce, N., Chandran, A., Theodoratou, E., Sutanto, A., Sedyaningsih, E.R., Ngama, M., Munywoki, P.K., Kartasasmita, C., Simões, E.A., Rudan, I., Weber, M.W., Campbell, H. 2010. Global burden of acute lower respiratory infections due to the respiratory syncytial virus in young children: a systematic review and meta-analysis. *The Lancet*, 375(9725), 1545–1555.

Nuanualsuwan, S. and Cliver, D. O. 2003. Infectivity of RNA from inactivated poliovirus. *Applied and environmental microbiology,* 69(3), 1629–32.

Olchanski, N., Hansen, R.N., Pope, E., D'Cruz, B., Fergie, J., Goldstein, M., Krilov, L.R., McLaurin, K.K., Nabrit-Stephens, B., Oster, G., Schaecher, K., Shaya, F.T., Neumann, P.J., and Sullivan, S.D. 2018. Palivizumab Prophylaxis for Respiratory Syncytial Virus: Examining the Evidence Around Value. *Open Forum Infectious Diseases*, 5(3), 031.

Openshaw, P. J. M. and Tregoning, J. S. 2005. Immune responses and disease enhancement during respiratory syncytial virus infection. *Clinical Microbiology Reviews*, 18(3), 541–55.

Pfaender, S., Brinkmann, J., Todt, D., Riebesehl, N., Steinmann, J., Steinmann, J., Pietschmann, T., Steinmann, E. 2015. Mechanisms of Methods for Hepatitis C Virus Inactivation. *Applied and Environmental Microbiology*, 81(5), 1616–1621.

Psarras, S., Papadopoulos, N. G. and Johnston, S. L. 2004. Pathogenesis of respiratory syncytial virus bronchiolitis-related wheezing. *Paediatric Respiratory Reviews*, 5 Suppl A, S179-84.

Ramakrishnan, M. A. 2016. Determination of 50% endpoint titer using a simple formula. *World Journal of Virology,* 5(2), 85–6.

Rosner, I.K., Welliver, R.C., Edelson, P.J., Geraci-Ciardullo, K., Sun, M. 1987. Effect of ribavirin therapy on respiratory syncytial virus-specific IgE and IgA responses after infection. *The Journal of Infectious Diseases*, 155(5), 1043–7.

Royall, E., Doyle, N., Abdul-Wahab, A., Emmott, E., Morley, S.J., Goodfellow, I., Roberts, L.O., and Locker, N. 2015. Murine norovirus 1 (MNV1) replication induces translational control of the host by regulating eIF4E activity during infection. *The Journal of Biological Chemistry*, 290(8), 4748–58.

Walsh, D. and Mohr, I. 2011. Viral subversion of the host protein synthesis machinery. *Nature Reviews Microbiology*, 9(12), 860–875.

Willcocks, M.M., Locker, N., Gomwalk, Z., Royall, E., Bakhshesh, M., Belsham, G.J., Idamakanti, N., Burroughs, K.D., Reddy, P.S., Hallenbeck, P.L., and Roberts, L.O. 2011. Structural features of the Seneca Valley virus internal ribosome entry site (IRES) element: a picornavirus with a pestivirus-like IRES. *Journal of Virology*, 85(9), 4452–61.