International Journal of Basic and Applied Virology 6(2): 19-23, 2017
ISSN 2222-1298

DOI: 10.5829/idosi.ijbav.2017.19.23

Oncolytic Activity of Bluetongue Virus

Kawther S. Zaher

Department of Microbiology and Immunology,
National Research Centre, Dokki, Egypt, 60014618

Abstract: Humans are sero-negative toward bluetongue viruses (BTVs) since BTVs do not infect normal human cells. Infection and selective degradation of HeLa cell line by BTV serotype 16 have been investigated. We determined the susceptibilities of both Vero and HeLa cells to BTV infections and developed analysis of their cytopathic effects, survival rates, ultra-structural changes, cellular apoptosis using MTT assay and progeny titers. The wild-type BTVs, was used without any genetic modifications, could preferentially infect and degrade both Vero and HeLa cells. BTV-degradation of these cells is viral dose-dependent, leading to effective viral replication and induced apoptosis. Xenograft tumors in mice were substantially reduced by a single intra-muscular BTV injection in *vivo* experiments. Thus, wild-type BTVs, without genetic modifications, have oncolytic potentials. They represent an attractive, next generation of oncolytic viral approach for potential human cancer therapy combined with current anti-cancer agents and irradiation.

Key words: Oncolytic bluetongue viruse · Cancer treatment · Selective cytotoxic effects · Virotherapeutics · Oncolytic · Anti-Cancer · Antitumor · Antineoplastic

INTRODUCTION

Pathogens cause many diseases and subsequently tissue damage and interaction with host immune systems. An oncolytic virus (OV) is a virus that has the ability to infect and kills cancer cells. As the infected cancer cells are destroyed by oncolysis, they release new infectious virus particles that destroy the remaining cancer cells. It represents a unique class of cancer therapeutics with distinct mechanisms of action [1]. In 1904 Dock attributed the decrease of the white blood cell count of a patient with chronic myelogenous leukemia (CML) to a flu-like illness [2], suggesting to use viral infection to treat tumors. This idea has been a dream to many scientists in this field as they tried to use wild-type viruses with genetic modifications such as selective genetic deletion of viral virulent domains or modification of viral genes critical for viral replication and the addition of immune enhancer genes [3]. Oncolytic viruses attacks cancer cells while normal cells invasion initiate interferon production which inhibits further replication of virons in normal cells. Several researches discovered the oncolytic potentials of some viruses in six major viral families, reovirus type 3, papillomaviruses, herpesviruses, hepadnaviruses, flaviviruses and retroviruses [4]. Except reovirus, all these viruses are “human cancer viruses” and they responsible for more than a fourth of all human cancers. This reason urge scientists to use innovative methods integrating molecular biology and genomics/proteomics to ulter or reduce pathogenicity of these viruses [5] and to improve their oncolytic activity [6].

Bluetongue (BT) is a viral belongs to genus Orbiviruss and family Reoviridae. Bluetongue virus (BTV) is a double stranded RNA divided into 10 linear segments. The RNA codes for 5 non-structure proteins (NS1, NS2, NS3, NS3A and NS4) and 7 structure proteins (VP1-VP7) [7]. Unlike current therapeutic modalities, BT as a model for OVs has many advantages such as it has no probability for generation of resistance as it targets multiple oncogenic pathways and employ multiple means for cytotoxicity, another advantage is that its dose increases by time contradicted with classical drug pharmacokinetics that decreases with time. BT does not infect humans [8] and thus, humans do not have pre-existing antibodies to BTVs [9] and subsequently it is safely used in human cancer. In addition it is RNA virus and not genotoxic which does not integrate its genome in host genome [10].

Corresponding Author: Kawther S. Zaher, Department of Microbiology and Immunology, Veterinary Research Division, National Research Centre, Dokki, Giza, Egypt. Postal cod: 12622.

This study aims for monitoring the *in-vitro* and *in-vivo* Oncolytic activity of BTV which considered safe and show very minor effect on exposure.

MATERIALS AND METHODS

Cell Lines: HeLa cervix carcinoma and Vero cells (green monkey kidney cell) were used. Both cells were obtained from Vaccine and Sera Institute (VACSERA) Dokki, Cairo, Egypt. Roswell Park Memorial Institute (RPMI) 1640 media was used to maintain both cells containing 100 µg/ml penicillin, 100µg/ml streptomycin and 10% fetal calf serum and maintained in a 5% CO_2 humidified incubator at 37°C.

Virus: Bluetongue virus (BTV- 16) was obtained from Virology Unit, Microbiology and Immunology Department, National Research Centre, Dokki, Egypt.

Virus Propagation and Titration: BT was propagated on both HeLa and VERO cells each was performed separately and supplemented with RPMI 1640 media, 10% fetal calf serum, 100 µg/ml penicillin and 100 µg/ml streptomycin in a 5% CO_2 humidified incubator at 37°C. Plaque forming test was performed according to**.** Virus titers were determined by inoculation of serial virus dilutions into both HeLa and VERO cells. The stored aliquots of BTV were taken from the -80°C freezer, thawed on ice and reconstituted to the appropriate volume with cold phosphate buffered saline (PBS). 5×10^4 cells/well in 96-well plates (Costar, Corning, NY, USA) and infected 6 h later at multiplicities of infection (MOIs, virus particles per tumor cell) of 0.01, 0.1 and 1. Cells were infected in 96-well plates for 1, 3, 5 and 7 days and the minimal cytotoxic concentration $_{50}$ (MCC)$_{50}$ was calculated.

***In vitro* Testing of Oncolytic Activity of BTV on Tumor Cell Line**

Cellular and Morphological Determination by Inverted Microscopy: After cells were infected with BTV-16 at MOI of 1.0 for 36 h, the morphological changes of all the cells were observed daily and determined by inverted microscope (Nikon eclipse Ti, Tokyo, Japan) supplemented with digital camera software (NIS-Elements F 3.2, Nikon, Tokyo, Japan).

Examination Using Electron Microscopy (EM): Cells were collected and fixed using glutaraldehyde (30 g/L) according to Hu *et al* [8]. The cells were examined under electron microscope for cellular ultrastructures.

Analysis of Cell Survival Rate Using MTT Assay: Survival rate of the five cell lines was assessed by MTT assay which measured the cytotoxicity and cellular apoptosis by the bio-reduction of a tetrazolium compound: 3-(4,5-dimethylthiazol-2-ly)-5- (3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium into a formazan that could be measured calorimetrically. Each well of the 96 well plates was seeded with $4x10^4$ cells and incubated with 200 µl of the growth media. After incubation for 24 - 48 h, cells were infected by BTV with MOI in series for 36 h. At the end of the incubation, the growth medium was removed and 20 µl MTT solution and 180 µl fresh medium was added to each well. Dimethyl sulfoxide (DMSO) was then added to each well and the optical density of each well was screened and determined with a Plate Reader (BioTek, ELX800, USA) at 570 nm.

The survival rate = (optical density of the tested group x100%)/ the mean optical density of control. The titer of original viral suspension used was $10^{5.05}$/ml TCID50. Based on the Poisson: Distribution, TCID50 = -In (0.5) = 0.693. Thus, 1

TCID50 = 0.693 IU (infection unit) [11]. Through data conversion, different viral multiplicity of infection (MOI) in five groups was adapted to 10^{-3}, 10^{-2}, 10^{-1}, 1 and 10 IU/cell, respectively.

***In vitro* Virus Replication Assay by BTV Titration after Infection:** In 24-well tissue culture plate 5×10^6 HeLa or VERO cells/well each was performed separately and was inoculated. Both HeLa and VERO cells were infected 6 h later with an MOI of 1.0 of BT at 1, 3, 5 and 7 days post infection, the supernatants were collected and the virus titers determined by serial dilution in Both HeLa and VERO cells. Where, the day before infection, both cells were plated to confluence in a 24-well plate. Both cells were infected with serial supernatant dilutions in a total volume of 20 µl. The titer of the virus was determined.

Statistical Analysis: Comparisons among groups and inside the groups were statistically analyzed by ANOVA (Analysis of Variants).

RESULTS AND DISCUSSION

BT, a *Orbivirus* and Reoviridae family member, known as an anticancer therapeutic agent more than 50 years ago as a result of its potent oncolytic and limited toxicity to normal cells. BT selectively replicates within tumor cells, making it an attractive anticancer therapeutic with a low toxicity profile [10].

Virus Propagation and Minimal Cytotoxic Concentration$_{50}$ (MTC$_{50}$): BT was propagated on Vero cell and the viral cultural morphology was monitored. Cells showed elongation and vaculation this may be attributed to necrotic effect of BT (Fig. 1) this result matches with the results of Hu *et al.* [8]. The MTC$_{50}$ was estimated to use minimal concentrations on cells. The ability of BTV to kill Vero cell line was tested in vitro at MOIs of 0.01, 0.1 and 1. In our initial experiments, we used the MOI in series dilution to determine the survival rates of these two cell lines using the MTT assay. Statistical analysis one-way ANOVA (Analysis of Variance) showed that there were significant differences among the two cell types in their survival rates (F= 95.635, p>0.01) (Figure 2 and 3). To further access whether survival rates of the Two cells could be affected by different MOIs, we used series MOIs of BTV-16 to infect these cell lines in multiple independent experiments and their survival rates were determined 36 h post infection by the MTT method. When the MOI of infection varied from 0.001 to 10, the by The virus effectively killed HeLa cells and a dose response was observed. At an MOI of 1, more than 80% of the cells were killed at 5 days. Even at the lowest MOI of 0.01 over 40% of the cells had been killed by day 7 (Fig. 3). The best result obtained was at MOI 1 which was used for HeLa cell this fact matches with Hu *et al.,* [8] on MKN-74 human gastric cancer cells. Other researchers found that the best result obtain was at MOI 0.1 on panel of human cancer cells included human pancreatic, breast, thyroid, head and neck and gastric cancers, as well as human and murine malignant melanoma cell lines [12].

***In vitro* Testing of Oncolytic Activity of BTV on Tumor Cell Line:** Over 90% cytopathic effects (CPE) were readily observed and detected in HeLa and Vero cells, infected with BTV 36 h post-infection at a MOI of 1 as shown by light microscopy in Figure 1. Cellular apoptosis was evident in these cell lines. Morphological characteristics and CPE of BTV-infected cells were recorded. They appeared to adhere to each other more tightly with some loss of their normal spindle shape. The efficiency of viral replication was monitored by collecting supernatants of infected HeLa cells at different time points (At 1, 3, 5 and 7 days post infection). The highest virus titer on Vero cells (10^4) was detected 5 day after infection of HeLa cells (Fig. 4).

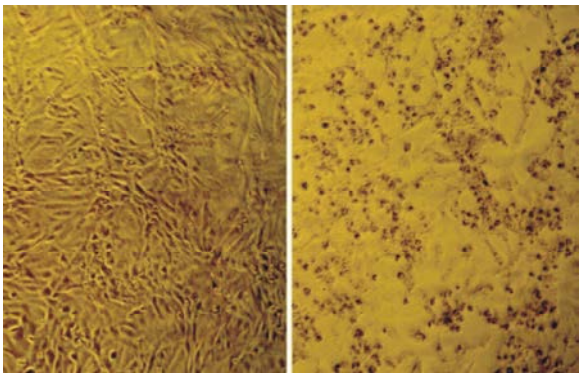

Fig. 1: Photo on left shows normal Vero cells. While on the right shows Vero cells four days post infection.

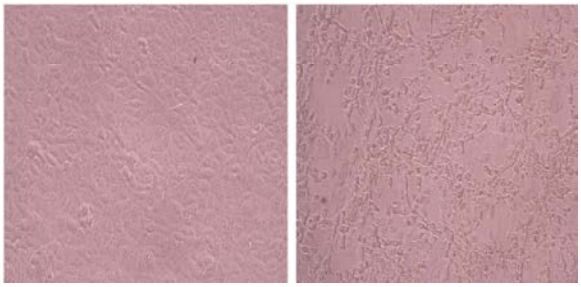

Fig. 2: Photo on the left shows normal HeLa cells. Photo on the right shows the cells four days post infection which shows necrosis and vaculation

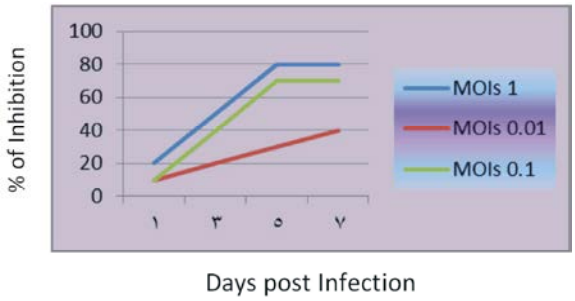

Fig. 3: Chart shows the percent of Vero cell destruction by BT at MOIs 1, 0.1 and 0.01 after 1, 3, 5and 7 days post infection. Best result obtained is at MOI 1.0.

The results of this study seems to be promising and the frequency of BTV treatment shows no significant results. Also these results suggested that the BTV may be an attractive candidate for oncolytic virotherapy of a variety of tumors and malignancy to investigate and determine the oncolytic activities and capacities of BTVs and their selective degradation of cancer cells as potential treatment of human cancers. All above data

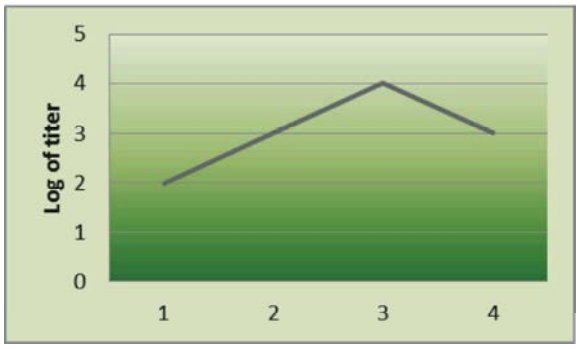

Fig. 4: In vitro BT replication in HeLa cell line after in vitro infection with an MOI of 1.0. Supernatants were collected 1, 3, 5 and 7 days post-infection and titered by serial dilution and on Vero cells. Peak viral titers measured 10^4 at 5 days after infection

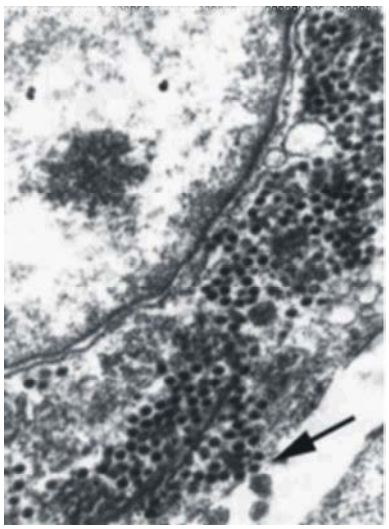

Fig. 5: Electron microscopy of HeLa cell shows dilated and condensed chromatin with accumulation of virus progeny in the cell cytoplasm

came in agreement with Hu *et al*. [8] and Xiao *et al*. [12]. Between 70 and 90% cytopathic effects (CPE) were readily observed and detected along with cellular apoptosis and necrosis in these cell lines with various degrees of morphological and cellular damage could be visualized by light microscopy.

EM Examination: Viral particles could be seen intra-cellularly and at the plasma membrane Figure 5. Dilated nuclear chromatin and their condensation and cytoplasmic shrinking were readily detected and easily observed in EM. BTV viral genomic ds-RNA fragments and viral mRNAs could be found in BTV- infected cells which is explained by Chen *et al*. [13] that the survival rate of cell lines was low if high MOI was used.

***In vitro* Assay:** After tumor cells were grown in culture and then injected as xenografts into mice (5 mice/group), tumors developed and in all positive group these mice died in less than 8 days. When 5×106 pfu of BTV in sterile phosphate-buffered saline (PBS) was injected i/m, the sizes of tumors in mice were reduced 60-85%. Most mice with the reduced tumors survived more than 35 days before they were sacrificed for tissue and organ samples (kidney, lung, liver, heart …. etc.) to detect the presence of BTVs. No BTVs and CPEs were found in these tissue and organ samples. There was also no weight loss. The tumor

The current mechanisms of oncolytic Reovirus towards human cancer cells have recently been summarized [14]. An activated Ras/RalGEF/p38 pathway is potentially involved with the permissiveness of host cells to Reovirus infection [15, 16]. Since PKR phosphorylation and activity are impaired in most cancer cells and tumor, Reoviruses can successfully evade the cellular antiviral defense system, resulting in efficient viral amplification that leads to apoptosis of the infected host cell.

Further in vivo researches and clinical studies are now in progress to document and investigate this unique oncolyitc potential of BTVs which will provide us better and safer application in humans either in monotherapy or combination therapy in the near future.

REFRENCES

1. Atherton, M.J. and B.D. Lichty, 2013. Evolution of oncolytic viruses: novel strategies for cancer treatment. Immunotherapy, 5(4): 1191-206.
2. Dock, G., 1904. Influence of complicat-ing diseases upon leukemia. American Journal of Medical Science, 127(2): 563-592.
3. Liu, T.C., E. Glanais and D. Kirn, 2007. Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. National Clinical Practical Oncology, 4(1): 101-117.
4. Huang, F., B.R. Wang, Y.Q. Wu, F.C. Wang, J. Zhang and Y.G. Wang, 2016. Oncolytic viruses against cancer stem cells: A promising approach for gastrointestinal cancer. World Journal of Gastroenterology, 22(2): 7999-8009.
5. Wildner, O., 2003. Oncolytic viruses as therapeutic agents. Annals of Medicine, 33(5): 542-556.

6. Vacchelli, E., A. Eggermont, C.S. Fridman, J. Galon, L. Zitvogel, G. Kroemer and L. Galluzzi, 2013. Oncolytic viruses for cancer therapy. OncoImmunology, 6(2): 19-25.
7. Zaher, K.S. and W.M. Ahmed, 2015. Molecular characterization of bluetongue disease. Middle-East Journal of Scientific Research, 23(11): 2689-2694.
8. Hu, J., C.Y. Dong, J.K.K. Li, J. Chen, D.E. Chen and K. Liang, 2008. Selective in vitro cytotoxic effect of human cancer cells by bluetongue virus 10. Acta Oncol., 48(1): 124-134.
9. Dong, C.Y., D.E. Chen, X. Chen, Z.H. Cao, J.R. Duan and Y.H. Liu, 1998. Isolation of Bluetongue Virus Strain HbC and its Biological Characters. The Clinic Medicine Research. Beijing: Chinese Population Publishing House, pp: 180-182.
10. Li, J., 2011. Oncolytic bluetongue viruses: promise, progress and Perspectives Frontiers in Microbiology, 2(46): 1-13.
11. Du, P., 1985. Medical experimental virology. Beijing: People's Military Medical Press, pp: 102-9.
12. Xiao, A.T., C.Y. Dong, JK.K Li, D.E. Chen, J. Liu and W.Y. Zhang, 2004. Studies on the infectivity of bluetongue virus strain HbC3 to several human and animal tumor cells. Virologica Sinica, 19(2): 349-52.
13. Chen, X., Y.F. Yan, C.Y. Dong, B.P. Chen, P. Tu and F.J. Wang, 1999. The characteristics of the propagation, purification and serology of bluetongue virus HbC Strain. Acta Acad Med HuBei, 2(3): 179- 91.
14. Vidal, L., T.A. Yap, C.L. White, K. Twigger, M. Hingorani and V. Agrawal, 2006. Reovirus and other oncolytic viruses for the targeted treatment of cancer. Target Oncol, 1(3): 30- 50.
15. Strong, J.E., M.C. Coffey, D. Tang, D. Sabinin and P.W.K. Lee, 1998. The molecular basis of viral oncolysis: Usurpation of the Ras signaling pathway by reovirus. EMBO, 17(4): 3351-62.
16. Norman, K.L., K. Kirasawa, A.D. Yang, M.A. Shields and P.W.K. Lee, 2004. Reovirus oncolysis: The Ras/RalGEF/p38 pathway dictates host cell permissiveness to reovirus infection. Proc Natl Acad Sci USA, 101(1): 11099-104.