ADENOVIRUS TYPE 5 REACTIVATION IN T- LYMPHOBLAST HUMAN CELL AFTER ETOPOSIDE TREATMENT

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ABSTRACT

Adenoviruses cause different types of human infections including, respiratory, conjunctivitis and gastrointestinal tract infection. Despite it causes infections in both immunocompetent and immunocompromised patients, it represents a real threat for the latter group which makes it necessary to detect the infection, reasons underlying reactivation and hence the appropriate treatment. The antineoplastic therapies such as etoposide could be a compromising agent that facilitates the spread of opportunistic infections such as adenovirus. The study aimed to assess adenovirus copy number/cell before and after etoposide treatment. The results showed that the etoposide treatment upregulated the replication of the virus to more than 2 fold as compared to the untreated samples. The results revealed that the etoposide has the ability to reactivate the virus when it starts to be latent.

INTRODUCTION

More than 50 adenovirus serotypes were identified with various types of human diseases such as respiratory tract infection, conjunctivitis, liver and gastrointestinal tract infection. . The virus infects both immunocompetent and immunocompromised patients (1)

Adenovirus type 5 can infect monocyte hybridoma cell line but to a lower extranet as compared to HeLa cells and the virus genome amplification was detected 2 days post infection. The culture survived the infection for one year with a 200copy/cell average (2).

Widespread adenovirus infection confirmed in viremia and respiratory samples from cancer patients using qPCR and the copy number of the virus genome was assessed. The virus resulted in 75% of the infected adults and this suggests that adenovirus could be a cause for death of immunocompromised patients (3).

In a case study, an under chemotherapy treated adult with leukemia, the patient developed bacteria chest infection which was cured and then followed by adenovirus pneumonia at 92 million adenovirus copy/ cell. The antiviral treatment failed to cure the infection since the patient died with 20 million copy of adenovirus/ ml (4). Adenovirus hepatitis caused a death for 3 and a child with acute lymphoblastic leukemia under chemotherapy when the antiviral failed in curing the infection (5). Other fatal conditions were reported after Almetuzumab immunotherapy for hematological malignancies adenovirus, enterovirus and Epstein Barr virus (6).

Un explained fever with Almetuzumab immunotherapy for acute lymphocytic leukemia might be due to a fatal activated adenovirus infection (7). In a Neuroblastoma cell line, treating cells with etoposide anti-cancer treatment induces the cellular DNA damage which is accompanied with inducing HSV-1 promoter and gene expression (8).Etopophos or etoposide phospahate and chemically is 4'-Demethyl-epipodophyllo-toxin 9-[4,6-O-(R)-ethylidene-β-D-glucopyranosideis is an antineoplastic material encouraged the reactivation of viruses such as HSV, the analysis investigated the effect of etoposide treatment on copy number of adenovirus type 5 in MOLT4 cell line .

MATERIALS AND METHODS

T- lymphoblast human cell line (MOLT4) was maintained at 37℃, 5% CO2 in 10% FCS- Dulbecco's Modified Eagle Medium DMEM. The cells were seeded 24 hours before infecting with human adenovirus type 5 . The cells were challenged with the virus at moi of 5 and left for 2 days before equivalent cultures were treated with 12.5 or 25 µM of Etoposide dissolved in DMSO and samples were harvested at 3 days post infection from treated or untreated samples to analyze the adenovirus copy number at both treated or untreated cultures before and after the treatment.

Absolute copy number assay

DNA isolation

DNA was purified by Sigma-Aldrich GenEluteTM extraction kit according the company's protocol and DNA yield was either directly used or stored at -20 ℃. The virus copy number was quantified by qPCR assay using specific primers for each target gene, adenovirus or GAPDH (9).

Real Time Quantitative Polymerase Chain reaction

For DNA copy number analysis, 5ng of total DNA was used per reaction for Ad5 and GAPDH plasmids, respectively and qPCR experiments were conducted using Stratagene MX3005P light cycler (Agilent Technologies) using the following cycling conditions: 95℃ for 3 mins, followed by 40 cycles of 1 min at 95℃, 30 sec at 55 ℃ and 30 sec at 95 ℃. The CTs was automatically determined by the machine software. Adenovirus DNA copy number was quantified from standard curves of viral genome and GAPDH plasmids.

Table-1 qPCR primers

	Forward primers 5'-3'	**Reverse Primer 5'-3'**	**Reference**
E1A	GTGCCCCATTAAACCAGTTG	GGCGTTTACAGCTCAAGTCC	**(10)**
GAPDH	CCCCACACACATGCACTTACC	CCTAGTCCCAGGGCTTTGATT	**(11)**

RESULTS

With the aim of analyzing the effect of etoposide treatment on adenovirus genome copy number in MOLT4 cells, after infecting the cells with adenovirus wt300 at moi = 5 and either treated or untreated with different concentrations of etoposide. The analysis relied on harvesting cells over a planned time course from wt300 or mock infected Molt4 cells.

Untreated culture and 6 days post infection showed an increase in adenovirus copy number while the mock infected samples showed no infection. Then the copy number started to decline in the 7^{th} day reached to more or less 150 copy/cell.

Treating the infected culture with DMSO only did not change the number of genome copies at different time points post infection but the etoposide treatment gave different pattern. Treating cells with 25 µM of etoposide slightly increased in the number of virus copies reached to 200 at 1 day after treatment. Leaving the cultures for another 24 hours boosted the number of adenovirus copies close to 500 copy/ml while DMSO or 12.5 µM did not change the number Fig 1.

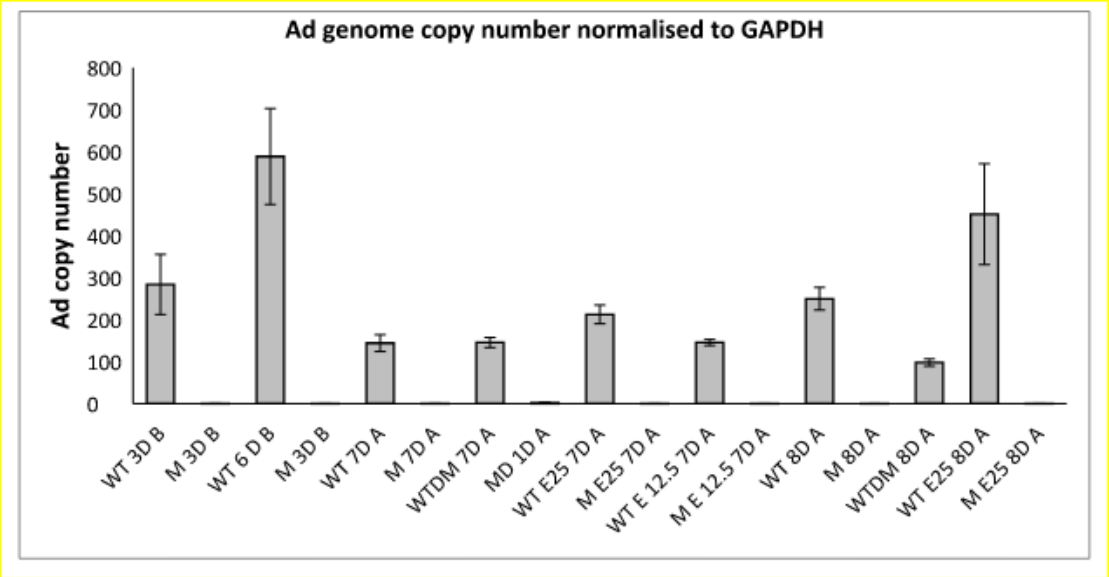

Fig.1. Ad5 genome replication. Molt 4 cells were seeded at a count of 1×105 /ml for 24 hours in 75 ml flasks. Ad5 wt300 infection was achieved at moi = 5. DNA samples were extracted from each time point (3, 6, 7,8 days post infection). Ad5 genomic copy number was quantified from standard curves of Ad5 and genomic GAPDH plasmids made by 10 fold serial dilutions and then normalized to the control sample value. Key labelling, WT= human adenovirus type; M= Mock infection; 5, 1D, 3D, 6D, 7D and 8D = number of days post infection; B= before treatment with etposide; A= after treatment with etoposide; DM= DMSO; E= Etoposide.

DISCUSSION

Adenoviruses have the ability to persist in lymphoid cells achieving appropriate modification to escape the immune response. The virus downregulates the expression of Major histocompatibility class I. The other support that the virus obtains is the A2 and DR53 human leukocyte antigens which provide the suitable environment for the virus to escape the immune response (12). Serotype 1 Spread infection was reported in a 45 years old-chronic lymphocytic leukemia woman.

Adenovirus was isolated from blood with a failure in developing appropriate anti-adenovirus antibodies (13).

Obviously treating adenovirus- infected lymphocytes upregulated the replication of the virus to more than two fold and such effect didn't belong to the effect of DMSO since the latter samples had more or less the same number of copies/ml compared to the corresponding samples before treatment. Topoisomerase inhibitors have two stages of toxicity, the short includes the gastric and myelosuppression while the long term includes the cardiac, hepatic or even secondary leukemia (14) .

Etoposide as a chemotherapeutic agent to inhibits the activity of topisomerase II, it was found to be a suppressor for the expression of the inflammatory cytokines and deficiency in number of activated T lymphocytes (15). Such effect on the immune response might ease the mission of the virus to be reactivated after treatment with etoposide and eventually spread of the virus infection in different tissues of the body.

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تنشيط العدوى بالفيروس الغدي في خلايا نوع ٥ بعد المعاملة بالايتوبوسايد في خلايا الارومة اللمفاوية بعد المعاملة بالايتوبوسايد

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الخلاصة

الفايروس الغدي يسبب انواع مختلفة من العدوى للإنسان مثل عدوى الجهاز التنفسي والقناه الهضمية وعدوى القرنية. وبالرغم من ان الفيروس الغدي يسبب العدوى في المرضى ذوي الاجهزة المناعية الطبيعة والمنخفضة الا انه يسبب تهديد حقيقي للمجموعة الثانية من المرضى مما يجعل من الضروري تشخيص الفيروس والاسباب التي تقف وراءها وايضا وصف العلاج المناسب.تسبب العقاقير المضادة للسرطانات مثل مثبطات التوبوايزوميريز كالايتوبوسايد تثبيط للجهاز المناعي للمرضى تحت العلاج لذا كان الهدف من الدراسة هو حساب عدد نسخ جينوم الفيروس الغدي قبل وبعد العلاج بتركيز مختلفة من الايتوبوسايد. اظهرت النتائج بان

معاملة الخلايا بالايتوبوسايد يزيد مستوى تضاعف الفيروس الى اكثر من الضعف بعد ان بدأ تضاعف الفيروس بالانخفاض. تظهر النتائج بان الايتوبوسايد له القابلية على اعادة تنشيط تضاعف الفيروس في الخلايا اللمفاوية .

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