Original Article

Comparison between Antitumor Activity of Live-Attenuated Measles Virus and Cisplatin on Ki-67 Expression of Colon Cancer Cell Line (SW-480) *In vitro*

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Abstract

Background: Previous studies declare that Ki-67 protein (Ki-67P) expression was shown to be correlated with cell proliferation and was considered as a prognostic marker for different cancer diseases. **Objective:** The aim of this study was to investigate the relation between Ki-67 and the cytotoxic effect of MV and cisplatin chemotherapy in inhibition of the proliferation of human colon cancer cell line. **Materials and Methods:** Colon cancer cell line (SW-480) was cultured in microtiter plates incubated in the presence of different titers of measles virus and different concentrations of cisplatin. The expression titer of Human Ki-67 protein was determined in cell culture supernatant by quantitative ELISA. The optical density (OD) was measured with spectrophotometry at a wavelength of 450 nm. The OD value is proportional to the concentration of human Ki-67P. The concentration of human Ki-67P in samples was calculated by comparing the OD of the samples with the standard curve. **Results:** The expression of Ki-67P was decreased in relation to the virus titer and concentration of cisplatin in treated cell groups. The results showed significant differences in the level of cellular expression of protein (Ki-67) between the virus-infected cells, cisplatin-treated, and the l untreated control cells group ($P \leq 0.001$). The lowest Ki-67P expression was recorded after treatment of SW-480 colon tumor cell with high concentration of measles virus. **Conclusion:** Both live-attenuated MV (Edmonton strain) and cisplatin could reduce Ki-67P expression in tumor cell line when treated with either one of them. Therefore, they have beneficial effects in reducing the resistance to chemotherapy and radiotherapy.

Keywords: Cisplatin, Ki-67, measles virus, SW-480

Introduction

Cancer is one of the worldwide leading causes of death. Although significant advances were made in cancer treatment, the mortality rates for most malignancies remain alarmingly high.[1] Colorectal cancer is the third common cancer after breast cancer in female and bronchial cancer in male and the second leading cause of death among cancers in western world.[2,3] Cancer chemotherapies were primarily selected through a panel for agents that killed the rapidly dividing cells. The cytotoxic drugs are still the cornerstone of current treatment, but the benefit was limited due to the narrow therapeutic index, harmful toxicities, and frequently arising resistance.[4] The treatment of cancers with oncolytic viruses offers a very promising approach.[5] These are "living" agents which specifically infect and kill tumor cells as part of the virus replication process. Huge numbers of progeny virions are released, which in turn attack further tumor cells.[6,7]

During the past decade, measles virus (MV) vaccine strains have emerged oncolytic platform virus, that may not be compromised by preexisting MV immunity.[8] MV vaccine Edmonston or Schwarz strains use mainly CD46 molecules to infect cells. During cancer development, tumor cells often express high levels of CD46 molecules, which inhibit the complement system.[9] This CD46 overexpression makes

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the tumor cells less sensitive to lysis by the complement but highly sensitive to MV vaccine infection.[10] These viruses, either naturally occurring or genetically engineered, are replication-competent viruses that are able to selectively infect and destroy cancer cells either intratumoral or systemic administration.[11] Oncolytic viruses display an anticancer mechanism in comparison to ordinary treatments (chemotherapy and radiotherapy), permitting the opportunity for synergistic effect in cancer treatments. Slowing the growth and progression of cancer cells is one of the major tasks that faced by modern medicine.[1] Ki-67 as a nuclear protein expressed in dividing mammalian cells is widely used in cancer biology as prognostic marker of cancer and has been widely investigated. Nishimura *et al.*[12] suggest that the Ki-67 titer before neoadjuvant chemotherapy is a sold diagnostic parameter for evaluation of the usefulness of the chemotherapies, also they found that the pathological documented response was significantly associated with Ki-67 values using different analysis. Patients with higher cell proliferation (Ki-67 >25%) may be considered better candidates for neoadjuvant chemotherapy. After neoadjuvant chemotherapy, lower Ki-67 values indicate a low chance for pathological complete response but a better prognosis.[13] The aim of this study was to investigate the relation between Ki-67 and the cytotoxic effect of MV and cisplatin chemotherapy in inhibition of the proliferation of human colon cancer cell line.

MATERIALS AND METHODS

Virus preparation

Live-attenuated MV (Edmonston) was obtained from vaccine and sera institute in Baghdad. Each vial vaccine contains 10 doses of lyophilized hyperattenuated MV (Edmonton strain); each dose contains at least 1000 CCID50. Virus was prepared as recommended by manufacturing company (serum institute of India Ltd (siil)) by adding 5 ml of sterile DDW to the lyophilized powder with continuous stirring. Then stored at -80o C till use.

Cell culture

The human colon cancer cell line (SW-480) was purchased from National Cell Bank of Iran, Pasteur Institute. They were cultured in medium 1640 (RPMI-1640, Gibco-BRL), with 10% heat-inactivated fetal bovine serum (Gibco) and penicillin/streptomycin (100 U/mL) (Sigma). Cell line was grown at 37°C incubation. Cells were plated in 25 cm^2 tissue culture flasks for the subsequent experiments. Information was recorded on data sheet and flask checked daily for observation of any changes in media and/or monolayer formation.[14]

Exposure stage

Colon cancer cell line (SW-480) was cultured in four microtiter plates of 96-well flat-bottomed and incubated in the presence of different titers of MV and different concentrations of cisplatin. The plated cells were divided into three groups: Group 1 was the untreated control group; Group 2 was MV-treated group; and Group 3 cell line that treated with cisplatin. Three

replicates were used for each dilution as well as the control wells which were treated with serum-free medium only. The cells were incubated at 37°C in CO_2 incubator for 24, 48, 72, and 96 h; then, the supernatants were collected, and the cells were detached for the estimation of Ki-67 protein (Ki-67P).

Estimation of human Ki-67 protein

Ki-67P concentrations were determined in cell culture supernatant by quantitative ELISA Kit (Abcam). The optical density (OD) could be measured with spectrophotometry at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of human Ki-67P. The concentration of human Ki-67P in samples was calculated by comparing the OD of the samples with the standard curve.

Statistical analysis

Statistical analysis was done using SPSS (statistical package for social sciences) version 20 in which we used mean and standard deviation as descriptive statistics and analysis of variance with least significant difference for comparison between groups. $P \leq 0.05$ was considered statistically significant.

RESULTS

Ki-67 profile was measured by ELISA test in supernatant of human colon cell line that was treated with different titer of MV (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}), different concentration of cisplatin (3.125, 6.25, 12.5, 25, and 50 μg/ml), and non-treated cell group for 24, 48, 72, and 96 h.

The study has shown that the Ki-67P expression in colon (SW-480) cell line that had treated with high dosage of MV was 63.529 ± 3.116 after 24 h of inoculation and then started to decrease gradually with increased postinoculation time intervals and reach its minimum value of Ki-67 expression at 96 h postinoculation which was 22.530 ± 1.167 in colonic tumor cells which was more lower than control nontreated cell group.
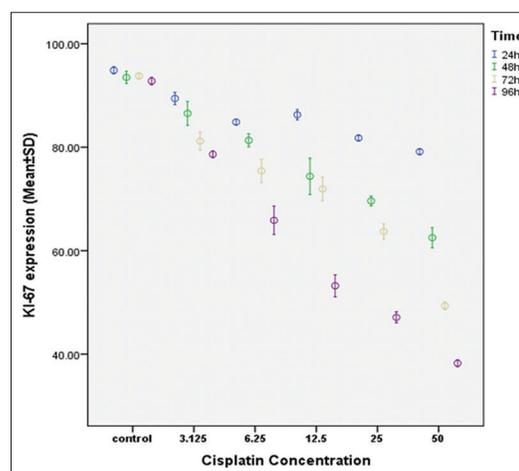

Figure 1: Influence of cisplatin on Ki-67 protein expression in SW-480 colon cancer cells. Data are represented as mean ± standard deviation (*n* = 3) (*P* < 0.001)

Table 1: Comparison between antitumor activity of live-attenuated measles virus and cisplatin on Ki-67 expression of colon cancer cell lines

Type of treatment	Concentration	Ki-67P expression (mean±SD)				*P*
		Hours posttreatment				
		24 h	48 h	72 h	96 h	
Control (SFM)	0	94.868±0.694	93.494±1.166	93.773±0.554	92.799±0.722	<0.001
MV + SFM	10^{-5}	89.279±1.455	86.513±3.325	78.037±1.559	61.947±5.604	
	10^{-4}	87.239±2.901	77.348±2.413	68.969±1.652	47.922±2.015	
	10^{-3}	81.115±1.465	68.055±0.642	62.112±2.409	40.943±1.094	
	10^{-2}	75.250±1.044	59.604±1.052	51.579±2.690	29.226±0.591	
	10^{-1}	63.529±3.116	48.174±1.387	36.763±1.113	22.530±1.167	
CIS + SFM	3.125	89.435±1.197	86.546±2.323	81.173±3.511	78.629±0.660	<0.001
	6.25	84.863±4.531	81.320±1.270	75.429±2.233	65.870±2.749	
	12.5	86.286±1.004	74.379±3.522	71.932±2.268	53.211±1.765	
	25	81.778±2.298	69.607±0.960	63.716±1.504	47.100±1.080	
	50	79.136±0.568	62.513±1.954	49.341±0.762	38.233±0.651	

SD: Standard deviation, SFM: Serum-free medium, MV: Measles virus, CIS: Cisplatin
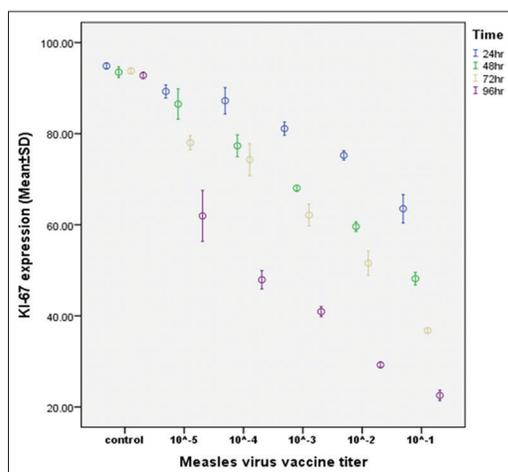

Figure 2: Influence of attenuated measles virus (Edmonton strain) on Ki-67 protein expression in SW-480 colon cancer cells. Data are represented as mean ± standard deviation ($n = 3$) ($P < 0.001$)

Whereas Ki-67P expression level after treatment with high concentration of cisplatin was 79.136 ± 0.568 at 24 h post treatment and then gradually decrease to reach its lowest mean value of Ki-67 (38.233 ± 0.651) at 96 h after treatment. [Table 1 and Figures 1 and 2] reflected significant differences in the level of KI-67P expression between cell that was treated with (measles virus or cisplatin) and untreated control group.

DISCUSSION

Beside that, Ki-67P expression titer reflects the biological activity of tumor cells; also, it clinically has clinical correlation with metastasis and stage of tumors. In addition, it is significantly higher in malignant tissues with poorly differentiated tumor cells in comparison with normal tissue.[15] It was used as a prognostic and predictive marker for cancer diagnosis and treatment, increasing the evidence which may indicate that Ki-67P may be an effective cellular target in cancer therapy. Ki-67P expression in proliferating cancer is a prognostic marker for various cancers.[16]

In the present study, the association between the Ki-67 expression and the therapeutic effects of MV and cisplatin was investigated in colon cancer cell line that treated with different titer of MV and different concentration of cisplatin at different periods of time. The results revealed a reduction in expression of Ki-67P proliferation marker in cancer cell line that was treated with MV and cisplatin in comparison with control group. Ki-67 expression was decreased with increasing titer of MV and concentration of cisplatin.

This result is consistent with other studies which were documented a reduced expression of Ki-67P proliferation markers in cancer cell line that treated with MV or cisplatin. Shi and Tian[17] reported that cisplatin can inhibit the growth of HeLa cell and decreased expression of Ki-67Ps during treatment with different concentrations of cisplatin. Zhao *et al*.[18] showed that Ki-67 expression was declined within the tumors which treated with 10^6 CCID50/ml MV, compared with the control group which treated with phosphate buffer saline. Furthermore, the result of this study is also consistent with previous studies which were documented that oncolytic virus inhibits Ki-67 expression during growth leading to inhibition of cancer cell line. Li *et al*.[19] found that adenovirus could inhibit Ki-67 expression and cellular proliferation beside induce apoptosis of prostate carcinoma *in vivo*, which is consistent with previous studies *in vitro* experiments. These observations indicated that Ki-67 may be an effective target in cancer therapy. The results documented that the inhibition of cell growth was associated with a decrement in the expression of Ki-67 protein and increased in the ratio apoptosis compared with control group. And the inhibition of cell growth an important mechanism of anti-tumor activity of measles virus and cisplatin.

CONCLUSIONS

Both live-attenuated MV (Edmonton strain) and cisplatin could reduce Ki-67P expression in tumor cell line when treated with either one of them. Therefore, they have beneficial effects in reducing the resistance to chemotherapy and radiotherapy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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