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INFLUENCE OF DEAE-DEXTRAN ON THE YIELD OF PARAINFLUENZA-3 (PI-3) VIRUS PROPAGATED ON DIFFERENT CELL CULTURE SYSTEMS

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Abstract

Infectivity titre and haemagglutination titre of PI-3 virus in MDBK cells using monolayer, suspension and roller systems revealed that the roller system produced the highest infectivity and haemagglutination inhibition titre with an increase of $\log_{10}^{8}$ than their corresponding monolayer cell culture. On the other hand, the infectivity and HA titres in cell suspension cultures were 1.0 $\log_{10}$ lower than their corresponding monolayer cell culture system.

Influence of DEAE-Dextran for the enhancement of PI-3 virus infectivity in different cell culture systems, 25ug of DEAE-Dextran per ml is considered the safest concentration to the MDBK and VERO cell lines and giving these results:

1. Infectivity titre of PI-3 virus was markedly increased under the effect of both serial passages, and showed an increase of 1.5 $\log_{10}$ and 1.3 $\log_{10}$, respectively, more than their respective non-treated passages.
2. In the roller system, DEAE-Dextran enhanced the growth of virus by about 1.0 $\log_{10}$ and 1.2 $\log_{10}$ more than growth of untreated virus in both MDBK and VERO cell lines, respectively.
3. The highest titre obtained when using DEAE-Dextran MDBK roller system was 9.5 $\log_{10}$.

INTRODUCTION

One the essential factors in preparing a good and highly immunizing inactivated vaccine is to get a virus of high titre, and consequently, more antigen in the resulting vaccine as stated by Anderson *et al.* (1971). The PI-3 virus is capable of producing great economical losses to live stock industry due to poor weight gains and stunted growth, which lead to loss in meat productions in addition to lengthening of the feeding period.

Taha (1992) studied some factors influencing the cultivation of PI-3 virus in cell culture as the sensitivity of different cell culture types. The cytopathic effect is considered the major signs of virus propagation and adaptation (Plowright and Ferris, 1959), as well as, its successful propagation of MDBK and VERO cell line in Egypt.

Polycations such as protamine and DEAE-Dextran have been used extensively to facilitate the uptake of infectious viral ribonucleic acid by cells in tissue culture (Pagano and Vaheri, 1965). Similarly, an enhancing effect was noticed with poliovirus monkey cell system rubella hamster kidney cell system and rabies virus in BHK21 cell culture (Kaplan *et al.,* 1967), as well as, respiratory syncytial virus in Hep-2 cell monolayer (Nomura, 1968), and plaque enhancement of Newcastle disease virus was recorded by Barchona and Hanson (1968). Such trials encouraged us to use DEAE-Dextran for enhancement of PI-3 virus infectivity in different cell culture systems.

The purpose of the present work is to: 1) study the highest infectivity titre and HI titre of PI-3 virus in both MDBK and VERO cells grown in various cultures (monolayer, roller and suspension) to help in choosing the best system; 2) investigate the influence of DEAE-Dextran on the yield of PI-3 virus propagated in different types of cells (MDBK, VERO) grown by different systems (monolayer, roller and suspension).

MATERIALS AND METHODS

1. Virus

PI-3 virus strain 45, the local strain was isolated and identified by Singh and B z (1966). It was used for vaccine production (Pneumo-3 vaccine).

2. Madin Darby Bovine Kidney (MDBK) cell line

It was obtained from Ames, Iowa Laboratories, USA. Cell monolayers were grown in Eagle's MEM supplemented with 10 percent newly born calf serum (Marcus and Moll, 1968) tested to be free from the non-cytopathic (NCP) BVD-MD virus.

3. VERO cell line

It was obtained from the African Horse Sickness Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The monolayer culture cells were grown in Eagle's 199 medium supplemented with 10% newly born calf serum.

4. Media and chemical reagents

a) Minimum essential medium Eagle's (MEM)

Modified with Eagle's salt without sodium bicarbonate was obtained from Flow Laboratories, UK. It was used for growing MDBK cell line culture.

b) Growth 199 medium:

Difco tissue culture medium 199 (Morgan *et al.*, 1950) could be used as a growth medium for the propagation of VERO as well as a maintenance medium.

5. Cell culture

a) Roller culture

MDBK and VERO cell cultures were propagated in 500ml and roller bottles (Strume blood plasma bottles, Arthur Thomas, Co., Philadelphia, Qa) rotated at 5 rpm. Minimum essential medium Eagle's and medium 199 were used for growing MDBK and VERO cell lines respectively, and after 48 hours, the total cell concentration in the average roller bottle was about 5×10^7 cells with 100ml growth medium supplemented with 10% calf serum, antibiotic and tryptose phosphate.

b) Cell suspension

MDBK and VERO cell cultures were grown as spinner culture in 500 ml volumes in bottles containing teflon coated magnetic bar and placed over a magnetic stirrer. Growth medium supplemented with 10% calf serum, antibiotics, tryptose phosphate were used. Viable cells as determined by trypan blue stain were counted in hemocytometer chambers.

c) Cell counting and viability

The cells were counted by using modified Fuchs-Rosenthal Chamber according to the following equation:

Cell number per one square x dilution rate x depth of the slide x 10^3 : cell number counted x 10^6 x ml. The cell viability was determined by using 0.05% trypan blue. The dead cells were stained blue, while, living ones remained unstained.

6. Diethyl amino ethyl (DEAE) dextran

It was obtained as powder from Pharmacia, Uppsaler, Sweden. It was prepared in a solution of 10 ug/ml by dissolving 0.1 gm of DEAE-Dextran (M.W. 500,000) in 100 ml of double distilled water. This was autoclaved and used at a final concentration of 25 ug/ml for both types of cells used in this study.

Formula

0.1 g DEAE-Dextran in 100 ml double distilled water (DDW) 1 x 10 x 1000 mg DEAE-Dextran in 100 ml DDW.

100 mg DEAE-Dextran in 100 ml DDW
100 x 1000 ug DEAE-Dextran in 100 ml DDW
1000 x 100 / 1000 : 1000 ug/ml
0.1 ml of DDW contains 100 ug DEAE-Dextran
0.25 ml of DEAE-Dextran + 0.75 ml of virus suspension.

RESULTS

1. Titration of PI-3 virus

MDBK and VERO cells were grown in monolayer, suspension and roller systems as previously described . The total calculated cell numbers were 5 x 10^7, 8 x 10^7 in both monolayer and roller systems, respectively, and about 2 x 10^6 per ml in suspension system. The cultures were infected with a fixed dose of virus. After 2 hours adsorption, maintenance medium was added to all cell culture systems and incubated at 37°C for about 72 hours. The presence of PI-3 virus in tissue cultures was determined by observation of characteristic cytopathic effect consisting of small highly granular giant cells, and vacuoles confirmation was obtained by haemoadsorption using the method of Vogel and Shekalov (1957). Inoculated control cultures were maintained and tested in parallel. Harvestation was done when the cytopathic effect (CPE) reached 50%, 70%, 90% and > 90% approximately. Then, the virus harvested was assayed for infectivity titres in the respective cell culture. The titre was expressed as $\log_{10}$ $TCID_{50}$ / ml virus using the formula of Reed and Muench (1938). Also, the haemagglutination unit of PI-3 was examined in the respective cell culture.

The result indicated that the virus titre increased respectively, parallel to the degree of CPE till reaching its higher level ($10^{7.3}$ and $10^{7.5}$ $TCID_{50}$/ml) at 70% and 90% CPE degrees at 72 and 96 hours post-inoculation, respectively and the titre began to decline when CPE was >90% ($10^{6.5}$ $TCID_{50}$/ml) at 120 hours post-inoculation. The maximum infectivity titres obtained at $10^{7.5}$ $TCID_{50}$/ml at 90% cytopathogenicity, but in the same time, we can say that harvested time began at 72 hours post-inoculation at 70% cytopathogenicity when the titre was more than 10^7 $TCID_{50}$/ml and avoided completely cytopathic effect which may affect the end titre.

2. Infectivity titres and haemagglutinating unit at PI-3 virus in MDBK and VERO cells using monolayer suspension and roller systems

Results were illustrated in Table 1 which demonstrated that, the roller system

produced the highest infectivity and haemagglutinating titre for both cell types. There is an increase of $\log_{10}$ 1.2 and 0.8 for MDBK and VERO cell types, respectively, than their stationary monolayer cell culture. On the other hand, the infectivity and HA titres of PI-3 virus in cell suspension culture were $\log_{10}$ 1.0 lower than their corresponding monolayer cell culture system.

3. Cytotoxic effect of different concentrations of DEAE-Dextran on both type of cell cultures

Different concentrations of DEAE-Dextran (ug/ml) were used with both types of cells chosen for virus propagation in order to determine the optimum quantity of the chemical needed to enhance the virus propagation without inducing toxic effect to cells used. Results are given in Table 2.

From the obtained data, it can be seen that,100% cytotoxic effect was noticed on treatment of MDBK and VERO cell cultures with 200 and 100 ug/ml while 50% and 25% cytotoxic effect appeared by other doses, respectively. A dose of 25 ug/ml DEAE-Dextran produced no cytotoxic effect.

4. Effect of DEAE-Dextran on the growth of PI-3 in 2 types of cells

1.5 ml of virus and 0.5ml of DEAE-Dextran (100ug/ml) were mixed together to get a final concentration 25 ug/ml of DEAE-Dextran, for both types of cell culture. Two prescription bottles were inoculated with the mixture and two prescription bottles of both types of cell, each was inoculated with 0.5 ml of virus alone. The virus harvested was assayed for virus infectivity 48 hours post-infection. Seven successive passages in the presence or absence of DEAE-Dextran were carried out and the results are given in Table 3. It is clear that the effect of serial passage of PI-3 in both cell cultures revealed an average of 7.2, 5.5 $\log_{10}$ in MDBK and VERO cell cultures, respectively, in fourth passage in the absence of DEAE-Dextran. When DEAE-Dextran was added to both cells in the first passage titres increased to $\log_{10}$ 7.8 and 5.4 at third passage and $\log_{10}$ 8.9 and 6.9 at 5th passage for MDBK and VERO cells respectively, and the titre was stable after 5th passage, since further passages provoked little or no increase in the virus titre.

5. The effect of DEAE-Dextran on the final titre of PI-3 virus propagated in different cell culture systems

In this experiment, both MDBK and VERO cell types were propagated under the

following systems: monolayer, suspension and the roller system using the previous recommended conditions with and without DEAE-Dextran.

Results of this investigation are illustrated in Table 4 where it was found that, MDBK gave higher titres than VERO cell whether propagated as monolayer or suspension or by means of the roller system. However, there was always difference of about more than 1 $\log_{10}$ when DEAE-Dextran was added to the VERO cells.

The results indicated that the roller system is much better than the other two systems whether DEAE-Dextran was added or not, since the roller system showed an increase of about 0.8 and 0.1 $\log_{10}$ than the monolayer system and about 2.0 $\log_{10}$ and 1.1 $\log_{10}$ than the suspension system of MDBK and VERO cell lines respectively.

DISCUSSION

The kinetics of virus cell interaction is a complex and implying considerable difficulties where we studied the effect of several factors on final virus yield to choose optimal conditions. This includes the ratio of the inoculated particles to the cells, the composition of the maintenance medium e.g. presence or absence of DEAE-Dextran, the conditions of virus yield and several others.

Concerning the influence of cell culture system on the final infectivity titre of PI-3 virus grown in MDBK and VERO cell lines, the results in Table 1 indicated that the roller system produced the highest infectivity titre with an increase of 0.8 $\log_{10}$ for MDBK and VERO cells than monolayer system, yet, the suspension system gave an infectivity titre of 0.6 to 1.0 $\log_{10}$ lower than the monolayer system.

The lower infectivity titres in suspended cell culture may be attributed to the action of two factors. The first was the decrease in the viability of the suspended cells, and the second factor is the rapid release of the virus from the cell, and virus stability may be affected by other culture medium and environment as confirmed by Crandell *et al.* (1975) and Ferrari *et al.* (1981).

The rapid loss of viability obtained with suspended culture does not correlate well with the development of cytopathological changes of the virus in infected monolayer on or it may be a result of temperature of incubation or by the action of culture medium as pH or presence of horse serum, and the degree of cytopathogenicity of PI-3 virus replicated in tissue culture was the essential indicator for virus yield.

DEAE-Dextran was used in this study with the aim of getting higher titres of PI-3 virus grown in both types of cells (MDBK) and VERO cells propagated by different technique. In a preliminary step, the non-cytotoxic dose of DEAE-Dextran for each type of cell was evaluated (Table 2), and a dose of 25 ug/ml was selected for virus enhancement according to Saber *et al.* (1984).

Table 3 indicated that, when PI-3 virus was propagated in MDBK and VERO cells for seven successive passages without using DEAE-Dextran, there was a progressive increase to reach a titre of 6.8 and 5.4 $\log_{10}$, respectively by the 3rd passage. When DEAE-Dextran was added, the respective titres were 8.7 and 6.8 after 4 passages, then, remained constant afterwards. The degree of cytopathogenicity suitable for harvestation of PI-3 virus was 72 hours post-inoculation at 70% cytopathogenicity according to Plowright and Ferris (1959) who reported that, total virus titre increased until about 50% CPE was reached, but despite containing destruction, virus titres did not increase significantly and avoided more progress of CPE due to the titre beginning to decline when CPE was > 90% . This may be attributed to the changes in the maintenance media including unsuitable pH, toxic destroyed cells depress, besides the long incubation time 5 days of the virus at 37°C.

Results of the effect of DEAE-Dextran on the final titre of PI-3 propagated in different cell culture systems revealed once more that DEAE-Dextran caused an increase in the final virus titre with the used system (monolayer or roller), yet, the increase was about 1.0 $\log_{10}$ for both monolayer and roller systems. This phenomenon was explained by Kaplan *et al.* (1967) as the polycations could possibility act by binding to the cellular surface, thereby, creating a favourable ionic charge for virus attachment. Another explanation is by competing with the virus particles, thereby, allowing them to attach to cell surface more efficiently (Pagano and Vaheri, 1965).

This result is very important in preparing inactivated vaccine according to Anderson *et al.* (1971) who indicated that, higher antigen doses maintained serum antibody for a longer period. This was associated with the change from IgM to IgG production. Many authors explained the role of DEAE-Dextran for improvement of the immune response of vaccines and suggested that the adjuvant activity of DEAE-Dextran could be due to a membrane effect on the immuno-component cells to become antibody producer (Wittmann, 1970, Anderson *et al.,* 1971).

Table 1. Influence of cell culture system on the final infectivity and HA titre of PI-3 virus grown in MDBK, Vero cell line.

Type of cell used	Titre expressed in $\log_{10}$ $TCID_{50}$/ml			Haemagglutination unit		
	Monolayer system	Suspension system	Roller system	Monolayer system	Suspension system	Roller system
MDBK	$10^{7.5}$	$10^{6.5}$	$10^{8.3}$	512	256	1024
Vero	$10^{6.6}$	$10^{6.0}$	$10^{7.4}$	256	128	512

Table 2. Effect of various concentrations of DEAE-dextran on both types of cell culture.

DEAE dextran	Types of cells		Percentage of cytotoxic effect
	MDBK	Vero	
200	++++	++++	100%
100	++++	++++	100%
75	++	++	50%
50	+	+	25%
30	+	-	25%
25	-	-	-
10	-	-	-

++++ 100% cytopathic effect. +++ 75 % cytopathic effect.

++ 50% cytopathic effect. + 25% no toxicity.

Table 3. Comparative titration of PI-3 virus passaged successively in both types of cells in presence and absence of DEAE-dextran.

No. of passage	Titre expressed in log_{10} $TCID_{50}$/ml in both cell type			
	MDBK		Vero	
	+ D	- D	+ D	- D
1	5.8	5	4.5	3.5
2	7	6	5.5	4.3
3	7.8	6.8	6.5	5.4
4	8.7	7.2	6.8	5.5
5	8.9	7.4	6.9	5.4
6	8.8	7.6	6.8	5.6
7	8.6	7.5	6.6	5.7

Table 4. Effect of cell culture system on the final virus titre in the presence and absence of DEAE-dextran.

Type of cells	Titre expressed in log_{10} $TCID_{50}$/ml					
	Monolayer system		Suspension system		Roller system	
	+ D	- D	+ D	- D	+ D	- D
MDBK	8.5	7.5	7.5	6.5	9.5	8.2
Vero	6.8	5.7	6.4	5.5	7.5	6.9

+ D : Presence of DEAE-dextran.

- D : No DEAE-dextran was added.

ND : Not Done.

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تأثير الديناديكستران على القوة العيارية لفيروس البارا أنفلونزا ٣ الممرد في الانظمة المختلفة لخلايا الزرع النسيجي

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في دراسة للقوة العيارية لفيروس البارا أنفلونزا-٣ في MDBK باستخدام نظم الزرع الثابت والمعلق الزرعي والزرع الدوار يتضح ان القوة العيارية للفيروس على الزرع الدوار قد زاد بمعدل عيار ٠,٨ لو.١ عن الزرع الثابت كذلك وجد انخفاض في المعيار العياري في خلايا المعلق الزرعي بمقدار ١,٠ لو.١ بالمقارنة بنظيره في نظام الزرعي الثابت عند دراسة مادة الديادكستران على زيادة القوة العيارية للفيروس المنتج.

ثبت ان اضافة ٢٥ ملليجرام لكل ملليلتر من مستحضر الديادكستران كان انسب تركيز واعطت النتائج الأتية:

١. المعيار المعدي لفيروس البارا أنفلونزا-٣ قد أرتفع بدرجة ملحوظة نتيجة لتأثير التمرير التتابعي والديادكستران في خلايا MDBK والفيرو بمقدار ١,٥ لو.١، ١,٣ لو.١ على الترتيب اكثر من نظيرها من الخلايا غير معاملة بالديادكستران.

٢. زاد نمو الفيروس باستعمال الديادكستران مستخدماً نظام الزرع الدوار بحولي ١,٠ لو.١، ١,٢لو.١ أكثر من الفيروس غير المعامل والممرر في MDBK والفيرو.

٣. تم الحصول على أعلى معيار وهو ٩,٥ لو.١ جرعة معدية بمقدار ٥٠ ٪ من الزرع النسيجي لكل ملليلتر وذلك باستخدام ديادكستران مع الفيروس المزروع في خلايا MDBK بطريقة الزرع الدوار.