

STUDIES ON DIFFERENT ADJUVANTS AND INACTIVATORS FOR RABIES VACCINE

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ABSTRACT

This work aims to determine the best inactivator which provides complete inactivation of cell culture adapted rabies virus. The use of 3 concentrations of β PL induced complete inactivation of rabies virus in final concentration ranged from 1:5000, 1:10000 and 1: 20000 at 4; 6 and 7 hours respectively. The rate of virus inactivation and complete absence of residual virus were confirmed by inoculation of BHK cell culture and weaned mice by intra cerebral route. On the other side, binary ethylenimine (BEI) was used for rabies virus inactivation by cyclization of 2-Bromoethylamine hydrobromide in alkaline solution (sodium hydroxide). Different molarities (0.001, 0.002 and 0.003) of BEI completely inactivated rabies cell culture virus after 18, 12 and 6 hours respectively and these results were confirmed by inoculation of BHK cell culture and mice observed for 15 days post inoculation to detect any signs of rabies. These findings revealed that the used concentration of β PL and molarities of BEI are the effective factors that determine the time within which complete inactivation of cell culture rabies vaccine could be obtained.

Key Words: BHK, BEI, β PL, Inactivation, Rabies virus

(BVMJ 23(2): 108-112, 2012)

1. INTRODUCTION

Rabies virus is the prototype of the genus *Lyssavirus* in the family *Rhabdoviridae*. All warm-blooded animals are vulnerable to infection with rabies virus, but mammals are the only known vectors and reservoirs in nature. Factors such as the viral variant, the quantity of virus inoculated and the bite site affects the host susceptibility. In addition, the degree of species susceptibility varies considerably [5].

Various methods, which are still valid, have been used to render the viruses non-pathogenic or essentially inactivated (Killed) as vaccines. These include, but are not limited to betapropiolacton (β PL), UV light, acetyl ethylamine as well as other amines. Phenol and formaldehyde are no longer recommended for virus inactivation

[12]. β -propiolactone, similarly to pepsin, can also reduce efficiently the complement activation by the tested sera nevertheless; the β -propiolactone treatment didn't alter neutralization titers, while considerable reduction was observed after treatment with pepsin [15].

Attyat [2] and Naglaa [8] prepared inactivated vaccines from mice brain using β -probiolactone (BPL) and binary ethyleneimine (BEI) respectively.

This work was designed as a golden goal to determine the best inactivator which provides complete inactivation of cell culture adapted rabies virus.

2. MATERIALS AND METHODS

2.1. Cell culture:

Baby hamster kidney cell line (BHK-21) established by [7] was for virus titration and determination of complete virus inactivation.

2.2. Rabies virus ERA strain:

ERA strain of rabies virus was kindly supplied by Prof. P. Sureau, the director of WHO Collaborating Centers for References and Researches in Rabies Institute Pasteur, Paris, France. It was supplied in a lyophilized form with a titer of $10^{3.5}$ TCID₅₀ / ml.

2.3. Preparation of rabies virus suspension:

In order to prepare the virus suspension, ERA virus was replicated at MOI rate of 2:1 of virus/BHK21 cells.

2.4. Virus titration:

Titration of the obtained virus was performed using the micro-titer technique and the virus titer was calculated according to [12].

2.5. Virus inactivation:

2.5.1. Virus inactivation by Binary ethylene amine (BEI):

BEI was added at 37°C to the viral suspension with a final concentration of 0.01M. The mixture was stirred continuously at 37°C according to former authors [14]. Inactivation process was stopped by addition of cold sodium thiosulphate with a final concentration of 2%.

2.5.2. Inactivation by Beta propiolactone (βPL):

The clarified harvested virus fluid was treated with βPL as 1:5000, on a magnetic stirrer for continuous stirring at 37°C according to previous authors [10]. Samples from treated virus suspensions were obtained on hour periods to estimate the rate of virus inactivation through the application of virus titration in BHK cell culture.

2.6. Mice:

Three hundred Albino Swiss mice, 3- 4 weeks old, were supplied by the Department of Pet Animal vaccine Research; Veterinary Serum and Vaccine Research Institute; Abassia, Cairo. These mice were used to determine the complete inactivation of rabies virus.

3. RESULTS AND DISCUSSION

It was demonstrated that virus inactivation by βPL is the result of alkylation of imidazol functional groups in the viral nucleic acid [4]. As shown in table (1) and figure (1); the use of 3 concentrations of βPL induced complete inactivation of rabies virus in final concentration ranged from 1:5000, 1:10000 and 1: 20000 at 4; 6 and 7 hours respectively. The rate of virus inactivation and complete absence of residual virus were confirmed by inoculation of BHK cell culture and weaned mice by intra cerebral route. In this respect; it was found that βPL inactivation rate of rabies virus differs according to source and nature of harvested material (either it is of brain or tissue culture origin) where earlier study [9] found that a dilution of 1:6000 βPL inactivated rabies virus in BHK within 2 hours, while they used βPL for vaccine of human use at concentration of 1: 4000 for 24 hours at 4°C and for 2 hours at 37°C while the virus was inactivated brain tissue with 1/4000 βPL for one hour at 37°C by [1, 6] used 1:1000 βPL to inactivate rabies virus after 6 hours at 37°C. So, it could be suggested that the required time to obtain complete inactivation of rabies virus; in addition to its origin; depends on the concentration of βPL and the process temperature. Also; as an important point of view; it was stated that βPL acts as nucleic acid denaturant and does not denaturant the virus protein but cross link nucleic acid [11]. On the other side, BEI is used in inactivation by cyclization of 2-Bromoethylamine hydro-bromide in alkaline solution (sodium hydroxide).

Table 1 Inactivation of rabies virus using β PL at 37°C

Time (hrs.)	Virus titer (\log_{10} TCID ₅₀ /ml) with different concentration of β PL		
	1/5000	1/10000	1/20000
Pre-inactivation	$10^{7.8}$	$10^{7.8}$	$10^{7.8}$
1	$10^{4.6}$	$10^{5.7}$	$10^{6.6}$
2	$10^{2.4}$	$10^{4.5}$	$10^{5.7}$
3	$10^{1.1}$	$10^{3.6}$	$10^{4.3}$
4	0	$10^{2.3}$	$10^{3.5}$
5	0	$10^{1.2}$	$10^{2.1}$
6	0	0	$10^{1.2}$
7	0	0	0

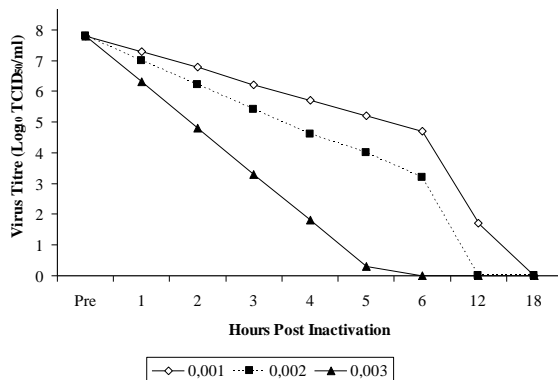


Fig. 1 Inactivation of rabies virus using β PL at 37°C

As shown in table (2) and fig (2) different molarities (0.001, 0.002 and 0.003) of BEI completely inactivated rabies cell culture virus after 18, 12 and 6 hours respectively and these results were confirmed by inoculation of BHK cell culture and mice observed for 15 days post inoculation to detect any signs of rabies. These findings revealed that the used molarities of BEI are the effective factor that determine the time within which complete inactivation of cell culture rabies vaccine could be obtained. Similar findings were recorded by former studies [1, 3, 8] using 0.003M of BEI for inactivation of CVS virus. The obtained present results indicated that both of β PL and BEI are effective agents could be used as inactivators to rabies virus with the faster one 1:5000 of β PL for 4 hours followed by 0.003M of BEI for 6 hours at 37°C.

Table 2 Inactivation of rabies virus using BEI at 37°C

Time (hrs.)	Virus titer (\log_{10} TCID ₅₀ /ml) with different molarities of BEI		
	0.001	0.002	0.003
Pre-inactivation	$10^{7.8}$	$10^{7.8}$	$10^{7.8}$
1	$10^{7.3}$	$10^{7.0}$	$10^{6.3}$
2	$10^{6.8}$	$10^{6.2}$	$10^{4.8}$
3	$10^{6.2}$	$10^{5.4}$	$10^{3.3}$
4	$10^{5.7}$	$10^{4.6}$	$10^{1.8}$
5	$10^{5.2}$	$10^{4.0}$	$10^{0.3}$
6	$10^{4.7}$	$10^{3.2}$	0
12	$10^{1.7}$	0	0
18	10	0	0

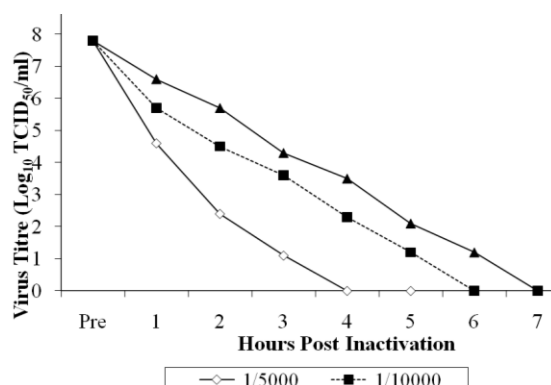


Fig. 2 Inactivation of rabies virus using BEI at 37°C.

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دراسات على تثبيط فيروس السعار باليناري إيثلين أمين والبيتا بروبيولاكتون

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الملخص العربي

تهدف هذه الدراسة لتحديد أفضل مثبط يعمل على التثبيط الكامل لفيروس السعار المرر على خلايا ، ذلك باستخدام ثلاثة توكيزات من مثبط بيتا بروبيولاكتون والذي أعطى التثبيط الكامل كان بتركيز نهائى 1 : 5000، ¼ : 10000 و 1 : 20000 عند 4، 6، 7 ساعات بالتوالى وتثبيط الفيروسى وعدم وجود بقايا فيروسى وأثبت ذلك بالحقن فى خلايا ال BHK والفئران السويسرية المفطومة بواسطة الحقن المعى. ومن الناحية الأخرى . وباستخدام اليناري إيثلين أمين لتثبيط فيروس السعار بالتحويل إلى 2-بروموايثلين أمين هيدروبرمايد فى محلول قلوئى (صوديوم هيدروكسيد)، بالتركيزات المولواتية المختلفة 0,001، 0,002 و 0,003 ثم تثبيط الفيروس المرر على الخلايا نهائيا بعد 18، 12، و 6 ساعات على التوالى وهذه النتائج تم تأكيدها بالحقن فى خلايا ال BHK₂₁ والفئران الصغيرة ومتابعتها فى خلال 15 يوم بعد الحقن لملاحظة أى علامات لمرض السعار. وهذه النتائج نجد أن التركيزات المختلفة لل BPL بيتا بروبيولاكتون والتركيزات المولاداتية لل بيناري إيثلين أمين عاملين مهمين لمعرفة الوقت للحصول على فيروس كامل التثبيط لفيروس السعار المرر على الخلايا لانتاج لقاح الفيروس المثبط.

(مجلة بنها للعلوم الطبية البيطرية: عدد 23(2)، ديسمبر 2012: 112-108)