BENHA VETERINARY MEDICAL JOURNAL, VOL. 23, NO. 2, DEC 2012: 108-112

STUDIES ON DIFFERENT ADJUVANTS AND INACTIVATORS FOR RABIES VACCINE

Amani A. Abass[a], El-Bagory, G.F.[b] and Khodeir, M.H.[a]

[a] Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, [b] Dept. of Virology, Fac. Vet. Med., Benha University.

A B S T R A C T

This work aims to determine the best inactivator which provides complete inactivation of cell culture adapted rabies virus. The use of 3 concentrations of ßPL 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 ethylenemine (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 ßPL 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, βPL, 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 (ßPL), 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 fomer 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 (ßPL):*
The clarified harvested virus fluid was treated with ßPL as 1:5000, on a magnetic stirrer for continuous stirring at 37ºc 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 ßPL 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 ßPL 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 ßPL 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 ßPL inactivated rabies virus in BHK within 2 hours, while they used ßPL 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 ßPL for one hour at 37˚C by [1, 6] used 1:1000 ßPL 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 ßPL and the process temperature. Also; as an important point of view; it was stated that ßPL BPL 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 ßPL at 37ºC

Time (hrs.)	Virus titer (log^{10} $TCID_{50}$/ml) with different concentration of ßPL		
	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

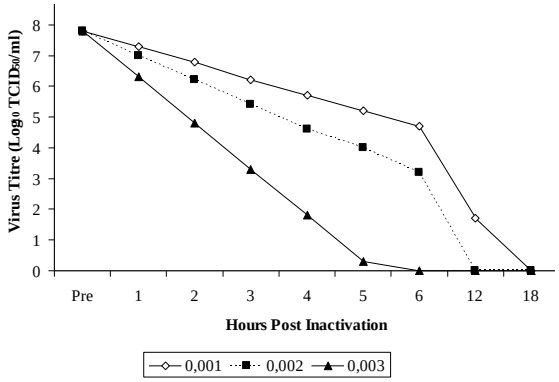

Fig. 1 Inactivation of rabies virus using βPL at 37ºC

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 ßPL and BEI are effective agents could be used as inactivators to rabies virus with the faster one 1:5000 of ßPL for 4 hours followed by 0.003M of BEI for 6 hours at 37˚C.

Table 2 Inactivation of rabies virus using BEI at 37ºC

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		

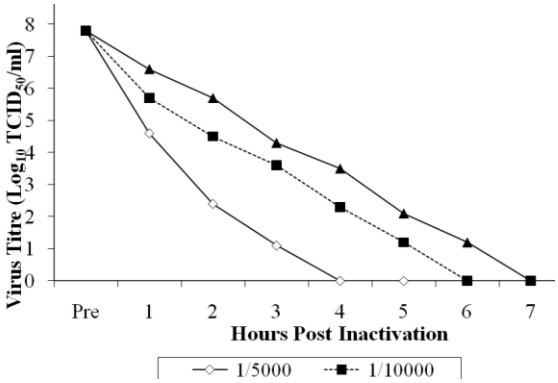

Fig. 2 Inactivation of rabies virus using BEI at 37ºC.

4. REFERENCES

1. Amany M. Abbas 2008. Effect of some inactivators on the immunogenicity of rabies virus vaccine. M.V.Sc., Fac. Vet. Med., Benha Univ.
2. Attyat, A.K. 1988. Studies on preparation of mice encephalon inactivated anti-rabies vaccine. M.V.Sci., Fac. Vet. Med., Cairo Univ.
3. Edries, S.M. 1994. Production of inactivated tissue culture rabies vaccine. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.
4. Fishbein, L., Flamm, W.G. and Flak, H.L 1970. Chemical mutagens. Academic Press INC., New York.
5. Green, C.E. and Rupprech, C.E. 2006. Rabies and other lyssa virus infections. Chapter 22. In: Infectious Diseases of the dog and cat. 3rd ed. Philadelphia, W.B. Saunders Co. Pp. 168-172
6. Gledel, I.J., Mered. B. and Sureau, P. 1968. Rabies vaccine inactivated by B-

propilactone prepared from kid brain and new born mouse brain. *Arch. S. Inst. Pasteur Algerie* **46**: 76-89.

7. Macpherson, I. and Stocker, M. 1962. Polyma transformation of hamster cell clones. An investigation of genetic factors affecting cell competence. *Nature* **201**: 1251-1256.
8. Naglaa, I. Aly 1996. Comparative studies on some adjuvants used in production of inactivated anti-rabies vaccines. M.V.Sc. , Fac. Vet. Med., Cairo Univ.
9. Nicolas, J., Falquet, R.B., Montognon, B.D. and Soulebot, J.P 1978. WHO/JABS symposium on the standariation of rabies vaccines for human use produced in tissue culture. *Develop Biol. Standard* **40**: 17-24.
10. OIE 2002. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2. 2.5. World Organization of Animal Health. 5th ed., Paris France. Vol.1. Pp. 328-346.
11. OIE 2004. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2.2.5. World Organization of Animal Health, 6th ed., Paris, France. Vol.1. Pp. 328-346.
12. Reculard, P. 1996. Cell culture vaccines for veterinary use. In: laboratory techniques in rabies 4th ed. (F-x Meslin; M.M. Kaplan and Koprowski, Eds.) Geneva: World Health Organization. Pp. 314-323.
13. Reed, L.J. and Muench, J. 1938. A simple method of estimating fifty percent points. *Am. J. Hyg*. **27**: 493- 497.
14. Rosalvo, G., Josef, M., Marco, A. Roberto Marcelino, I and Hisako, C. 2007. Effect of B-propiolactone treatment and the complement activation mediated by equine antisera.Rev. *Inst. Med. Trop. S. Paulo* **39**: 4665.
15. WHO 1973. Expert Committees on Rabies. 6th report, Technical Report Series no.523. Pp. 29-50.

مجلة بنها للعلوم الطبية البيطرية
BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

دراسات على تثبيط فيروس السعار بالينارى إيثلين آمين والبيتابروبيولاكتون

أمانى محمد عباس[1]، جبر فكرى الباجورى[2]، محمد حسن خضير[1]

[1] معهد بحوث الأمصال واللقاحات البيطرية– العباسية– القاهرة، [2] قسم الفيرولوجيا– كلية الطب البيطرى– جامعة بنها

الملخص العربى

تهدف هذه الدراسة لتحديد أفضل مثبط يعمل على التثبيط الكامل لفيروس السعار المرر على خلايا ، ذلك باستخدام ثلاثة توكيزات من مثبط بيتابروبيولاكتون والذى أعطى التثبيط الكامل كان بيتركيز نهائى 1 : 5000، ¼ : 10000 و 1 : 20000 عند 4، 6، و7 ساعات بالتوالى وتتثبيط الفيروسى وعدم وجود بقايا فيروسى وأثبت ذلك بالحقن فى خلايا ال BHK والفئران السويسرية المفطومة بواسطة الحقن المخى. ومن الناحية الأخري . وباستخدام البينارى إيثلين امين لتثبيط فيروس السعار بالتحويل إلى 2–بروموايثلين امين هيدروبرمايد في محلول قلوى (صوديوم هيدروكسيد)، بالتركيزات المولواتية المختلفة 0,001، 0,002 و0,003 ثم تثبيط الفيروس الممرر على الخلايا نهائيا بعد 18، 12، و6 ساعات على التوالى وهذة النتائج تم تأكيدها بالحقن فى خلايا الـ BHK_{21} والفئران الصغيرة ومتابعتها فى خلال 15 يوم بعد الحقن لملاحظة أى علامات لمرض السعار. وهذة النتائج نجد أن التركيزات المختلفة لـ BPL بيتا بربيولاكتون والتركيزات المولداتية لل بينارى إيثلين أمين عاملين مهمين لمعرفة الوقت للحصول على فيروس كامل التثبيط لفيروس السعار الممرر على الخلايا لانتاج لقاح الفيروس المثبط.

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