EFFECT OF ASCORBIC ACID AND RIBAVIRIN SUBSTANCES ON RIFT VALLEY FEVER VIRUS

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ABSTRACT

The present work aimed to study the potentiality of two antiviral agents; ascorbic acid and ribavirin on Rift Valley Fever virus in vivo and vitro. The study was done on BHK-21 cell line and swiss albino mice. The results revealed that the optimal concentration of ascorbic acid which gave the highest percentage of protection against RVF virus inoculation in mice was 5 µg which were detected on 3 rd and 7 Th day post injection then declined rapidly on the 10 Th day due to the rapid excretion of the drug.All concentrations of ascorbic acid (A. A.) used to study the CPE in T.c. revealed complete inhibition of the virus.Concerning ribavirin (R.n.) the best concentration injected in mice and showed the highest protection was 0.16 µg and that was clear on the 3 rd and 7 th day post treatment. Complete suppresion of the virus was also detected on infected BHK cells which was treated with various concentration of ribavirin drug. Accordingly, the two drugs can be used prophylacticaly or chemotheraputicaly for sheep and cattle subjected to RFV virus exposure or infection. Also the drugs may be used to lessen the side effects on using the living RVF virus vaccine.

INTRODUCTION

Rift Valley Fever (RVF) is a disease chiefly affecting live stock causing epizootics being responsible for great losses due to abortion of pregnant animals and heavy mortalities among young animals (**Imam *et al.*, 1978). The weekly epidemiological record (1994)** reported RVF virus infection among human and animals in Aswan Governorate.

Kadymov and Kulibekov (1975) studied the effect of vitamin C. (Ascorbic Acid)" A-A" on the immunological response of animals and they showed that utilization of Vit. C. increased during vaccination and animals, which are given additional A. A. acquired prolonged immunity. **Siegel (1975)** reported that supplemental A. A. in mice

enhanced interferon production and significantly increases T. lymphocytes. Ascorbic acid (A. A) is accepted as a general antioxidant and is a potential anti-immunosuppressive agent (**Pardue and Thaxton , 1984**).

Ribavirin (Rn.) a (1-B-D-Ribofuranosyl-1-2-4-triazole-3, 1-Carboxamide) has been shown to exhibit a potent antiviral effect against many DNA and RNA viruses both in vitro and vivo (**Sidwell *et al.*, 1972**). Toxicological studies (**Hauffman *et al.*, 1973**) have presented clues for the safety and efficacy of Rn. Both in vitro and in vivo in ribonucleic acid viruses.

The present work aimed to study the potentiality of two antiviral agents, ascorbic and ribavirin on Rift Valley Fever virus both in vivo and vitro.

Tissue culture and mice were inoculated with different non-toxic concentrations of the two drugs, then tested for cytopathic effect in tissue culture or signs of illness in mice, after RVF virus inoculation to detect the least concentration which can be used as antiviral agent.

MATERIAL AND METHODS

Materials:

1. RVF virus (ZH_{501}) strain:

It was obtained from RVF Department, Vet. Serum and Vaccine Production and Research Inst., Abbasia, Cairo. It has a titer of $10^{6.5}$ $TCID_{50}$/ml (**Taha, 1982**).

2. Cell culture (T. C.):

Monolayer BHK-21 cell culture were grown and maintained for virus titration according to **El-Nimr *et al.*, (1981)** for RVF virus titration as well as toxicity of Ascorbic acid and Ribavirin.

3- Mice:

Weaned Swiss albino mice, 21-28 days old weighing (20-30 gm) of both sex were used for titration of RVF virus as well as the toxicity of Ascorbic acid and Ribavirin.

4- Ascorbic Acid (A. A.):

It was obtained from Memphis Co., Cairo. Dilutions of A. A were made in sterile media (MEM) to get the following concentrations 1000, 100, 50, 25, 12.5, 6.25, 3.12, 1.66, 0.8 and 0.4 µg / ml.

5- Ribavirin (Rn.):

It was obtained from October Pharma Co., as a virazole capsules. Dilutions of Rn. were made in sterile media (MEM) to get the following concentrations 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.66, 0.8 and 0.4 µg /ml.

Methods:

1- Determination of Ascorbic acid and Ribavirin cytotoxicity in tissue culture:

Cytotoxicity was assessed by the addition of A. A in MEM media using concentrations of 1000, 100, 50, 25, 12.5, 6.25, 3.12, 1.66, 0.8 and 0.4 µg /ml. Five cell culture tubes of BHK- 21 cells were used for each dilution as well as control tubes. Tissue culture tubes were incubated at 37^0C for 7 days. Culture tubes were examined daily for detection of cytotoxicity compared with control T. C. tubes. The cytotoxicity of Rn. was done following the same technique of A. A. using dilutions of 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.66, 0.8 and 0.4 µg / ml.

2- Determination of A. A. and Rn. cytotoxicity in mice:

The same dilutions of A. A. and Rn. mentioned before were inoculated (0.2 ml I/P) into adult mice. 10 mice were used for each dilution as well as non-inoculated control mice. They were observed for 10 days for detection of cytotoxicity (manifested by death of mice).

3- Determination of antiviral activity in tissue culture:

Predetermined non-toxic concentrations of A. A and Rn. were added in amount of 0.1 ml to each 5 tissue culture tubes per dilution and incubated at 37^0C for 24 hours. Then the same tubes were inoculated with RVF virus (0.1 ml containing $10^{6.5}$ $TCID_{50}$/ml). Observation was done daily for 7 days for detection of C. P. E. controls included non-treated non-infected cells and cells only infected with the virus.

4- Determination of antiviral activity in adult mice:

Groups of mice were inoculated I/P with non-toxic dilutions of either A. A. or Rn. and each group contained 10 mice. Mice were challenged at 3rd, 7th, and 10th days respectively with 0.1 of 10^4 $MIPLD_{50}$/ml of RVF virus, observation was done daily for 10 days post challenge for detection of the percent of protection. Control mice either infected with RVF virus only or non-infected were included.

RESULTS

1- Determination of cytotoxicity of ascorbic acid (A. A.)and ribavirin (Rn.):

1.1. In BHK cell culture:

Results indicated that the drug at all the used concentrations, did not exhibit any cytotoxic changes in the treated cells except at 1000 µg /ml for A. A. and 200 and 100 µg /ml for Rn. respectively (Table, 1).

1.2. In adult mice:

Results of mice treated with A. A. or Rn. at the before mentioned concentrations showed that no signs of illness or deaths occurred in all the concentrations except at 1000 µg /ml for A. A. and 200 and 100 µg /ml for Rn. respectively (Table, 1).

2- Antiviral activity:

2.1. In BHK cell culture:

Ascorbic acid and ribavirin are potent antiviral agents against RVF virus. Culture did not show any C. P. E. while the non-treated virus infected culture showed clear C. P. E. (Table, 2).

2.2. In adult mice:

Results of mice treated with A. A. or Rn. that the percentages of protection at different concentrations by using A. A. or Rn. as illustrated in (Table, 3).

DISCUSSION

Vaccination is always considered as the best tool for protection and/or combating viral and bacterial diseases with respect to highly infectious and zoonotic diseases. The use of inactivated vaccines is recommended. However, the short immunization period of inactivated vaccines and the need of boostering dose open the gate for using attenuated vaccine in some occasions. Antiviral agents are now used as biotheraputic agents to stimulate the immune system in animals to enhance resistance to infectious diseases **Mouaz, *et al.*, (1995)**. These agents could be used as emergency, interfering or as a therapy for highly communicable and dangerous diseases as in case of RVF.

This work present the application of two agents A. A. and Rn. on RVF virus using BHK cells and in vivo using adult mice. Result of A. A. proved it as a potent antiviral agent against RVF virus in tissue culture (T.C.) neither inhibiting growth nor

cytotoxic on BHK cells. In mice there was no signs of illness and the percent of protection were 100% and 100% at concentration of 25μg/ml given on the 3rd and 7th days post-challenge respectively. These results agreed with **White *et al.*, (1986)** who reported that A. A. is important for the optimal functioning of the immune system. It has a direct virucidal and bactericidal activity against number of pathogens in vitro. Also A. A. enhances the production of interferon by cells infected with Newcastle disease (**Dahl and Degre, 1976**).

In case of Rn. the results were also efficient and it proved to be antiviral as it inhibit the appearance of CPE in infected BHK cells with RVF virus. In mice the percent of protection were 80% and 100% at concentration of 0.8 μg/ml given in 3rd and 7th days post-challenge respectively. Similar results were also noticed by (**Browne, 1979 & 1981**) whom found that the drug Rn. has inhibited the replication of influenza and parainfluenza viruses, so it could be considered that these results are of Rn. against many viruses.

The antiviral effect of ribavirin can be explained by the ability of the drug to inhibit cell division as reported by **Steer *et al.*, (1973)** that Rn. inhibits the host cell enzyme inosine monophosphate dehydrogenases, thus causing a depletion of the cellular guanosine nucleotide pool, or may be due to the inhibition of attachment and penetration of the virus to the cell as its effect on surface glyco-protein responsible for these processes.

Ribavirin showed no effect on growth and viability of both cell cultures (in a range of concentration of 0.4 -200 μg/ml) when such Rn. concentrations were applied to BK and VERO cell cultures infected with Rinderpest virus (RPV), no CPE were noticed for 12 days of incubation. The, in vitro, efficacy of Rn. against RPV has been confirmed by the back titration test (**Mouaz *et al.*, 1995**).

But in 10^{th} day mice treated with drugs either Ascorbic acid or Ribavirin and challenged with RVF virus were low protected by 50%-20% and this denote to the minimal concentration of these drugs inside the body and/or may be excreted during this period (10 days). Where as at high concentration of A. A. found that the level of 330 mg/kg diet for broiler chicks increases its resistance against virulent infectious bursal disease (IBD) virus (**El-Zanaty, 1994**).

However the obtained results are encouraging to use of ascorbic acid and ribavirin prophylactically or chemotherapeutically for sheep and cattle subjected to RVF virus exposure or infection. Also it may be used in vaccinated animals with living RVF virus vaccine as a trial to lessen the side effects of the virus on the general condition of vaccinated animals.

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Table (1): Cytotoxicity of Ascorbic acid (A. A.) and Ribavirin (Rn.) on BHK cell culture and in adult mice.

Concentrations of A.A (Injected amount) µg /ml	Cytotoxicity	(%) of mice death	Concentrations of Rn. (Injected amount) µg /ml	Cytotoxicity	(%) of mice death
1000 (200)	Toxic	100	200 (40)	Toxic	100
100 (20)	non-toxic	0	100 (20)	Toxic	100
50 (10)	non-toxic	0	50 (10)	Non-toxic	0
25 (5)	non-toxic	0	25 (5)	Non-toxic	0
12.5 (2.5)	non-toxic	0	12.5 (2.5)	Non-toxic	0
6.25 (1.25)	non-toxic	0	6.25 (1.25)	Non-toxic	0
3.12 (0.62)	non-toxic	0	3.12 (0.62)	Non-toxic	0
1.66 (0.33)	non-toxic	0	1.66 (0.33)	Non-toxic	0
0.8 (0.16)	non-toxic	0	0. 8 (0.16)	Non-toxic	0
0.4 (0.08)	non-toxic	0	0.4 (0.08)	Non-toxic	0
Cell control	Healthy	-	Cell control	Healthy	-
Mice control	-	no signs of illness-no death	Mice control	-	no signs of illness-no death

Table (2): Effect of Ascorbic acid and Ribavirin on BHK cells after inoculation with RVF virus.

Non-toxic concentrations of A. A. µg /ml	CPE %	Non-toxic concentrations of Rn. µg /ml	CPE %
100	0	50	0
50	0	25	0
25	0	12.5	0
12.5	0	6.25	0
6.25	0	3.12	0
3.12	0	1.66	0
1.66	0	0.8	0
0.8	0	0.4	0
0.4	0	-	-
Non-treated BHK cells and infected with RVF virus	100	Non-treated BHK cells and infected with RVF virus	100
cell control	0	cell control	0

Table (3): Effect of Ascorbic acid and Ribavirin in adult mice after inoculation with RVF virus.

Treated mice with A. A.				Treated mice with Rn.				Control inoculated mice +	control mice *
Concentrations of A. A (Injected amount) µg/ml	% of protection post virus inoculation			Concentrations of Rn. (Injected Amount) µg/ml	% of protection post virus inoculation				
	3rd day	7th day	10th day		3rd day	7th day	10th day		
100 (25)	40	100	25	50 (10)	20	100	0	Deaths (no protection)	No deaths (alive)
50 (10)	60	100	50	25 (5)	40	100	20		
25 (5)	100	100	20	12.5 (2.5)	60	100	25		
12.5 (2.5)	60	100	20	6.25 (1.25)	60	100	20		
6.25 (1.25)	60	100	40	3.12 (0.62)	40	100	40		
3.12 (0.62)	60	100	20	1.66 (0.33)	60	100	33		
1.66 (0.33)	40	100	25	0.8 (0.16)	80	100	40		
0.8 (0.16)	20	100	25	0.4 (0.08)	80	100	40		
0.4 (0.08)	20	100	20	-	-	-	-		

* Non treated and non-inoculated mice.
\+ Non treated mice inoculated with RVF virus

الملخص العربي

تأثير حمض الأسكوربيك والريبافيرين على فيروس حمي الوادي المتصدع

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في هذه الدراسة تم اختيار عقارين هما : حمض الأسكوربيك و الريبافيرين كمواد مانعة لنشاط فيروس حمى الوادي المتصدع المحقونة في خلايا كلية الجربوع الرضيعة وكذلك في فئران الألبينو السويسرية بعد معالجتها بتركيزات مختلفة من العقارين وذلك لمعرفة التأثير الباثولوجي في الخلايا أو ظهور أي أعراض مرضية في الفئران من الفيروس .

أظهرت النتائج أن التركيز المناسب لحمض الاسكوربيك والذي أعطى نسبة وقائية عالية ضد فيروس حمى الوادي المتصدع في الفئران كان ٥ ميكروجرام عند اليوم الثالث والسابع من الحقن وفى اليوم العاشر انخفضت النسبة الوقائية بسرعة ، ويرجع ذلك إلى سرعة إفراز العقار .

ولوحظ أيضا التأثير المثبط لعقار حمض الاسكوربيك على الفيروس في الخلايا المحقونة وذلك بعدم ظهور التأثير الباثولوجي على الخلايا . وبمتابعة احسن تركيز بالنسبة لعقار الريبافيرين المحقونة في الفئران والتى حقنت بالفيروس من قبل وجد ان أعلى نسبة وقائية بتأثير العقار كانت ٠.١٦ ميكروجرام وذلك عند اليوم الثالث والسابع من الحقن بالعلاج ولوحظ أن هناك أيضا تأثير مثبط لعقار الريبافيرين على الخلايا المحقونة بالفيروس .

مما سبق نستنتج أن كلا العقارين من المحتمل عند استخدامهما للوقاية أو كعلاج كيميائي مضاد لفيروس حمى الوادي المتصدع في الأغنام والماشية المصابة بالفيروس وأيضا يمكن استخدامهما للحد من الآثار الجانبية عند استخدام اللقاح الحي لفيروس حمى الوادي المتصدع .