Studies On Potential Activity Of Amantadine On Rift Valley Fever Virus

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ABSTRACT

Evaluation of the antiviral activity of Amantadine Hydrochloride (AH) against Rift Valley Fever virus (RVFV) was performed in BHK cells (monolayer and cell suspension), baby, adult mice and sheep. AH had cytotoxic effect on BHK cell suspension, while it had 50% cytotoxicity on BHK monolayer at conc. of 3 mg/ml and no cytotoxic effect on baby mice till 5mg/ml. AH stop the replication of RVFV when inoculated at the same time and before inoculation of RVFV on BHK monolayer cells at concentration of 200ug/ml. Using AH before and at the same time with RVFV inoculation in adult mice cause high protection (100%), while using AH after 24, 48 and 72 hours of RVFV inoculation in adult mice leading to 90, 60 and 20% protection respectively. In sheep using the therapeutic dose of AH before and at the same time with RVFV infection showed no elevation of body temperature, while after 24 and 48 hrs it minimize the virus titer in sheep sera.

INTRODUCTION

Rift Valley Fever (RVF) is peracute or acute disease of domestic ruminants in Africa, caused by mosquito borne virus causing high mortality rate in new born animals and abortion in pregnant ones. It is a zoonotic disease, human become infected by contact with tissue of infected animals or by mosquito bite causing severe influenza – like illness. Encephalitis and haemorrhagic disease occur and deaths *(1,2)*.

Recently, several antiviral agents have been developed for treatment of viral infection by inhibiting specific steps in the process of viral replication *(3)*.

Amantadine hydrochloride (AH) plays an important role as a broad spectrum antiviral agent which has virostatic activities against both DNA and RNA viruses *(4,5)* including equine influenza virus, hepatitis C virus, Herpes virus, respiratory equine syncytial virus infection in children and rabies in foxes.

The current work aimed to study the potential activity of Amantadine hydrochloride against the replication of Rift Valley Fever virus.

MATERIALS AND METHODS

Animals

Sheep

Twenty one adult sheep about 6 – 8 months old tested by SNT and ELISA and found to be anti body free for RVFV were used.

Swiss albino mice

Eighty adult mice about 20 gm bodyweight and Groups of 3 – 5 days old suckling mice supplied by the breeding unit, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt were used.

Tissue culture (BHK)

Baby Hamster Kidney cell line (BHK), supplied by Rift Valley Fever, (VSVRI), Abbassia, Cairo, Egypt were used.

RVF virus

ZH 501 (isolated from a human patient in Zagazig province in 1977), supplied by RVF Department, (VSVRI), Abbassia, Cairo, Egypt was used.

Antiviral agent

Amantadine hydrochloride (AH) capsule (Adamine) 100mg/capsule, manufactured by RAMEDA Company – Egypt. Ten mg / ml stock solution in MEM media, was prepared, sterilized by filtration and used in different concentrations of; 25, 50, 100, 200, 400, 500, 1000, 2000, 3000, 4000 and 5000ug/ml.

Amantadine Toxicity

Tissue culture

The different concentrations of (AH) were added to confluent BHK monolayer cells in 96 wells tissue culture plate prepared before 24 hours as well as BHK cell suspension, the

plates were incubated at 37°C and examined microscopically for 5 days.

Baby mice

Baby mice were inoculated Intraperitoneally (I/P) with 0.1ml by the different concentrations of (AH), then kept under observation for 15 days for detection of deaths or any sign of toxicity. Some of baby mice slaughtered for post mortem examination (PM).

Antiviral assay of Amantadine

Tissue culture

Confluent BHK 96 wells tissue culture plates were inoculated with nontoxic concentrations of (AH) and 10^3 $TCID_{50}$/ml of RVFV at a multiplicity of infection MOI of 1:5 of serum free media *(6)* as follows:-

1- Adding of AH to BHK plates 24 hours before inoculation of RVFV.

2- Adding of AH to BHK plates with RVFV at the same time.

3- Adding of AH to BHK plates 24 hours after RVFV inoculation.

The plates were incubated at 37°C and examined microscopically for 5 days for cytopathic effect (CPE).

Adult mice

Eight groups of adult mice (10 mice for each) were inoculated with AH for successive 10 days at concentration of 300ug/ml (therapeutic dose according to manufacturer pamphlet) and single dose of 10^4 $MIPLD_{50}$/ml of RVFV *(7)* as follows:-

1- Inoculation of mice with AH)24 hours before inoculation of RVFV.

2- Inoculation of mice with AH and RVFV at the same time.

3- Inoculation of 3 groups of mice with AH at 24, 48 and 72 hours after RVFV inoculation.

4- Three groups of mice kept as control for RVFV, AH and negative one.

Mice were kept under observation for 15 days for detection of any symptom or deaths. Dead mice were collected for post mortem examination (PM).

Sheep

Seven groups of sheep (3 for each) were inoculated with 10^5 $MIPLD_{50}$/ml of RVFV and treated with AH for 10 successive days as 15mg/kg body weight (therapeutic dose according to manufacture pamphlet) and as follows:-

1- Inoculation of 3 sheep with AH 24 hours before inoculation of RVFV.

2- Inoculation of 3 sheep with AH and RVFV at the same time.

3- Inoculation of 3 groups (3 sheep for each) with AH at 24, 48 and 72 hours after RVFV inoculation.

4- Three groups of sheep (2 for each) kept as control for RVFV, AH and negative one.

Sheep were kept under observation with daily recording of body temperature and any signs of RVF disease. Sera samples were collected from sheep groups at the peak of temperature for virus isolation.

RESULTS AND DISCUSSION

Table 1. Toxicity of Amantadine Hydrochloride on normal BHK cell and baby mice

Amantadine conc. µg/ml	BHK		Baby mice
	monolayer	Suspension	
25	Non toxic	Toxic	Non toxic
50	Non toxic	Toxic	Non toxic
100	Non toxic	Toxic	Non toxic
200	Non toxic	Toxic	Non toxic
400	Non toxic	Toxic	Non toxic
500	Non toxic	Toxic	Non toxic
1000	Non toxic	Toxic	Non toxic
2000	Non toxic	Toxic	Non toxic
3000	50% Toxic	Toxic	Non toxic
4000	100%Toxic	Toxic	Non toxic
5000	100% Toxic	Toxic	Non toxic
Cell control	Non toxic	Non toxic	Non toxic

Table 2. Effect of Amantadine Hydrochloride on RVF infected BHK monolayer cells

Amantadine conc. µg /ml	Time RVFV inoculation		
	Before	At the same time	After 24 hour
25	*CPE	CPE	CPE
50	CPE	CPE	CPE
100	CPE	CPE	CPE
200	NO CPE	NO CPE	CPE
400	NO CPE	NO CPE	CPE
500	NO CPE	NO CPE	CPE
1000	NO CPE	NO CPE	CPE
2000	NO CPE	NO CPE	CPE
3000	NO CPE	NO CPE	CPE
Cell control	NO CPE	NO CPE	NO CPE
Virus control	CPE	CPE	CPE

CPE = cytopathic effect

Table 3. Effect of Amantadine Hydrochloride on RVF infected adult mice

Groups of mice	No .of mice	Number of mice at Days post infection											Survival. %	Mortality.%
		1	2	3	4	5	6	7	8	9	10	15		
24 h Before RVFV	10	10	10	10	10	10	10	10	10	10	10	10	100	0
With RVFV	10	10	10	10	10	10	10	10	10	10	10	10	100	0
24h After RVFV	10	10	10	10	10	10	10	10	10	9	9	9	90	10
48h After RVFV	10	10	10	10	8	8	7	6	6	6	6	6	60	40
72h After RVFV	10	10	10	9	7	6	4	2	2	2	2	2	20	80
Control +ve	10	10	10	10	8	5	2	1	0	0	0	0	0	100
Control – ve	10	10	10	10	10	10	10	10	10	10	10	10	100	0
AH control	10	10	10	10	10	10	10	10	10	10	10	10	100	0

Table 4. Mean body temperature of RVF infected sheep treated with Amantadine Hydrochloride

Groups of sheep	No .of sheep	Days post infection										
		0	1	2	3	4	5	6	7	8	9	10
24 h Before RVFV	3	38.4	38.4	38.5	39	38.5	38	38	38.3	38.5	38.5	38.2
With RVFV	3	38.5	38.4	38.9	39	38.5	38.1	38	38.3	38.5	38.7	38.2
24h After RVFV	3	38.6	38.8	39.3	39.5	39.3	39	39.4	39	38.5	38.5	38.3
48h After RVFV	3	38.5	38.5	39.7	40	39.5	39	39	39.4	39	38.8	38.5
72h After RVFV	3	38.3	39.2	40.1	41.3	40.2	39.5	39.7	39.8	39.5	39.3	39.1
Control +ve	2	38.5	39.3	40.5	41.7	40.5	39.7	39.8	40	39.8	39.5	39.2
Control – ve	2	38.3	38.5	38.2	38.2	38.3	38.5	38.4	38.1	38.3	38.5	38.2
AH control	2	38.2	38.4	38.3	38.2	38.1	38.3	38.1	38.3	38.2	38.1	38.2

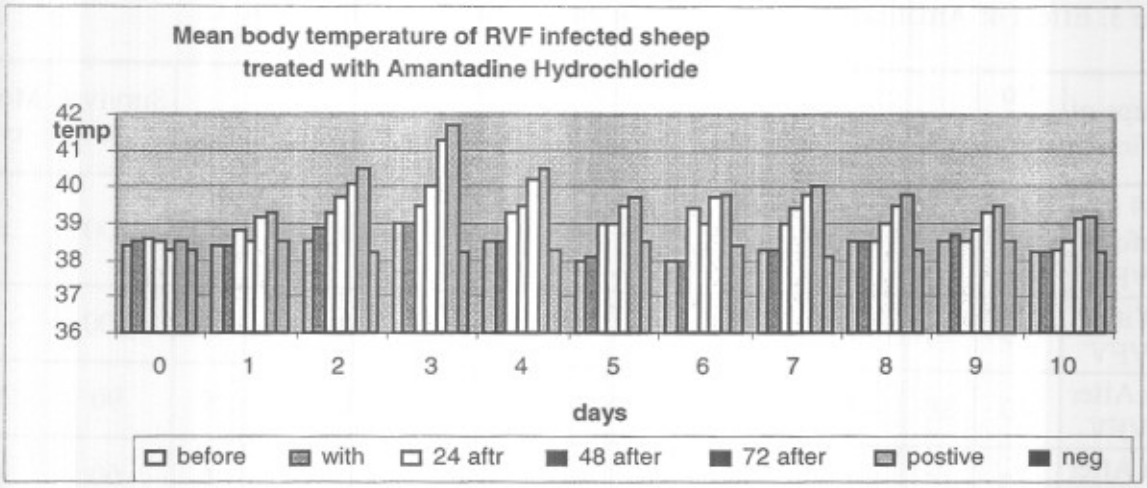

Table 5. Virus isolation and titration from sera of different sheep groups at 3rd day post RVFV inoculation

Sheep groups	24 h Before RVFV	RVT	With RVFV	RVT	24h After RVFV	RVT	48h After RVFV	RVT	72h After RVFV	RVT	Control	
											RVFV	AH
Virus titer*	1.2	6.6	2.3	5.5	2.7	5.1	2.9	4.9	7.5	0.3	7.8	0.4

* Virus titer expressed as log 10 $TCID_{50}$ / ml. RVT=reduction of virus titer.

Amantadine hydrochloride (AH) is effective in the prophylaxis of equine influenza A virus *(4)* Borna disease virus affect horses, sheep, cattle, cats, dogs and ostriches *(8)* and herpes virus *(3)*.

AH was the first highly specific potent antiviral drug effective against any virus (9) at low conc. specifically inhibits influenza A virus.

Table 1 showed results of cytotoxicity of different concentrations of Amantadine on monolayer, cell suspension of BHK and baby mice. AH had cytotoxic effect on BHK cell suspension, while it had no cytotoxicity effect till 3000ug/ml and 5000ug / ml for BHK monolayer and baby mice respectively. These results are consistent with those previously reported *(10)* who reported that AH had 50% cytotoxic effect on BHK monolayer at conc. of 3000 ug/ml. PM examination of slaughtered mice cleared normal liver, spleen, intestine, kidney and brain. Similar results reported that antiviral agent had minimum toxicity for normal host cell *(11)*.

Table 2 revealed that AH stop the replication of RVFV when inoculated at the same time and before inoculation of RVFV on BHK monolayer cells at conc. of 200 ug/ml (50% inhibitory concentration) "IC50" till 3mg/ml . It has been cited previously that AH had antiviral effect on infected tissue culture *(12)*. AH had antiviral effect at conc. of 100 ug/ml till 3mg/ml when inoculated with FMD virus at the same time on monolayer BHK *(10)*. While using AH after 24 hours of RVFV inoculation on BHK monolayer had no effect on RVFV replication.

Table 3 explained that using AH 24h before and at the same time with RVFV inoculation in adult mice had high protection (100% survival percent till 10 days observation), while using AH after 24, 48 and

72 hours of RVFV inoculation in adult mice leading to 90, 60 and 20% protection respectively, AH had antiviral effect on influenza A virus when administered in the first 24 hours to 48 hours of infection and be given at high dose for at least 10 days *(9)*. PM examination of dead mice showed typical RVFV hepatic lesion and severe congestion of internal organ. Similar PM lesions were recorded previous study *(13)*.

Table 4 revealed that using AH as a therapeutic dose for sheep for 10 days at different times; 24h before and at the same time with RVFV infection showed no elevation of body temperature for 10 days, while when used after 24 and 48 hours of RVFV infection showed mild elevation of temperature (39 and 39.5^{O}C respectively), while high elevation of body temperature (41.3^{O}C) was recorded after 72 hours with watery nasal discharges and diarrhea, this picture of symptoms were similar to that of positive control sheep. Similar findings were previously cited *(14)*.

Table 5 recorded the titer of RVFV isolated from sera of different sheep groups using BHK cell line at the 3rd day (peak of temperature) which cleared that high virus titer was 7.5 and 7.8 log 10 $TCID_{50}$/ml of sheep group treated with AH after 72 hours post RVFV infection and positive RVFV sheep control respectively.

It can be concluded that Amantadine Hydrochloride could be used as prophylactic and treatment of infected animals during RVF outbreak by reduction of RVFV titer in serum of infected animals.

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الملخص العربي

دراسات لمعرفة تأثير نشاط الأمنتادين على فيروس حمى الوادي المتصدع

تراضي عبد الفتاح سيد ، ألفونس مينا إبراهيم ، كريم الدين زكى حسن

تم تقييم الفعل المضاد لمادة الأمنتادين هيدروكلوريد ضد فيروس حمى الوادي المتصدع في كل من خلايا كلية الجربوع الرضيع والفئران السويسرية الرضيعة والبالغة والأغنام.

وُجد أن: ٣ ميلليجرام/مل من الأمنتادين له ٥٠% تأثير سام على خلايا كلية الجربوع الرضيعة وليس له تأثير سام على الفئران السويسرية الرضيعة حتى ٥ مجم / مل و٢٠٠ميكروجرام / مل يثبط فيروس حمى الوادي المتصدع على خلايا كلية الجربوع الرضيعة عند حقنه في آن واحد أو قبل حقن الفيروس على الخلايا.

أما في الفئرا ن السويسرية البالغة عند استخدامه كجرعة علاجية ٣٠٠ميكروجرام/مل لمدة عشرة أيام قبل ومع الحقن بالفيروس كانت نسبة الوقاية ١٠٠%.

لم يظهر إرتفاع في درجة حرارة الأغنام عند حقن الجرعة العلاجية للأمنتادين قبل ومع حقن فيروس حمى الوادي المتصدع بينما إستخدامه بعد ٢٤و٤٨ساعة من حقن فيروس حمى الوادي المتصدع أدى إلى تقليل القوة العيارية للفيروس في المصل الأغنام.