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RINDERPEST VIRUS INHIBITION IN VITRO BY RIBAVIRIN

By

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SUMMARY

The effect of the broad spectrum antivirus ribavirin has been tested against the replication of rinderpest virus (RPV) in bovine kidney cells (BK) as aprimary culture and vero cell line culture.

Ribavirin showed no effect on growth and viability of both cell cultures in a range of 0.1-200 Ug/ml. When such ribavirin concentrations were applied to BK and vero cell cultures, infected with RPV, no cytopathic effects were detected for 12 days of incubation. The efficacy of ribavirin against RPV in vitro has been confirmed by the back titration test.

INTRODUTION

Ribavirin (1-B-D- Ribofuranosyl -1,2,4-triazole-3,1 carboxamide) has ben shown to exhibit a potent antiviral effect against many DNA and RNA viruses in vitro and in vivo (Sidwell et. al., 1972 and 1979) Ribavirin has been used in clinical trials in the United States of America, Mexico and Brazil with promising results in treating measles (Mannon and Arroyo, 1977), herpetic gingivostomatitis (Dib et. al., 1977), hepatitis A (Galvao & Castro 1975) , influenza (Magnussen et .al., 1977) and genital infections caused by herpes virus -2 (Sidwell et. al., 1979)

Toxicological studies (Hauffman et al., 1973; Sidwell et al., 1972 and 1973) have presented clues for the safety and efficacy of ribavirin both in vitro and in vivo.

Rinderpest virus (RPV) is a member of the Paramyxovirdae family and causes fatal disease in cattle. Egypt was considered to be free from Rinderpest (Abdel-Ghaffar et al. 1977) , until the devastating epidemic which commenced in 1982. In addition, the disease is still endemic and epidemic in most of the Near and Far East countries and most of the African countries.

The present work aimed to study the poteniality of the antiviral ribavirin (Virazole) on rinderpest virus (Live atttenuated vaccinal strain) in vitro.

MATERIAL AND METHODS

1- Ribavirin:

Ribavirin was obtained from Dr.Roberts (Viratek, Inc. U.S.A.) Stock solution of ribavirin was prepared by dissolving 100 mg in 10 ml Hank's balanced salt solution (HBSS, Difco) and sterilized by filtration through a 22 um membrane (Millipore Inc.). Dilutions of ribavirin were made in sterile HBSS at the following concentrations: 200, 100,50,10,5,1, and 0.1 Ug/ml.

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2- Rinderpest virus:

The rinderpest bovine old Kabete (RBOK) strain of virus was used at its 101 st passage on bovine kidney cells (BK), being propagated on vero cells for one passage. The virus titre was 10^6 $TCID_{50}$/ml. (Osman et al., 1990).

3- Cell cultures:

3-1 Bovine kidney cell culture:

Primary boine kidney cell culture (BK) were prepared routinely according to method of Hancock et al .,(1959) as modified by Singh et al., (1964). Bovine kidneys were obtained aseptically from one year old calves, promptly after slaughtering at Cairo abbatoir.

3-2 Vero cells:

African green monkey kidney cells (yero) established by Yasumura and Kawatika (1963) were maintained as stock cultures. Cells were propagated as monolayers throughout the study.

4- Cell culture medium and supplements:

Minimum Essential Medium (MEM) with Hank's salts (Eagle, 1959) was used for cell culture preparation and cell passages. It was supplemented with new born calf serum (mycoplasma and virus , free) as 10%.

The medium contained 50 Ug of streptomycin and 100 units of penicillin/ml of growth medium. Cell monolyers were established in Roux bottles at 37°C. Monolayers were dispersed in a fresh medium and dispensed automatically in tissue culture tubes (160 x 15 mm) at a density of approximately 10^4 cell /tube.

5- Determination of ribavirin cytoxicity:

Tube cultures of BK and vero were incubated at 37°C till semiconfluent monolyers were reached. Growth medium was replaced by a fresh one before the addition of ribavirin. cytotoxicity was assessed by the addition of 0.1 ml of ribavirin solution in HBSS per tissue culture tube using concentrations of 200, 100, 50, 10,5,1 and 0.1 Ug/ml. Five cell culture tubes of BK and vero cells were used for each dilution as well as control. Tissue culture tubes were incubated at 37°C for 12 days with changing the medium at three days interval.

Cutures were examined daily for detection of cytoxicity compared to control for 12 days.

6- Determination of the antiviral activiy:

The procedure included the infection of semi confluent BK and vero cell culture tubes with (RBOK) virus strain using 0.1 ml inoculum of virus (10^6 $TCID_{50}$ ml). Following 1 hour adsorption of virus; infected tubes were washed to remove unadsorbed virus and then supplied with a freshly prepared complete growth medium in one ml volumes. Non toxic concentrations from ribavirin were added in 0.1 ml to each of 5 culture tubes per dilution. Controls included non-treated unifected cells and cells infected with the virus. For each control tube, 0.1 ml of HBSS was added. All culture tubes were incubated at 37°C for 12 days with daily inspection for cytopathic effect (C.P.E.).

7- Back titration:

At the end of the incubation period, all cell culture tubes were maintained at- 70°C and then frozen and thawed for three cycles. Cell homogenates were then centrifuged for 30 min. at 2000 r.p.m. at 4°C. Supernatant fluids of both cells infected with virus, and virus infected cells, co-treated with

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ribavirin, were used for the assay, Virus titer was estimated according to Read and Muench method (1938), for all used dilutions. The titer was expressed in log_{10} $TCID_{50}$ per ml homogenate.

RESULTS

1- Cytotoxicity:

BK and vero cell monoloyers were treated with ribavirin concentrations in doses of 200,100,50,10,5,1 and 0.1 Ug/ml. The incubated cell cultures were inspected daily for detection of any sign of cell toxicity up to the 7th day post treatment. During the incubation period growth medium was changed by a fresh one at day 4. Comparisons were made between controls and ribavirin - treated cultures. Results indicated that the drug at all concentrations used , did not exhibit any cytoxicity changes in the treated cells. Therefore, all the used concentrations were applied for the examination of their effect on rinderpest virus infection of cells.

2- Antiviral activities:

Results in table 1 show data of two experiments, clearly, ribavirin is a potent antiviral agent against rinderpest virus. Compared to RPV-infected cultures, the ribavirin-treated and infected cultures did not show any cytopathic effect. Virus infected non treated cultures commenced to produce C.P.E at the third day post infection (+). Maximum C.P.E. accompanied by polykaryocyte formations was reached at day 6p.i. (++++).

3- Back titration of RPV:

Virus infected cell cultures, treated as well as non treated with different concentrations of ribavirin, were frozen and thawed three times. Each set was pooled together, centrifuged and the supernatant was used in 0.1ml/cell culture tube of BK as well

Table 1: Cytotoxicity and percentage of cytopathic effect (C.P.E.) of ribavirin treated and rinderpest virus infected bovine kidney and vero cells . Cultures,incubated at 37°C for 12 days .

Treatment (Ug/ml)	Cytotoxicity		% C.P. E.	
	BK	VERO	BK	VERO
Control cells	healthy	healthy	0	0
Infected cells+0.1Ug ribavirin	"	"	0	0
" " + 1 Ug "	"	"	0	0
" " + 5 Ug "	"	"	0	0
" " + 10 Ug "	"	"	0	0
" " + 50 Ug "	"	"	0	0
" " + 100 Ug "	"	"	0	0
" " + 200 Ug "	"	"	0	0
Cells + Virus alone	CPE		100	100

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Table 2 : Back titration and C.P.E. of rinderpest virus (RPV) in BK and vero cell cultures previously infected with RPV alone (control) or treated with different concentrations of ribavirin

Treatment	C.P.E		Titer(Mean Log $TCID_{50}$ /ml)	
	BK	VERO	BK	VERO
RPV alone "(Control)	+	+	6.5	6
RPV + 0.1 Ug/ml	-	-	0	0
RPV + 1 Ug/ml	-	-	0	0
RPV + 5 Ug/ml	-	-	0	0
RPV + 10 Ug/ml	-	-	0	0
RPV + 50 Ug/ml	-	-	0	0
RPV + 100 Ug/ml	-	-	0	0
RPV + 200 Ug/ml	-	-	0	0

as vero cells. Results obtained throughout 12 days of incubation are shown in table 2. All homogenates of RPV-infected cultures and treated with ribavirin did not show any sign of cytopathology.

This effect was true for all used concentrations. However, homogenate of virus-infected cells showed C.P.E. and the titre was 10^6 $TCID_{50}$ /ml. Accordingly, back titration confirmed the antiviral activity of ribavirin and even the inactivation of the RPV inoculum.

DISCUSSION

This paper presents the first report on the application of the antivitus ribavirin on rinderpest virus in vitro using bovine kidney and vero cell cultures.

Our results indicated the efficacy and safety of ribavirin as a potent anti RPV in both cell cultures. Neither inhibition of growth nor cytotoxicity, have been produced, on BK and vero cells.

It could be considered that these results are extra addition to the broad spectrum of ribavirin against many viruses. This drug has been reported to inhibit the replication of influenza and parainfluenza viruses (Browne 1979, Browne et al. 1980, and 1981).

It has been reported that ribavirin inhibits the host cell enzyme inosine monophosphate dehydrogenase, thus causing a depletion of the cellular quanosine nucleotide pool (Streeter et al., 1973) . However, some authors have claimed that

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the antviral action is similar to the role of actinomycin D which causes inhibition of cellular m RNA synthesis (Barry, 1964).

Actinomycin D affects Orthomyxoviruses including influenza virus but has no inhibitory effect on Paramyxoviruses including RPV.

As it may be the mechanism of action of ribavirin on either rinderpest virus , BK or vero cells, it is worth elucidation of the cause (s) of its differential effect.

However, the obtained results are encouraging to test the use of ribavirin, prophylactically or chemotherapeutically for cattle subjected to rinderpest exposure or infection.

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