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INHIBITORY EFFECT OF AMANTADINE HYDROCHLORIDE ON BOVINE VIRUS DIARRHEA AND SF-4 VIRUSES

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Certain myxoviruss and several strains of human influenza and parainfluenza viruses are inhibited by amantadine hydrochloride (1-adamantanamine hydrochloride) both **in vitro** and **in vivo.**[1,2,5] The evaluation of amantadine in the prevention of influenza in humans has been discussed. Other viruses, such as rubella, pseudorabies, and fowl plague viruses are also sensitive to this drug.[3,4] Recently, the drug was shown to inhibit murine sarcoma viruses in cell cultures.[6]

The effect of amantadine hydrocloride on the multiplication of bovine virus diarrhea (BVD) and SF-4 (bovine myxovirus parainfluenza-3) viruses in primary bovine embryonic kidney (BEK) cell culture are presented in this report.

MATERIALS AND METHODS

Viruses. Cytopathic strain of BVD virus (NADL) and a strain of SF-4 virus had undergone many passages in BEK cell cultures in our laboratory. The BVD virus had a 50% tissue culture infective dose ($TCID_{50}$) titer of 10^6/ml and the SF-4 virus had a $TCID_{50}$ titer of 10^5/ml.

Cell cultures and media. Routine maintenance medium (MM) for primary BEK cell cultures was Eagle's basal medium containing Earle's salts and 2% bovine fetal serum (free of adventitious viruses) and the usual concentration of antibiotics.

Amantadine preparation. Amantadine hydrocloride** (symmetrel) stock solutions were prepared as 1 mg/ml in MM. Further dilutions of the drug into different concentrations were made using MM as diluent.

Infectivity titration. Virus titrations were made in BEK cell cultures by two methods. In one method, the BEK cells were washed with Hank's balanced salt solution (BSS) and 1 ml of MM containing various concentrations of amantadine was added. The monolayers were infected with undiluted virus using 0.1 ml per tube. Five tubes were inoculated for each dilution of the drug and cultures were then incubated at 37 C for 24 hours. At the end of this incubation period, the drug treated and control cultures were frozen at-70 C, thawed once and pooled. After low centrifugation, viral analysis was made on the supernatant

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fluid. Three to four BEK culture tubes were used for each virus dilution using 0.1 ml inoculum per tube. After 7 day incubation at 37 C, the cells were examined for cytopathic effects (CPE).

*Courtesy of Dr. J. H. Gillespie, Cornell Veterinary College, Ithaca, N. Y.

**Courtesy of E. I. DuPont Co., Wilmington, Del.

Any evidence of CPE, even if a small focus, was considered as a criterion of positive response. In addition, hemadsorption test was used for SF-4 virus assay.

In the second method, the cells were washed with BSS, and 1 ml of MM containing various concentrations of amantadine was added to each tube. Ten-fold dilutions of virus was made in BSS and three to four tubes were infected with each dilution using 0.1 ml inoculum per tube. The cultures were incubated and scored as above. The drug was present throughout the incubation period.

Viricidal effect. The possibility of a viricidal effect by amantadine was examined by mixing undiluted virus with various concentrations of the drug in MM. The mixture was held at 37 C for 2 hours and then titrated in drug-free MM as described above.

RESULTS AND DISCUSSION

The tests were repeated at least twice to ensure validity of the results. The effect of amantadine hydrochloride on the multiplication of BVD and SF-4 viruses is summarized in Table 1.

At 50 and 100 μg/ml amantadine inhibited the production of BVD and SF-4 viruses being most effective at 100 μg/ml. The drug was ineffective at 25 μg/ml. Compared to nontreated control cultures, the BVD virus was inhibited approximately 30 to 100 times in the presence of 100 μg/ml of the drug and there was only 3 to 10-fold loss in infectivity titer at 50 μg/ml. The SF-4 virus was inhibited 30-fold at 100 μg and 10-fold at 50 μg levels. Cultures with 100 μg/ml exhibited slight cytotoxicity as the treatment period lengthened. The drug was extremely cytotoxic at 200 μg/ml. No viricidal effect on BVD and SF-4 viruses was demonstrated in direct contact for 2 hours with amantadine at different concentrations. The infectivity titers of drug treated and untreated viruses were the same, indicating that the antiviral activity is not due to direct inactivation of virus.

There is evidence to show that amantadine acts by blocking the penetration of virus into the cells.[3] However, antiviral activity of amantadine due to inhibition of uncoating of virus in the cells has also been demonstrated.[4] It has also been shown that the drug is most effective when the compound is added to cell cultures at the time of virus infection.[3-5]

Our experiments indicate that amantadine inhibits the production of BVD, a RNA helical virus and SF-4, a paramyxovirus in BEK cell cultures. Optimal conditions for this inhibitory effect and the effect of this drug on these viruses **in vivo** are under further investigation.

SUMMARY

Amantadine hydrochloride (1-adamantanamine hydrochloride) inhibited the production of BVD and SF-4 viruses in primary embryonic kidney cell cultures. The antiviral activity was not due to direct inactivation of the virus.

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TABLE I

Effect of Amantadine hydrochloride on bovine virus diarrhea virus and SF-4 virus production

Virus	Amantadine HCl (μg/ml)	Virus yield* ($TCID_{50}$/ml)	Virus yield** ($TCID_{50}$/ml)
BVD	100	3.2×10^3	3.2×10^3
	50	3.2×10^4	3.2×10^4
	25	3.2×10^5	1.0×10^5
	0	3.2×10^5	1.0×10^5
SF-4	100	1.0×10^4	3.2×10^4
	50	3.2×10^4	1.0×10^5
	25	3.2×10^5	1.0×10^6
	0	3.2×10^5	1.0×10^6

*Determined 24 hours after beginning of incubation in contact with amantadine HCl.

**Determined in the presence of the drug throughout the incubation period.

BVD=Bovine virus diarrhea virus.

SF-4=Bovine myxovirus parainfluenza-3 virus.