CHEMOTHERAPEUTIC STUDIES ON MOUSE HEPATITIS VIRUS (3)

Antiviral Effect of Some Pharmacodynamic Drugs

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Introduction

There have been published many reports concerning the antiviral agents, but none of them has proved to be effective until recently in clinical trials (TAMM 1962, KAUFMANN 1963). The relationship between mouse hapatitis virus (MHV) and human hepatitis virus is one of the most important problems to apporoach to the chemotherapy of human viral hepatitis.

The chemotherapeutic effect of some drugs on MHV have been published elsewhere but none of them possessed the complete effectiveness *in vivo*. Xenaldial and its derivatives have been reported as a most potent antiviral agent by MAGRASSI, *et al.* (1961), Benadryl also reported as effective on MHV *in vivo* by JUDAH (1965), and *p*-carboxy-N-methylacetyl nicotinic acid (CDO3) is a synthetic drug derived from e-amino-nicotinic acid which possessed many pharmacological activities. Formycin is one of the antibiotics which has been found by UMEZAWA (1965), but it have never been tested on the antiviral activity on mouse hepatitis virus.

Present report described on the comparative chemotherapeutic studies of these drugs on MHV *in vitro* and *in vivo*.

Materials and Methods

1) Virus ;

Mouse hepatitis virus EHF-120 strain was used throguhout the experiments. This strain was first isolated in Japan by BUCHER (1952). Virus solution was prepared from the infected liver mice as 10% of homogenate in sterile saline. Virus was inoculated 0.2 ml per mouse intraperitonealy.

2) Mice ;

White swiss mice weighing 9~10 g were used in all experiments.

3) Drugs ;

Xenaldial, Benadryl hydlochloride, *p*-carboxy-N-methylacetylnicotinic acid (CDO3) and Formycin were dissolved in distilled sterilized water, and they were injected 0.1 ml per mouse intraperitoneally or subcutaneously. After infection mice were observed for 7 days and mortality was estimated.

Results

I) Direct inactivating effect of Xenaldial on MHV *in vitro*.

One hundred μg of Xenaldial were added to the 10^{-1} and 10^{-2} dilutions of virus and incubated at 8°C for 2 days ; after the incubation, these solutions were individually injected 0.2 ml per mouse to every 10 mice intraperitonealy and mortality by infection was observed. As the control 100 μg and 10 μg of formalin were also used. After the injection mean survival time of dead mice (days) was estimated. The results are ahown in Table 1. As shown in the table 100 μg of Xenaldial has a more potent inactivating effect than formalin to both dilutions of virus. One hundred μg of Xenaldial completely inactivate the virus to both dilutions of virus.

II) Chemotherapeutic effect of Xenaldial, Formycin and CDO3 on mice infected with MHV

To test the chemotherapeutic effect, after infection of 10^2 dilution of virus, these three drugs were administered at the first, second and third days of infection subcutaneously. The mean survival time

Table 1. Direct inactivating effect of Xenaldial and Formycin on MHV *in vitro*.

Virus diln.	Drugs	Conc. (γ/ml)	Treatment	Death/ treated	Mean survival time(days)
10^{-1}	Xenaldial	100	8°C-2days	0/10	—
//	Formalin	100	//	2/10	4.5
//	//	100	//	5/10	4.3
//	Control	—	//	8/10	4.0
10^{-2}	Xenaldial	100	//	0/10	—
//	Formalin	100	//	3/10	6.0
//	//	100	//	4/10	4.0
//	Control	—	//	4/10	3.9

(days) and mortality for infection were estimated. The results are shown in Table 2.

As shown in the table, Xenaldial did not show the significant difference as compared to the control. By the administration of 20 mg of Formycin, the mean survival time was more prolonged than the control and the mortality was also decreased. In the case of CDO3 large dosis (500 mg/kg) mortality was also decreased, but complete chemotherapeutic effects were not shown in every case.

III) Chemotherapeutic effect of Xenaldial, Formycin and CDO3 on mice infected with 10^{-2} dilutions of MHV

As to test the chemotherapeutic effect of these three drugs, the same experiments were repeated under the higher infectious doses of virus. After infection of 10^{-2} dilution of virus, these drugs were also administered subcutaneously with three times and the mean survival time and mortality were also estimated. The results are shown in Table 3. Xenaldial also showed no effect but Formycin prolonged the survival time and decreased the mortality rate, as described in the above experiment.

IV) Chemoprophylaxic and chemotherapeutic effect of Xenaldial, Formycin, Benadryl and CDO3 on mice infected with 10^{-3} dilution of MHV

These four drugs have never been reported on the effect of chemotherapeutic and chemoprophylaxic effect of MHV, one tenth of the dosis of the previous experiments were administered subcutaneously for 2 days before and after the infection. The mean survival time and mortality were also estimated. The results are shown in Table 4.

As shown in the table, only Benadryl decreased the mortality, but other three drugs did not influenced the mortality and survival time.

V) Chemoprophylaxic and chemotherapeutic effect of Xenaldial, Formycin, Benadryl and CDO3 on mice infected with 10^{-2} dilution of MHV

As to test the same experiment on the higher infectious doses, same doses were administered subcutaneously for 2 days before and after the infection. The mean survival time and mortality were also estimated. The results are shown in Table 5.

As shown in the table, Benadryl also decreased the mortality but the survival time of mice has been rather shortened. These might be influenced to the toxicity of Benadryl.

Other three drugs did not influence the survival time and mortality.

Table 2. Chemotherapeutic effect of Xenaldial, Formycin and CDO3 on mice infected with MHV

Drugs	Dose (mg/kg)	Treatment ↓ ↓ ↓ ↑ Infection	Mean survival time (days)	Mortality (%)	Note
Xenaldial	125	three times after infection	6.0	57.0	no significant
//	50	//	4.6	42.8	//
Formycin	20	//	5.3	42.8	//
//	10	//	5.3	42.8	//
CDO3	500	//	4.6	37.5	//
//	250	//	5.2	37.5	//
//	125	//	3.7	37.5	//
Control	—	—	4.0	57.0	—

All mice were injected intraperitoneally with 0.2 ml of 10^{-3} dilution of virus.

Table 3. Chemotherapeutic effect of Xenaldial, Formycin and CDO3 on mice infected with MHV.

Drugs	Dose (mg/kg)	Treatment ↓ ↓ ↓ ↑ Infection	Mean survival time (days)	Mortality (%)	Note
Xenaldial	125	three times after infection	3.1	57.0	no significant
//	50	//	3.6	57.0	//
Formycin	20	//	6.8	42.9	//
//	10	//	5.5	28.6	//
CDO3	500	//	3.6	28.6	//
//	250	//	4.0	28.9	//
//	125	//	3.9	57.2	//
Control	—	//	3.7	37.5	—

All mice were injected with 0.2 ml 0f 10^{-2} dilution of virus intraperitonealy.

Discussion

Chemotherapy of virus diseases has been remained as one of the most important problems of modern medicine. On the other hand, there have been published many reports concerning the chemotherapeutic agents until recently *in vitro* and *in vivo*, but none of them showed the clinical effectiveness.

It has been reported that Xenaldial

Table 4. Chemotherapeutic effect of Xenaldial, Formycin and CDO3 on mice infected with MHV.

Drugs	Dose (mg/kg)	Treatment ↓ ↓ ↓ ↓ ↓ / ↑ Infection	Mean survival time (days)	Mortality (%)	Note
Xenaldial	12.5	four times injections	3.7	43.0	no significant
Benadryl	0.5	//	3.5	28.5	//
Formycin	1.0	//	2.3	43.0	//
CDO3	12.5	//	5.0	57.0	//
Control	—	saline as cont.	4.0	43.0	—

All mice were injected with 0.2 ml of $10^{-2.5}$ dilution of virus intraperitonealy.

Table 5. Chemotherapeutic effect of Xenaldial, Formycin Benadryl and CDO3 on mice infected with MHV.

Drugs	Dose (mg/kg)	Treatment ↓ ↓ ↓ ↓ ↓ / ↑ Infection	Mean survival time (days)	Mortality (%)	Note
Xenaldial	12.5	four times injections	4.5	85.0	no significant
Benadryl	0.5	//	3.0	24.3	//
Formycin	1.0	//	5.0	85.0	//
CDO3	12.5	//	4.3	43.0	//
Control	—	saline as cont.	3.7	54.3	—

All mice were injected with 0.2 ml of 10^{-2} dilution of virus intraperitoneally.

possessed a broad antiviral spectrum by MAGRASSI *et al.* (1961), and Benadryl was also reported as effective to mice hepatitis virus by JUDAH (1965).

In our experiments, Xenaldial showed the most potent virus-inactivating activity on MHV (EHF-120), but did not show the therapeutic effect *in vivo*. Benadryl was effective *in vitro*, but not completely. Formycin, one of the antibiotics derived in Japan (UMEZAWA 1966), was effective *in vivo*, it did not decrease the mortality but the survival time was prolonged. CDO3 was also effective *in vivo*, but only at larger dose. The antiviral mechanism of these drugs has been unknown. Formycin has been described that it might be related with the disturbance of purine bioysnthesis as shown in the case of cordycepin (OVERGAAED-HANSEN 1964). But the inhibitory effect is still unknown in case of virus infection. It should be necessary to conduct the biochemical researches *in vitro*.

Summary

Antiviral effect of Xenaldial, Benadryl, *p*-carboxyl N-methyl, acetyl nicotinic acid, and formycin on the MHV (EHF-120) strain of mice have been studied *in vitro* and *in vivo* comparatively.

Xenaldial showed a potent virus-inactivating effect *in vitro*, but showed no therapeutic effect *in vivo*. Benadryl, *p*-carboxyl N-methyl, acetyl nicotinic acid and Formycin showed a slight therapeutic effect *in vivo*.

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