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Influenza Infections of Mice. I. Curative Activity of Amantadine HCl (33395)

J. W. McGAHEN AND C. E. HOFFMANN
(Introduced by J. A. Schneider)
Pharmaceuticals Division, Industrial and Biochemicals Department, E. I. du Pont de Nemours and Co., Wilmington, Delaware 19898

The reports of anti-influenza activity of amantadine HCl[1] (Symmetrel) have been concerned chiefly with the prophylactic action of this compound. However, Davies *et al.* (1) and Grunert *et al.* (2) reported that treatment with amantadine HCl as late as 72 hr, but not 96 hr, after infection time reduced mortality and increased survival time of mice infected with influenza A2/AA/2/60. Similarly, Solovyov and Tolmacheva (3, 4) showed that amantadine HCl was effective when given to mice 24 hr after infection with influenza A2/Frunze and with influenza A2/Lvov. Floor-Wieringa *et al.* (5) indicated that amantadine HCl reduced disease duration and some symptoms in some, but not all, human therapeutic trials during an influenza A2 epidemic in the Netherlands.

Although the mouse studies showed that postinfection treatment with amantadine HCl was protective, no curative action could be claimed since it was not shown that dosing began after the appearance of influenza A2 disease symptoms. Our studies of influenza A strains in mice have shown that a decrease of water consumption is the earliest observable disease symptom or sign. The present studies were undertaken to determine the effects of amantadine HCl on influenza A2 mouse infections when dosing was begun after the appearnce of this symptom.

Materials and Methods. Female white mice 28 to 30 days old (t. 18 ± 2 g) which had been caged on wire in continuous light 5 days before use were used throughout. Such mice, under light ether anesthesia, were infected intranasally with 0.05 ml of a mouse lung preparation of either influenza A2/Bethesda/10/63 or influenza A2/AA/2/60 diluted to a concentration calculated to cause 85% mortality on the tenth day after infection (3–5 LD_{50}).

The water consumption of virus-infected and sham-infected mice was measured to the nearest 1 ml at 4-hr intervals beginning 4 hr after infection. The data were analyzed as the cumulative milliliters of water consumed by groups of 60–80 mice.

Amantadine HCl was administered 12 hr after the first measurement period of significant water consumption difference. With influenza A2/Bethesda/10/63, amantadine HCl was dosed by two methods, singly and in combination: *ad libitum* in the drinking water as a 0.5 mg/ml solution from 48 hr

[1] 1-Adamantanamine HCl, EXP 105-1.

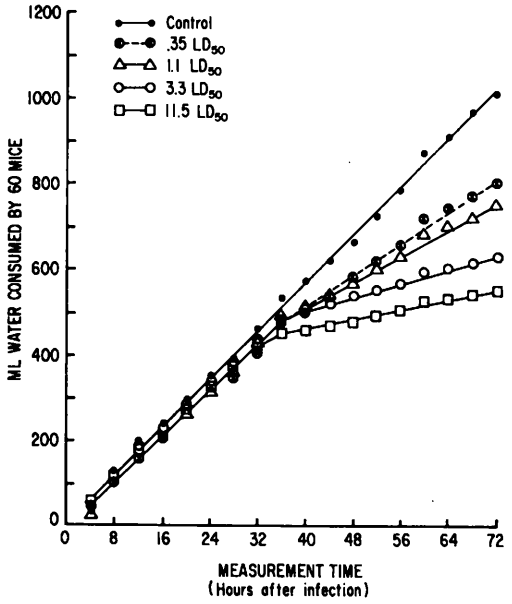

FIG. 1. Water consumption of mice infected with influenza A2/Bethesda/10/63.

through 240 hr after infection, and by oral intubation at 100 mg/kg in 0.2 ml of water as a single dose at 48 hr, as two doses at 48 and 72 hr and as three doses at 48, 72, and 96 hr after infection. In order to approximate the studies of Grunert *et al.*, treatment of influenza A2/AA/2/60 infections was by oral intubation in 0.2 ml of water at 4-hr intervals beginning 64 hr and continuing through 124 hr after infection at 10, 5, 2.5, and 1.25 mg/kg. Mortality was recorded daily through the tenth day after infection at which time the tests were terminated. The data were analyzed as the regression of percentage survivors per day of the data set.

On day 10 the mice were rated according to the following subjective disease severity classes: 0 = apparently healthy; 1 = reduced activity, weight loss or no gain, rough fur, respiratory difficulties; and 2 = moribund or dead.

The mean survival day was taken from the regression curves, by extrapolation when necessary, as the day when 50% of the total number of test animals (N) were alive.

Results. Water consumption studies. The decrease in total water consumption of influenza A2/Bethesda/10/63-infected mice was found to be virus-dose related as shown in Fig. 1. However, the time of decrease in water consumption, as measured by slope change, occurred at the 36-hr measurement time with each of the four virus infection levels used: 0.35, 1.1, 3.3, and 11.5 LD_{50}. The time of slope divergence as determined by regression was 34 hr after infection for the virus level of the experiment shown in Table I.

TABLE I. Survival and Disease Severity Ratings of Mice Infected with Influenza A2/Bethesda /10/63 and Treated with Amantadine HCl.

Treatment schedule (hr postinfection)						
Drinking water[a]	Oral intubation[b]	N	Survival day 10 (%)	Mean survival day	Mean disease severity rating	Healthy animals (%)
Control		77	14.3	6.2	1.88	2.6
48–240	None	78	42.3[d]	7.9[c]	1.32[d]	25.6[d]
48–240	48	76	44.8[d]	8.0[c]	1.32[d]	27.6[d]
48–240	48, 72	76	52.6[d]	10.2[d]	1.3 [d]	26.3[d]
48–240	48, 72, 96	79	50.6[d]	10.2[d]	1.26[d]	26.6[d]
None	48	80	17.5	7.0	1.74	10.0
None	48, 72	76	23.7	7.5	1.80	5.3
None	48, 72, 96	78	27.0	7.7	1.58[c]	14.3[c]

[a] Dose: 0.5 mg/ml in water.
[b] Dose: 100 mg/kg in 0.2 ml of water.
[c] $p = .05$.
[d] $p = .01$.

TABLE II. Survival and Disease Severity Ratings of Mice Infected with Influenza A2/AA/2/60 and Treated with Amantadine HCl.

Dose (mg/kg)[a]	N	Survival day 10 (%)	Mean survival day	Mean disease severity rating	Healthy animals (%)
Control	60	15.0	6.2	1.82	3.3
10	60	43.3[c]	8.2[c]	1.22[c]	35.0[c]
5	58	37.9[c]	8.4[c]	1.34[c]	27.6[c]
2.5	60	30.0	6.8	1.57[b]	16.7[b]
1.25	60	26.7	6.8	1.60	16.7[b]

[a] Treatment by oral intubation beginning 64 hr and continuing through 124 hr after infection at 4-hr intervals.

[b] $p = .05$.

[c] $p = .01$.

The decrease in water consumption of mice infected with influenza A2/AA/2/60 occurred later than that of mice infected with influenza A2/Bethesda/10/63. The time of slope divergence was 52 hr after infection with the virus level of the experiment shown in Table II.

Amantadine HCl curative trials. Influenza A2/Bethesda/10/63. As shown in Table I, significant ($p = <.05$)) increases of both percentage of survivors and survival time occurred only in those groups of mice which received amantadine HCl in the drinking water; a significant increase in survival time, but not in survivors, occurred in the group of mice given three oral doses of amantadine HCl at 48, 72, and 96 hr after infection. The single 48-hr oral dose was not additive to the drinking water treatment, while two doses at 48 and 72 hr were additive and the third oral dose at 96 hr caused no additional effects. Significant reduction in disease severity and increase in the number of healthy animals occurred among those groups of mice which received amantadine HCl in the drinking water and in the group which received three oral doses. The oral dosing afforded no additional protection when given with the drinking water treatment.

Influenza A2/AA/2/60. Significant increases in percentage of survivors and in survival time were effected by 10 and 5 mg/kg but not with 2.5 or 1.25 mg/kg of amantadine HCl treatments (Table II). There were no differences among the 10- and 5-mg/kg amantadine HCl treatment groups. Significant reduction in disease severity occurred in the 10-, 5-, and 2.5 mg/kg groups but not the 1.25-mg/kg group. There was a significant increase in the percentage of healthy animals in all treated groups. In addition, the percentage of healthy animals of the survivors (classes 0 and 1) in the 10- and 5-mg/kg groups was significantly greater than in the control group: 81% at 10 mg/kg ($p = .006$) and 73% at 5 mg/kg $p = .03$) as compared to 22% of the controls. Thus amantadine HCl at 10 and 5 mg/kg reduced mortality, increased survival time, decreased disease severity, and increased the proportion of healthy animals among the survivors.

Discussion. A decrease in water consumption has been the earliest indication of disease among mice infected with pneumotropic influenza strains which we have studied. With influenza A2/Bethesda/10/63, the reduced water consumption occurs by hour 36 after infection. With other influenza strains both the times of occurrence of this first disease symptom and the time between the first evidence of disease and of survival time may differ. This is shown in the experiments reported here with influenza A2/Bethesda/10/63 and influenza A2/AA/2/60. The mean survival time, 6.2 days, was the same for the mice infected with either virus strain, the percentage mortality on day 10 was the same (85.7 and 85%), but the water consumption reduction times differed by 18 hr (34 and 52 hr).

The degree of water consumption reduction was related to the amount of virus given in

the inoculum and was correlated with the severity of the disease as measured by LD_{50}. The time at which the decrease in water consumption began did not appear to be correlated with the inoculum LD_{50} within a range of 1.5 $\log_{10}$ virus concentrations. Thus, the appearance of disease symptoms with a virus concentration which caused 15% mortality and 33% reduction in water consumption occurred at the same time as with a virus concentration which caused 96% mortality and 78% reduction in water consumption.

On the assumption that water consumption reduction is a valid criterion for disease onset, the curative action of amantadine HCl appears to be adequately demonstrated by the trials of this study. This is reflected by the decrease in mortality, increase in survival time and increase in apparently healthy animals in influenza A2/Bethesda/10/63, as well as in an increased proportion of healthy animals among the survivors of influenza A2/AA/2/60 mouse infections.

Summary. The first disease symptom of influenza A2-infected mice was a reduction in water consumption. The degree of reduction with influenza A2/Bethesda/10/63 was virus-dose related whereas the time of appearance of this symptom was not. Amantadine HCl treatment of mice infected with influenza A2/Bethesda/10/63 and with influenza A2/AA/2/60 beginning 12 hr after the appearance of this disease symptom resulted in an increase in the percentage of survivors, an increase in survival time, an increase in the number of healthy animals, and an increase in the proportion of healthy to diseased animals of those which survived.

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Identification of Human Hepatic Glucokinase and Some Properties of the Enzyme* (33396)

S. J. PILKIS
(Introduced by M. E. Krahl)
Department of Physiology, University of Chicago, Chicago, Illinois 60637

Glucokinase, a hexokinase type enzyme with a high K_m toward glucose, has been established as an important factor in glucose phosphorylation by rat liver (1–4). It was at first reported that glucokinase did not occur in human liver (5); subsequently, it was detected there by starch gel electrophoresis (6). The present study deals with the definite identification of a glucokinase in human liver and with some of the properties of the enzyme.

Methods. Liver tissue was obtained from a 24-year-old Negro male during gastrointestinal surgery for vagotomy and pyloroplasty. He had been fasted overnight but was otherwise well-nourished and had no signs of liver disease; he received intravenous glucose during operation. A 1-g wedge of liver was removed after hemostatic sutures had been placed but not tightened.

The tissue was placed in ice immediately after excision and homogenized within 5 min

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