Prednisolone in Hepatitis by MHV-3 Virus: Study of the Dynamics of Virus Multiplication in Relation to Time of Prednisolone Injection (36072)

M. Piazza, M. Visconti, D. Lista, and F. De Ritis
(Introduced by J. B. Nelson)
Institute of Clinica Medica II, University of Naples Medical School, Naples, Italy

MHV-3 virus (1, 2), when inoculated into susceptible mice, induces a severe hepatitis, characterized by a histologic picture resembling that of viral hepatitis of man. In a recent work MHV-3 virus was inoculated intravenously into susceptible mice and virus concentration was determined in the blood, liver, spleen, kidney, and brain at different time intervals after virus injection. Interesting data were thus obtained on the evolution of the infectious process by MHV-3 (3).

A number of compounds are capable of influencing the course and mortality of MHV-3 hepatitis (2). Particularly interesting seems the action of prednisolone because of the widespread use of corticosteroids in the treatment of human viral hepatitis. It has been shown that prednisolone, if administered at high doses for several days to MHV-3 virus-infected mice, induces a marked increase on the mortality with early death of treated animals (4). Also, the administration of cortisone makes C_3H mice susceptible to MHV(Pr) hepatitis virus (5–7).

The present work was aimed at better defining some aspects of the biological action of prednisolone in mice with MHV-3 virus hepatitis. In a first series of experiments we have investigated: (a) whether prednisolone would still exhibit its effect when inoculated as a single dose at the time of virus injection; (b) in which stage of virus multiplication prednisolone exerts its action; (c) whether the effect of prednisolone would be different in the liver, spleen, and blood.

In subsequent experiments we have studied the effect of prednisolone inoculated at varying time intervals before and after virus injection.

Materials and Methods. MHV-3 was maintained in weanling Swiss mice weighing 10–11 g (21–22 days old) infected by the intraperitoneal route. A 10% homogenate of infected liver in broth saline was centrifuged at 4° (6000*g* for 15 min) and the supernate was used. Swiss mice 30–32 days old (13–14 g) were used for experimental infection studies. All infectivity titrations were carried out in slightly younger and smaller mice (21–22 days old, 10–11 g). Prednisolone ($\Delta^{1,4}$-pregnadien-11β,17α,21-trione,3,20-dione) was used throughout. Eighteen groups of 6 mice each were inoculated in the dorsal tail vein with stock virus, each animal receiving 10^6 LD_{50} of virus and 2 mg of prednisolone in 0.2 ml.

Comparable groups of 6 mice each were inoculated with virus plus saline and they represented the controls. The animals of each group were killed by decapitation and bled at varying time intervals, after intravenous inoculation, as indicated in Fig. 1.

The blood was collected from each group for viral assay. Livers and spleens were excised; pooled; and the respective pools, prepared as 10% tissue homogenates in Tris buffer solution at pH 7, were frozen at —70° for subsequent infectivity titration. The Spearman-Kärber method was employed to determine the virus LD_{50} and the relative standard error (8). The experiment was done in triplicate with identical results. In a second series of 8 experiments, we investigated the effect of prednisolone, still used as a single 2 mg dose, given not at the time of virus injection, but instead 2, 4, 6, 10, 12, 16 hr after and 12, 16 hr before it. Both the virus and prednisolone were inoculated by the intravenous route. The injected mice, divided in groups of 6 animals each, were killed

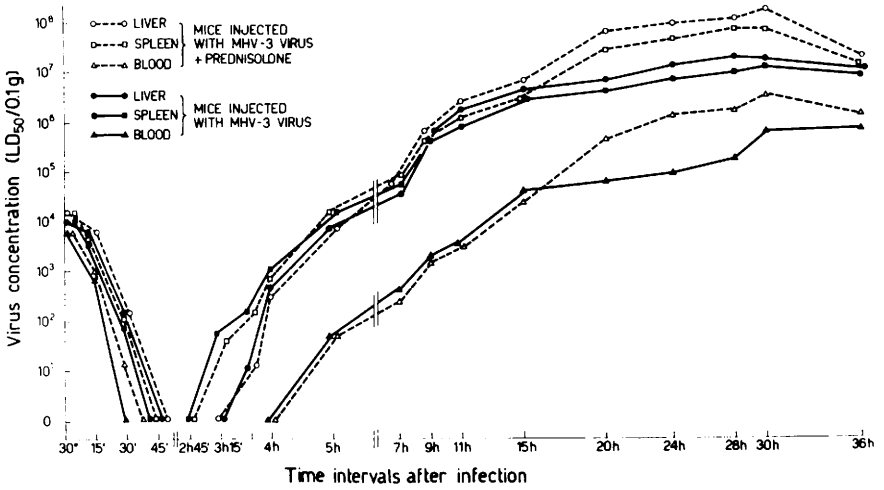

FIG. 1. Virus concentration in liver, spleen, and blood of control mice (mice infected with 10^6 LD_{50} of MHV-3) and of mice injected simultaneously with 10^6 LD_{50} of MHV-3 and 2 mg of prednisolone, at different time intervals after infection. For the titration of virus, mice were injected intraperitoneally with 10-fold dilutions of each material from 10^{-1} to 10^{-11} (10 mice for each dilution).

at time intervals, after virus infection, as shown in Figs. 2 and 3. The liver of each mouse was removed and livers of the animals in the same group were pooled. From each

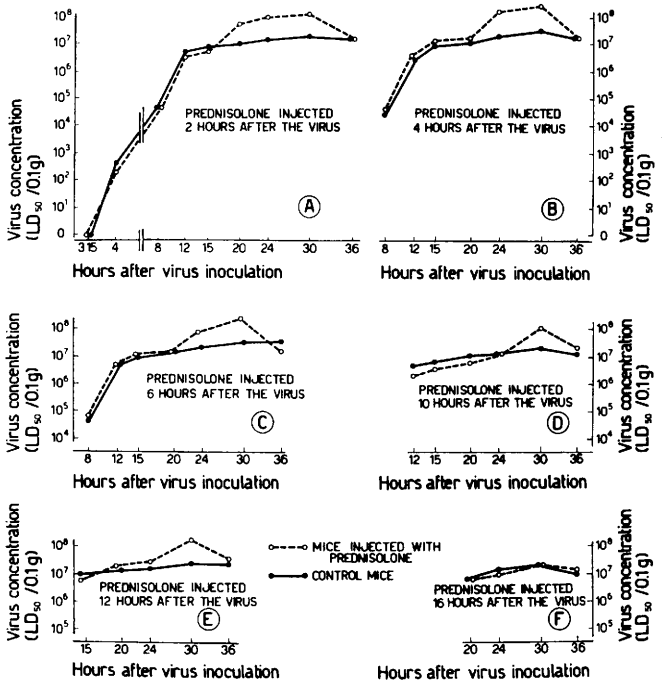

FIG. 2. Virus concentration in liver of control mice (mice injected with 10^6 LD_{50} of MHV-3) at different time intervals after inoculation; and of mice injected with 2 mg of prednisolone at the following time intervals after virus injection (10^6 LD_{50} of MVH-3 intravenously) (hr after virus injection): (A) 2; (B) 4; (C) 6; (D) 10; (E) 12; and (F) 16.

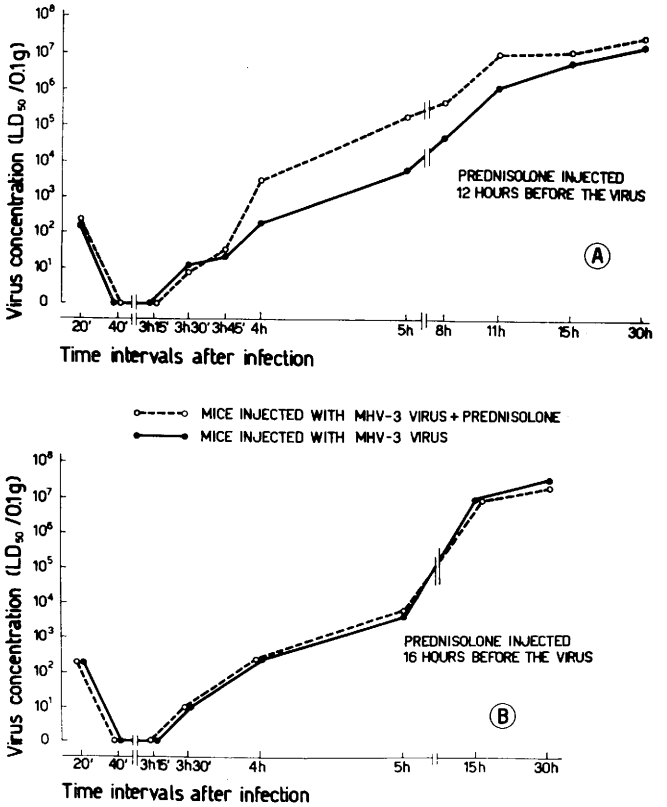

FIG. 3. Virus concentration in liver of control mice (mice injected with 10^6 LD_{50} of MHV-3) at different time intervals after inoculation; and of mice injected with 2 mg of prednisolone, at the following time intervals before virus injection (10^6 LD_{50} of MHV-3 intravenously) (hr before virus injection): (A) 12; and (B) 16.

pool, a 10% homogenate in sterile broth was prepared and immediately frozen at —70°. Biological titration of each liver pool homogenate was carried out, and the LD_{50} and the respective standard error was determined according to Spearman-Kärber.

Control mice were inoculated with saline instead of prednisolone. Each experiment was repeated three times with comparable results.

Results. Figure 1 shows that in the first series of experiments the data relative to liver, spleen, and blood are identical with respect to the effect of prednisolone on the LD_{50} as well as to the time of its appearance. Therefore, in the subsequent experiments, only viral concentration of liver was determined.

When prednisolone was injected 16 hr before or after the inoculation of virus no significant increase of virus concentration was detected in the liver of treated animals as compared with the controls. In the remaining experimental groups the effect of prednisolone, as reflected in a higher virus concentration in the liver of treated mice with respect to the controls, always became apparent 15–20 hr after inoculation of the drug (Figs. 2, 3).

The administration of prednisolone 12 hr before virus inoculation results in an increase of virus concentration in treated animals, which becomes significant at the fourth hour postvirus infection, to disappear by hour 15 (Fig. 3). In no case was a significant difference in viral LD_{50} observed at 36 hr after virus infection.

Discussion. The prednisolone-induced increase of virus concentration of liver (spleen and blood) of MHV-3 mice is of limited

extent. However, calculation of the standard error and the consistency of the data (each experiment was repeated three times with identical results) indicate that the differences observed are significant.

It is interesting to note that the effect of prednisolone always becomes apparent 15–20 hr after its administration, even if the drug is injected as a single dose and regardless of the time of virus infection.

The disappearance of a significant difference of viral LD_{50} at hour 36 is the result of the acute atrophy of the liver that, in the case of intravenous inoculation of the virus, is already marked at 30 hr. However, the observation that, in the experiment in which prednisolone was injected 12 hr before the virus, the difference of LD_{50} was detectable as early as hour 4 and disappeared at 15 hr indicates that the effects of a single dose of prednisolone last for about 10 hr.

Finally, it should be pointed out that when prednisolone was inoculated 16 hr before the virus, the time at which the drug was expected to exert its effect coincided with the very early stage of virus infection. Since in this experiment no significant increase of virus LD_{50} in the liver of treated animals was obtained, we may suppose that prednisolone has no effect on the eclipse phase of the virus.

Summary. Swiss mice were injected intravenously with 10^6 LD_{50} of MHV-3 virus and, simultaneously, with 2 mg of prednisolone. Control mice were injected with physiological saline and virus, respectively. The animals were killed at different time intervals after the injection and the Spearman-Kärber method was used to determine viral LD_{50} (and relative standard error) in blood, liver, and spleen.

Prednisolone produces an increase of viral LD_{50}. This effect occurs after 15–20 hr, with more or less identical intensity in liver, spleen, and blood.

Further studies were done by injecting prednisolone intravenously (single dose of 2 mg), not simultaneously with the virus, but 2, 4, 6, 10, 12, and 16 hr afterward and also 12 and 6 hr before.

Determination of LD_{50} in liver, at different time intervals after the injection of virus, demonstrated that the effect of prednisolone always occurred 15–20 hr after its administration. This effect was temporary and disappeared in about 10 hr. Furthermore, prednisolone did not appear to influence the eclipse phase of the virus.

1. Dick, G. W. A., Niven, J. S. F., and Gledhill, A. W., Brit. J. Exp. Pathol. **37**, 90 (1956).

2. Piazza, M, "Experimental Viral Hepatitis." Thomas, Springfield, IL (1969).

3. Piazza, M., Pane, G., and De Ritis, F., Arch. Gesamte Virusforsch. **22**, 472 (1967).

4. De Ritis, F., Coraggio, F., Villari, V., and Bianco, R. G., Mal. Infett. **9**, 240 (1957).

5. Nelson, J. B., J. Exp. Med. **96**, 293 (1952).

6. Nelson, J. B., J. Exp. Med. **96**, 303 (1952).

7. Gallily, R., Warwick, A., and Bang, F. B., Proc. Nat. Acad. Sci. U.S.A. **51**, 1158 (1964).

8. Finney, D. J., "Statistical Methods in Biological Assay", p. 524. Griffin, London (1964).

Received July 9, 1971. P.S.E.B.M., 1972, Vol. 139.