Protective Effects of a Novel Quinone Derivative, E3330, on Mouse Hepatitis Virus (MHV)-Induced Chronic Hepatitis in Athymic Nude Mice

Koji UETSUKA1, Michio SUZUKI1, Chieko KAI2, and Naoaki GOTO3

1Department of Veterinary Pathology and 2Department of Veterinary Microbiology, Faculty of Agriculture, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, and 3Shin Nippon Biomedical Laboratories, Ltd., Tokyo Byouri Center, 2-7-7 Shinkawa, Chuo-ku, Tokyo 104, Japan

Abstract: In this experiment, we examined the protective effects of a novel quinone derivative, E3330, on MHV-2cc-induced chronic hepatitis in athymic nude mice for up to 3 weeks after virus infection. The daily dose of 25 mg/kg b.w. suppressed the viral replication in the liver and the progression of hepatic lesions. The expansion of small focal lesions at 1 week after viral inoculation (WAI) was suppressed at 2 WAI, and the lesions were still small at 3 WAI in E3330-administered group, whereas small focal lesions at 1 WAI were expanded at 2 WAI to fuse with each other at 3 WAI in the control group. E3330 therefore showed protective effects on MHV-2cc-induced chronic hepatitis in athymic nude mice, but further studies are needed to analyze the mechanism.

Key words: athymic nude mice, chronic viral hepatitis, E3330, MHV-2cc, protective effect

A new novel quinone derivative, E3330 ((2E)-3-[5-(2,3-dimethoxy-6-methyl-1,4-benzoquinolyl)]-2-nonyl-2-propenoic acid; MW: 378.47) was synthesized at Eisai Tsukuba Research Laboratory (Eisai Co. Ltd., Ibaraki, Japan). E3330 is an orange crystalline powder and practically insoluble in water [11, 12]. It has been reported that E3330 has a protective effect on lipopolysaccharide (LPS)/galactosamine hepatitis and tumor necrosis factor (TNF)/galactosamine hepatitis [11, 12] in both of which massive hepatic necrosis is elicited within 24 hr in C3H/HeN mice.

It is uncertain, however, that E3330 exerts the protective effect on viral hepatitis. The authors have reported the protective effect of interferon (IFN) on mouse hepatitis virus (MHV)-induced chronic hepatitis [15]. In this study, with the same experimental model, the protective effect of E3330 on viral hepatitis was examined.

In our preliminary in vitro examinations with the DBT cell line, it was clarified that E3330 effectively inhibited the proliferation of MHV-2, a hepatotropic and highly virulent variant of MHV, in a dose-dependent manner. The addition of E3330 to medium at a dose of 50–150 μM resulted in a reduction in viral plaque in a dose-dependent manner, compared with the control group, although the 150 μM of E3330 was ob-

(Received 7 August 1996 ; Accepted 5 December 1996)
Address corresponding: K. Uetsuka, Department of Pathology, Faculty of Agriculture, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan
served to damage the DBT cells. After the addition of 100 μM of E3330 to the medium, the viral plaque had been reduced to 50%, and the viral replication started 4 hr later than in the control group. This suggests that E3330 may also have a protective effect on MHV-infection in vivo. In this study we therefore tried to examine a protective effect of E3330 on one of the models of human chronic viral hepatitis, i.e. MHV-2cc-induced chronic hepatitis in athymic nude mice [3, 4].

Six-week-old female athymic BALB/c-nu/nu mice [23–27 g] were purchased from CLEA Japan, Inc (Tokyo). They have been confirmed to be free from MHV-infection serologically. They were kept under controlled conditions (temperature, 23 ± 2°C; relative humidity, 55 ± 5%; lighting, 12 hr (8:00–20:00); ventilation, 20 times an hr) in an isolator casing system (Niki Shoji Co., Ltd., Tokyo) and fed autoclaved pellets, MF (Oriental Yeast Co. Ltd., Tokyo) and water ad libitum. The mice were equally divided into 7 groups (4 groups for Experiment 1 and 3 for 2). MHV-2cc [5], a low virulence variant of MHV-2, was diluted with Eagle’s minimum essential medium (MEM) (Nissui Co. Ltd., Tokyo) to the concentration of 1 × 10^5 plaque forming units (PFU)/0.2 ml. All the mice were then inoculated intraperitoneally with 1 × 10^5 PFU/0.2 ml of MHV-2cc.

In Experiment 1, the object of which was to determine the adequate concentration of E3330, 3 of 4 groups were orally administered with E3330 at daily dose levels of 5, 15 and 25 mg/kg b.w. suspended in 0.2 ml of 0.5% methyl cellulose (MC) solution for 3 consecutive weeks from the day of MHV-2cc-inoculation, respectively. The remaining one group was given MC solution alone in the same way and served as controls. Three mice in each group were killed by exanguination under ether anesthesia at 1, 2 and 3 weeks after MHV-2cc-inoculation (WAI), respectively. In Experiment 2, the object of which was to compare the progression of MHV-2cc-induced chronic hepatitis among E3330-administered, interferon (IFN)-administered and control groups, one group was given a with daily oral dose of 25 mg/kg b.w. of E3330 suspended in 0.2 ml of 0.5% MC solution and one group intraperitoneally given a daily dose of 1 × 10^5 IU of mouse recombinant interferon-α/β (IFN-α/β, Japan Chemical Research, Kobe) in 0.1 ml of phosphate buffered saline and orally 0.2 ml of 0.5% MC solution for 3 consecutive weeks from the day of MHV-2cc-inoculation, respectively. The remaining one group was given MC solution alone in the same way and served as controls. Three mice in each group were killed by exanguination under ether anesthesia at 1, 2 and 3 WAI, respectively. The dose of E3330 was set according to the results of Experiment 1, and that of IFN was chosen on the basis of a previous report on the effect of IFN on fulminant hepatitis induced by MHV-2 [7].

Virus titers were assayed with DBT cells [6]. Liver samples for this assay collected from each mouse at necropsy were stored at −80°C until used. Tissue samples of the liver were taken from all mice at necropsy in Experiments 1 and 2 and fixed in 10% neutral buffered formalin. Paraffin sections (4 μm) were stained with hematoxylin and eosin (HE). All data are expressed as the mean ± standard error (SE). Statistical analysis was done with Student’s t-test.

In Experiment 1, although a slight to moderate reduction in body weight gain was observed in the control, 5, 15 and 25 mg/kg-groups, no mice died during the experiment period. In the liver virus titer, there were no significant differences between control, 5 and 15 mg/kg-groups at any point of examination (Fig. 1). On the other hand, the liver virus titer was significantly
lower in the 25 mg/kg-group than in the other 3 groups at 3 WAI (Fig. 1). Histopathologically, the progression of inflammatory lesions in the liver was significantly delayed and inhibited in the 25 mg/kg-group (data not shown). Neither the 5 nor 15 mg/kg-group showed any apparent protective effects on the hepatic lesions as compared with the control group. 25 mg/kg b.w. of E3330 therefore significantly suppressed the viral replication and the progression of the lesions in the liver.

In Experiment 2, no mice died during the experiment period, though a moderate reduction in body weight gain was recorded in the control group. The liver virus titer in the control group increased linearly with time (Fig. 2). In the E3330- and IFN-groups, the titer was not significantly different from that in the control group at 1 and 2 WAI, though the titer in the former was lower than that in the latter. At 3 WAI, the titer was significantly lower in the E3330- and IFN-groups than in the control group (Fig. 2). In the control group, focal lesions which were characterized by hepatocyte necrosis with inflammatory cell infiltration occurred at 1 WAI (Fig. 3). The lesions showed prominent expansion at 2 WAI, and were fused with each other and occupied large areas of the liver section at 3 WAI (Fig. 4). At 3 WAI, mild organization of the lesions was also observed. In the E3330-group, small focal lesions similar to those in the control group developed at 1 WAI (Fig. 5). After that, the lesions did not show...
prominent expansion at 2WAI and were almost organized and did not progress at 3 WAI (Fig. 6). In the IFN-group, except for one animal showing marked enlargement of lesions at 2 WAI, the lesions remained focal even at 3 WAI (Fig. 7). E3330 (daily dose of 25 mg/kg b.w.) therefore also exerted a protective effect on MHV-induced chronic hepatitis.

E3330 influenced viral replication of MHV in vitro, and the hepatic lesions were not shown to progress after 2 WAI and the virus titer in the liver was decreased at 3 WAI in the E3330-administered group in the in vivo experimental system for MHV-2cc-induced chronic hepatitis. From these results, it is considered that the inhibitory effect on viral replication seen in vitro might not be exerted so effectively in vivo, and the protective effect seen in vivo would be caused by not only the inhibitory effect on viral replication but also some other effect of E3330.

It is reported that the protective effects of E3330 on LPS/galactosamine hepatitis may be related to the inhibition of TNF- and reactive oxygen-production [12]. It is also suggested that the protective effects of E3330 on TNF/galactosamine hepatitis may be related to the inhibition of the production of leukotrien or thromboxan B2 and to the promotion of prostaglandin E2-production [11]. A significant increase in serum TNF was also reported in MHV-induced hepatitis [2], which suggested that the suppressive effect of E3330 on TNF-production may play a role in the protection against MHV-2cc-induced chronic hepatitis in athymic nude mice.

In this experiment, although we administered IFN to a group of mice, it is difficult to directly compare the protective effects of E3330 and IFN, because IFN was administered together with MC solution in this experiment. The reason for this is that it is reported that MC solution delayed recovery from CCl4-induced liver fibrosis and cirrhosis in rats [14], and we previously reported using the same experimental hepatitis model and finding that the administration of IFN alone was more effective [15].

It is well known that IFN inhibits viral protein synthesis in vivo [9, 10] and in vitro [1, 8] and activates natural killer (NK) cells [13]. As mentioned above, E3330 effectively suppressed the proliferation of MHV-2 in vitro in our preliminary examinations, but it is uncertain that the present protective effect of E3330 in vivo has a relationship to its inhibitory effect on viral replication.

Further studies are in progress to clarify the exact protective mechanisms of E3330 in MHV-2cc-induced chronic hepatitis in athymic nude mice.

References