Inhibition of Concanavalin A-Induced Mice Hepatitis by Coumarin Derivatives

Toshihiro Okamoto¹*, Shinichi Yoshida¹, Tadashi Kobayashi¹ and Susumu Okabe²

¹Research Laboratories, Nippon Chemiphar Co., Ltd., 1-22 Hikokawato, Misato, Saitama 341-0005, Japan
²Department of Applied Pharmacology, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan

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ABSTRACT—The effects of coumarin derivatives, osthole, imperatorin, Pd-Ia, Pd-II and Pd-III, on mice concanavalin A (Con A) (0.2 mg/mouse, i.v.)-induced hepatitis were studied. At the dose of 200 mg/kg (i.p.), these coumarins inhibited more than 90% of the Con A-induced elevation of plasma alanine aminotransferase activity, but glycyrrhizin (200 mg/kg, i.p.) caused only 45% inhibition. At the dose of 100 mg/kg (i.p.), osthole produced the strongest inhibition among these coumarins. The inhibitory activity of osthole is lost when its 7-methoxy group is replaced by a 7-hydroxy group to form osthenol. The present results showed that coumarin derivatives inhibited Con A-induced hepatitis, with osthole being the most inhibitory.

Keywords: Coumarin, Concanavalin A, Liver

Coumarin derivatives are widely present in many plants used as herbal medicine. Many pharmacological properties of coumarins have been reported, including an antiproliferative effect on smooth muscle cells (1), inhibition of prostaglandin synthesis (2), anti-tumor activity (3) and inhibition of protein kinase (4). Coumarin derivatives are active components of herbal medicine and are known not to exhibit cellular toxicity. Although coumarins are valuable for many clinical applications, their effects on hepatitis have not been studied.

The administration of concanavalin A (Con A) to mice activated their T-cells and caused the release of proinflammatory cytokines such as interferon-γ and tumor necrosis factor-α (TNF-α) (5), and these proinflammatory cytokines contribute to the development of hepatitis. This Con A-induced mouse hepatitis model has been well used for the evaluation of drugs used to treat hepatitis.

In the present study, we examined the effects of coumarin derivatives on Con A-induced mouse hepatitis. Female BALB/c mice obtained from Charles River Japan, Inc. (Atsugi) were used at 7 – 10 weeks of age. The animals were kept in an air-conditioned room and were given standard chow and water ad libitum. Con A was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Con A dissolved in pyrogen-free saline was administered to mice via a tail vein (injection vol, 100 μl). To measure plasma transaminase activity, mice were anesthetized with ether, and then blood was collected from an abdominal vein in a syringe with a sodium heparin film. Plasma transaminase activity, i.e., that of alanine aminotransferase (ALT), was measured as described previously (6). The chemical structures of the coumarins are presented in Fig. 1. Osthole and imperatorin were isolated from the dried fruit of Cnidium monnieri. Pd-Ia, Pd-II and Pd-III were isolated from dried roots of Angelica decursiva Maxim. Osthenol was synthesized in the Research Laboratories of Nippon Chemiphar Co., Ltd. Glycyrrhizin was obtained from Tokyo Kasei Kogyo (Tokyo). Reverse-transcription polymerase chain reaction (RT-PCR) analysis was performed as described previously (6).

Statistical analyses were performed by means of the Dunnett multiple comparison test. Glycyrrhizin is a main ingredient of licorice (Glycyrrhiza glabra) and reportedly inhibits several forms of animal hepatitis (7 – 9). Administration of glycyrrhizin to patients with chronic hepatitis caused by hepatitis C virus infection reportedly lowers elevated plasma transaminase activity (10). Under the experimental conditions used in our laboratory, more than 200 mg/kg (i.p.) of glycyrrhizin was required to inhibit Con A-induced hepatitis. Thus, to compare the effects of coumarins with that of glycyrrhizin on Con A-induced hepatitis, we first examined the effects of coumarins at the dose of 200 mg/kg (i.p.). The chemical structures of the coumarins used are presented in Fig. 1A. Since elevation of plasma ALT and morpho-
logical changes in the liver are correlated (11, 12), the effect of coumarins on hepatitis was evaluated by measuring plasma ALT. Mice were pretreated with 200 mg/kg (i.p.) of osthole, imperatorin, Pd-Ia, Pd-II, Pd-III or glycyrrhizin at 1 h before Con A administration (n = 5). Con A (0.2 mg/mouse, i.v.) was administered, and at 24 h after the treatment, plasma was sampled for ALT measurement. These coumarins, at the dose of 200 mg/kg (i.p.), inhibited more than 90% of the Con A-induced elevation of plasma ALT (Fig. 2A). However, the same dose of glycyrrhizin caused only 45% inhibition of the Con A-induced elevation of plasma ALT. Furthermore, Con A-induced elevation of plasma aspartate aminotransferase was also significantly inhibited by the coumarins (not shown). These results clearly indicated that coumarins have a greater ability to inhibit Con A-induced hepatitis than glycyrrhizin.

As the next step, the inhibitory effects of coumarins were compared using the lower dose of 100 mg/kg. Mice were pretreated with osthole, imperatorin, Pd-Ia, Pd-II or Pd-III at the dose of 100 mg/kg (i.p.) at 1 h before Con A (20 mg/kg, i.v.) administration. Con A was injected, and at 24 h after the treatment, plasma was sampled for ALT measurement. Osthole inhibited more than 90% of the Con A-

![Fig. 1. Chemical structures of coumarins. A: Chemical structures of osthole, imperatorin, Pd-Ia, Pd-II and Pd-III. B: Chemical structure of osthanol.](image1)

![Fig. 2. Effects of coumarins on Con A-induced hepatitis. A: Effects of coumarins and glycyrrhizin on Con A-induced hepatitis. Mice were pretreated with osthole, imperatorin, Pd-Ia, Pd-II, Pd-III or glycyrrhizin at the dose of 200 mg/kg (i.p.) at 1 h before Con A administration (n = 5). Con A (0.2 mg/mouse, i.v.) was administered, and at 24 h, plasma was sampled for measurement of plasma ALT. *P<0.01 vs Con A Cont. Numbers of animals used for the normal control and Con A control were 3 and 7, respectively. B: Comparison of the inhibitory effects of coumarins. Mice were pretreated with osthole (n = 5), imperatorin (n = 5), Pd-Ia (n = 3), Pd-II (n = 5) or Pd-III (n = 6) at the dose of 100 mg/kg (i.p.) at 1 h before Con A administration (n = 5). Con A (20.0 mg/kg, i.v.) was administered and at 24 h after treatment plasma was sampled for measurement of plasma ALT. *P<0.01 vs Con A Cont. C: Effect of osthole on Con A-induced hepatitis. Mice were pretreated with osthole (25, 50 and 100 mg/kg, i.p.) at 1 h before Con A treatment. Con A (20.0 mg/kg, i.v.) was administered, and at 24 h, plasma was sampled for measurement of plasma ALT. Number of animals used for the normal control and Con A control were 3 and 7, respectively. *P<0.01 vs Con A Cont.)
induced elevation of plasma ALT and thus showed the strongest inhibitory effect among these coumarins (Fig. 2B). Since osthole produced the strongest inhibition, its effect on Con A-induced hepatitis was examined at 3 different doses. Osthole inhibited Con A-induced hepatitis in a dose-dependent manner (Fig. 2C). Osthelenol is formed by substituting a 7-hydroxy group for the 7-methoxy group of osthole (Fig. 1B). The effect of ostholenol on Con A-induced hepatitis was compared with that of osthole. Mice were pretreated with osthole or ostholenol at the dose of 100 mg/kg (i.p.) at 1 h before Con A administration. Con A was injected, and at 24 h after treatment, plasma was sampled for ALT measurement. Osthole produced greater than 90% inhibition of Con A-induced hepatitis. However, ostholenol inhibited Con A-induced hepatitis by only 32%. Thus, the 7-methoxy group may play a critical role in the inhibitory effect of osthole. Since Con A-induced hepatitis is cytokine-dependent, the effect of osthole on Con A-induced cytokine expression in the liver was measured by RT-PCR analysis. Interleukin-2 is commonly measured as a marker of T-cell activation and TNF-α play a critical role in the development of Con A-induced hepatitis. As previously reported (13), treatment of mice with Con A induced interleukin-2 and TNF-α mRNA expressions in the liver at 2 h. However, osthole did not affect Con A-induced interleukin-2 mRNA expression and only slightly inhibited Con A-induced TNF-α mRNA in the liver (Fig. 3). Thus, osthole may inhibit Con A-induced hepatitis by a mechanism other than cytokine inhibition.

Coumarin derivatives are present in plants belonging to families such as the Rutaceae and Umbelliferae. These plants have long been used as medicines and the safety of coumarins has been established (14, 15). The present study showed that coumarin derivatives inhibit Con A-induced hepatitis more strongly than glycyrrhizin. Furthermore, osthole was revealed to have a stronger ability to inhibit hepatitis.

Taking all the results together, we conclude that coumarin derivatives, especially osthole, produce strong inhibition of Con A-induced hepatitis, and the possible application of osthole for the treatment of hepatitis is suggested.

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