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Effects of Cholane Compounds on the Development of Morphine Tolerance

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Abstract □ The present study was undertaken to determine the inhibitory effects of cholane compounds, ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) on the development of morphine-induced tolerance and physical dependence, and also to determine the hepatic glutathione contents. UDCA and CDCA inhibited the development of morphine-induced tolerance and physical dependence significantly. UDCA inhibited the hepatic glutathione decrease induced by morphine multiple injections, while this effect was not observed in CDCA treated mice. It was throught that the inhibitiory effects of hepatic glutathione decrease in morphine-treated mice by UDCA and CDCA showed a tendency of inhibitory effects of development of morphine tolerance and dependence.

Keywords □ Ursodeoxycholic acid, chenodeoxycholic acid, morphine, tolerance and dependence, glutathione.

The principal pharmacological and therapeutic action of morphine is the relief of pain. But morphine has a tendency to induce addiction and tolerance which are some of the most challenging problem in clinical fields.

Cholane compounds including ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) have inhibitory effects on the morphine 6-dehydrogenase which catalyzed morphine to morphinone[1,2]. UDCA and CDCA are major components present in bile[3] and increase the hepatic glutathione level. Meanwhile, it has been reported that the dual action of the drugs, the inhibition of morphinone production and the activation in morphinone-glutathione conjugation due to the increased glutathione level for detoxication plays an important role in the inhibition of the development of morphine tolerance and dependence.[1,2,4-6]

Even though the inhibitory effects of UDCA and CDCA on morphine 6-dehydrogenase as well as the increasing effects of hepatic glutathione have been reported, no clinical and experimental reports have been published where the effects of UDCA and CDCA on the development of tolerance and dependence to morphine have been discussed.

The present study was undertaken to determined the inhibitory effects of orally administered UDCA and CDCA on the development of morphine-induced tolerance and dependence in mice, and also to determine the glutathione contents to examine any relationships between the extent of tolerance development and the hepatic glutathione contents.

MATERIALS AND METHODS

ICR male mice weighing 18-22 g in a group of 10-15, were used in all experiments. UDCA (Dae Woong Pharm. Co.) and CDCA (Choong Wae Pharm. Co.) 50, 100 and 200 mg/kg suspended separately in 0.5% CMC solution were administered orally to mice. Morphine hydrochloride (Dae Won Pharm. Co.) and naloxone hydrochloride (Endo Lab Co., Ltd) were injected subcutaneously (s.c.) and intraperitoneally (i.p.), respectively.

Induction of morphine tolerance and dependence

Morphine hydrochloride 10 mg/kg was administered to mice every 24 hr for a period of 6 days by Kaneto's method[7]. UDCA and CDCA 50, 100 and 200 mg/kg were administered to mice once a day 2 hr prior to the injection of morphine, respectively.

Measurement of analgesic tolerance

The inhibitory degree of morphine tolerance development of the test drugs by oral administration was evidenced by the increase in analgesic

response to morphine hydrochloride 5 mg/kg. The analgesic response was estimated every 30 min for 90 min by the tail flick method[7] 24 hr after the final injection of morphine and calculated as an area under the curve (AUC)[8]. The tail flick latencies to thermal stimulation were determined in seconds prior to and 30, 60 and at 90 min after the injection of morphine. A value of 10 sec was used as a cut-off time to avoid damage to the tail. All data for the dose-response curves were converted to percent of analgesia as follows;

$$\text{Percent of Analgesia (\%)} = \frac{\text{Tt-To}}{\text{Tc-To}} \times 100$$

where To is the base line or pre-morphine reaction time, Tt is the reaction time at t min after morphine injection, and Tc is cut-off time. For the dose-response data, animals were examined at 0, 30, 60 and 90 min after morphine injection. The effects were calculated as an AUC that was obtained by plotting the analgesic percent on the ordinate and the time intervals (min) on the abscissa, and expressed as a percent of the effects obtained in control animals treated only with morphine as reported previously[9,10]

Measurement of naloxone-inducedwithdrawal

The Inhibition of withdrawal induced by naloxone (1 mg/kg, i.p.) to the morphine dependent mice as well as test drugs treated mice was estimated by the decreased scores of the withdrawal for 30 min, 24 hr after the final injection of morphine on the 7th day. The abstinence syndrome was quantified by placing subjects on diaphanous circular cylinder of 35 cm in diameter and 70 cm in height, and by scording the withdrawal induced by naloxone as follows; jumping and diarrhea 2 points, defecation, wetdog shake, writhing syndrome, rearing, grooming and ptosis 1 point according to all or none response by the modified Tagashira and Dewey's methods[11]. And then the withdrawal scores were expressed as a percent of the control as reported previously[9,10].

Measurement of the hepatic glutathione contents in mice

Test drugs were administered orally daily for 6 days 2 hr prior to morphine injection. The mice treated with morphine only and the mice treated with the test drugs regularly for 6 days were sacrificed by decapitation on the 7th day, 24hr after the final injection of morphine. The liver was removed immediately and the glutathione concentration was

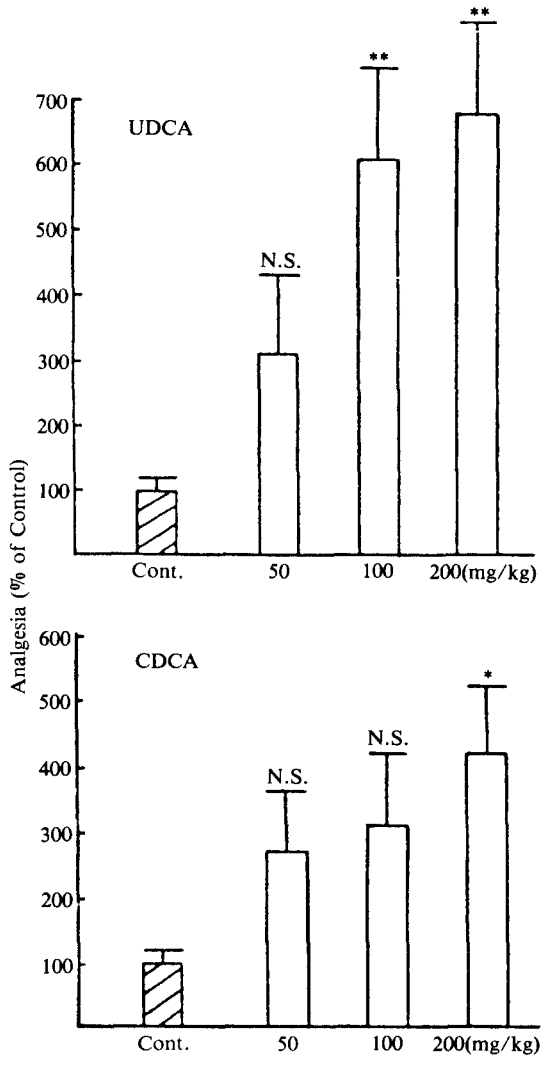

Fig. 1. Effects of UDCA and CDCA on the development of morphine analgesic tolerance.
Morphine (10 mg/kg s.c.) was injected to mice daily for 6 days. Analgesic tolerance test to morphine (5 mg/kg s.c.) was determined every 30 min for 90 min and calculated as % of control by AUC 24 hr after the final injection of morphine. Cont. Control: N.S., Not Significant
*$p<0.05$, **$p<0.01$

determined by Ellman's method as follows[12]. The isolated liver were homogenized in 4 volume of ice-cold phosphate buffer (0.5 M, pH 7.4) to give a suspension equivalent to 250 mg/kg of wet liver. For an estimation of reduced glutathione, an aliquot of the liver homogenate was deproteinized by addition of equal volume of 4% trichloroacetic acid contain-

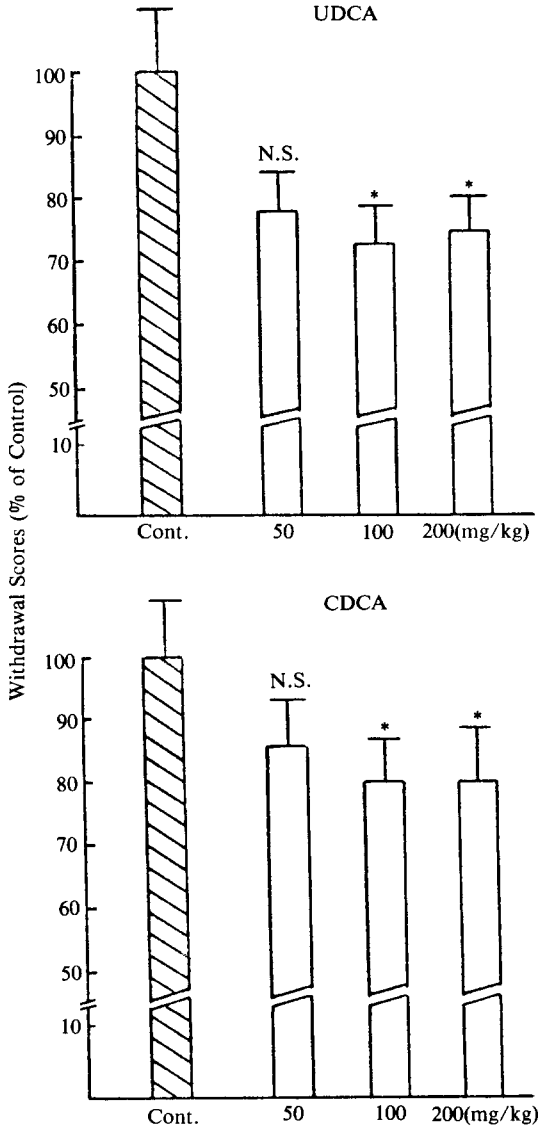

Fig. 2. Effects of UDCA and CDCA on the development of morphine induced withdrawal syndrome.
Withdrawal scoring by naloxone (1 mg/kg i.p.) was made for 30 min immediately after injection of naloxone in mice and compared with that of morphine control group.
Cont., Control; N.S., Not Significant

ing 1 mM Na-EDTA and centrifuged at 2000 × g for 5 min at 4 °C. The supernatant 0.5 m*l* was added to 4.5 m*l* of 5,5'-dithiobis(2-nitrobenzoic acid) and allowed to stand for 20 min at room temperature. The reaction mixture was measured at 412 nm against reagent blank.

Statistics

The data were expressed as means of changes ± S.E.. The differences in the means for different responses in different treatment groups were analyzed by the Student's t-test.

RESULTS

The base line of each group in analgesia changes was determined to check the residual effect of test drugs and morphine 30 min prior to the tolerance test. There were no difference in the base line of tail flick latencies in the different groups.

Inhibition of analgesic tolerance

Daily administration of UDCA or CDCA to mice resulted in the inhibition of the development of morphine tolerance to its analgesic effect, depending upon the doses as shown in Fig. 1.

The analgesia of each group calculated by the AUC to morphine 5 mg/kg showed the comparative value of 6 times in UDCA 100 mg/kg, 6.7 in UDCA 200 mg/kg, respectively compared with that of morphine control group, but no significant differences were observed in UDCA or CDCA 50 mg/kg and CDCA 100 mg/kg groups (Fig. 1).

Inhibition of naloxone induced withdrawal

The inhibitory degrees of naloxone induced withdrawal scores were 27% in UDCA 100 mg/kg, 20% in CDCA 100 mg/kg, 25% UDCA 200 mg/kg and 20% in CDCA 200 mg/kg group, respectively compared with that of the morphine control group, but no significant differences were observed in UDCA or CDCA 50 mg/kg group (Fig. 2).

Inhibition of the hepatic glutathione contents decrease

The heptic glutathione levels (μg/100 mg of tissue) in the mice treated with UDCA and CDCA were 101 ± 1.2 in UDCA 60 mg/kg, 96.5 ± 2.6 in CDCA 50 mg/kg, 114 ± 3.5 in UDCA 100 mg/kg, 96.5 ± 3.1 in CDCA 100 mg/kg, 121 ± 3.5 in UDCA 200 mg/kg and 98.5 ± 2.8 in CDCA 200 mg/kg group, compared with 94.5 ± 4.9 of the morphine control group.

Glutathione contents in the mice treated with saline alone were 126 ± 5.8 μg/100 mg of tissue. And glutathione contents (μg/100 mg of tissue) of the mice treated with both saline and UDCA were observed from 128 ± 8.0 in UDCA 50 mg/kg group to 135 ± 9.3 in UDCA 200 mg/kg group. In the UDCA treated mice, there were significant in-

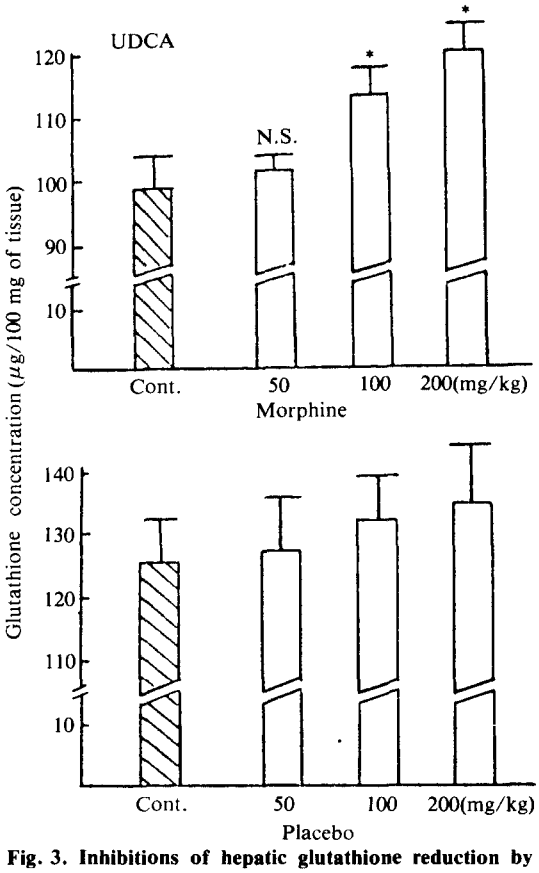

Fig. 3. Inhibitions of hepatic glutathione reduction by daily injection of morphine 10 mg/kg for 6 days.
UDCA was administered orally to mice once a day 2 hr prior to morphine injection for 6 days. The measurement was made on the 7th day 24 hr after the final injection of morphine.
Cont., Control; N.s., Not Significant
*$p<0.05$

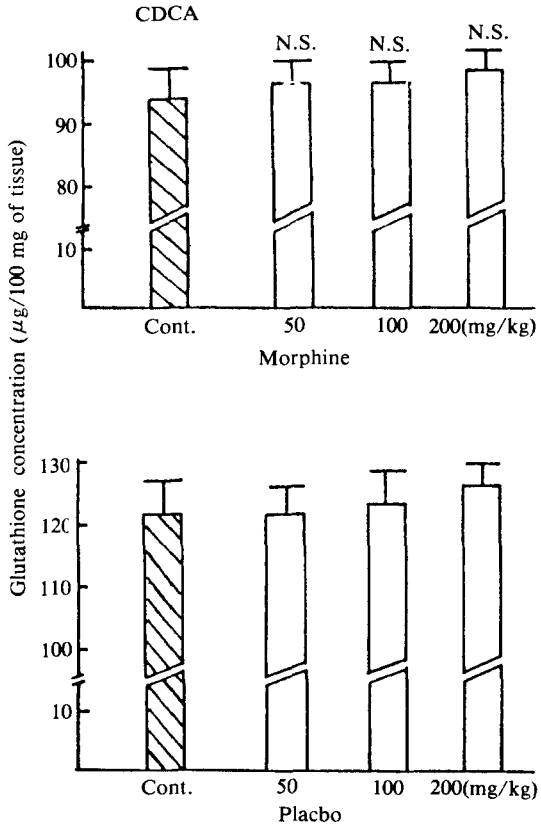

Fig. 4. Inhibitions of hepatic glutathione reduction by daily injection of morphine 10 mg/kg for 6 days.
CDCA was administered orally to mice once a days 2 hr prior to morphine injection for 6 days. The measurement was made on the 7th day 24 hr after the final injection of morphine.
Cont., Control; N.S. Not Significant

hibitory effects on the hepatic glutathione contents compared with that of morphine control group. However, no significant differences were observed in the groups treated with CDCA, compared with that of morphine control group (Fig. 3 and 4).

DISCUSSION

In this experiment UDCA and CDCA administered orally are found to inhibit the development of morphine-induced tolerance and physical dependence, and the hepatic glutathione level decrease in mice. In the mice livers, a portion of morphine administered is metabolized into morphinone which has the 9 times toxicity and one half the analgesic activity of morphine, based on LD_{50} and ED_{50} value in each mouse (s.c.)[4]. Morphine 6-dehydrogenase which catalyzes morphinone production from morphine is inhibited by 20(s)-protopanaxadiol and 20(s)-protopanaxatriol *in vitro*[13]. UDCA and CDCA have the parent chemical structure of 20(s)-protopanaxadiol[14], a kind of cholane compound. Both UDCA and CDCA have inhibitory effects on morphine 6-dehydrogenase[1]. UDCA increases slightly the hepatic glutathione content in this experiment.

Glutathione depletion is increased on morphine-induced hepatotoxicity and glutathione conjugate with the metabolite of morphine has been proved to be closely related to the detoxication process[5,6]. A part of morphinone has been reported to be meta-

bolized into morphinone-protein SH conjugate concerned with the development of morphine induced tolerance and physical dependence by covalent binding to the sulfhydryl group of opiate receptor[4].

In this experiment, the inhibition of the hepatic glutathione level decrease in morphine treated mice with UDCA shows an inhibitory tendency of the development of morphine induced tolerance and physical depenence. It is assumed that such results are, in part, due to inhibition of morphine 6-dehydrogenase as well as the increasing effects of hepatic glutathione contents.

ACKNOWLEDGEMENTS

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LITERATURE CITED

1. Nishida, F., Yamano, S. and Taki, S.: Inhibition of guinea pig liver naloxone reductase by steroids, Abstracts, The 104th Annual Meeting of Pharmaceutical Society of Japan, p. 537 (1985).
2. Yamano, S., Nishida, F. and Toki, S.: Guinea-pig liver morphine 6-dehydrogenase as a naloxone reductase, *Biochem. Pharmacol.*, **35**, 431 (1986).
3. Bacharch, W.H. and Hoffmann, A.E.: Ursodeoxycholic acid in the teatment of cholesterol cholethiasis, *Dig. Sci.*, **27**, 737 (1982).
4. Nagamatsu, K., Kido, Y., Terao, T., Ishida, T. and Toki, S.: Protective effects of sulfhydryl compounds on acute tocixity of morphine, *Life Sci.*, **30**, 1121 (1982).
5. Nagamatsu, K., Kido, Y., Terao, T., Ishida, T. and Toki, S.: Studies on the mechanism of covalent binding of morphine metabolites to proteins in mouse, *Drug. Metabol. Dispos.*, **11**, 190 (1983).
6. McCartney, M.A.: Effect of glutathione depletion on morphine tocixity in mice, *Biochem. Pharmacol.*, **38**, 207 (1989).
7. Kaneto, H., Kasaka, N. and Hirota, N.: Timing of cycloheximide administration and inhibition of the development of analgesic tolerance to morphine, *Life Sci.*, **31**, 2351 (1982).
8. D'Amour, F.E. and Smith, D.L.: A method for determining loss of pain sensation, *J. Pharmacol. Exp. Ther.*, **72**, 74 (1942).
9. Kim, H.S., Oh, K.W. Park, W.K., Choi, J.W. and Bae, D.S.: Effect of panax ginseng on the development of morphine induced tolerance and dependence (VI). On the oral administration of ginseng ther fraction and saponons. *Arch. Pharm. Res.*, **10**, 188 (1987).
10. Kim, H.S., Jang, C.G. and Lee, M.K.: Antinarcotic Effects of the Standardized Ginseng Extract G115 on Morphine, Planta Medica (1989), in press.
11. Tagashira, E. and Dewey, W.L.: Pharmacological effects of T'S and blue on narcotic-induced antinociception and physical dependence on morphine. Abstracts, The 16th Symposium of Drug Metabolism, Phamacology and Toxicology in Japan, p. 165 (1984).
12. Ellman, G.L.: Tissue sulfhydryl group. *Arch. Biochem. Biophys.*, **82**, 70 (1959).
13. Kim, H.S., Oh, K.W., Park, W.K., Toki, S. and Yamano, S.: Effects of panax ginseng on the development of morphine tolerance and dependence. *Kor. J. Ginseng Sci.*, **11**, 182 (1987).
14. Kim, H.S. and Oh, S.K.: Effects of panax ginseng on the development of morphine induced tolerance and dependence (I), *Yakhak Hoeji*, **29**, 27 (1985).