ANTIHYPERTENSIVE ACTIVITY OF ORALLY ADMINISTERED
METHYL O-(4-HYDROXY-3-METHOXYCINNAMOYL)
RESERPATE (CD-3400) IN CONSCIOUS HYPERTENSIVE RATS

Hitoshi KATO, Kenichiro NAKAO, Keijiro TAKAGI, Terumi TAMADA*,
Toshio KAMISHIRO* and Yasuo FUJIMOTO*

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences,
University of Tokyo, Bunkyo-ku, Tokyo 113 and
*Department of Research Institute, Nippon Chemiphar Co., Ltd.
Hikokawato 1-22-1, Misato-shi, Saitama 341, Japan

Accepted September 16, 1976

Abstract—Antihypertensive activity of orally administered methyl O-(4-hydroxy-3-
methoxycinnamoyl)reserpate (CD-3400) was examined and compared with such activity
of reserpine and its related agents in conscious hypertensive rats. CD-3400, 4-16
mg/kg p.o., produced a marked antihypertensive effect lasting 6 hr and a slight decrease
in heart rate in spontaneously hypertensive rats (SHR). Reserpine, 1-4 mg/kg, rescinn-
amine, 4-16 mg/kg, and syrosingopine, 8-32 mg/kg p.o., produced effects similar to
those of CD-3400. The order of antihypertensive activities of the agents was as follows:
reserpine > CD-3400 > rescinnamine > syrosingopine. In renal hypertensive rats and
deoxytocoristosterone hypertensive rats, CD-3400 and rescinnamine, 4-16 mg/kg, and
reserpine, 1-4 mg/kg p.o., also produced an antihypertensive effect and the order of
potency was as follows: reserpine > rescinnamine > CD-3400. Repeated adminis-
trations of CD-3400, 2-12 mg/kg/day p.o., for 14 days, to SHR produced the marked
constant antihypertensive effects during the administration period. The order of the
potency was as follows: reserpine > rescinnamine > CD-3400. CD-3400 possessing
a sustained antihypertensive activity may be prescribed in cases when long-term therapy
is required for hypertensive patients.

Reserpine, one of the Rauwolffia alkaloids, has been used as an antihypertensive agent.
Rescinnamine, which has a weaker activity than reserpine on the sympathetic nervous
system, has been also extensively used for long-term treatment of hypertensive patients.

Methyl O-(4-hydroxy-3-methoxycinnamoyl)reserpate (CD-3400) was originally de-
veloped by Kametani et al. (1) during a search for new derivatives without adverse effects
such as mental depression, peptic ulcer, diarrhea and ptosis, and was found to possess a
more potent antihypertensive activity than rescinnamine in spontaneously hypertensive
rats when administered i.v. (1).

In the present study, antihypertensive activities of orally administered CD-3400 in
conscious hypertensive rats were examined and compared with those of reserpine and its
related agents.

MATERIALS AND METHODS

Blood pressure and heart rate measurements in spontaneously hypertensive rats

Blood pressure and heart rate of spontaneously hypertensive rats (SHR) were measured
according to the method described by Nakao et al. (2). Male SHR which originated from the Okamoto strain, F₂₁₋₂₆ generations, between 20 and 30 weeks old, having a mean blood pressure higher than 140 mmHg, were anesthetized with ether for catheterization of the tail median coccygeal artery. The tip of a polyethylene catheter was positioned in the abdominal aorta. The animal was transferred into a small wire net cage and allowed to regain consciousness there. Arterial blood pressure was continuously recorded through the catheterized artery by a pressure transducer (Nihon Kohden, MPU-0.5). Heart rate was measured by a cardiotachometer (Nihon Kohden, RT-2) triggered by blood pressure pulses. The recordings were made on an ink-writing oscillograph (Nihon Kohden, WI-260). Agents were orally administered at least 60 min after the catheterization when the animal had regained consciousness.

In the experiments for repeated administration for 14 days, arterial blood pressure was measured indirectly according to a modified plethysmographic tail method described by Okamoto et al. (3). Systolic blood pressure measurements were made once a day (9:00–10:00 a.m.) for 5 days before the onset of the administrations, for 14 days during repeated administrations and for 11 days following withdrawal of agents. Changes in blood pressure induced by the administrations were calculated from the difference between mean values of blood pressure for 5 days before treatments and blood pressure during treatments. Agents were administered p.o. daily 1 hr after the pressure measurements.

Production of renal hypertensive rats and blood pressure measurement

Male Wistar strain rats weighing 200 to 300 g were anesthetized with ether. Renal hypertension was produced according to the technique described by Sokabe and Grollman (4). The right kidney was removed and the left one was wrapped with a cotton thread in a figure-8 ligation. Rats having a mean blood pressure higher than 140 mmHg (RHR) were used for the experiments 4 to 5 weeks after the surgical treatment. The blood pressure measurement of RHR was made according to the method described by Nakao et al. (2) except that the catheter was inserted into the femoral artery.

Production of deoxycorticosterone hypertensive rats and blood pressure measurement

Male Wistar rats weighing 200 to 300 g were anesthetized with ether and the left kidney was extirpated. The animals were provided 1% NaCl aqueous solution for drinking ad libitum and were given deoxycorticosterone acetate (DOCA, Tokyo Kasei Co.), 15 mg/kg s.c. once a week. After 4 to 5 weeks, rats having a mean blood pressure higher than 170 mmHg (DHR) were used for the experiments. The blood pressure measurement of DHR was made according to the method described by Nakao et al. (2) except that the catheter was inserted into the femoral artery.

Drugs examined

Methyl O-(4-hydroxy-3-methoxycinnamoyl) reserpate (CD-3400).

FIG. 1 Chemical structure of methyl O-(4-hydroxy-3-methoxycinnamoyl) reserpate (CD-3400).
namoyl)reserpate (CD-3400), as shown in Fig. 1, is a light yellow powder which is insoluble in water and was crystallized from dioxane. In addition, the following drugs were used: reserpine (Inverni and Della Beffo, Italy), rescinnamine (Inverni and Della Beffo, Italy) and syrosingopine (Inverni and Della Beffo, Italy). In all experiments, the agents suspended in 0.2% carboxymethylcellulose (CMC) aqueous solution were administered orally via a stomach tube.

RESULTS

Effect of single administration on mean blood pressure and heart rate in spontaneously hypertensive rats

Oral administration of 0.2% CMC solution, 2 ml/kg, produced virtually no effect on the mean arterial blood pressure and heart rate of SHR for 6 hr (Fig. 2).

CD-3400, 4, 8 and 16 mg/kg p.o., 6 rats for each dose produced a dose-related and progressive fall in mean blood pressure during a 6 hr measurement, as shown in Fig. 2. The maximum falls produced by 8 and 16 mg/kg p.o. were 23±4 mmHg and 37±4 mmHg, respectively, 6 hr after the administrations. However, heart rate was scarcely affected by the agent.

Reserpine, 2 to 4 mg/kg p.o., showed marked antihypertensive effects in a dose-related
manner, which lasted more than 6 hr, and a gradual fall in heart rate. Heart rate was significantly elevated immediately after administration of reserpine, 1 mg/kg p.o., and pretreatment levels were reverted 1 to 2 hr after the administration.

Rescinnamine, 4, 8 and 16 mg/kg p.o., produced a dose-related fall in mean blood pressure and a decrease in heart rate. The fall in mean blood pressure by rescinnamine, 4 to 16 mg/kg p.o., was similar to that induced by CD-3400 in the same dose level.

Syrosingopine, 8 and 16 mg/kg p.o., caused a dose-related fall in mean blood pressure at a period of 5 to 6 hr after the administrations. Heart rate was also decreased in a dose-related manner. The dose-response curves for the hypotensive effect of four agents, as shown in Fig. 3, were obtained from the maximum fall in mean arterial blood pressure within 6 hr following the administrations. The antihypertensive activity of CD-3400 in SHR was similar to that of rescinnamine and was about half that of reserpine.

Effect of single administration on mean blood pressure in renal hypertensive rats

In RHR, the administration of 0.2% CMC solution, 2 ml/kg p.o., induced no significant change in mean arterial blood pressure. CD-3400, 4, 8 and 16 mg/kg p.o., exerted a considerable antihypertensive effect lasting for over 6 hr, in a dose-related manner: 4 mg/kg p.o., produced a maximum fall in blood pressure by about 20 mmHg, the maximum falls produced by 8 and 16 mg/kg p.o. were 42.0±4.5 and 52.5±6.0 mmHg, respectively, 6 hr after the administrations, as shown in Fig. 4.

Oral administrations of reserpine, 1, 2 and 4 mg/kg p.o., and rescinnamine, 4, 8 and 16 mg/kg p.o., provoked a gradual fall in mean blood pressure, which was dose-related over the observation period. The fall in mean blood pressure in RHR produced by these agents was significantly greater than that in SHR.

The dose-response curves for the maximum fall in mean arterial blood pressure induced by CD-3400, reserpine and rescinnamine during a measurement period are shown in Fig. 5. The order of hypotensive effects induced by the agents used was as follows: reserpine > rescinnamine ≥ CD-3400.
Effect of single administration on mean blood pressure in deoxycorticosterone hypertensive rats

In DHR, 0.2% CMC solution caused no significant change in mean arterial pressure. CD-3400, 8 and 16 mg/kg p.o., resulted in a fall in mean blood pressure immediately after the administrations and progressively lowered in a dose-related manner, as shown in Fig. 6. The maximum fall produced by CD-3400, 16 mg/kg p.o., in DHR was 61.0 ± 5.5 mmHg 6 hr after the administration and was greater than that with the same dose of the agent in SHR and RHR.

The oral administrations of reserpine, 1, 2 and 4 mg/kg, and rescinnamine, 4, 8 and 16 mg/kg, caused a dose-related fall in mean blood pressure. The antihypertensive activity of reserpine in DHR was greater than that in SHR and RHR. The dose-response curves for the maximum fall in mean blood pressure by these agents indicated that the fall in mean blood pressure by CD-3400 was slightly greater than that by rescinnamine, as shown in Fig. 7.
Effect of repeated administrations for 14 days on systolic blood pressure in spontaneously hypertensive rats

Fig. 8 illustrates the antihypertensive effects of repeated administrations of CD-3400, 2, 5 and 12 mg/kg/day p.o. for 14 days, on systolic blood pressure in SHR. No significant changes in systolic blood pressure in rats treated with 0.2% CMC solution, 2 ml/kg p.o., were observed.

CD-3400, 5 mg/kg/day p.o. for 14 days, produced 25 to 30 mmHg fall in systolic blood pressure during the administrations, and blood pressure was returned to the pretreatment level within 5 days following withdrawal of the agent. The higher dose of CD-3400, 12 mg/
Effect of repeated oral administrations for 14 days of CD-3400 on systolic blood pressure of conscious spontaneously hypertensive rats. Arrows indicate the points at which CD-3400 was administered. Each value is the mean obtained from 6 rats. ▲, 0.2% CMC; ○, 2 mg/kg p.o.; ×, 5 mg/kg p.o.; ●, 12 mg/kg p.o.

Dose-response curves for antihypertensive effects of CD-3400, reserpine and rescinnamine on systolic blood pressure of conscious spontaneously hypertensive rats. Each point represents the maximum fall in systolic blood pressure during the oral administration of each agent for 14 days. ○, CD-3400; ●, reserpine; ×, rescinnamine.

kg/day p.o., caused a more marked fall in systolic blood pressure by 40 to 50 mmHg during repeated administrations and the pressure returned to the pretreatment level more than 9 days following the withdrawal of the agent.

Reserpine, 0.5, 1.5 and 4 mg/kg/day p.o., 14 days, and rescinnamine, 1.5, 4 and 10 mg/kg/day p.o. for 14 days, produced a dose-related fall in systolic blood pressure. The responses to CD-3400, 2, 5 and 12 mg/kg/day p.o., were slightly less than those to rescinnamine, 1.5, 4 and 10 mg/kg/day p.o.

**DISCUSSION**

The antihypertensive activity of CD-3400 by oral administration was compared with activities of reserpine and its related agents in conscious rats. In a previous paper, Kametani et al. (1) reported that in SHR the i.v. injection of CD-3400 caused a fall in blood pressure.
and that the agent was 1.75 times as potent as rescinnamine. In the present study, oral administrations of the agent also elicited a significantly sustained fall in arterial blood pressure, which was pronounced in doses of 4 mg/kg or more. A comparison of the antihypertensive activity of CD-3400 with that of rescinnamine in various types of hypertensive rats revealed the following order: CD-3400 = rescinnamine in SHR, rescinnamine ≥ CD-3400 in RHR, CD-3400 > rescinnamine in DHR, and in repeated administrations for 14 days, the antihypertensive activity of CD-3400 was slightly less effective in SHR. Reserpine caused a marked fall in mean blood pressure of SHR in doses of 2 mg/kg p.o. or more. All agents examined provoked a decrease in heart rate of SHR, which was significantly greater than that of 0.2% CMC treated rats more than 6 hr following the administrations. A tendency toward bradycardia was seen with CD-3400, 4, 8 and 16 mg/kg p.o.

There is evidence which suggests the role of catecholamines in the etiology of essential hypertension (5-9). Reserpine depletes norepinephrine content in the brain and various peripheral tissues of normotensive and hypertensive rats (10-12). CD-3400 as well as rescinnamine and syrosingopine remarkably depleted norepinephrine stores in the cardiac muscle but slightly in the brainstem when administered i.p. to normotensive rats, and the order of potency was as follows: CD-3400 = rescinnamine > syrosingopine in the cardiac muscle, rescinnamine > CD-3400 = syrosingopine in the brainstem (Tabei, personal communication). It seems likely that the fall in blood pressure by CD-3400 is closely related to catecholamine depletion in the various tissues as is the case with reserpine and its related compounds. The depletion of norepinephrine stores in the brainstem by CD-3400 was equal to that by syrosingopine which was demonstrated to have a weak inhibitory effect on the central nervous system (13). Plummer et al. (14) and Orlans et al. (15) reported that the low doses of syrosingopine which exhibited no inhibitory effect on conditioned avoidance response caused norepinephrine depletion in the heart and, therefore, syrosingopine appeared to cause a fall in blood pressure by depleting catecholamines in the peripheral tissues. Garattini et al. (16) suggested that the fall in blood pressure caused by reserpine and bietaserpine could be correlated with the norepinephrine level in the heart. Furthermore, the central actions of CD-3400 such as a tranquillization, hypothermia or hypnosis and the depletion of serotonin level in the brain were weaker than those of rescinnamine and reserpine (17). Thus, CD-3400 appears to cause a fall in blood pressure by acting mainly on the peripheral tissues rather than the central nervous system. In the experiments on the development of gastric lesions and increase in gastric secretion in rats, reserpine was the most potent, followed by rescinnamine, and CD-3400, in that order (18).

In the present experiments, repeated administrations for 14 days of CD-3400 resulted in a constant fall in blood pressure during the administration period.

CD-3400 has a sustained antihypertensive activity and a weak central action with less potency to cause gastric lesions, therefore, this agent may be prescribed in cases when long-term antihypertensive chemotherapy is required.

Acknowledgements: Thanks are due to Messrs. K. Ishii and S. Iwata, Dept. of Chemical Pharmacology, University of Tokyo, for technical assistance.
REFERENCES

1) Kametani, T., Ihara, M., Suzuki, T., Takahashi, T., Iwaki, R., Takei, H., Miyake, N., Yoshida, M., Hasegawa, Y. and Kitagawa, H.: J. med. Chem. 15, 686 (1972)
2) Nakao, K., Kato, H. and Takagi, K.: Japan. J. Pharmacol. 25, 25 (1975)
3) Okamoto, K. and Aoki, K.: Japan. Circulation J. 27, 282 (1963)
4) Sokabe, H. and Grollman, A.: Tex. Rep. Biol. Med. 21, 93 (1963)
5) Engelman, K., Portnoy, B. and Soferdisma, A.: Circulation Res. 27, Suppl. 1, 141 (1970)
6) Esler, M.D. and Nestel, P.J.: Aust. N. Z. J. Med. 3, 117 (1973)
7) Louis, W.J., Doyle, A.E. and Anavekar, S.: New Engl. J. Med. 288, 599 (1973)
8) Nestel, P.J. and Esler, M.D.: Circulation Res. 27, Suppl. 2, 75 (1970)
9) Doyle, A.E. and Fraser, J.R.E.: Circulation Res. 9, 755 (1961)
10) Berkowitz, B.A., Tarser, J.H. and Spector, S.: J. Pharmacol. exp. Ther. 190, 21 (1974)
11) Vetadzokoska, D., Gudeska, S., Glavas, E., Sukarova, M. and Nikodijevic, B.: Spontaneous Hypertension. Edited by Okamoto, K., p. 46, Igaku Shoin Press, Tokyo (1972)
12) Kohler, C., Berkowitz, B.A. and Spector, S.: J. Pharmacol. exp. Ther. 193, 443 (1975)
13) Lucas, R.A., Kuehne, M.E., Cegłowski, M.J., Dzieman, R.L. and MacPhillamy, H.B.: J. Am. Chem. Soc. 81, 1928 (1959)
14) Plummer, A.J., Barrett, W.E., Maxwell, R.A., Finocchio, D., Lucas, R.A. and Earl, A.E.: Archs int. Pharmacodyn. Thér. 119, 245 (1959)
15) Orlans, F.B.H., Finger, K.F. and Brodie, B.B.: J. Pharmacol. exp. Ther. 128, 131 (1960)
16) Garattini, S., Lamieta, L., Valzelli, L. and Mortari, A.: J. Pharm. Pharmacol. 13, 548 (1961)
17) Sorimachi, M.: Tokyo Ika Daigaku Zasshi, 34, 677 (1976) (in Japanese)
18) Chiba, M., Miyake, N., Tamada, T., Fujimoto, Y. and Okabe, S.: Folia pharmacol. japon. (in Japanese) (in press)