Arch. Pharm. Res. Vol. 18, No. 1 pp. 38-40, 1995

Analgesic and Hypothermic Activities of Melandrin

Eun Bang Lee, Seon Ah Cheon, Young Soon Lee, Ha Sook Chung and Won Sick Woo

Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

(Received August 30, 1994)

Melandrin exhibited an analgesic activity as assessed by inhibition of writhing syndrome in mice and by pressure method of inflamed paws of rats and inflamed tails of mice, and showed a hypothermic effect. However, no anti-inflammatory activity was shown.

Key words: Melandrin, *Melandrium firmum*, Analgesic, Hypothermic activity

INTRODUCTION

Melandrin is a phenolic acid amide isolated from *Melandrium firmum* (Caryophyllaceae) (Woo and Choi, 1987) which is used traditionally in cases of bleeding, pain and carbuncle and to treat gonorrhea and to normalize blood circulation (Huh, 1984). Melandrin was considerd as an phytoalexin but exhibited no antibacterial, antifungal nor antiviral effects (Moon *et al.*, 19 93).

The aim of the present study is to evaluate analgesic, hypothermic and antiinflammatory activities of melandrin.

MATERIALS AND METHODS

Materials and animals

Melandrin (mol. wt. 273) synthesized by the coupling reaction from 5-hydroxyanthranilic acid and p-hydroxybenzoic acid (Moon *et al.*, 1991) was suspended in 2% CMC solution for administration to animals. The animals used were male ddY mice (19-24 g) and male Sprague-Dawley rats (150-230 g). All animals were kept under a constant 12-hr day and 12-hr night cycle with temperature between 21-25 ℃.

Analgesic effect

Antiwrithing method: The experiment was carried out according to the method of Whittle (1964). Writhing syndrome in mice was induced by *i.p.* injection of 0.7% acetic acid-saline solution. The sample and aminopyrine as a reference drug were administered subcutaneously 1 hr prior to injection of the irritant to each group of mice. The writhing syndrome was counted for 10 min from 10 min after induction of writhing syndrome.

Randall-Selitto method: The experiment was achieved according to the method of Randall and Selitto (1957) and improved by Winter and Flataker (1965). The right hind paw was inflamed by a subplantar injection (0.1ml) of a 20% brewer's yeast suspension in 0.9% saline. Thirty min after injection of yeast suspension, the vehicle, compound and aminopyrine were administered subcutaneously in each group of 8 rats. Measurement of pain threshold was carried out 0.5, 1.5 and 2.5 hr after administration, using Analgesy meter (Ugo Basile).

Tail pressure method: The analgesic response was measured by a modification (Lee, 1992) of tail pressure method(Takagi *et al.*, 1958). Briefly, the analgesia was determined with reaction threshold to pressure given on the mouse tail by exerting a force which suppressed at a constant rate of 20 mmHg/sec using electrosphygmomanometer and was recorded on a Narco Physiograph. In order to increase the sensitivity of pain, the tails of mice were injected subcutaneously 0.1ml of 20% brewer's yeast suspension in 0.9% saline at a position of 1cm distance from the tail root 2 hr before oral administration of the compound.

The animals in the treated groups were designated as "protected" if the individual reaction thresholds to pressure exceeded the control group mean threshold by two standard deviations of that mean, according to Swingle *et al.* (1971). The reaction thresholds to the pressure were determined at 30 and 90 min after administration of the compounds and the ED50 values were calculated graphically.

Correspondence to: Eun Bang Lee, Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

Hypothermic effect

Normal mice were used. After subcutaneous treatment of the compound and aminopyrine as a reference drug, the rectal temperatures of a group of 6 animals were measured using digital thermometer at the stated intervals.

Anti-inflammatory effect

Carrageenin-induced edema test was performed using male Sprague-Dawley rats according to the method of Winter *et al.* (1963). The rat was given drugs orally 1 hr prior to injection of 0.1ml of 1% carrageenin into the tissue of the subplantar surface of the hind paw of the rat. The volume of the foot was measured with plethysmometer (Ugo Basile) before injection of carrageenin solution and every 1 hr after the injection for 3 times.

Seven rats of control and treated groups were used and aspirin was given as a reference drug in this experiment.

The acute toxicity

Following oral administration of melandrin to groups of 6 mice, the lethality was observed.

Statistical analysis

Values are expressed as the mean standard errors. Differences between groups were evaluated by analysis of variance followed by student's *t*-test, and $p< 0.05$ was considered significant.

RESULTS AND DISCUSSION

The inhibitory effect of melandrin in acetic acid-induced writhing was shown in Table I. Melandrin given subcutaneously at a dose of 50mg showed 56% inhibition of writhing syndrome in mice. This effect may be roughly considered to be equipotent to that of aminopyrine.

The pain thresholds in inflamed rat paws in control and drug-treated group as measured by Randall-Selitto method were shown in Table II. At the doses of 100 and 200 mg/kg s.c. of melandrin, the thresholds were significantly elevated 1.5 and 2.5 hr after its administration as compared to control group. The effect of aminopyrine used as reference drug was much more elevated at the same doses. This indicates that melandrin has analgesic effect and shows less potent activity than that of aminopyrine in weight basis.

The analgesic effect as assayed by tail pressure of mice is shown in Table III. The ED_{50} values of melandrin in mice were 440 mg/kg 1.5 hr after oral administration and 234.3 mg/kg 2.5 hr after the administration. The data indicate that the analgesic potency of melandrin 2.5 hr after its administration corresponds to about one half that of aminopyrine.

The effect of melandrin on normal temperature of mice was shown in Fig. 1. One hour after subcutaneous administration of 200 mg/kg of melandrin a 2℃ decrease in rectal temperature was observed. Aminopyrine at a dose of 200 mg/kg s.c. induced 3.5℃ decrease 30min after the administration. It is noted that the effect of melandrin showed a moderate decrease with delayed effect as compared to that of aminopyrine. The result might suggest that melandrin has an antipyretic effect.

At the dose of 500 mg/kg, *p.o.*, no anti-inflammatory

Table I. The effect of melandrin in acetic acid-induced writhing in mice

Drug	Dose (mg/kg, s.c.)	No. of mice	No. of writhing	Inhibition (%)
Saline	–	7	18.9± 1.9	–
Melandrin	50	6	8.3± 1.8*	56.1
Aminopyrine	50	6	8.2± 1.8*	56.6

Significantly different from the saline group (*; $p<0.01$)

Table III. Analgesic effect of melrandrin in pressure method of inflamed mouse tail

Compound	ED_{50} (mg/kg, *p.o.*)	
	1 hr	2 hr*
Melandrin	440	234.3
Aminopyrine	50	140.0

*The time after treatment of the compounds

Table II. Analgesic effect of melandrin in Randall-Selitto method in rats

Drug	Dose (mg/kg, s.c.)	No. of rats	Pain threshold (g, M± S.E.) 0.5	1.5	2.5 hr
Saline	–	7	152.9± 18.5	125.7± 6.8	117.1± 12.0
Melandrin	100	7	177.1± 32.0	231.4± 32.3*	201.1± 21.8**
	200	7	213.6± 34.9	222.9± 21.0**	203.6± 10.2**
Aminopyrine	100	7	204.6± 31.0**	350.0± 37.9**	241.8± 32.3**
	200	7	666.4± 30.5**	702.9± 15.8**	540.0± 55.8**

Significantly different from the saline group (*; $p<0.05$, **; $p<0.01$)

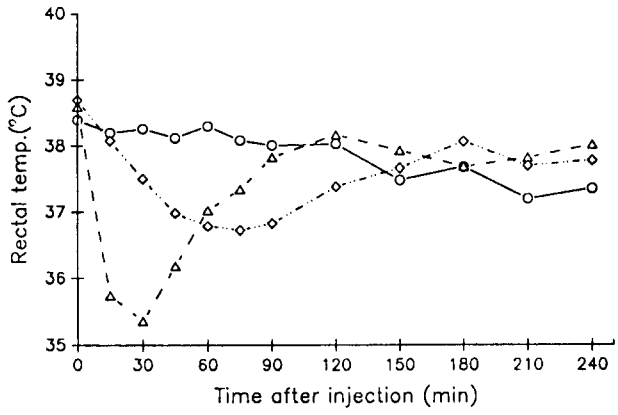

Fig 1. Hypothermic effect of melandrin.
—◎—; Saline, ---◇---; Melandrin (200 mg/kg), -·-△-·-; aminopyrine (200 mg/kg)

effect on the carrageenin-induced paw edema in rats was observed (data not shown). The oral doses of melandrin up to 6000 mg/kg to 6 mice did not influence their lethality in 3 days.

In conclusion, these results indicate that melandrin exhibits potent inhibition of writhing syndrome induced by acetic acid, and showed analgesic activity in pressure method of inflamed rat paws and inflamed mouse tail, and it also showed inhibition of normal body temperature in mice. The acute toxicity of melandrin is noted to be very weak.

REFERENCES CITED

Huh, J., *Dongeui-bogam,* Translated edition by a committee for translation, Namsan-dang, Seoul, pp. 1190 (1984).

Lee, E. B., An evaluation method of analgesic activity, In Yun-Choi, H. S. and Chang, I. M. (Eds'), "*Method in new drug development from traditional medicinal materials*". Natural Products Res. Inst., Seoul National University, Seoul, pp. 24-27 (1992).

Moon, G. S., Lee, K. R., Lim, G. K. and Woo, W. S., Synthesis of melandrin. *Yakhak Hoeji,* 35, 332-334 (1991).

Moon, G. S., Lee, K. R., Lim, G. K., Woo, W. S. and Park. S. W., Synthesis of N-substituted 5-hydroxyanthranilic acid. *Yakhak Hoeji,* 37, 243-246 (1993).

Randall, L. O. and Selitto, J. J., A method for measurement of analgesic activity on inflamed tissue. *Arch. Int. Pharmacodyn.,* 61, 409-419 (1957).

Swingle, K. F., Grant, T. J. and Kvam, D. C., Quantal responses in the Randall-Selitto assay. *Proc. Soc. Exp. Biol. Med.,* 137, 536-538 (1971).

Takagi, K., Kameyama, T. and Yano, K., A study on analgesic agent(III). A test method using a pressure apparatus. *Yakugaku Zasshi,* 78, 553-558 (1958).

Whittle, B. A., The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *Br. J. Pharmacol.,* 22, 246-253 (1964).

Winter, C. A. and Flataker, L., Reaction thresholds to pressure in edematous hind paws of rats and responses to analgesic drugs. *J. Pharmacol. Exptl. Therap.,* 150, 165-171 (1965).

Winter, C. A., Risley, E. A. and Nuss, G. W., Anti-inflammatory and antipyretic activities of indomethacin. *J. Pharmacol. Exptl. Therap.,* 141, 369-376 (1963).

Woo, W. S. and Choi, J. S., A phenolic amide and other constituents of *Melandrium firmum. Phytochemistry,* 26, 2099-2100 (1987).