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Anxiolytic-like effect of paeonol in mice

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Abstract

The present study in mice compared the putative anxiolytic-like effect of paeonol, a phenolic component from the root bark of *Paeonia moutan*, with the benzodiazepine diazepam in the elevated plus maze and the light/dark box-test. The comparison was also with regard to locomotor activity (open-field test) and myorelaxant potential (inclined plane test). As with 2 mg/kg diazepam, paeonol (at 17.5 mg/kg) increased the percentage of time spent on open arms in the elevated plus maze and increased the time spent in the light area of the light/dark box (at 8.75 and 17.5 mg/kg). Since paeonol, in contrast to diazepam, had no effect on either the number of squares entered in the open-field test or in the inclined plane test, its side-effect profile is considered as superior to the benzodiazepine.

Keywords: Paeonol; Diazepam; Anxiolytic; Elevated plus maze; Light/dark box; Mice

1. Introduction

Paeonol, a major phenolic component of Moutan Cortex, the root bark of *Paeonia moutan*, is described to have antiaggregatory, antioxidant and anti-inflammatory activities. Chou (2003) reported that the mechanisms by which paeonol exerts its anti-inflammatory and analgesic effects may be associated with decreased production of proinflammatory cytokines, NO and PGE_2 and increased production of IL-10, an anti-inflammatory cytokine. In addition, in carrageenan-injected rat paws, attenuation of the elevated iNOS and COX-2 protein expression as well as neutrophil infiltration in carrageenan-injected paws may also be involved in the beneficial effects of paeonol. Paeonol significantly inhibited histamine release from the rat peritoneal mast cells (RPMCs) treated with compound 48/80, a mast cell deregulator (Kim et al., 2004). Paeonol was found to be inhibitory against MAO-A and MAO-B in a dose-dependent manner (Kong et al., 2004). Zhang et al. (2003) reported that the protection by paeonol against myocardial injury is due to its blocking effect on L-type Ca^{2+} channel current (Fig. 1).

In traditional Chinese medicine, *Cynanchum paniculatum* Radix (the main component is paeonol) was used to treat insomnia caused by neurasthenia. Paeonol also has sedative, antiepileptic, analgesic effects. For MAOIs, such as moclobemide, an anxiolytic-like effect after single and multiple treatments has been reported (Luisa and Chiara, 2000). Considering paeonol's pharmacological profile, we have investigated here its anxiolytic potential by using animal models sensitive to clinically effective anti-anxiety compounds.

2. Material and methods

2.1. Animals

Male Swiss mice (bred at the Experimental Animal center of Shenyang Pharmaceutical University) weighing 16–18 g were housed in groups of five in polycarbonate cages (cage size: $25 \times 14 \times 12$ cm) for at least 10 days prior to testing under a reversed L12:D12 cycle (lights off 07:00) in a temperature-controlled (22±2 °C) animal

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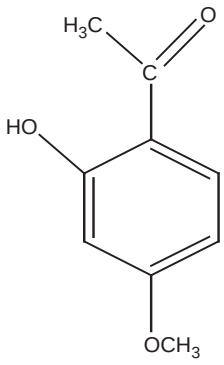

Fig. 1. Structure of paeonol.

facility. Food and water were freely available with the exception of the brief test periods. Animals were handled gently every day for 7 days. All mice were experimentally naive.

The experiments were performed following approval of the Committee of Experimental Animal Administration of the University and were in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals.

2.2. Drugs and treatments

Paeonol was purchased from Xuanchengbaicao Botanic Department (Anhui, China). Diazepam was purchased from Hubei Pharmaceutical Factory (Hubei, China). Tween-80 was obtained from Shenyang Dongxing Reagent Factory (Shenyang, China). Diazepam and paeonol were both ultrasonically dispersed in distilled water to which Tween-80 (4 drops/10 ml) had been added. All drugs were prepared immediately before use and were administered PO in a volume of 10 ml/kg body weight. Control group received the corresponding vehicle.

3. Behavioral tests

3.1. Open-field test

To study potential effects on locomotor activity, each animal was placed in the center of a rectangular area (40×60 cm) which was surrounded by 35-cm walls; the black floor was divided into 16 equally sized (10×15 cm) squares by white lines. The apparatus was illuminated with four 25-W red bulbs mounted 350 cm above the floor, giving a light intensity of 10 lx at floor level. Mice ($n = 10$ per group) were randomly assigned to seven experimental groups (vehicle control, 2 mg/kg diazepam or 8.75, 17.5, 35, 70 or 140 mg/kg paeonol). Drug administration was oral and 60 min prior to the test. Recording of the number of squares entered during the 5-min trial occurred during the dark phase of the L12:D12 cycle.

3.2. Elevated plus maze test

The elevated plus maze consists of four arms (30×5 cm) elevated 45 cm above the floor, with each arm positioned at 90° relative to the adjacent arms. The two enclosed arms had 30 cm walls and to facilitate grip on the open arms these included a raised edge of 0.25 cm. Open and closed arms were connected via a central area (5×5 cm) to form a plus sign. The maze floor was constructed of black Plexiglas and the wall of the enclosed arms was constructed of clear Plexiglas (Chen et al., 2003). The room was illuminated with four 25-W red bulbs giving a light intensity if 12 lx on the arms. Mice ($n = 9–10$ per group) were randomly assigned (with a slight adjustment for matched body weight) to five experimental groups (vehicle control, 2 mg/kg diazepam or 8.75, 17.5, 35 mg/kg paeonol). Drug administration was oral and 60 min prior to the test. The number of entries into and the time spent on each of the two types of arms and the latency to enter open arms were recorded during the 5-min trial. Thereby, an arm entry was defined as all four paws having crossed the dividing line between an arm and the central area. Test sessions were recorded via an overhead video camera linked to a monitor and video-recorder in an adjacent room and, recordings were quantified by an observed unaware of the treatment. The plus maze was thoroughly cleaned after each animal. All behavioral recordings were carried out with the observer unaware of the treatment of the mice.

3.3. Light/dark transition test

The light/dark box consists of two compartments: one light area (27 L × 27 W × 27 H cm, 400 lx) illuminated by 100-W desk lamp was painted white, and the other dark area (18 L × 27 W × 27 H cm, 4 lx) was painted black. The floor of the light area was divided into nine equal squares (9×9 cm) by black lines and the dark area was divided into six equal squares (9×9 cm) by white lines. The two compartments were separated by a partition with a tunnel (7.5×7.5 cm) to allow passage from one compartment to the other. Mice ($n = 9–10$ per group) were randomly assigned (with a slight adjustment for matched body weight) to five experimental groups (vehicle control, 2 mg/kg diazepam or 8.75, 17.5, 35 paeonol). Drug administration was oral and 60 min prior to the test. The experiments were performed between 09:00 and 14:00, i.e., in the middle of the dark phase. Animals were placed in the center of the lit area facing the wall opposite to the tunnel. The following parameters were recorded during 5 min: (1) latency time for the first crossing to the dark compartment, (2) the number of crossings between the light and the dark compartment, (3) the total time spent in the illuminated part of the cage, (4) the overall movements (squares entered) in both areas. The apparatus was cleaned thoroughly between trials. All behavioral recordings were

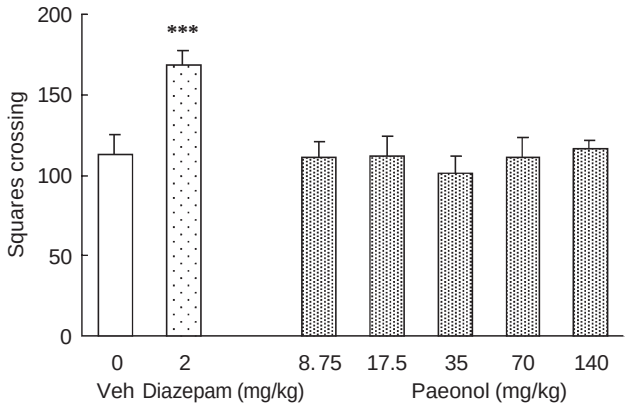

Fig. 2. Number of squares entered in the open-field test following treatment with diazepam or paeonol. Drugs were administered orally 60 min before the mice were exposed for 5 min to the open-field. Data are expressed as mean±SEM. ***$P<0.001$ compared to control group (one-way ANOVA followed by two-tailed Dunnett's t-test). $n=10$.

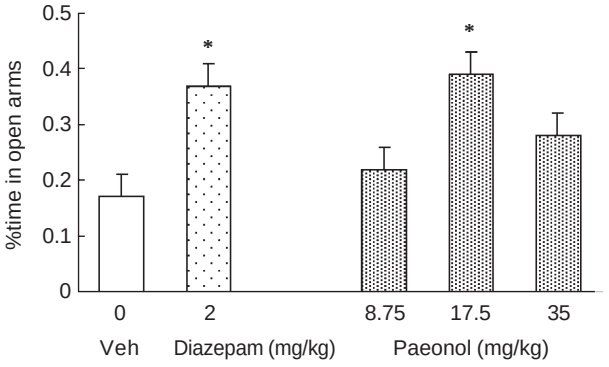

Fig. 3. Percentage of time spent on open arms in the mouse elevated plus maze following treatment with vehicle, 2 mg/kg diazepam or 8.75, 17.5 or 35 mg/kg paeonol. Drugs were administered orally 60 min before the mice were tested for 5 min in the elevated plus maze. Data are expressed as mean±SEM. *$P<0.05$ compared to control group (one-way ANOVA followed by two-tailed Dunnett's t-test). $n=10$.

carried out with the observer unaware of the treatments of the mice.

3.4. Inclined plane test

By using the inclined plane test, the motor function of mice after administration of diazepam or paeonol was evaluated. The plane consists of two rectangular steel boards connected at one end by a hinge. One of the boards serves as the base and the other as the movable inclined plane. Two protractor-like steel side panels with degrees marked on their faces are fixed on the base. A rubber mat with ridges 0.6 mm in height was fixed to the surface of the moveable plane. Mice were placed in such a position on the mat that their body axis was perpendicular to the axis of the inclined plane. The maximum inclination (each mouse was tested twice and the mean value was recorded as the maximum inclination) of the plane at which a mouse could maintain itself for 5 s was recorded and taken to represent the mouse's functional ability. In practice, the angle was either increased at about 5° intervals or decreased at about 5° intervals until the mouse could maintain its position for 5 s without falling. 70 mice were randomly assigned to seven experimental groups (control group, diazepam 1, 3, 9 mg/kg groups, paeonol 8.75, 17.5, 35 mg/kg groups, $n=10$).

3.5. Statistical analyses

All analyses were performed using the software SPSS V11.5 for windows. All data are represented as mean±SEM values. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparisons between vehicle- and drug-treatment groups were performed using the Dunnett's t-tests. The level of statistical significance adopted was $P<0.05$.

4. Results

4.1. Open-field test

The behavior effects of treatment with paeonol and diazepam, respectively, on the behavior of mice in the open-field test are summarized in Fig. 2. ANOVA for number of squares entered yielded [$F(6,63)=4.692$, $P<0.001$]. Control and experimental groups were compared with Dunnett's procedure. Diazepam, 2 mg/kg, increased the number of squares entered ($P<0.01$).

Table 1
Effect of acute paeonol on the behavior of male mice in the 5-min elevated plus maze test

Parameter	Vehicle	Diazepam (mg/kg)	Paeonol (mg/kg)		
		2	8.75	17.5	35.0
Open arm entries	3.7±0.9	7.8±1.6*	3.8±0.8	7.3±1.5	5.1±1.2
Closed arm entries	10.7±1.3	12.8±1.2	9.9±0.9	10.6±1.1	10.2±0.9
Total arm entries	14.4±1.7	20.6±1.5*	13.7±1.0	17.9±2.3	15.3±1.0
Open arm time (s)	34.5±8.7	77.5±17.7	41.9±11.1	76.6±11.0	57.7±15.8
Closed arm time (s)	160.8±7.4	122.6±12.5	150.6±13.5	116.7±11.2*	133.9±11.2
Latency to enter open arm (s)	95.0±34.8	25.8±7.4	27.7±8.0	20.9±8.4	38.4±17.4

Values represent mean±SEM. *$P<0.05$ drug vs. control groups (one-way ANOVA followed by two-tailed Dunnett's t-test). See Fig. 3 for complementary data. $n=10$.

Paeonol induced no significant increase in the number of squares entered at any tested dose.

4.2. The elevated plus maze test

The effect of 2 mg/kg diazepam and the paeonol doses on the behavior in mice in the elevated plus maze is summarized in Table 1 and Fig. 3. ANOVA for open arm entries yielded [$F(4,43)=2.519$], for total arm entries [$F(4,43)=3.394$, $P<0.05$], for time spent in the open arm [$F(4,43)=2.212$], for time spent in the close arm [$F(4,43)=2.682$, $P<0.05$], for the ratio open/total time [$F(4,43)=2.773$, $P<0.05$]. Control and experimental groups were compared with Dunnett's procedure. Diazepam, 2 mg/kg, increased total arm entries ($P<0.05$) and the ratio open/total arm time ($P<0.05$). The dose of 17.5 mg/kg paeonol significantly increased and decreased the ratio open/total arm time and the time spent on closed arms, respectively ($P<0.05$ in both cases). None of the additional parameters was significantly affected.

4.3. The light/dark transition test

Results of the light/dark test are shown in Table 2. ANOVA indicated a significant effect for time in the light area [$F(4,45)=4.451$, $P<0.01$] but not for the overall number of entered squares [$F(4,45)=1.951$], the latency to enter the dark area [$F(4,45)=2.212$] or the number of inter-compartment transitions [$F(4,45)=2.288$]. Comparisons between the vehicle control group and experimental groups (Dunnett's tests) indicated that 2 mg/kg diazepam had significantly increased the time spent in the light area and the number of transitions between the two compartments ($P<0.01$ and $P<0.05$, respectively). Paeonol, at the dose of 8.75 and 17.5 mg/kg, significantly increased the time spent in the light area ($P<0.01$) but was not significantly affecting any of the other parameters.

4.4. The inclined plane test

Results of the inclined plane test are shown in Fig. 4. ANOVA for degrees of inclined plane test yielded [$F(6,63)=15.450$, $P<0.001$]. Control and experimental groups were compared with Dunnett's procedure. Diazepam, 3 mg/kg ($P<0.05$) and 9 mg/kg ($P<0.01$), had myorelaxant effect in mice. Paeonol had no effect on motor function at any of the three tested doses.

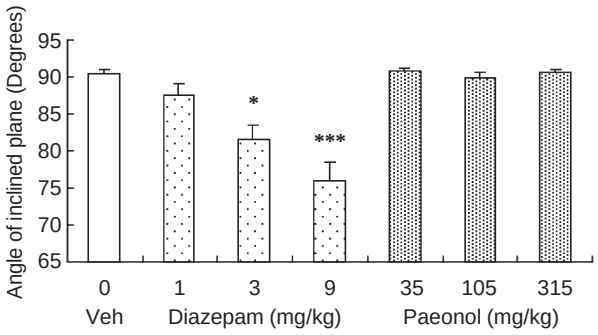

Fig. 4. Effect of vehicle, 1, 3 or 9 mg/kg diazepam or 35, 105 or 315 mg/kg paeonol on the inclined plane test. Data are expressed as mean ± SEM. *$P<0.05$, ***$P<0.001$ compared to control group (one-way ANOVA followed by two-tailed Dunnett's t-test). $n=10$.

5. Discussion

The results of the present study demonstrate that paeonol has an anxiolytic-like effect at some of the tested doses in the elevated plus maze and light/dark box.

The open-field test, which was used to evaluate the drug effect on motor activity by many authors (Hooi and Hung, 1999; Fathi et al., 1994), was used in the present study as well. Diazepam (2 mg/kg) induced a significant increase in the number of squares entered whereas paeonol did not affect the number of squares entered at any of the doses tested. We found that mice administered with diazepam (2 mg/kg) were quiet and sleepy in their home-cage. While in the open-field, the mouse's activity increased greatly. This phenomenon demonstrated that diazepam could increase the mouse's exploratory behavior in a novel environment. Paeonol induced no significant increase in the number of squares entered indicating that paeonol, at the doses tested, had no effect on motor activity.

The elevated plus maze (EPM), in which mice are allowed to freely explore the open and closed arms, has been extensively validated in both rats and mice (Hogg, 1996). In the present study, diazepam increased, as expected for this typical anxiolytic compound, the number of open arm entries, the absolute time as well as the percent time spent on open arms and, as a direct consequence, decreased the time spent in

Table 2
Effect of acute paeonol on the behavior of male mice in the 5-min light/dark transition test

Parameter	Vehicle	Diazepam (mg/kg)	Paeonol (mg/kg)		
		2	8.75	17.5	35.0
Time in light area (s)	90.0±8.4	128.8±7.7**	124.2±8.3**	125.1±7.1**	109.7±5.2
Overall movements	99.8±13.1	141.6±10.8	119.1±10.9	136.9±13.3	114.2±12.7
Latency time (s)	12.1±3.8	7.9±1.6	19.9±6.1	6.3±1.3	7.9±3.4
Number of transitions	12.4±2.4	22.8±1.7*	17.1±3.0	19.8±2.9	20.9±3.0

Values represent mean ± SEM. *$P<0.05$, **$P<0.01$ drug vs. control group (one-way ANOVA followed by two-tailed Dunnett's t-test). $n=10$.

closed arms. Paeonol, at least trendwise, also increased the number of open arm entries and the time spent on open arms, the effect-size of these changes did, however, not reach the level of significance. The latency to enter the dark compartment tended to be reduced following both diazepam and paeonol (no significant level was reached). Paeonol significantly increased and decreased the percent time spent on open and closed arms, respectively, without altering the number of total arm entries. Both diazepam and paeonol did not significantly change the number of closed arm entries, a parameter thought to reflect locomotor activity.

Light/dark box is also widely used in rodents as a model for screening anxiolytic or anxiogenic drugs, based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, novel environment and light (Crawley and Goodwin, 1980). Both diazepam and paeonol can increase time in the light area. Both diazepam and paeonol could also increase transitions between two compartments and squares entered but both failed significantly. Both diazepam and paeonol failed to increase the latency time. Few data were available concerning the latency time parameter. This index was used by Crawley (Blumstein and Crawley, 1983; Crawley, 1981; Crawley and Goodwin, 1981; Crawley and Davis, 1982), in contrast to some other authors (Costall et al., 1989). Increase in latency time could be the result of disinhibitory behavior and decreased anxiolysis, where animals spent more time in exploring the white area. The other explanation is the influence of sedation, where animals are unable to move quickly to the dark compartment (Hascöet and Bourin, 1998). Paeonol (8.75 and 17.5 mg/kg) can increase time in the light area without affecting locomotion suggesting an anxiolytic-like effect.

The dose–response relation with paeonol is bell-shaped in both the elevated plus maze and the light/dark paradigm. This indicates that the active dose-range is limited but also, as indicated by data from the open-field test as well as from the inclined plane test, that the lack of an anxiolytic effect at higher doses not seem to be prevented by any motor-disturbing effects kicking in with higher doses of paeonol. It might be mentioned here that also for other non-classical anxiolytic compounds such as 5-HT_3 and 5-HT_4 receptor antagonists (Vasar et al., 1993 and Jordi et al., 1996, respectively) bell-shaped dose–response curves have been reported. With regard to robustness, an anxiolytic-like effect with paeonol was seen at one dose in the elevated plus maze and two doses in the light/dark test, only and also quantitatively, the anxiolytic-like effect of paeonol appears limited when compared to the benzodiazepine diazepam, used here as a positive standard: since a significant effect was seen for the percent time spent on open arms, only. Again, this may reflect what has been previously seen with 5-HT_3 and 5-HT_4 receptor antagonists (Vasar et al., 1993 and Jordi et al., 1996). With regard to potential side-effects, paeonol can however be considered as superior to diazepam given that it had no effect on motor activity in either the open-field test or the inclined plane test, also at doses well above the compound's anxiolytic dose-range.

To summarize, some doses within the dose-range tested of paeonol exhibited in mice an anxiolytic-like effect in the elevated plus maze and the light/dark test. The mechanism of action via which paeonol may cause this remains at present unsolved and this certainly needs to be addressed in future investigations.

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