DESIGN OF A PHENCYCLIDINE IMPLANTATION PELLET; SUITABLE FOR TOLERANCE DEVELOPMENT

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When using laboratory animals (e.g., mice) for phencyclidine (PCP) tolerance studies, an essential part of the procedure is to administer the PCP in such a way that the animals received adequate doses of the drug at frequent enough intervals to reach and maintain the desired levels of tolerance or employ a osmotic minipump which is either suitable or convenient to develop a high degree of tolerance to PCP in a large number of animals in a short period. However, these methods are unfit for routine work because of repeated daily injections consume too much time and osmotic minipump comes expensive. Therefore, in this paper we attempted to develop PCP pellet suitable for tolerance development. The s.c. implantation of a 10 or 20 mg PCP pellet in the back of a conscious mouse resulted in a much more rapid development of tolerance to PCP than that produced in mice receiving daily i.p. injection of, 10 or 20 mg/kg, PCP-HCl. Assessment of and degree of tolerance to PCP by PCP pellet implantation and daily injection of PCP-HCl were evidenced by a degree of decrease in the duration of motor incoordination after the challenge with, 20 mg/kg, PCP-HCl 24 h after removal of PCP pellets or a last injection of PCP-HCl. These studies may demonstrate a substantial methodological improvement in producing a high degree of tolerance to PCP in a short period of time by means of the s.c. pellet implantation technique.

Keywords — motor incoordination; mouse; osmotic minipump; phencyclidine; pellet implantation; routine work; tolerance

INTRODUCTION

Phencyclidine (PCP) is a phenylcyclohexyl derivative of piperidine with an unusual spectrum of pharmacological actions. It sometimes induces behavioral changes that mimic the primary symptoms of schizophrenia in drug abusers or exacerbates symptoms in chronic schizophrenics.1,2) Furthermore, PCP is known to be a major drug of abuse.3,4) Like many other drugs of abuse, PCP has been reported to induce tolerance to its pharmacological actions.5,6) Repeated administration of PCP in primates has been shown to decrease periods of effective anesthesia for several weeks or months following administration.7) Tolerance to the pharmacological actions of PCP has also been reported in humans8,9) and mice.8,9) In a previous study, daily injection of PCP (10–40 mg/kg) is employed to induce tolerance.8) However, such high doses of PCP administered as a bolus, can not maintain effective drug concentration for a prolonged period of time, due to metabolism and/or elimination. As a result, this method is neither suitable nor convenient to develop a high degree of tolerance to PCP in a large number of animals. Therefore, Nabeshima et al.10) have employed Alzet osmotic minipump containing PCP to develop tolerance in mice. As a result of using this method, mice are exposed to high concentration of PCP continuously so that a high degree of tolerance to PCP is developed in a short period. In addition, a qualitative and quantitative
assessment of the tolerance is possible. Thus, this method gives consistent results with respect to dose- and time-related tolerance development. However, the Alzet osmotic minipump comes expensive for routine work to investigate a mechanism of tolerance development to PCP.

On the other hand, it is well known that the researches in morphine and pentobarbital tolerance are progressing using the pellet-implantation methods which result in a rapid development of tolerance and a much greater degree of tolerance at low costs.11,12) Therefore, in this paper we attempted to develop PCP pellet suitable for tolerance development like the morphine and pentobarbital pellets.

MATERIALS AND METHODS

**Animals** — Male ICR mice weighing 30±2 g (Charles River Japan, Inc., Atsugi, Japan) were used for all experiments. These animals were maintained on standard laboratory chow and tap water at all time. They were housed in a temperature (23±1°C) and humidity (55%) regulated room with a constant lighting conditions (lights were on 8:00−20:00 h).

**Reagents** — Phencyclidine [1-(1-phenylcyclohexyl)piperidine; PCP] and PCP-HCl were synthesized by us. The other reagents used were microcrystalline cellulose [Avicel](Funakoshi Medicines, Kanda, Japan), silica fumed (Sigma, St Louis, Mo.) and calcium stearate (Katayama Chemical Co., Nagoya Japan).

**Preparation of PCP Pellet** — PCP pellet were made by the method of Gibson and Tingstad13 with minor modifications.

**Formation of PCP Pellets:**

<table>
<thead>
<tr>
<th>PCP free base particle</th>
<th>10 mg</th>
<th>15 mg</th>
<th>20 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCP free base particle</td>
<td>10.0 mg</td>
<td>15.0 mg</td>
<td>20.0 mg</td>
</tr>
<tr>
<td>microcrystalline cellulose</td>
<td>285.5 mg</td>
<td>280.5 mg</td>
<td>275.5 mg</td>
</tr>
<tr>
<td>silica fumed</td>
<td>1.5 mg</td>
<td>1.5 mg</td>
<td>1.5 mg</td>
</tr>
<tr>
<td>calcium stearate</td>
<td>3.0 mg</td>
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Directions: Screen the microcrystalline cellulose, fumed silica and calcium stearate through 64 to 100 mesh screens. They were mixed well in a porcelain mortar. At first, PCP free base was ground to powder in a small agate mortar and then the PCP powder of 300 mg was compressed to prepare a tablet of 13 mm diameter by an apparatus of tablet former for infrared (IR) analysis at 30 kg/cm² for 15 s. To prepare PCP particles of proper size, this tablet was pounded and screened through 12 to 16 mesh screens. According to the formulation described above, the obtained PCP-small particles of fixed weight were embedded in the center of mixed vehicles and then compressed by the tablet former at 30 kg/cm² for 15 s, to make final PCP pellet. The sizes of resultant pellet are as follows: weight; 300 mg, thickness; 20 mm, diameter; 15 mm. We employed PCP base particles instead of PCP powder or small PCP core to prepare PCP pellets by following reasons: 1) When PCP base powder was mixed evenly with the vehicles and made pellets and then mice were implanted subcutaneously (s.c.) with these pellets, even 2 mg PCP-pellet killed all of the animals at early time of implantation since the release of the drug from the pellet was too fast. 2) By the same reason, PCP-HCl which easily dissolves in an aqueous solution was not used. 3) On the contrary, the pellet containing small hard PCP core did not show any pharmacological effect of PCP until 10 d after implantation. Placebo pellets containing microcrystalline cellulose 295.5 mg, silica fumed 1.5 mg and calcium stearate 3.0 mg were prepared as same as PCP pellets.

**Body Weight and Cumulative Mortality after PCP Pellet Implantation** — Four groups of 5 to 101 mice each were used and each animal was implanted with a placebo or a PCP pellet (10, 15 or 20 mg), s.c. for 5 d. Body weight and cumulative mortality were recorded every day.

**Motor Incoordination after PCP Pellet Implantation** — Motor incoordination induced by an implanted PCP pellet was determined according to the method of Flint and Ho8 with modifications. A wooden box (62×43×15 cm) was constructed. Wooden rods (30 cm long and 6 mm square) were cut and inserted 15 cm apart into
the box. Prior to PCP pellet implantation, the mice were positioned and observed for their ability to stay on the rods for at least 30 s. A scoring system was made to quantitatively analyze the loss of ability to balance on the rods. The following scores were given based on the duration of time that each mouse stayed on the rod: 5 = 0–10 s, 4 = 11–15 s, 3 = 16–20 s, 2 = 21–25 s, 1 = 26–30 s, 0 = over 31 s. This index was used for measuring the duration of effect by PCP on the animals.

Assessment of and Degree of Tolerance to PCP by PCP Pellet Implantation and Daily Injection of PCP-HCl — In order to assess a possible dose and time dependency in the development of PCP tolerance, 12 groups of 3 to 8 mice each were implanted with a placebo or a PCP pellet (10, 15 or 20 mg), s.c. for 1, 3 or 5 d. For a comparison of the degree of tolerance to PCP between PCP pellet implantation and daily injection of PCP-HCl, other 8 groups of 6 to 8 mice each were injected daily with saline or PCP-HCl (10 or 20 mg/kg), i.p. for 1, 3 or 5 d. In preliminary experiments an additive effect on motor incoordination was observed when mice with implanted PCP pellets were challenged with PCP-HCl. Therefore, 24 h after removal of PCP pellets or a last injection of PCP-HCl, each mouse was challenged with PCP-HCl, 20 mg/kg, i.p. and the motor incoordination was observed every 30 min after the challenge of PCP-HCl until total recovery. The animals were not placed on the wooden rods for observation until after PCP-HCl challenge. This was to eliminate the possibility of a learned response to the testing procedure.

All data were subjected to statistical analyses and the statistical significance was determined using Student’s t-test for body weight and Mann–Whitney’s U-test for motor incoordination.

RESULTS

1) Effects of PCP Pellet Implantation of Body Weight and Cumulative Mortality

As shown in Fig. 1, all the mice implanted with pellet containing PCP 10, 15 and 20 mg showed significant reduction in body weight, 1 to 5 d after the implantation as compared to the placebo pellet group. The effects of 10 and 15 mg PCP pellet-implantation reached a plateau 1 d after the implantation and continued for the whole experimental period (5 d) at the similar degree. The degree of decrease of body weight in the 20 mg PCP pellet-group was larger than those in the 10 and 15 mg pellet-groups 1 to 4 d after the implantation while no difference was observed among 3 groups 5 d after the implantation.

Mortality was also observed in all groups implanted PCP pellet (Fig. 2). Percent of cumulative mortality of the 15 and 20 mg PCP pellet-groups

![Figure 1](image_url)

**FIG. 1. Effect of PCP Pellet Implantation on Body Weight in Mice**

Four groups of 5 to 101 mice each were implanted, s.c., with a placebo or a PCP pellet (10, 15 or 20 mg) for 5 d. The body weight was recorded daily for 5 d. Data are means ± SEM; a) p<0.001 compared to placebo group, b) p<0.05 c) p<0.01, d) p<0.001 compared to 10 mg PCP pellet group, e) p<0.05, f) p<0.01 compared to 15 mg PCP pellet group. (Student's t-test).

- ○ placebo, ○ ○ ○ 10 mg PCP pellet, △ △ △ 15 mg PCP pellet, □ □ □ 20 mg PCP pellet.
reached to 25 and 37.5% respectively, 5 d after the implantation. However, in the 10 mg PCP pellet-group, only one out of 25 mice died.

2) Motor Incoordination Produced by PCP Pellet Implantation

As shown in Fig. 3, the 20 mg PCP pellet had an effect on 100% of the animals 1.5 h after the implantation. This group showed only few recovery by 120 h and full recovery 240 h after the implantation (data not shown). The 10 and 15 mg PCP pellet-groups showed the maximum score of motor incoordination 2 h after the implantation and then started to recover. These groups had almost full recovery 120 h after the implantation.

3) Quantitative Assessment of Tolerance Development to PCP after PCP Pellet Implantation

PCP pellet implanted groups showed a decrease in duration of PCP effect on motor coordination when challenged with a 20 mg/kg dose of PCP-HCl (Fig. 4). Four days after the implantation, the duration of motor incoordination was decreased by about 62.5, 50 and 50% in the 20, 15 and 10 mg PCP pellet-groups, respectively. The degree of decrease in the duration of motor incoordination in the 10 mg PCP pellet-group was almost the same as 15 mg PCP pellet-group. While the 20 mg PCP pellet showed slightly more potent effect than the 10 and 15 mg PCP pellets.

4) Time-Course of Development of Tolerance to PCP after PCP Pellet Implantation and Daily Injection of PCP-HCl

The 10, 15 and 20 mg PCP pellets were implanted and the subsequent motor incoordination produced by a challenge dose of PCP-HCl, 20 mg/kg, was assessed 1, 3 and 5 d after the implantation. As shown in Fig. 5, the duration of motor incoordination was decreased by 37.5, 50 and 62.5% 1, 3 and 5 d after the implantation of 20 mg PCP pellet, respectively. The degree of decrease in the duration of motor incoordination was dependent on the length of time after the PCP pellet implantation. In the 10 and 15 mg PCP pellet-groups, the durations of motor incoor-

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**FIG. 2. Effect of PCP Pellet Implantation on Mortality in Mice**

Four groups of 8 to 25 mice each were implanted, s.c., with a placebo or a PCP pellet (10, 15 or 20 mg) for 5 d. Percent of cumulative mortality was recorded daily for 5 d.

- ● placebo, ○ 10 mg PCP pellet, △ 15 mg PCP pellet, □ 20 mg PCP pellet.

**FIG. 3. Motor Incoordination Produced by PCP Pellet Implantation**

Four groups of mice were implanted with a placebo or a PCP pellet (10, 15 or 20 mg) for 5 d. The score of fall at time after PCP pellet implantation was recorded as described in Materials and Methods. The numbers in parentheses indicate the number of animals.

- ● placebo, ○ 10 mg PCP pellet, △ 15 mg PCP pellet, □ 20 mg PCP pellet.
dination were also decreased 1 d after the implantation. However, the degree of decrease did not depend on the length of time after PCP pellet implantation. Furthermore, in the PCP-HCl, 10 mg/kg daily injection group, the duration of motor incoordination was decreased to 87.5% of control on day 1 and 3 (Fig. 6), and to 75% of control on day 5. In the case of the PCP-HCl, 20 mg/kg daily injection group, the duration of motor incoordination was also decreased to 87.5, 75 and 50% of control on day 1, 3 and 5, respectively. The durations of motor incoordination induced by the challenged PCP in PCP-pellet implanted groups were less than those in PCP daily injected groups on day 1 and/or 3 (Fig. 6).

DISCUSSION

The s.c. pellet implantation technique was

**FIG. 4. Dose-Response Relationship of PCP Pellet Implantation on Tolerance Development in Relation to Motor Incoordination**

Four groups of mice were implanted with a placebo or a PCP pellet (10, 15 or 20 mg) for 4 d, after which time the pellets were removed. 24 h after removal, each mouse was challenged with PCP-HCl, 20 mg/kg, i.p. and the score of motor incoordination was recorded every 30 min after the injection. The numbers in parentheses indicate the number of animals per group. * p<0.05, ** p<0.01 compared to placebo group (Mann-Whitney's U-test).

- ●● placebo, ○ ○ 10 mg PCP pellet, △ △ △ 15 mg PCP pellet, □ □ □ 20 mg PCP pellet.

**FIG. 5. Time Course of Effect of PCP Pellet Implantation on Tolerance Development in Relation to Motor Incoordination**

Twelve groups of mice were implanted with a placebo or a PCP pellet (10, 15 or 20 mg) for 1, 3 or 5 d, after which time the pellets were removed. 24 h after removal, each mouse was challenged with PCP-HCl, 20 mg/kg, i.p. and the score of motor incoordination was recorded. The numbers in parentheses indicate the number of animals per group. * p<0.05, ** p<0.01 compared to placebo group. a) p<0.05 compared to 5 d implantation group. (Mann-Whitney's U-test)

- ●● placebo 5 d, ○ ○ 10 mg PCP 1 d, △ △ △ 15 mg PCP 3 d, □ □ □ 20 mg PCP 5 d.
FIG. 6. Comparison of Degree of Tolerance Development between PCP Pellet Implanted and PCP-HCl Daily Injected Animals

The group receiving PCP pellet was rendered tolerant by s.c. implantation of a 10 or 20 mg pellet. The motor incoordination after a challenge dose of PCP-HCl, 20 mg/kg, i.p. was measured at various day intervals following PCP pellet implantation as described in Materials and Methods. The score of motor incoordination of the animals implanted PCP were compared to those of animals that had received daily injections of PCP-HCl, 10 or 20 mg/kg, i.p. The numbers in parentheses indicate the number of animals per group. 

\[ p < 0.05, \quad p < 0.01 \] compared to control group.

a) \[ p < 0.05 \], b) \[ p < 0.01 \] compared to daily injection group (Mann–Whitney’s U-test).

A) ● ● control, △ △ daily injection 10 mg/kg, □ □ 10 mg PCP pellet.
B) ● ● control, △ △ daily injection 20 mg/kg, □ □ 20 mg PCP pellet.

originally used by Huidobro and Maggiolo for producing tolerance to morphine. By using a modified peller by Gibson and Tingstad, Way et al. have been able to produce a much greater degree of tolerance within a much briefer time period. While, Ho et al. have formulated a pentobarbital pellet, which is prepared similarly except pentobarbital replaced morphine. The pellet is useful for developing tolerance to barbiturates. Therefore, we have attempted to formulate a modified PCP pellet for investigation of PCP tolerance according to the method of pre-
Phencyclidine Implantation Pellet

To assess tolerance to the pharmacological effects of PCP in rodent, motor incoordinations were used by previous authors. It is observed that the development of tolerance to PCP is dose dependent by both qualitative and quantitative assessments in terms of motor incoordination. In addition, a decrease of PCP-induced stereotyped behavior was also observed in the mice rendered tolerance to PCP-induced motor incoordination (unpublished results). Furthermore, it is well known that a functional tolerance to PCP in the brain enkephalinergic and GABAergic systems correlates the development of tolerance to PCP-induced motor incoordination. Therefore, PCP-induced motor incoordination was used for an index of tolerance.

As shown in Fig. 5, the duration of PCP-induced motor incoordination in PCP pellet-implanted mice were significantly shorter than those of placebo pellet-implanted mice. However, there was no difference in the onsets of motor incoordination induced by PCP between PCP and placebo pellet-implanted mice in our experimental schedule. In order to assess the degree of tolerance to PCP, the duration of PCP induced motor incoordination was employed. A tolerance to PCP-induced motor incoordination was already developed pretty well in 10 and 15 mg PCP pellet implanted groups 1 d after the implantation. The degrees of tolerance in 20 mg PCP pellet-implanted group was dependent on the length of time after the implantation. However, the degree of tolerance in 10 and 15 mg PCP pellet-implanted groups did not depend on length of time after the implantation. That is, tolerance to PCP induced by the pellet containing low doses of PCP reached a plateau on the first day of implantation and remained at almost the same degree until the fifth day of implantation. These results suggest that the amount of PCP release from 10 and 15 mg PCP pellet maintains the same degree of tolerance, but this amount is not enough to develop higher degree of tolerance.

The animals have almost full recovery from motor incoordination 5 d after the implantation of 10 and 15 mg PCP pellets (Fig. 3). These results suggest two possibilities: 1) Tolerance to PCP released from PCP pellet is developed though the same amount of PCP is continuously releasing during 5 d. 2) The release of PCP from the 10 and 15 mg PCP pellets is terminated until 5 d after the implantation. To clarify these points described above, an investigation of kinetics in PCP release from pellets should be done. Though the cumulative mortality in mice implanted with 10 mg PCP pellet was much less than those of mice implanted with 15 and 20 mg PCP pellets (Fig. 2), the degree of tolerance in the former was similar to those of the latter (Fig. 4). Therefore, the dose of 10 mg may be proper to develop tolerance to PCP. While, daily PCP-HCl injected group also decreased the duration of motor incoordination induced by challenged PCP-HCl. However, in the case of daily injection of PCP-HCl, 10 mg/kg, the decrease of duration of motor incoordination was only 25% at the maximum. Furthermore, daily injection of PCP-HCl, 20 mg/kg, the decrease of duration of motor incoordination was 50% of control. The degrees of tolerance in daily injected group were less than those in pellet implanted group at any time points. It appears that PCP pellet implantation method develops higher degree of tolerance rapidly than successive injection method. Furthermore, this technique was low cost. However, it is a bit too complicate that to manufacture the pellets. If an effective PCP complex is synthesized, which releases slowly from pellet and induces PCP pharmacological actions, a more simple method will be developed.

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