Safety Assessment of a Prostacyclin Derivative (Beraprost Sodium) in Healthy Cats

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(Received 1 July 2011 / Accepted 1 December 2011)

SUMMARY: Objective: Since the sensitivity to prostacyclin differs widely depending on the animal species, it is essential to investigate the dose setting from the safety point of view before using beraprost (BPS) in diseased cats. Design: One week multiple administration toxicity study of BPS (10, 30, 70, 100 μg/kg, twice a day) compared with vehicle as control. Procedure: BPS group (n=6) at 10, 30, 70 and 100 μg/kg and vehicle group (n=5) were administered to healthy cats twice a day for 7 days. Both in BPS and vehicle groups, cessation period of the drug was provided 2 weeks between each doses of BPS. In addition hemodynamic parameters were also noted at 0.5 hour before initial administration on the first day and at 1 hour after final administration on the 7th day of each dose of BPS and vehicle. Results: Though BPS from 30 μg/kg upward transiently and dose-dependently increased the heart rate but had no influence on the blood pressure. Compared with the vehicle group, serum creatinine slightly but significantly decreased at 70 and 100 μg/kg and this decrease was also significant against the pre-administration value at the same dose. The influence of BPS on the blood parameters was nil or negligible, if any. Diarrhea were sporadically noted at 70 μg/kg, and mild and transient vomiting and sedation occurred at 100 μg/kg. However, each symptom soonerly disappeared without treatment. Conclusion: The pharmacological action and types of adverse effects of BPS in the cats were almost similar to those observed in other animals and the repeated oral administration of BPS can be safely conducted in cats at least up to 30 μg/kg twice a day. KEY WORDS: adverse effect, beraprost sodium, feline, prostacyclin, safety assessment

Introduction

Beraprost sodium (BPS) is a stable prostacyclin (PGI₂) derivative, and demonstrates higher oral bioavailability and longer biological half life [1-3] so that it has long been clinically applied in the treatment of peripheral arterial obstruction [4, 5] and pulmonary arterial hypertension [6, 7]. Other than that, the usefulness of BPS in various chronic kidney diseases model rat[8-10] and in chronic renal failure patients[11] has been reported in addition to the usefulness in the animal models of heart disease[12, 13], hepatic disease[14], diabetes mellitus[15]. PGI₂[16-18] that is produced in the vascular endothelial cells plays central role in the maintenance of tissue blood flow through its various physiological
effects including anti-platelet effect, vasodilation, inhibition on the constriction and proliferation of smooth muscle, protection of vascular endothelial cell, inhibition on inflammatory cytokine production, etc. Since the sensitivity to prostaglandin drugs differs widely depending on animal species[19], it is considered essential to investigate the dose setting from the safety point of view before using BPS in diseased cats. However, no report has been made in this regard up to present. Accordingly, we administered BPS twice a day for one week to healthy cats and assessed the safety of BPS based on the blood parameters and clinical symptoms. Furthermore, we also assessed the time course changes in blood pressure and heart rate up to 3 hours after administration because the hemodynamic changes attributable to BPS reportedly occur in the early stage after administration.

Materials and Methods

Animals
Eleven mongrel cats (6 males and 5 females) that were recognized as normal by clinical sign, urinalysis and blood tests were used. These animals were 1 to 6 years old, and weighed 2.4 kg to 4.5 kg and 6 cats were assigned to BPS group and 5 cats were assigned to vehicle group. The experiment was conducted in compliance with “Guide for Care and Use of Laboratory Animals” established at Animal Clinical Research Foundation, Tottori, Japan (“DORINKEN”). The cats were individually placed in cages. They were given food (Hill’s Science Diet Adult, Hill’s Colgate Japan) at 1 hour before the test substance administration. They were allowed free access to water all the time.

Drug and reagent
Beraprost sodium (BPS: sodium-2,3,3a,8b-tetrahydro-2-hydroxy-1-[(E)-3S)-3-hydroxy-4-methyl-1-oxet-6-ynyl]-1H-cyclopent[a](b) benzo[furan-5-butrate) was obtained from Toray Industries Inc. (Tokyo). Polyethylene glycol 400 (PEG-400, Wako Pure Chemical Industries Inc, Osaka) was used as the vehicle. BPS solution was stored in the freezer. It was diluted to prescribed concentration with PEG-400 and filled into capsules (50 μL/capsule) (size 5, Shionogi & Co., Ltd., Osaka) at the time of use. For the control group, capsules filled with only 50 μL of PEG-400 were used.

Administration method and dosage for multiple dose study
To reduce the number of animals and the individual differences as much as possible, 6 cats were assigned to BPS group and BPS was administered to the same cats by ascending dose method with an interval of 2 weeks between the doses[22]. The administration period of 1 week and drug withdrawal period of 2 weeks were prescribed with reference to the clinical studies of BPS in human22. BPS at 10 μg/kg bid. (bid. = bis in die; twice a day) was administered for 7 days in the first dosages. With the drug withdrawal period between the dosages, BPS at 30 μg/kg bid., 70 μg/kg bid. and 100 μg/kg bid. was administered in the second, third and fourth dosages respectively. On the other hand, vehicle was administered instead of BPS to the control group (n=5) for 10 weeks with the withdrawal period between the dosages as was the case in the BPS group. BPS was administered within 1 hour after feeding because it was administered after meal in the case of human.

Clinical sign and body weight measurement
The clinical sign of animals in question were observed every day at 0.5, 3 and 12 hours after test substance administration. Particular attention was paid to the sign whose onset was reported in the toxicity studies in rat and dog, that is, walking difficulty, sedation, lateral position, hot flushes, lacrimation, salivation, vomiting, diarrhea and incontinence. The body weight was measured before meal on the initial day and last day of test substance administration.

Hemodynamic assessment
Systolic and diastolic blood pressure as well as heart rate were determined at the initial oral administration of each dose, that is, 10, 30, 70 and 100 μg/kg, in the multiple dose study. Total 5 time points including before administration (0 hour), and at 0.5, 1, 2 and 3 hours after administration were prescribed for measurement which was conducted using a sphygmometer (osilograph, Life Scope 9, Nihon Koden) wound
to the forearm of awake cat.

**Blood collection, blood count and serum chemistry panel**
The blood was collected from the median vein of forearm at 0.5 hour before initial administration on the first day and at 1 hour after final administration on the 7th day of each of 4 dosages. EDTA tube and serum isolation tube were employed for the purpose of blood cell count and biochemical determination respectively.

The parameters determined were red blood cell, white blood cell, platelet, hemoglobin, blood urea nitrogen (BUN), creatinine, ALT, AST, Na, K and Cl.

**Statistic analysis**
The results are indicated in mean ± standard deviation. JMP 4.05J (SAS Institute Japan Ltd., Tokyo) was employed for statistic analysis. For the blood parameters, t-test was employed for comparison between each vehicle and BPS group respectively. In addition, paired t-test was done for comparison before and after administration at the same dose, only in the case statistically significant change was observed between vehicle and BPS group. For the comparison of blood pressure and heart rate, Dunnett’s test was used only at the point 0.5 hr after administration, at which the maximum response was noted. The p value of below 0.05 was judged to show a significant difference.

**Results**

**Clinical sign and body weight**
No noteworthy changes in clinical sign were observed in any of the cats after administration of BPS at 10 and 30 μg/kg b.i.d. Diarrhea were sporadically observed in 4/6 cats after administration at 70 μg/kg. Mild vomiting also occurred in 1 cat. At 100 μg/kg, mild diarrhea were noted in all the cats. In addition, mild vomiting in 5/6 cat and sedation in 3/6 cat were also noted. However, these effects were transient and disappeared after a short time without any treatment. The appetite did not decrease at all by the next BPS administration (12 hours afterwards). These adverse effects were hardly noted on the first day of BPS administration but they were concentrated from the second to the fifth day while the incidence markedly decreased in the sixth and seventh day. Throughout the study period, none of the above-mentioned adverse effects occurred in the vehicle group.

No change in body weight was observed before and after BPS administration and no significant difference was observed during the study period, in comparison with the vehicle group.

**Hemodynamics**
A dose-dependent increase in heart rate was observed after single administration of BPS (Table 1, Fig.2). Regardless of the dose, the maximum response was noted at 0.5 hour after administration, and the increase in the response became significant at 30 μg/kg or higher. The rates of increase at 30, 70 and 100 μg/kg were 10, 19 and 23% respectively higher in comparison with the level in the vehicle group. However, the recovery to the pre-administration level was

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after BPS administration (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>132 ± 6</td>
</tr>
<tr>
<td>10 μg/kg</td>
<td>130 ± 5</td>
</tr>
<tr>
<td>30 μg/kg</td>
<td>131 ± 3</td>
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<tr>
<td>70 μg/kg</td>
<td>129 ± 6</td>
</tr>
<tr>
<td>100 μg/kg</td>
<td>129 ± 4</td>
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</table>

All values are expressed mean ± SD

*: p<0.05, **: p<0.01 versus vehicle group by Dunnett’s test

![Fig.2 Effect of single dose of BPS on heart rate](image)
noted at 2 hours after administration regardless of the dose. On the other hand, no significant changes in the systolic and diastolic blood pressure. No change was noted in the heart rate and blood pressure in the vehicle group throughout the study period.

**Blood count and serum chemistry panel**

Table 2 summarizes the results. Significant difference was observed before administration between the vehicle and BPS group in RBC (10 µg/kg bid) and Hb (70 µg/kg bid). However both values in the vehicle and BPS group at these points were remained within the normal range. Red blood cell count in the 100 µg/kg bid BPS treated group showed a significant high (18%) compared to the vehicle group. However no change was observed before and after BPS administration. No significant difference was observed throughout the study period in the white blood cell and platelet count in comparison with the values in vehicle group. As to electrolytes concentrations in the blood, the Na concentration significantly increased and significantly decreased in comparison with the vehicle group after administration of BPS at 10 µg/kg bid and at 100 µg/kg bid respectively. But these changes were all as small as 1%. Compared with the vehicle group, a significant decrease in K concentration (7%) at 70 µg/kg bid and increase in Cl (3%) at 10 µg/kg bid were observed. However, no significant difference was noted compared with the value before administration in both cases.

As shown in Table 2, both AST and ALT were high in BPS (10 µg/kg bid) group compared with vehicle group before treatment. However values both in the vehicle and BPS group at this point were remained within the normal range. Regardless of the dose, no significant changes in AST and ALT were observed after administration of BPS in comparison with the vehicle group and with the value before administration at each dose.

BPS at 70 µg/kg bid upward decreased the serum creatinine concentration (Fig.3). Statistically significant difference from the vehicle group was observed at 70 µg/kg bid upward. In addition, these values were also

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment Group</th>
<th>Point in Time of Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µg/kg</td>
<td>30 µg/kg</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>Vehicle</td>
<td>2.8 ± 0.5</td>
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<tr>
<td>RBC (×10^6/µL)</td>
<td>Vehicle</td>
<td>728 ± 35</td>
</tr>
<tr>
<td>RBC (×10^6/µL)</td>
<td>BPS</td>
<td>838 ± 96*</td>
</tr>
<tr>
<td>WBC (×10^6/µL)</td>
<td>Vehicle</td>
<td>124 ± 59</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>Vehicle</td>
<td>12.2 ± 1.4</td>
</tr>
<tr>
<td>Na (mM)</td>
<td>Vehicle</td>
<td>154 ± 1</td>
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<td>K (mM)</td>
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<tr>
<td>Cl (mM)</td>
<td>Vehicle</td>
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</tr>
<tr>
<td>AST (units/L)</td>
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<td>24 ± 5</td>
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<td>ALT (units/L)</td>
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</tr>
<tr>
<td>Cre (mg/dL)</td>
<td>Vehicle</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>Vehicle</td>
<td>20.3 ± 4.4</td>
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</tbody>
</table>

*P<0.05, **P<0.01 versus vehicle treatment-group by unpaired t-test
†P<0.05, ††: P<0.01 versus before treatment value of same dosage by paired t-test
significantly lower compared with the pre-administration level. But these decrease in serum creatinine were relatively small, that is 14% and 16% in 70 μg/kg bid and 100 μg/kg bid group respectively. Although the value in the BPS group before administration at 100 μg/kg bid was significantly lower than that in the vehicle group, these change was small and within the normal range. As to BUN, a significant increase was observed in comparison with the vehicle group before administration of BPS at 70 μg/kg bid. However values both in the vehicle and BPS group at this point were remained within the normal range. No other change was noted in both BPS and vehicle groups respectively.

Discussion

We orally administered BPS to non-anesthetized healthy cats to investigate the safety of BPS. As a result, we discovered (1) induction of adverse effects (diarrhea, vomiting, sedation), (2) the heart rate increase that was not accompanied with the changes in blood pressure, (3) a slightly decrease in serum creatinine as well as the dose to induce each of these effects. No substantial influence on the blood cell count, blood Na, K Cl concentration and AST and ALT was observed.

The doses of BPS in this study were set with reference to the results of safety studies in rat[20], dog[21] and human[22]. That is, it was reported that the non-observed adverse effect level (NOAEL) in dogs was at 25 μg/kg/day in the 3-month multiple administration toxicity study of BPS, and that digestive symptoms including loose stools and diarrhea were observed at 250 μg/kg/day[21]. Hardly any report has been made on the pharmacologic effect of BPS in cat but the sole report in this regard referred to the inhibitory effect on platelet aggregation reporting that the 50% effective concentration (EC50) increases in the order of human, cat, dog and rat[19]. Accordingly, we decided to start administration to cats from 10 μg/kg that corresponded to about 1/3 of NOAEL in dogs, and to increase the dose up to 100 μg/kg/day while paying sufficient attention to adverse effects. The administration method of twice a day was selected because the efficacy at this administration method has been confirmed in the chronic renal failure model of rat[8] as well as in various animal models of diabetic nephropathy[15], peripheral circulation disorder[23], atherosclerosis[24].

![Graph 1](image1.png)

**Graph 1** Decrease of Serum creatinine and unvariable level of Blood urea nitrogen During the Experiment

![Graph 2](image2.png)

**Graph 2** Decrease of Serum creatinine and unvariable level of Blood urea nitrogen During the Experiment

"*P<0.05, **P<0.01 versus vehicle treatment-group by unpaired t-test
†P<0.05, ††P<0.01 versus before treatment value of same dosage by paired t-test"
etc., and therefore it is expected that this administration method is also employed in the treatment of diseased cat in the future.

We set BPS group (n=6) and vehicle group (n=5) to reduce the number of animals and the individual differences as much as possible.

The adverse effects observed in cat were mainly digestive symptom (diarrhea) that occurred at 70 μg/kg bid. (140 μg/kg/day), and digestive symptoms (vomiting) and central nerve symptom (sedation) that occurred at 100 μg/kg bid. (200 μg/kg/day). However, these symptoms were all mild in severity and recovery was observed without any treatment. In the 3-month subcutaneous toxicity study of BPS in rat[25], the inhibition on spontaneous movement, prone position arrest, lacrimation, hot flushes, loose stools and diarrhea occurred after oral administration at 1000~5000 μg/kg. In addition, in the 3-month subcutaneous toxicity study of BPS in dog[21], the findings of sedation, vomiting, diarrhea and lacrimation were reported in the 250 and 2500 μg/kg groups. As to the single BPS administration to human, transient and mild flushed face, dull headache, fuzzy head, anorexia and feeling queasy occurred at 200 μg/day[22]. In the 10-day continuous oral administration to human at 50 μg/day three times a day, facial hot flushes, dull headache, fuzzy head were reported as adverse effects[26]. As described in the foregoing, diarrhea, vomiting and sedation among the above-mentioned symptoms were observed as adverse effects in cat. Other symptoms were not observed within the dose range investigated this time. The adverse effect on the digestive system of human, dog and rat occurred at the oral doses of 200 μg/kg/day[22], 250 μg/kg[21] and 5000 μg/kg/day[20] respectively. As in the case of above-mentioned anti-platelet effect, these adverse effects on digestive symptoms were observed at the lowest dose in human, followed by the doses in ascending order in cat, dog and rat. As was reported in dog[21], the sedation is considered to be attributable to the decrease in blood pressure or the increase in heart rate due to the transient vasodilatation. Similarly, these symptoms occurred in various animal species at the doses that reflected the differences in the sensitivity as in the case of other symptoms. These pharmacologic effects and harmful actions observed in cat after administration of BPS were commonly observed with prostaglandin I2 itself[27] even though there is a difference in the onset dose. Therefore, it is considered that these effects are not characteristic to BPS only but they are common to other prostaglandin I2 receptor agonists.

The results[28] obtained from rat and dog indicated that the blood pressure decreased by BPS administration but no change occurred in the heart rate under the anesthetized condition. On the other hand, the heart rate increased and no change occurred in the blood pressure under the awake condition[28]. The difference was thought to be attributed to the presence or absence of anesthesia. Under the awake condition, the increase in heart rate was observed caused by vagus nerve reflex to the blood pressure decrease due to vasodilating effect of BPS. The effects of BPS on the cardiovascular system of cat corresponded well with that in other animal species. The increase in heart rate of unanesthetized rat[28] observed only from 100 μg/kg upward and this was three times higher compared to the dose in cat. As described in the foregoing, it was observed in vitro that the cat is more sensitive than rat in regard to the platelet aggregation inhibition by BPS[19]. Similar difference in sensitivity was noted in the heart rate. It was reported in the studies conducted in rat[29] and dog[30] that the serum concentration reached a peak about 0.5 hour after oral administration of BPS, followed by a gradual decline. Similar changes in blood concentration are expected to occur in cat, and the vasodilating effect of BPS is also expected to occur in parallel with the changes in the serum concentration. As to the blood parameters, some were already different between BPS and vehicle group before treatment. However, as these differences were small and all values were remained within the normal range, they might not affect to evaluate the effect of BPS. BPS substantially did not influence the blood cell count and hemoglobin concentration of cat. These results corresponded to those obtained in the 3-month subcutaneous toxicity study in dog[21] and phase I 10 days repeated administration study in human[26]. On the other hand, in the subcutaneous toxicity study in rat[27], the platelet count decreased and neutrophil increased at a high dose (1,000 μg/kg/day) of BPS, and red blood cell count, hemoglobin decreased in the males at 5000 μg/kg/day. However, all these reactions were observed at a very high dose that is expected to demonstrate toxic actions, and these changes are not handled as the clinically problematic changes.

BPS changed the blood Na, K and Cl concentrations
in cat at some doses. However, the changing rate was small and remained as a change within the normal range. Furthermore, in view of the absence of dose-dependency, these changes were considered as accidental. In the case of rat, dog and human, no particular changes in blood ion were observed. Based on the above results, it was considered that BPS hardly influenced the blood cell count and electrolytes in cat, rat, dog and in human.

As to AST and ALT, no significant changes were observed in this study by comparison with the vehicle group and by comparison before and after BPS administration at the same dose. AST significantly decreased in the female rat after oral administration at 1000~5000 μg/kg/day in the 3-month subacute toxicity study in rat[27]. However, this dose is a level expected to cause toxic reactions in rat. Since the change in AST did not occur in dog[21] and human[26], it is considered that this action is characteristic to the high dose of BPS.

As to serum creatinine slightly but significantly decreased at a high dose of 70 μg/kg bid and 100 μg/kg bid in comparison with the level in the vehicle group. This change was also significant in comparison with the pre-administration value at the same dose. As to serum creatinine, only a slight decrease in female rat was reported at a high dose of 5 mg/kg/day in the 3-month administration[25]. Though the doses up to 2.5 mg/kg/day and 200 μg/kg as adverse effects appeared were administered to dog[21] and human[26] respectively, no particular change was observed. On the other hand, the decrease in serum creatinine in cat occurred at which the adverse reactions of BPS administration at 70 μg/kg were comparatively mild, may suggest the difference from other animal species in the effect on the kidney function. However, considering that detailed mechanism and physiological meaning are still unclear. As to BUN, a decrease was not observed and it was within the normal range like other animal species. The cause of difference in mechanism of BPS for both parameters indicating the renal function is also unclear, and further investigation in the future is necessary.

Further, when observing the effect of BPS medication on each patient, there were two cases where a parameter changed by 50% or more after the medication; one case concerned the increase of platelets after medicating 10 μg/kg bid and the other case concerned the increase of ALT after medicating 100 μg/kg bid. However, these increases remained within normal range, and could not be observed in the same patient when different amounts were medicated. Thus, we consider that these changes are not clinically significant, and that the clinical effect of BPS medication does not differ with individual patient.

To sum up, the results of safety study of BPS in cat are basically similar to those of preceding studies in rat, dog and human. It was clarified that continuous oral administration of BPS in cat can be safely conducted at least up to the dose of 30 μg/kg bid.

Reference


