Effects of Beraprost with or without NOS Inhibitor on Aldosterone and Hemodynamics in Healthy Cats

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Article

Effects of Beraprost with or without NOS Inhibitor on Aldosterone and Hemodynamics in Healthy Cats

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Simple Summary: Beraprost is a stable prostacyclin analogue that has been shown to be effective and safe for the treatment of chronic kidney disease (CKD) in cats. However, the pharmacological effects of beraprost on renin-aldosterone axis and hemodynamics in cats were largely unknown, and the prescription of beraprost has been often limited in feline patients with related disorders. This study in healthy cats demonstrated that the clinical dose of beraprost for feline CKD reduces plasma aldosterone, which is reversed by nitric oxide synthase (NOS) inhibition, without changes in plasma renin and hemodynamics. These findings may help understand pharmacological mechanism, and also clinical safety of beraprost in the treatment of feline CKD.

Abstract: Objectives: The aim of the study was to evaluate the effects of oral administration of clinical dose of beraprost for feline CKD in healthy cats, and also to examine whether NOS inhibition reversed them. Methods: A placebo-controlled pharmacological sequential design study was carried out to assess plasma aldosterone and renin concentrations, mean blood pressure, heart rate, renal plasma flow, and renal vascular resistance. Results: Beraprost reduced plasma aldosterone when compared to the placebo ($P < 0.05$); this was reversed when NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME) was added to beraprost treatment ($P < 0.01$). No differences in the plasma renin concentration or hemodynamic parameters were detected between beraprost and placebo. The correlation ratios (η^2) showed opposite relationships between beraprost and added L-NAME effects on aldosterone concentration, mean blood pressure, heart rate, renal plasma flow, and renal vascular resistance ($P < 0.001$). Conclusions: In healthy cats, the clinical dose of beraprost suppresses plasma concentration of aldosterone, which can be reversed by inhibition of NOS.

Keywords: beraprost; feline; chronic kidney disease; aldosterone; NOS

1. Introduction

Aldosterone and the mineralocorticoid receptor play an essential role in hemodynamics[1-3] and pathomechanisms of feline kidney disease [4]. From substantial and ever-increasing evidence in laboratory animals and humans, chronic kidney disease (CKD) can be considered as a state of relative hyperaldosteronism [2,3]. In cats with hypertensive CKD, increased plasma aldosterone levels have been reported [5-7].

Beraprost is a chemically and metabolically stable prostacyclin analogue with high and long-lasting oral activity [8]. Beraprost induces endothelial NO synthase (eNOS), increases levels of intracellular NO [9], and also mediates endothelium-independent vasodilation [10,11]. In rats, beraprost has been shown to protect kidney endothelium and microvasculature, to slow down loss of kidney function [12], and to prolong survival in rodent models of CKD [13]. In a randomized, double-blind, placebo-controlled trial with feline CKD patients, beraprost suppressed the deterioration of kidney function, assessed by change in serum creatinine, without any adverse effects [14]. A retrospective cohort study also showed beraprost is associated with better overall survival of cats with CKD [15]; however, the pharmacological mode of action of beraprost on feline renin-aldosterone axis and hemodynamics are not known.

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The purpose of this placebo-controlled sequential design study in the target species was to describe the pharmacological effects of beraprost when orally administered to healthy adult male and female cats at dose levels used in clinical practice for treatment of feline CKD, and to examine whether NOS inhibition with NG-nitro-L-arginine methyl ester (L-NAME) could reverse these effects.

2. Materials and Methods

2.1. Subjects

Seven Domestic Shorthair cats were obtained from a commercial laboratory animal breeder. their baseline characteristics are shown in Table 1. The cats were kept under specified pathogen- free conditions and single housed during the conduct of study. Room temperatures was maintained at 21 ± 2 °C with 50 ± 20% humidity and 12 hours light and dark cycle. Cats were allowed to exercise outside the cages for 30 minutes once daily, and fed with a standard pellet diet Prostage Le Chat (Yeaster, Co., Ltd., Hyogo, Japan) twice daily.

Table 1. Baseline characteristics of all cats.

No.	Age (years)	Weight (kg)	Sex	Breeds
1	3.4	2.7	Intact Female	Domestic Shorthair
2	3.7	2.6		
3	3.4	3.1		
4	3.4	3.0		
5	2.0	3.8	Intact Male	
6	3.4	2.8		
7	2.0	3.6		

2.2. Study design

This was a prospective placebo-controlled laboratory study conducted in Tokyo University of Agriculture and Technology, Tokyo, Japan. The time schedule in Table 2 describes all study interventions and assessments. All seven cats underwent identical sequential treatments in each of the three study phases, 1) placebo, 2) beraprost, and 3) beraprost + L-NAME. Study animals were evaluated for body weight, hematology, blood pressure, heart rate, para-aminohippuric acid (PAH) plasma clearance, and plasma renin and aldosterone levels. The same data were collected for each cat twice in each study phase – once in the first and once in the second week of each phase.

Table 2. Time schedule of study procedures.

	Phase 1					Phase 2					Phase 3			
	Placebo					Beraprost					Beraprost + L-NAME			
	Day 1	Day 7	Day 8	Day 14	Day 15	Day 16	Day 22	Day 23	Day 29	Day 30	Day 36	Day 37	Day 42	Day 43
Treatments														
Placebo administration[1]	D1-15 inclusive													
Beraprost administration[1]						D16-43 inclusive								
L-NAME administration[2]											X	X	X	X
Assessments														
Plasma PAH clearance[3]		X		X			X		X		X		X	
Blood pressure and heart rate[4]			X		X			X		X		X		X
Plasma aldosterone and renin[5]			X		X			X		X		X		X

2.3. Materials

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Tablets containing 55 µg beraprost (RAPROS®) or placebo were provided in blister packages by Toray Industries, Inc. Placebo tablets had the same appearance and composition as the beraprost tablets, except that beraprost was replaced by lactose. The tablets were stored according to the manufacturer's instruction. L-NAME (NG-Nitro-L-arginine methyl ester hydrochloride) was from Fujifilm Wako Pure Chemical Co., Ltd., Tokyo, Japan. It was dissolved in aqua ad injectabilia for intravenous infusion. PAH (10% sodium para-aminohippurate injection) was obtained from Daiichi-Sankyo Co., Ltd., Tokyo, Japan and was diluted in saline for intravenous injection.

2.4. Treatments

Depending on study phase (Table 2), one tablet per cat of either placebo or beraprost was administered twice daily at feeding. The dosage of beraprost (55 µg per cat, twice daily) corresponds to the dose level which is clinically prescribed for cats with CKD.

Administration of L-NAME followed previous description for NOS inhibition in cats [16]: each cat received intravenous infusion of L-NAME at a dose rate of 100 µg/kg/min, starting with the beraprost tablet administration and lasting for 360 min once daily on days 36, 37, 42 and 43.

2.5. Methods

For determination of aldosterone and renin plasma concentrations, blood was taken from a peripheral vein at 350 min after tablet administration into vials containing anti-coagulant EDTA-2K. Vials were immediately centrifuged at 1510g for 10 min. Plasma samples were kept at -80 °C until determination of aldosterone and renin levels with standard chemiluminescent enzyme immunoassay at Showa Medical Science Co., Ltd., Tokyo, Japan. The limit of detection for aldosterone and renin were 25 pg/ml and 0.15 ng/ml.

Blood pressure and heart rate were measured at 340 min after tablet administration using the oscillometric device petMAP graphic II, Ramsey Medical, Inc., Tampa, FL, USA following the manufacturers instruction. All procedures of blood pressure measurements were in accordance with the ACVIM consensus statement on management of systemic hypertension in dogs and cats [17].

PAH was intravenously injected at a dose of 40 mg/kg at baseline and at 260 min after placebo or beraprost tablet administration on days 7, 14, 22, 29, 36, and 42. Blood samples for determination of PAH clearance were collected via a peripheral vein at 300 and 320 min after tablet administration. Plasma PAH clearance was examined as previously described [18,19]: PAH concentration was determined by standard colorimetric method, and the initial concentration (C_0) and half-value period ($T_{1/2}$) of PAH using the 1-compartment model built from measured data. Renal plasma flow (RPF) and renal vascular resistance (RVR) were calculated by the following formulas: RPF = 40(mg/kg) × Body weight (kg) × loge2 ÷ [C_0(mg/ml) × $T_{1/2}$(min)], and RVR = Mean blood pressure (MBP)/RPF.

2.6. Data management

Data were collected after treatment from a total of seven cats, each providing two sets of data for each study phase. This allowed reduction of the number of cats used in this study, while a data set of n=14 per study phase covered a broader spectrum of variance.

Aldosterone and renin concentration [pg/ml and ng/ml], mean blood pressure (mmHg), heart rate (bpm), renal plasma flow (ml/kg/min), and renal vascular resistance (mmHg-min/ml) were presented as dot plots and mean ± standard error (SE). Aldosterone and renin values below the limit of detection were set to the half of the limit value.

The differences among three groups: placebo, beraprost, and beraprost combined with L-NAME (NOS inhibitor), were tested by Friedman's test or one-way ANOVA followed by Holm–Bonferroni-corrected t test. Effects were calculated by differences between the placebo and beraprost groups for beraprost, and between the beraprost and beraprost + L-NAME for added L-NAME, respectively. Correlation ratios (η^2) were obtained and tested by one-way ANOVA. A commercial statistical software program was used for all analyses (BellCurve for Excel; Social Survey Research Information Co., Ltd., Tokyo, Japan). Statistical significance was set at 2-sided level * $p < 0.05$, ** $p < 0.01$.

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3. Results

3.1. Aldosterone and renin plasma concentrations

Mean aldosterone plasma concentrations [pg/ml] ± SE were 79.2 ± 9.5 in the placebo group, 48.1 ± 6.8 in the beraprost group and 110.4 ± 10.0 in the beraprost + L-NAME group. Thus, aldosterone levels were lowered to statistically significantly ($p < 0.05$) amounts by treatment with beraprost, which was fully reversible ($p < 0.01$) when adding L-NAME to the beraprost treatment.

Mean renin plasma concentrations [ng/ml] ± SE were 1.6 ± 0.2 in the placebo group, 1.9 ± 0.2 in the beraprost group and 1.5 ± 0.3 in the beraprost + L-NAME group. Renin concentrations were similar between all three groups with no significant difference (Figure 1).

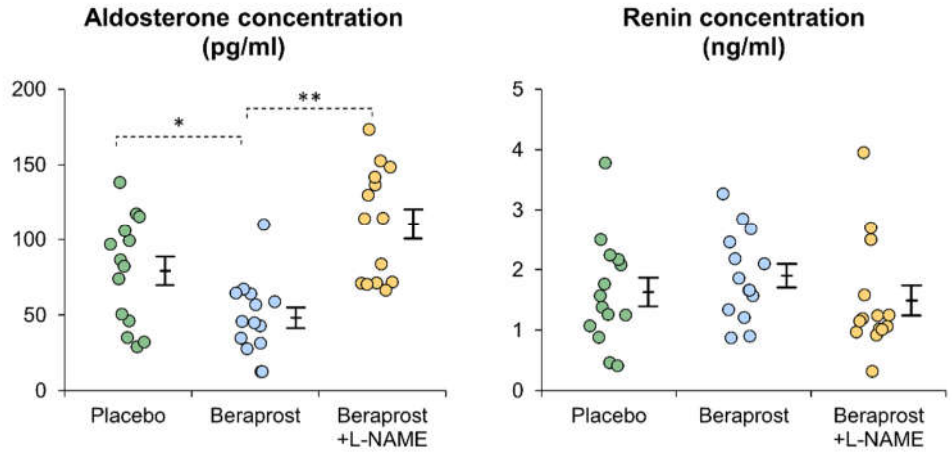

Figure 1. Comparison of plasma aldosterone and renin concentration in cats administered placebo, beraprost, and beraprost combined with L-NAME (NOS inhibitor). Data represent dot plots and mean ± SE of n=14 data sets from two experiments with seven cats per study phase. * $p < 0.05$, ** $p < 0.01$ by Friedman's test.

3.2. Measurements of hemodynamics

Mean blood pressure [mm Hg] ± SE was 120.7 ± 2.7 in the placebo group, 117.8 ± 3.1 in the beraprost group and 159.0 ± 5.2 in the beraprost + L-NAME group. Mean blood pressure was similar between the placebo and beraprost groups, while it was higher in the beraprost + L-NAME group than other groups ($p < 0.01$).

Mean heart rate [bpm] ± SE was 177.1 ± 4.3 in the placebo group, 178.0 ± 4.6 in the beraprost group and 138.1 ± 5.6 in the beraprost + L-NAME group. Heart rate was similar between the placebo and beraprost groups, while it was lower in the beraprost + L-NAME group ($p < 0.01$) (**Figure 2**).

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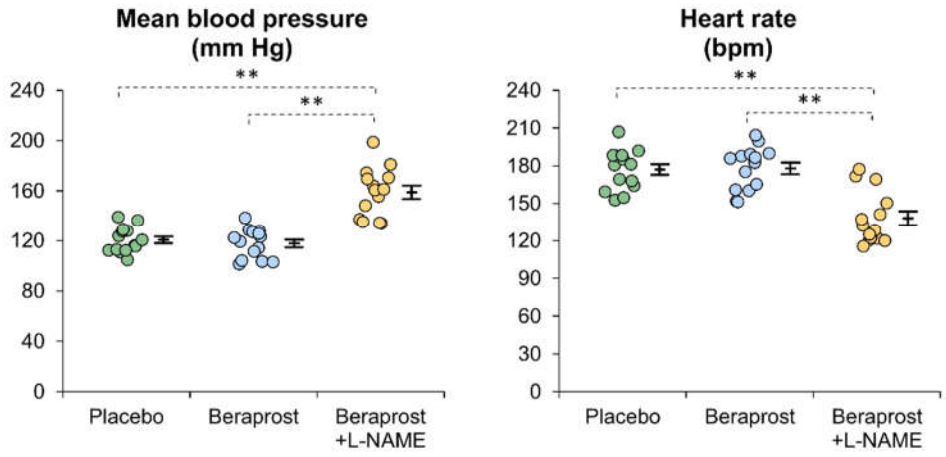

Figure 2. Comparison of mean blood pressure and heart rate in cats administered placebo, beraprost, and beraprost combined with L-NAME (NOS inhibitor). Data represent dot plots and mean ± SE of n=14 data sets from two experiments with seven cats per study phase. ** $p < 0.01$ by one-way ANOVA followed by Holm–Bonferroni-corrected t test for mean blood pressure, ** $p < 0.01$ by Friedman's test for heart rate.

Mean renal plasma flow [ml/kg/min] ± SE was 11.3 ± 0.6 in the placebo group, 12.4 ± 0.6 in the beraprost group and 9.3 ± 0.6 in the beraprost + L-NAME group. Renal plasma flow did not differ significantly between the placebo and beraprost groups, while it was lower in the beraprost + L-NAME group ($p < 0.01$ vs beraprost, $p < 0.05$ vs placebo).

Mean renal vascular resistance [mmHg-min/ml] ± SE was 2.2 ± 0.2 in the placebo group, 2.0 ± 0.1 in the beraprost group and 3.8 ± 0.5 in the beraprost + L-NAME group. Renal vascular resistance did not differ significantly between the placebo and beraprost groups, while it was higher in the beraprost + L-NAME group ($p < 0.01$) (**Figure 3**).

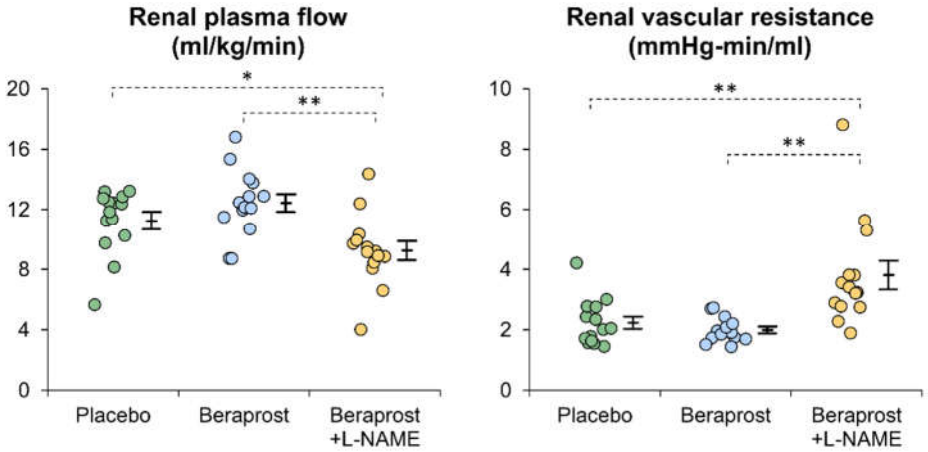

Figure 3. Comparison of renal plasma flow and renal vascular resistance in cats administered placebo, beraprost, and beraprost combined with L-NAME (NOS inhibitor). Data represent dot plots and mean ± SE of n=14 data sets from two experiments with seven cats per study phase * $p < 0.05$, ** $p < 0.01$ by Friedman's test.

3.3. Assessments of the effects of beraprost and added L-NAME (NOS inhibitor)

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In order to compare the effects of beraprost and added L-NAME on aldosterone and renin concentration, and hemodynamics, effects were calculated by differences between the placebo and beraprost groups for beraprost, and between the beraprost and beraprost + L-NAME for added L-NAME, respectively. The effects, correlation ratios, and p-values are presented in **Table 3**. The effects of beraprost on aldosterone concentration and hemodynamic parameters were counteracted by added L-NAME ($p < 0.001$), while neither drug had a substantial effect on renin concentration.

Table 3. Effects of beraprost and added L-NAME (NOS inhibitor) and their correlation ratios.

	Aldosterone (pg/ml)	Renin (ng/ml)	Mean blood pressure (mmHg)	Heart rate (bpm)	Renal plasma flow (ml/kg/min)	Renal vascular resistance (mmHg-min/ml)
Beraprost	- 31.1 ± 9.1	+ 0.3 ± 0.2	- 2.8 ± 2.7	+ 0.9 ± 3.3	+ 1.2 ± 0.5	- 0.2 ± 0.2
Added L-NAME	+ 62.3 ± 9.6	- 0.4 ± 0.3	+ 41.1 ± 5.1	- 39.9 ± 6.0	- 3.2 ± 0.6	+ 1.8 ± 0.4
Correlation ratio (η^2)	0.657	0.138	0.693	0.579	0.527	0.478
p-value	$^{**}p < 0.001$	0.0517	$^{**}p < 0.001$	$^{**}p < 0.001$	$^{**}p < 0.001$	$^{**}p < 0.001$

Data represent mean ± SE of n=7 data sets of seven cats in week 1 and week 2 with seven cats. ** $p < 0.01$ by one-way ANOVA.

4. Discussion

Results of this study in healthy cats show that oral administration of the clinical dose of beraprost used for feline CKD reduces plasma concentration of aldosterone and this effect is reversed by NOS inhibition with L-NAME. These findings indicate that beraprost may pharmacologically enhance NOS activity in cats. CKD has been associated with impaired bioavailability of NO and endothelial dysfunction. In feline CKD, endogenous eNOS inhibitor asymmetric dimethylarginine (ADMA) is increased in plasma [20]. Kidney endothelial cell loss detected by endothelial marker CD34 is present and is positively correlated with kidney dysfunction [21]. Beraprost has a protective effect on injured endothelial cells [22], restores impaired endothelial dysfunction in diabetes mellitus rats [10], and induces eNOS and elevated NO production in various animal endothelial cells to improve endothelial function [9]. Our present findings support the assumption that the pharmacological mechanism of beraprost on feline CKD may be endothelial protection through its activation of eNOS and NO production.

Previous studies have described the role of NO in the regulation of aldosterone synthesis. NO donors including nitroprusside inhibit angiotensin II, potassium, and ACTH-induced aldosterone synthesis in adrenal cells [23,24]. On the contrary, NOS inhibitors cause aldosterone synthesis in adrenal cells [25,26], and increase serum aldosterone in humans [27]. Therefore, the results of the present study in cats with the observed effects of beraprost decreasing plasma aldosterone levels, it can be hypothesized that beraprost modulates aldosterone synthesis in cats via increased NO production. At the same time, clinical dosages of beraprost did not affect plasma renin levels in healthy cats. This was unexpected as beraprost is a synthetic analog of the prostaglandin prostacyclin, and there are previous reports about effects of prostaglandins on renin secretion. *In vitro* experiments in isolated rat kidneys and glomeruli indicate that prostaglandins stimulate renin secretion [28,29].

In human pulmonary hypertension, beraprost above a certain dose exerted short-term hemodynamic effect [30], and the human maximal tolerated dose of beraprost, four times a day, is reported to improve exercise capacity and symptoms [31]. In feline patients with CKD there were no reports of hemodynamic-related adverse events in two studies [14,15]. The present finding that clinical dose of beraprost for feline CKD did not show change in hemodynamic parameters may be helpful for clinical practice regarding expected hemodynamic effects.

The effects of L-NAME on hemodynamics were previously examined in two studies with anesthetized cats with inconsistent findings; in the earlier study, L-NAME did not modify systemic hemodynamic variables [32], while L-NAME increased systemic arterial pressure and renal vascular resistance in a later study [16]. The authors suspected that the inconsistency might have resulted from the different types of anesthetics used: ketamine vs pentobarbitone [16]. In this study the

pharmacology of L-NAME alone in healthy cats remained unexplored, while it was shown that L-NAME reversed the effects of beraprost on plasma aldosterone and hemodynamics. Another limitation in this study is that all collected data were from a single time point during an interval of 300 to 360 min after oral administration of beraprost. There are two reasons for this: first, the clinical dosing frequency of beraprost for feline CKD twice daily and the 300 to 360 min post-dose interval is approximately midway between two doses, so was thought to be appropriate for measuring the average effects of beraprost; second, this study design involved several measurements in parallel and repetition of measurements had to be limited for reasons of practicality.

Cats with CKD often have various complications and co-morbidities [33-35], which includes disorders can affect renin-aldosterone axis and hemodynamics. Thus, it would be necessary to examine the current effects of beraprost on aldosterone and hemodynamics also in feline CKD patients to affirm the clinical relevance.

5. Conclusions

In healthy cats, the dose of beraprost used clinically for feline CKD reduced plasma aldosterone concentration, and this was reversed by NOS inhibition. Beraprost did not affect plasma renin concentration or hemodynamic parameters. These findings may be beneficial for understanding pharmacological mechanism, and also clinical safety of beraprost in feline CKD with renin-aldosterone axis and hemodynamics related disorders.

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Institutional Review Board Statement: All experiments were performed in accordance with the institutional guidelines and the Guide for the Care and Use of Laboratory Animals, which was approved by the Institutional Review Board of Tokyo University of Agriculture and Technology Animal Experiment Committee (protocol code: R02-53).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Conflicts of Interest: T.M. works for Toray Industries Inc., Tokyo, Japan. None of the authors have any personal or financial relationships that could have inappropriately influenced or biased the content of the paper.

References

1. Duprez, D.A. Aldosterone and the vasculature: mechanisms mediating resistant hypertension. *J Clin Hypertens (Greenwich)* **2007**, *9*, 13-18, doi:10.1111/j.1524-6175.2007.06367.x.
2. Bertocchio, J.P.; Warnock, D.G.; Jaisser, F. Mineralocorticoid receptor activation and blockade: an emerging paradigm in chronic kidney disease. *Kidney Int* **2011**, *79*, 1051-1060, doi:10.1038/ki.2011.48.
3. Shavit, L.; Lifschitz, M.D.; Epstein, M. Aldosterone blockade and the mineralocorticoid receptor in the management of chronic kidney disease: current concepts and emerging treatment paradigms. *Kidney Int* **2012**, *81*, 955-968, doi:10.1038/ki.2011.505.
4. Spencer, S.; Wheeler-Jones, C.; Elliott, J. Aldosterone and the mineralocorticoid receptor in renal injury: A potential therapeutic target in feline chronic kidney disease. *J Vet Pharmacol Ther* **2020**, *43*, 243-267, doi:10.1111/jvp.12848.
5. Jensen, J.; Henik, R.A.; Brownfield, M.; Armstrong, J. Plasma renin activity and angiotensin I and aldosterone concentrations in cats with hypertension associated with chronic renal disease. *Am J Vet Res* **1997**, *58*, 535-540.

6. Mishina, M.; Watanabe, T.; Fujii, K.; Maeda, H.; Wakao, Y.; Takahashi, M. Non-invasive blood pressure measurements in cats: clinical significance of hypertension associated with chronic renal failure. *J Vet Med Sci* **1998**, *60*, 805-808, doi:10.1292/jvms.60.805.
7. Jepson, R.E.; Syme, H.M.; Elliott, J. Plasma renin activity and aldosterone concentrations in hypertensive cats with and without azotemia and in response to treatment with amlodipine besylate. *J Vet Intern Med* **2014**, *28*, 144-153, doi:10.1111/jvim.12240.
8. Toda, N. Beraprost sodium. *Cardiovascular Drug Reviews* **1988**, *6*, 222-238.
9. Niwano, K.; Arai, M.; Tomaru, K.; Uchiyama, T.; Ohyama, Y.; Kurabayashi, M. Transcriptional stimulation of the eNOS gene by the stable prostacyclin analogue beraprost is mediated through cAMP-responsive element in vascular endothelial cells: close link between PGI2 signal and NO pathways. *Circ Res* **2003**, *93*, 523-530, doi:10.1161/01.RES.0000091336.55487.F7.
10. Matsumoto, K.; Morishita, R.; Tomita, N.; Moriguchi, A.; Yamasaki, K.; Aoki, M.; Matsumoto, K.; Nakamura, T.; Higaki, J.; Ogihara, T. Impaired endothelial dysfunction in diabetes mellitus rats was restored by oral administration of prostaglandin I2 analogue. *J Endocrinol* **2002**, *175*, 217-223, doi:10.1677/joe.0.1750217.
11. Fuchikami, C.; Murakami, K.; Tajima, K.; Homan, J.; Kosugi, K.; Kuramoto, K.; Oka, M.; Kuwano, K. A comparison of vasodilation mode among selexipag (NS-304; [2-4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy-N-(methylsulfonyl)acetamide]), its active metabolite MRE-269 and various prostacyclin receptor agonists in rat, porcine and human pulmonary arteries. *Eur J Pharmacol* **2017**, *795*, 75-83, doi:10.1016/j.ejphar.2016.11.057.
12. Goto, Y.; Yamaguchi, S.; Tamura, M.; Mochizuki, H.; Kurumatani, H.; Okano, K.; Miyamoto, M. A prostacyclin analog prevents the regression of renal microvascular network by inhibiting mitochondria-dependent apoptosis in the kidney of rat progressive glomerulonephritis. *Prostaglandins Other Lipid Mediat* **2014**, *112*, 16-26, doi:10.1016/j.prostaglandins.2014.06.001.
13. Yamaguchi, S.; Inada, C.; Tamura, M.; Sato, N.; Yamada, M.; Itaba, S.; Okazaki, S.; Matsuura, H.; Fujii, S.; Matsuda, F.; et al. Beraprost sodium improves survival rates in anti-glomerular basement membrane glomerulonephritis and 5/6 nephrectomized chronic kidney disease rats. *Eur J Pharmacol* **2013**, *714*, 325-331, doi:10.1016/j.ejphar.2013.07.032.
14. Takenaka, M.; Iio, A.; Sato, R.; Sakamoto, T.; Kurumatani, H.; Group, K.T.C.S. A Double-blind, Placebo-controlled, Multicenter, Prospective, Randomized Study of Beraprost Sodium Treatment for Cats with Chronic Kidney Disease. *J Vet Intern Med* **2018**, *32*, 236-248, doi:10.1111/jvim.14839.
15. Ito, H.; Matsuura, T.; Sano, T. Beraprost and Overall Survival in Cats with Chronic Kidney Disease. *Vet Sci* **2023**, *10*, doi:10.3390/vetsci10070459.
16. Brown, S.A. Effects of inhibition of nitric oxide synthase on systemic arterial pressure and renal vascular resistance in cats. *Res Vet Sci* **1993**, *55*, 398-400, doi:10.1016/0034-5288(93)90119-z.
17. Acierno, M.J.; Brown, S.; Coleman, A.E.; Jepson, R.E.; Papich, M.; Stepien, R.L.; Syme, H.M. ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *J Vet Intern Med* **2018**, *32*, 1803-1822, doi:10.1111/jvim.15331.
18. Brun, C. A rapid method for the determination of para-aminohippuric acid in kidney function tests. *J Lab Clin Med* **1951**, *37*, 955-958.
19. Yoshimura, A.; Ohmori, T.; Yamada, S.; Kawaguchi, T.; Kishimoto, M.; Iwanaga, T.; Miura, N.; Fukushima, R. Comparison of pancreatic and renal blood flow in a canine tachycardia-induced cardiomyopathy model. *J Vet Med Sci* **2020**, *82*, 836-845, doi:10.1292/jvms.19-0694.
20. Jepson, R.; Syme, H.; Vallance, C.; Elliott, J. Plasma asymmetric dimethylarginine, symmetric dimethylarginine, l-arginine, and nitrite/nitrate concentrations in cats with chronic kidney disease and hypertension. *Journal of veterinary internal medicine* **2008**, *22*, 317-324.
21. Paschall, R.E.; Quimby, J.M.; Cianciolo, R.E.; McLeland, S.M.; Lunn, K.F.; Elliott, J. Assessment of peritubular capillary rarefaction in kidneys of cats with chronic kidney disease. *J Vet Intern Med* **2023**, *37*, 556-566, doi:10.1111/jvim.16656.
22. Kainoh M, N.S., Nakadate T. Cytoprotective Action of Beraprost Sodium against Peroxide-Induced Damage in Vascular Endothelial Cells. *Pharmacology* **1992**, *45*, 61-70.
23. Okamoto, M. Effects of alpha-human atrial natriuretic polypeptide, sodium nitroprusside and dibutyryl cyclic GMP on aldosterone production in bovine zona glomerulosa cells. *Acta Endocrinol (Copenh)* **1988**, *119*, 358-366, doi:10.1530/acta.0.1190358.
24. Natarajan, R.; Lanting, L.; Bai, W.; Bravo, E.L.; Nadler, J. The role of nitric oxide in the regulation of aldosterone synthesis by adrenal glomerulosa cells. *J Steroid Biochem Mol Biol* **1997**, *61*, 47-53, doi:10.1016/s0960-0760(97)00004-6.
25. Hanke, C.J.; Drewett, J.G.; Myers, C.R.; Campbell, W.B. Nitric oxide inhibits aldosterone synthesis by a guanylyl cyclase-independent effect. *Endocrinology* **1998**, *139*, 4053-4060, doi:10.1210/endo.139.10.6252.
26. Hanke, C.J.; O'Brien, T.; Pritchard, K.A., Jr.; Campbell, W.B. Inhibition of adrenal cell aldosterone synthesis by endogenous nitric oxide release. *Hypertension* **2000**, *35*, 324-328, doi:10.1161/01.hyp.35.1.324.

27. Muldowney, J.A., 3rd; Davis, S.N.; Vaughan, D.E.; Brown, N.J. NO synthase inhibition increases aldosterone in humans. *Hypertension* **2004**, *44*, 739-745, doi:10.1161/01.HYP.0000143852.48258.f1.
28. Linas, S.L. Role of prostaglandins in renin secretion in the isolated kidney. *Am J Physiol* **1984**, *246*, F811-818, doi:10.1152/ajprenal.1984.246.6.F811.
29. Beierwaltes, W.H.; Schryver, S.; Sanders, E.; Strand, J.; Romero, J.C. Renin release selectively stimulated by prostaglandin I2 in isolated rat glomeruli. *Am J Physiol* **1982**, *243*, F276-283, doi:10.1152/ajprenal.1982.243.3.F276.
30. Saji, T.; Ozawa, Y.; Ishikita, T.; Matsuura, H.; Matsuo, N. Short-term hemodynamic effect of a new oral PGI2 analogue, beraprost, in primary and secondary pulmonary hypertension. *Am J Cardiol* **1996**, *78*, 244-247, doi:10.1016/s0002-9149(96)90408-7.
31. Galie, N.; Humbert, M.; Vachiery, J.L.; Vizza, C.D.; Kneussl, M.; Manes, A.; Sitbon, O.; Torbicki, A.; Delcroix, M.; Naeije, R.; et al. Effects of beraprost sodium, an oral prostacyclin analogue, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. *J Am Coll Cardiol* **2002**, *39*, 1496-1502, doi:10.1016/s0735-1097(02)01786-2.
32. Marcel van Gelderen, E.; Heiligers, J.P.; Saxena, P.R. Haemodynamic changes and acetylcholine-induced hypotensive responses after NG-nitro-L-arginine methyl ester in rats and cats. *Br J Pharmacol* **1991**, *103*, 1899-1904, doi:10.1111/j.1476-5381.1991.tb12349.x.
33. Sparkes, A.H.; Caney, S.; Chalhoub, S.; Elliott, J.; Finch, N.; Gajanayake, I.; Langston, C.; Lefebvre, H.P.; White, J.; Quimby, J. ISFM Consensus Guidelines on the Diagnosis and Management of Feline Chronic Kidney Disease. *J Feline Med Surg* **2016**, *18*, 219-239, doi:10.1177/1098612X16631234.
34. Williams, T.L.; Peak, K.J.; Brodbelt, D.; Elliott, J.; Syme, H.M. Survival and the development of azotemia after treatment of hyperthyroid cats. *J Vet Intern Med* **2010**, *24*, 863-869, doi:10.1111/j.1939-1676.2010.0550.x.
35. Perez-Lopez, L.; Boronat, M.; Melian, C.; Saavedra, P.; Brito-Casillas, Y.; Wagner, A.M. Assessment of the association between diabetes mellitus and chronic kidney disease in adult cats. *J Vet Intern Med* **2019**, *33*, 1921-1925, doi:10.1111/jvim.15559.