

Study on relationship between right heart echocardiographic parameters and pulmonary artery pressure, and pharmacokinetics / pharmacodynamics of oral sildenafil in a canine model of chronic embolic pulmonary hypertension

Summary of Doctoral Thesis

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Pulmonary hypertension (PH) is a progressive disorder characterized by the elevation of pulmonary artery pressure (PAP), often leading to right heart failure and poor prognosis. PH in dogs is usually diagnosed and evaluated based on an estimate of PAP obtained from echocardiography though right heart catheterization (RHC) remains the gold standard for detecting PH. However, estimation of PAP via echocardiography have been revealed insufficient, and several conventional echocardiographic parameters related to the right ventricle or pulmonary artery have been advocated recently. In addition, sildenafil, a highly selective phosphodiesterase (PDE)-5 inhibitor, is administered to dogs with PH in order to block the inactivation of cyclic guanosine monophosphate by PDE-5. However, the relationship between right heart echocardiographic parameters and pulmonary artery pressure measured by RHC, and basic information regarding the pharmacokinetics and pharmacodynamics of oral sildenafil at different doses in dogs with PH have not been evaluated fully.

Therefore, the present study aimed to evaluate the relationship between right heart echocardiographic parameters and pulmonary artery pressure in canine models of chronic PH, and examine the pharmacokinetics and pharmacodynamics of oral sildenafil.

## Chapter 2 Relationship between right heart echocardiographic parameters and invasive pulmonary artery pressure in a canine model of chronic embolic pulmonary hypertension

The aim of this study was to examine the association between conventional right heart echocardiographic parameters and invasive PAP by RHC before and after PH. Chronic embolic pulmonary hypertension (CEPH) models were created by repeatedly injecting 100–300  $\mu\text{m}$  microspheres into the pulmonary arteries in five female beagle dogs regarded as clinically healthy. Echocardiography and RHC were conducted before and after creating (CEPH) models.

As a result, the normalized right ventricular internal diameter in diastole (RVIDdn), the ratio of the pulmonary artery and aortic diameter in diastole (PA/Ao), the acceleration time to ejection time ratio in pulmonary artery flow profile (AT/ET), and the normalized tricuspid annular plane systolic excursion (TAPSEn) were correlated with the invasive systolic (sPAP) and mean PAP

(mPAP). In contrast, the PA/Ao was correlated with invasive diastolic PAP (dPAP). Multiple linear regression analysis identified AT/ET and RVIDdn as independent predictors of sPAP, and PA/Ao and RVIDdn as independent predictors of mPAP. AT/ET and PA/Ao had sufficient sensitivity and specificity for predicting CEPH. In the present study a significant correlation between our estimated pressure gradient and each PAP value was not observed, although tricuspid and pulmonary regurgitation (TR and PR) were confirmed and measured in some dogs.

Based on our findings, PH should be suspected in dogs in the presence of an increased PA/Ao or a decrease in pulmonary artery AT/ET, even if TR or PR are not observed. In addition, alteration in these echocardiographic parameters enable us to evaluate pathological condition related to elevated PAP.

### Chapter 3 Pharmacokinetics of single dose sildenafil administered orally in clinically healthy dogs : effect of feeding and dose proportionality

Basic information related to the pharmacokinetics of sildenafil in clinically healthy dogs is scarce. The present study aimed to describe the pharmacokinetic properties of oral sildenafil and determine the effect of feeding and dose proportionality. The effect of feeding on pharmacokinetics of sildenafil (1 mg/kg) was investigated using a cross-over study with six dogs. In addition, the dose proportionality of sildenafil ranging 1–4 mg/kg was evaluated using five dogs in the fasted states. The plasma concentrations of sildenafil were determined using high performance liquid chromatography (HPLC), and pharmacokinetic parameters were calculated using a moment analysis.

Sildenafil administrations were well tolerated in all studies. The time to maximum plasma concentration ( $T_{max}$ ) were 1–2 hours. Feeding reduced the maximum plasma concentration ( $C_{max}$ ) and the area under the curve extrapolated to infinity ( $AUC_{inf}$ ) significantly. The elimination half-life ( $t_{1/2}$ ) did not differ between the fasted and the fed states, which were 2.8 and 3.2 hours, respectively. For dose proportionality, nonproportional increases in  $C_{max}$  and  $AUC_{inf}$  at 1–4 mg/kg doses were detected by a power model analysis.

These results show that it is beneficial to administer sildenafil without food. In addition,

this nonproportionality may reflect saturation of the metabolism in the liver because sildenafil administered in dogs is mainly metabolized by hepatic microsomal isoenzymes. These findings are beneficial for detecting changes in pharmacokinetic properties in dogs with PH, and provide basic information for deciding dose and frequency.

#### Chapter 4 Pharmacokinetics of single dose sildenafil orally administered in a canine model of chronic embolic pulmonary hypertension

Information regarding the pharmacokinetics of oral sildenafil in dogs with PH is limited. In this study, we examined the pharmacokinetics of oral sildenafil in a canine model of CEPH. The CEPH model was developed by repeatedly injecting microspheres into the pulmonary arteries. The pharmacokinetics of oral sildenafil at 1, 2 and 4 mg/kg was evaluated using four dogs with CEPH in the fasted state. The plasma concentrations of sildenafil were determined using HPLC, and pharmacokinetic parameters were calculated using a moment analysis.

PAP increased and cardiac output (CO) decreased significantly in CEPH models. Proportional increments in  $C_{max}$  and  $AUC_{inf}$  at drug doses of 1, 2 and 4 mg/kg were detected using a power model analysis. No significant differences were observed among the three doses in  $T_{max}$ . The mean residence time (MRT) and  $t_{1/2}$  were slightly but significantly higher at a dose of 4 mg/kg than at a dose of 1 mg/kg.

It is likely that the non-proportionality of sildenafil observed in healthy dogs disappeared in dogs with CEPH. In addition, the disappearance of non-proportionality for sildenafil in CEPH models appears attributable to impaired drug absorption due to hypoperfusion of the gastrointestinal tract resulting from reduced CO. However, concerning the elimination of sildenafil, the extent of the decrement in the elimination rate is unlikely to be pharmacokinetically and clinically significant because even prolonged MRT and  $t_{1/2}$  at 4 mg/kg were equivalent to those obtained from healthy dogs.

#### Chapter 5 The effect of sildenafil on pulmonary haemodynamics in a canine model of chronic embolic pulmonary hypertension

The effects of different doses of orally administered sildenafil on pulmonary haemodynamics in dogs with PH have not been documented in an invasive and quantitative manner because RHC is rarely performed due to its cost, anesthetic risk and high invasiveness with complications. In this study, we examined the effects of oral sildenafil using a canine model of CEPH. This CEPH model was created by repeatedly injecting microspheres through a catheter into the pulmonary arteries. The CEPH models received 1, 2 or 4 mg/kg of sildenafil orally twice a day for seven days. After washout period, the dogs were randomly assigned to receive one of the remaining doses. This continued until each dog had been given all doses. Haemodynamic measurements including PAP, systemic artery pressure (SAP), pulmonary artery wedge pressure (PAWP), right atrial pressure (RAP) and CO were obtained after seven days of each sildenafil administration via RHC and oscillometric blood pressure measurements.

Sildenafil administered at doses of 2 and 4 mg/kg significantly decreased systolic PAP compared with before administration. In addition, all doses of sildenafil significantly decreased the mean and diastolic PAP. Furthermore, 4 mg/kg of sildenafil significantly decreased PAP compared with 1 mg/kg. Sildenafil also significantly decreased pulmonary vascular resistance without notable changes in SAP or systemic vascular resistance. PAWP, RAP and CO did not increase significantly at any doses.

These findings confirm that oral sildenafil mainly dilates the pulmonary artery rather than the systemic artery in dogs with PH. Our results also suggest that 4 mg/kg oral sildenafil may enhance the effect of treatment compared with 1 mg/kg sildenafil.

In conclusion, alteration in echocardiographic parameters of right-sided heart enable us to evaluate pathological condition related to elevated PAP, even if TR or PR are not observed. In addition, concerning the treatment of PH, it may be beneficial to administer sildenafil 1–2 hours before feeding. Furthermore, we showed that the non-proportionality of sildenafil observed in healthy dogs disappeared in dogs with PH. Therefore, altered pharmacokinetics of oral sildenafil in dogs with PH should be considered for providing maximal therapeutic response because it is revealed that oral sildenafil decreased PAP in a dose-dependent manner.