Effect of glucagon like peptide-1 receptor agonists on gastrointestinal transit in healthy dogs

Summary

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Glucagon like peptide-1 (GLP-1) receptor agonists have major pharmacological activities, such as increasing glucose-dependent insulin secretion, suppressing glucagon secretion under hyperglycemic conditions, and delay of gastrointestinal transit rate, thereby which ameliorate glycemic control in patients with diabetes. Especially, delay of gastrointestinal transit rate is important for decreasing postprandial hyperglycemia. However, few studies have reported the effect of GLP-1 receptor agonists on gastrointestinal transit time (gastric emptying time and small intestine transit time) in healthy dogs. In addition, the acetaminophen (APAP) method and the salazosulfapyridine (SASP) method may be useful for the determination of gastrointestinal motility in healthy dogs because of its simple protocol. Although APAP is poorly absorbed from the stomach, it is rapidly absorbed from the small intestine. As such the rate of APAP absorption reflects the rate of gastric emptying. Meanwhile, oral administration of SASP is not absorbed from the small intestine and is metabolized to form sulfapyridine (SP) in the colon. Timing of the first appearance of sulfapyridine (SP) in plasma corresponds to the arrival of SASP in the colon. Therefore, the rate of SASP absorption reflects the rate of small intestine transit time. In this study, we first examined whether APAP method (evaluation of gastric emptying) and SASP method (evaluation of small bowel movement) can be accurately used for evaluating gastrointestinal motility as in dogs. Furthermore, final objective of this study was to evaluate the influence of GLP-1 receptor agonists on canine gastric emptying time and small intestine transit time using the APAP and SASP method.

Chapter 1　Basic research on measurement of serum APAP concentration and serum SP concentration

First, we evaluated the validity of commercial APAP detection kit, measuring canine serum levels after the administration of APAP. Furthermore, canine appropriate APAP dose was examined. Analytical evaluation of serum APAP concentration indicated acceptable precision, with intra-assay and inter-assay coefficients of variation (CV) not exceeding the commonly accepted 20% limit. A linear relationship was demonstrated with all dilutions tested, up to a 16-fold dilution. However, the dilution linearity graph did not pass through the origin. Furthermore, serum concentration below 5 μg/ml were not able to evaluate accurately. Therefore, this commercial kit is able to measure serum APAP concentration in dogs, but is not suitable for measuring low concentrations. Next, to determine the detectable APAP dose, 6 dogs were administered APAP with the dose of 10mg/kg and 20mg/kg. A significant increase was observed in serum APAP concentration with dose of 20 mg/kg, as compared to dose of 10 mg/kg. In addition, side effects were not observed at any tested dose in six dogs. Therefore, the minimum detectable APAP dose in dogs is approximately 20 mg/kg.

We evaluated the accuracy and reproducibility of serum APAP and SP concentration using HPLC method in dogs. In addition, recovery test reproducibility of serum APAP and SP concentration was estimated with serum samples of healthy dogs. Recovery test reproducibility of both serum APAP and SP concentration were within 80-120% of FDA standards in the United States. Therefore, there are no substances that interfere with the sample. Intra-assay and inter-assay reproducibility of serum APAP and SP concentration was estimated with 3 serum samples (high, middle, and low)

of healthy dogs. Intra-assay coefficients of variation (CV) did not exceed 20% limit of the FDA standards accepted with all the concentration. Inter-assay did not exceed 20% limit with middle and low serum APAP concentration, but the inter-assay CV of high serum APAP concentration sample was 35.59%. We suggest that it would be better to measure the serum APAP concentration within 7 days, since stored serum APAP sample did not show stable results over 10 days. A linear relationship was demonstrated with all dilutions tested, up to a 16-fold dilution. From the above results, serum APAP and SP concentration in dogs can measure using HPLC method.

Chapter 2 Comparison of the APAP and SASP method and the radiopaque marker method for the gastrointestinal motility in healthy dogs

For investigating canine gastrointestinal motility, accuracy of the APAP and SASP method and the barium impregnated polyethylene spheres (BIPS) were compared. Four healthy dogs were administered BIPS, APAP and SASP mixed with a wet food. X-ray photography and serum APAP and SP concentration taken by blood sampling at each time point were compared. APAP was emptied from the stomach at 0.5 hours after administration, and SASP was reach at the colon at 4 hours after administration. By contrast, BIPS was not excreted from the stomach over 10 hours after administration. Furthermore, it is difficult to discriminate between small and large intestine using BIPS. Therefore, BIPS could not use for the evaluating gastrointestinal motility in dogs.

Next, accuracy of the APAP and SASP method and the liquid contrast medium on canine gastrointestinal motility were compared. Additionally,

blood metabolites (Glucose, Insulin, NEFA, and TG) were also compared. Four healthy dogs were administered liquid contrast medium (barium sulfate suspension) and APAP and SASP mixed with a wet food. X-ray photography and blood sampling obtained at each time point were compared. APAP was emptied from the stomach at 0.5 hours after administration, and the liquid contrast medium was moved into the duodenum at 0.5 hours. As such, gastric emptying time measured by the X-ray photography and APAP method is similar. The liquid contrast medium appeared small intestine 2 hours earlier than the SASP method in 2 of 4 dogs. Although a time lag of about 2 hours was observed, we considered that the SASP method and X-ray photography showed similar small intestine transit time. There was no change in postprandial serum glucose and insulin concentration. Meanwhile, changes in serum NEFA and TG concentration corresponded to the results of the APAP and SASP method. Therefore, we considered that the APAP and SASP method is a reliable to evaluate the rate of gastric emptying and small intestine transit time without photographing X-ray.

Chapter 3 Effect of glucagon like peptide-1 receptor agonists on gastrointestinal transit time in healthy dogs

In humans, the capacity for GLP-1 receptor agonists, to slow gastrointestinal transit the dominant mechanism by which they reduce postprandial glycemic excursions. However, it is unclear in healthy dogs. In this chapter, we investigated the effect of GLP-1 receptor agonists on the gastrointestinal transit time and blood metabolic marker using the APAP and SASP method in healthy dogs. Three treatments were administered to five dogs. Treatments were consisted of subcutaneous

administration of saline solution (control treatment), exenatide, and liraglutide. GLP-1 receptor agonists (exenatide and liraglutide) and saline solution were administered 1 hour prior to meal with administering APAP and SASP. After blood sampling, serum APAP, SP, Glucose, Insulin, NEFA, TG, plasma GIP and GLP-1 concentration obtained at each time point were compared. As a result, exenatide and liraglutide delayed gastric emptying time. Furthermore, delayed gastric emptying induced lowering postprandial glycemia, Insulin, TG, and GIP. Meanwhile, side effect (loose stool) was observed after administration of liraglutide.

In conclusion, we established a simultaneous assay of serum APAP and SP concentration using HPLC method in dogs. Additionally, we confirmed that the APAP and SASP method is a reliable to evaluate the rate of gastric emptying and small intestine transit time. Furthermore, we investigated the effect of GLP-1 receptor agonists on gastrointestinal transit time in healthy dogs using the APAP and SASP method. We found that GLP-1 receptor agonists delay gastric emptying time and small intestinal transit time in healthy dogs. These results indicate that the APAP and SASP method can use for monitoring the gastrointestinal motility in dogs and GLP-1 receptor agonists would be useful for glycemic control in diabetic dogs in the future.