In vitro study of the effect of dog food on the adsorptive capacity of activated charcoal

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Abstract

Objective – To evaluate the effect of dog food on the adsorptive capacity of activated charcoal.
Design – In vitro laboratory study.
Setting – University veterinary teaching hospital.
Animals – None.
Interventions – None.
Materials and Methods – A fixed quantity of acetaminophen (50 mg) was added to a fixed quantity of activated charcoal (1 g), mixed with varying amounts of dog food (2–14 g). The admixture was agitated for 5 minutes, incubated at 38.5°C for 1 hour and then centrifuged for 30 minutes. The concentration of residual, nonadsorbed acetaminophen in the supernatant was quantitatively assayed by reverse phase high-pressure liquid chromatography with ultraviolet detection. Data were tested by linear regression analysis and statistical significance was set at $P < 0.05$.

Measurements and Main Results – A statistically significant reduction in the adsorptive capacity of activated charcoal was demonstrated with increasing amounts of dog food ($R^2 = 0.54; P = 0.0018$). However, all measurements of residual acetaminophen were less than 100 mg/L, representing a reduction in acetaminophen concentration of more than 98.6%.

Conclusions – The addition of dog food to activated charcoal reduces its total adsorptive capacity for acetaminophen. However, this reduction in adsorptive capacity is unlikely to be clinically significant in the presence of both the formulation of dog food and the ratio of dog food to charcoal used in this study.


Keywords: acetaminophen, decontamination, toxicology

Introduction

Intoxications are a common presenting complaint of small animals to emergency veterinary clinics and gastrointestinal decontamination is often the most appropriate first-line treatment. Activated charcoal is a carbonaceous, porous adsorptive agent with a large internal surface area of approximately 1000 m$^2$/g in commercial preparations, onto which organic matter will readily bind by van der Waals forces. Activated charcoal is widely used in both veterinary and human medicine to minimize the gastrointestinal absorption of ingested toxic agents. Various forms of powder or slurry are available, but are generally unpalatable and poorly accepted by patients, leading to charcoal often being administered by syringe feeding or drenching, where a large volume of liquid charcoal is administered orally from a bottle. These methods of administration potentially put patients at risk of aspiration pneumonia. In increasing the palatability of charcoal admixtures to encourage voluntary ingestion by patients could reduce this risk. Several studies in people have investigated the effect of increasing the palatability of activated charcoal by the addition of food and flavorings. Some food...
Acetaminophen has commonly been used in toxicological studies, both in vitro and in vivo. Previous pharmacodynamic studies have assessed the interaction of acetaminophen with activated charcoal and the adsorption characteristics of the drug have been well documented. Acetaminophen intoxication is relatively common in dogs, with a previous report of 79 cases recorded at the Georgia Animal Poison Information Center over a 19-month period, representing 1.7% of all toxicological enquiries. Clinical signs were reported in 32.4% of the dogs with acetaminophen exposure and the most common findings included lethargy and gastrointestinal tract disturbances. Fatalities can occur due to fulminant hepatic failure and, less commonly, acute kidney failure. These effects have been noted at doses exceeding 200 mg/kg in dogs.

It is common practice among veterinary professionals to mix charcoal with dog food in order to improve the palatability and encourage dogs to eat the medication. Homogenous canned food is most commonly used for this purpose, due to the ease of mixing with charcoal and the high palatability of such diets. However, it is not known if dog food will adsorb onto the active sites of the charcoal and thus reduce its efficacy for adsorbing toxins within the gastrointestinal tract. The aim of this study was to evaluate the effect of dog food on the adsorptive capacity of activated charcoal. It was hypothesized that dog food would significantly reduce the adsorptive capacity of activated charcoal.

**Materials and Methods**

The study was conducted using commercially available suspensions of both activated charcoal and acetaminophen and a commercial canned dog food. Fifty milligrams of acetaminophen (2.1 mL) was placed into a sterile centrifuge tube and diluted with 10 mL distilled water. One gram of activated charcoal (2.9 mL of activated charcoal suspension) was mixed with varying amounts of dog food, from 2 g through to 14 g, in 2 g increments. The premixed dog food and charcoal were then introduced to the centrifuge tube containing the diluted acetaminophen solution. The samples were manually agitated for 5 minutes to ensure thorough mixing and then incubated at 38.5 °C for 1 hour, while being gently agitated at 1.7 Hz in a shaker bath. The samples were then immediately centrifuged for 30 minutes at 2400 x g and 38°C. The resultant supernatant was siphoned off and submitted to a reference laboratory for quantitative acetaminophen analysis by reverse phase high-pressure liquid chromatography (HPLC) with ultraviolet detection. The limit of quantification of the assay was 20 mg/L. No special transport media were required because acetaminophen is stable in suspension. Two separate samples were assayed from each admixture of dog food.

A number of samples were assayed to validate the methodology used. One sample of 50 mg acetaminophen diluted in 5 mL distilled water, without the addition of charcoal or dog food, was assayed to confirm the concentration of acetaminophen present in suspension. One sample of 50 mg acetaminophen diluted in 5 mL distilled water, with 1 g activated charcoal, but without any dog food, was assayed to confirm the efficacy of activated charcoal for adsorbing acetaminophen. Two samples were also processed in addition to the validation samples, containing 50 mg acetaminophen diluted in 10 mL distilled water, with 14 g of dog food, but no activated charcoal, to determine if the food alone had an intrinsic-binding capacity for acetaminophen.

**Statistical Methods**

All analyses were performed using commercial statistical software. A linear regression analysis was used to test the association between the amount of dog food added to 1 g activated charcoal and the residual acetaminophen in the supernatant. Any samples with an acetaminophen concentration of <20 mg/L were classified as 0 mg/L for statistical purposes. Statistical significance was set at \( P < 0.05 \).

**Results**

**Validation samples**

The sample of diluted acetaminophen contained 7000 mg/L, thus confirming the accuracy of the HPLC assay and that the commercial acetaminophen solution contained the reported concentration of drug, which was evenly dispersed throughout the suspension.

The sample containing only acetaminophen and activated charcoal, without any dog food, had a residual acetaminophen concentration of 30 mg/L, thus confirming the efficacy of the activated charcoal, by reducing the acetaminophen concentration from 7000 mg/L to 30 mg/L. This represents a reduction in acetaminophen concentration of 99.6%.

**Adsorptive capacity of dog food**

The 2 samples containing 14 g of dog food and no activated charcoal demonstrated a reduction in residual acetaminophen concentration in the
supernatant of 74.9% and 74.4%, respectively, with a mean residual acetaminophen concentration of 1775 mg/L, indicating that the dog food itself was capable of adsorbing a significant proportion of the acetaminophen.

Main study results

There was a positive linear correlation between increasing quantities of dog food added to the activated charcoal and a greater residual acetaminophen concentration in the supernatant ($r^2 = 0.54$, $P = 0.0018$; Figure 1). This indicates a statistically significant reduction in the adsorptive capacity of the activated charcoal with increasing quantities of dog food added. However, the decrease in adsorptive capacity was small, with all samples demonstrating a reduction in total acetaminophen concentration in excess of 98.6%. Application of the linear regression equation indicated that for every 1 g increase in dog food, a 5.15 mg/L increase in residual acetaminophen concentration could be expected.

Discussion

The results of this experiment have demonstrated that the addition of dog food to activated charcoal decreases the adsorptive capacity of the charcoal and that this effect is greater with increasing amounts of dog food. However, the absolute reduction in adsorptive capacity of activated charcoal demonstrated in this study was small. Even with large ratios of dog food relative to charcoal (up to 14:1, weight for weight), more than 98.6% of the acetaminophen was adsorbed in all of the samples. The reduction in the adsorptive capacity of activated charcoal caused by the dog food is therefore unlikely to be clinically significant. Although the reduction in adsorptive capacity detected in this study was small, there was a good linear correlation, as indicated by an $r^2$ value of 0.54. Two separate samples were performed for each quantity of dog food, to improve the validity of the statistical analysis.

The experiment was designed to reflect common clinical practice. Acetaminophen was the chosen drug for this in vitro model, as it is a common toxin of dogs and its use has been well described in previous toxicological models. The dog food was chosen because it is considered to be a highly palatable food and therefore commonly used by veterinary practitioners to encourage oral intake of activated charcoal in dogs. The preparation of activated charcoal used is readily available to small animal practitioners.

As the current study was an in vitro experiment, the results may not correlate with the in vivo situation. However, the study was designed to represent an in vivo scenario as closely as possible and was based on previously established methods for toxicological studies investigating charcoal. The samples were mixed in the order they would be introduced to a dog’s stomach in a clinical situation; the acetaminophen was diluted first and the charcoal and dog food were premixed, before addition to the acetaminophen solution. Dilution was necessary to enable subsequent centrifugation with production of a supernatant and 10 mL of distilled water was found to be the optimal volume of diluent. Previous in vitro adsorption studies of activated charcoal have also used water dilution. The validation sample assaying the diluted acetaminophen alone confirmed the predicted acetaminophen concentration, thus indicating that the acetaminophen was evenly dispersed throughout the dilute solution and the dilution procedure had not adversely affected the acetaminophen assay. The volume of supernatant recovered from each sample in the main study was fixed, thus it is unlikely that the dilution of the samples introduced error to the results. The samples were manually agitated initially, to ensure thorough mixing and then gently agitated throughout the incubation period, in a temperature-controlled shaker bath, to mimic gastric mixing from peristalsis and normal patient movement. Although there is no biomechanical data available to ratify that this procedure replicates in vivo gastric mixing, it was considered that some motion during the incubation period was more representative of an in vivo scenario than static incubation. The rate of oscillation selected was low, to avoid excessively vigorous ongoing sample mixing. The incubation period was 1 hour, to ensure that the acetaminophen had reached equilibrium with the activated charcoal. The samples were not acidified, as pH has been previously shown to have no effect on the adsorption characteristics of acetaminophen.

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activated charcoal over the pH range of 1.2–7.5. Normal gastric pH in dogs after feeding has been documented to be in the range 1.3–2.5, therefore the adsorption characteristics of activated charcoal are unlikely to be affected at any physiologic gastrointestinal pH. The pH of the test solutions, containing the acetaminophen, activated charcoal, distilled water, and dog food, was measured with the minimum and maximum quantities of dog food used. The pH was 7.34 with 2 g of dog food and 6.47 with 14 g of dog food, thus the solutions used were within the pH range that has no effect on charcoal adsorption kinetics.

In the study presented here, some intrinsic-binding capacity of the dog food for acetaminophen was demonstrated. Although the overall reduction in acetaminophen concentration was large in the samples containing both activated charcoal and dog food, the proportion of reduction due to binding to the activated charcoal, compared with the proportion of reduction due to binding directly to the dog food could not be determined, as the pharmacodynamics are not known regarding which substance will more readily adsorb the acetaminophen. The in vivo effects of the ability of dog food to adsorb acetaminophen are similarly unknown. It can be assumed that the adsorbed acetaminophen would be later released to the surrounding milieu when the food is digested. However, it is unknown whether it would then be adsorbed onto surrounding activated charcoal in the gastrointestinal lumen, or systemically absorbed. It could be hypothesized that if a sufficiently large quantity of activated charcoal is used, then the drug may be more likely to be adsorbed onto the surrounding charcoal, instead of being absorbed systemically. Similarly, it could be hypothesized that more active sites on the charcoal will become available for binding toxins as the food is digested, assuming that food bound to charcoal will still be enzymatically degraded in the intestinal lumen. In vivo studies would be required to accurately determine and differentiate these effects.

It has been previously shown that at least 4X as much activated charcoal as acetaminophen, on a by-weight basis, must be used for effective adsorption. Furthermore, desorption of acetaminophen is minimal when 8X as much activated charcoal as acetaminophen, on a by-weight basis, is present. The dose of activated charcoal used in the study reported here was 20X the dose of acetaminophen, on a by-weight basis, in order to ensure both maximal adsorption and minimal desorption of acetaminophen with the activated charcoal, as the aim was to investigate the effect on the adsorptive capacity of the charcoal attributable to the presence of the dog food. In this way, any residual acetaminophen in the supernatant could be assumed to be a result of the presence of the dog food and not due to the intrinsic pharmacokinetics of the charcoal.

The dog food to charcoal ratio chosen for this study was extrapolated from perceived clinical scenarios, although data on such ratios used commonly in veterinary practice is not available. The recommended dose of activated charcoal for dogs is 1–4 g/kg.12 It islogistically difficult to administer more than 1 g/kg to a patient of the commercial activated charcoal slurry used in this study, as large volumes would be required relative to the patient size (2.9 mL slurry per 1 g activated charcoal). Based on a 10 kg dog receiving 1 can of the commercial dog food used in this study (156 g), mixed with activated charcoal at a dose of 1 g/kg, a ratio of dog food to charcoal, on a by-weight basis, of 15:1 was derived. The authors believe that for most clinical purposes, veterinary practitioners use a lower dog food to charcoal ratio, with an aim of providing some flavor to the charcoal, to encourage voluntary oral intake and this is reflected in the range of dog food to charcoal ratios used in this study, ranging from 2:1 to 14:1. It was initially thought that small changes in the quantity of dog food would have a large effect on the adsorptive capacity of the charcoal, although this was not the case.

Most commercially available methods of assaying acetaminophen involve colorimetric technology, which could not be used in this study due to interference from the charcoal particles. Quantitative HPLC was used instead, to avoid this colorimetric interference and for quantitative accuracy. HPLC has been shown to have a correlation coefficient of 0.9999 with serum acetaminophen concentrations, making it a very accurate assay method. The lower limit of quantification of the HPLC assay was 20 mg/L, which corresponds to the maximum therapeutic serum concentration in people. This serum concentration is not clinically significant in dogs, where oral administration of 60 mg/kg, a dose less than the reported toxic threshold of 200 mg/kg, produced maximum serum concentrations of 28–90 mg/L and no reported clinical signs of toxicity. The maximum possible concentration of residual acetaminophen from the samples in the main study presented here was 3333 mg/L, assuming no adsorption of the drug occurred onto food or charcoal, thus the HPLC assay was capable of detecting a reduction in adsorptive capacity of up to 99.4%. The HPLC assays were run on the sample supernatant to detect the residual acetaminophen concentration, as the concentration adsorbed onto the activated charcoal could not be directly measured. Thus, by subtracting the residual concentration from the known initial concentration of acetaminophen, the concentration of adsorbed acetaminophen could be derived.
However, for accuracy, the measured values of residual acetaminophen concentration were used in the statistical analysis.

The findings of this study are consistent with those of previous toxicological studies, which have investigated the effect of the addition of various human foods to activated charcoal, in order to improve the palatability.3,4 Dairy products have reduced the adsorptive capacity of activated charcoal to a greater extent than carbohydrate-based food substances in the human studies conducted previously.3,5 The results of the current study relate only to the combination of food type, charcoal, and acetaminophen used and should not be extrapolated to other food types or toxins. The results may also differ with higher ratios of dog food to activated charcoal. Future veterinary studies could investigate the effect of different types of dog food on the absorptive capacity of activated charcoal, as well as the effect of dog food on the absorptive capacity of activated charcoal in the presence of different toxic agents. The study described here used a commercial suspension of activated charcoal and further studies could assess for differences in the absorptive properties compared with powdered charcoal, as comparative data between the 2 are also lacking in the currently available literature.

We conclude that activated charcoal is likely to remain efficacious in the presence of dog food, in light of the minimal reduction in absorptive capacity caused by dog food in this study. However, in vivo investigation would be required to validate these results in a clinically at risk population.

Acknowledgments

The authors would like to gratefully acknowledge the help of Frank Quinlivan for his technical expertise with HPLC and Yu-Mei Chang for assistance with the statistical analysis.

Footnotes

a Activated charcoal suspension (1 g/2.9 mL), Kruuse, Langeskov, Denmark.
b Calpol Infant Suspension (120 mg/5 mL), McNeil Healthcare, Wokingham, Berkshire, UK.
c Prescription Diet a/d, Hill’s Pet Nutrition, Watford, Hertfordshire, UK.
d Clifton NES-10D, Nickle Electro Ltd., Weston-Super-Mare, North Somerset, UK.
e Medical Toxicology Laboratory, St Thomas’ Hospital, London, UK.
f GraphPad Prism 5.00, GraphPad Software, San Diego, CA, 2007.

References


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