The effect of cryopoor plasma addition to adsorbed plasma to correct prothrombin time in dogs: an *in vitro* study

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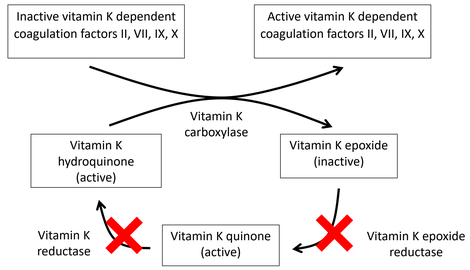
Introduction

- Anticoagulant rodenticide intoxication reduces activation of vitamin K dependent coagulation factors II, VII, IX, and X and is one of the most common toxicities in dogs that present to emergency veterinary clinics.
- The mechanism of toxicity is inhibition of vitamin K reductase and vitamin K epoxide reductase, which play an essential role in recycling of vitamin K. This leads to decreased carboxylation or activation of the specific coagulation factors[1,2].



- Due to the short biological half lives of factors II, VII, IX, and X, patients often present to emergency clinics 2-5 days after rodenticide ingestion with nonspecific clinical signs including lethargy, pale mucous membranes, and inappetence, as well as more severe signs of coagulopathies including hemoabdomen and hematochezia[1,2,3,4].
- Current treatments may include: vitamin K supplements, activated charcoal, hospitalized care, and transfusion of blood products[3,4].
- Cryopoor plasma, which is the byproduct of plasma processing of cryoprecipitate, has been shown to contain similar concentrations of the vitamin K dependent coagulation factors when compared to fresh frozen plasma[5].
- Cryopoor plasma is less expensive than fresh frozen plasma and may offer clinicians and clients another potential treatment option for rodenticide toxicity.

Anticoagulant Rodenticides	Toxic Dose in Dogs (mg/kg)
Brodifacoum	0.2 – 4.0
Bromadiolone	11 - 15
Diphacinone	0.9-8.0
Pindone	5-75
Warfarin	20-300

Table 1: Anticoagulant rodenticides commonly ingested by dogs
Adapted from Murphy, M.E. 2002

Objective

Determine if increasing amounts of cryopoor plasma added to barium adsorbed canine plasma is as effective as fresh frozen plasma to correct the prothrombin time and the concentrations of vitamin K dependent coagulation factors II, VII, IX, and X.

Significance

We seek to prove the principle that cryopoor plasma may be a more cost-effective treatment option than fresh frozen plasma in dogs without experimental induction of the intoxication or placement of clinical patients at risk. These data may be extrapolated in the future to treat anticoagulant rodenticide-intoxicated clinical patients using a predicted amount of cryopoor plasma.

There is currently no designated protocol to study canine coagulopathies *in vitro*. We aim to create a barium sulfate protocol that can be used in other coagulopathy studies such as anticoagulant rodenticide toxicities and hemophilia B.

Methods and Materials

Phase 1: Determine protocol to adsorb canine plasma
- Canine fresh frozen plasma was acquired through the UC Davis Veterinary Blood Bank
- Completed different trials with various forms and concentrations of barium to maximize the increase in PT and PTT.
- Oral barium sulfate suspension at a dose of 200mg $BaSO_4$ per mL of fresh frozen plasma adsorbed the plasma of vitamin K dependent coagulation factors.

Phase 2: Determine the optimal transfusion value
- Mix cryopoor plasma and fresh frozen plasma at various doses to mimic those below, including, and above that often used clinically (10mL-20mL of FFP/kg BW) for the treatment of rodenticide intoxication[1].
- Each sample will be analyzed at the UC Davis VMTH for PT, INR, PTT, and fibrinogen.
- Frozen samples will be sent to the Comparative Coagulation Lab at Cornell University Animal Health Diagnostic Center for complete analysis of the concentrations of coagulation factors II, VII, IX, and X.

Preliminary Data

Phase 1: Determine protocol to adsorb canine plasma

Contents	PT Ref: 7.0-9.3s	INR Ref: 0.8-1.2	PTT Ref: 10.4-12.9s	Fibrinogen Ref: 109-311mg/dl
Original plasma	9.5	1.2	12.6	146
Adsorbed plasma	>120	N/A	>140	115
2mL AP + 0.2mL FFP	15.9	2.4	83.3	123
2mL AP + 0.4mL FFP	12.6	1.8	61.8	131
2mL AP + 0.2mL CPP	13.8	2	70.4	123
2mL PA + 0.4mL CPP	12	1.7	51.1	126

Note: AP = adsorbed plasma, FFP = fresh frozen plasma, CPP = cryopoor plasma

Phase 2: Determine the optimal transfusion value

Contents	FII Ref: 50-150%	FVII Ref: 50-150%	FIX Ref: 50-150%	FX Ref: 80-175%	PTT Ref: 8.5-15.5s	PT Ref: 11-15.5s	Fibrinogen Ref: 150-490mg/dL
Original plasma	114	58	88	75	13.6	14.5	266
Adsorbed plasma	2	<1	1.40	<1	>180	>90	195
Fresh frozen plasma	77	46	108	56	11.6	13.6	292
Cryopoor plasma	149	94	80	129	13.6	14.2	259
2mL AP + 0.2mL FFP	6	2	8	<1	>180	38.5	
2mL AP + 0.4mL FFP	13	4	13	<1	112.3	31.0	
2mL AP + 0.6mL FFP	15	8	18	<1	85.9	28.9	
2mL AP + 0.8mL FFP	22	9	22	<1	67.7	26.3	
2mL AP + 1.0mL FFP	22	14	24	<1	57.4	26.4	
2mL AP + 0.2mL CPP	11	5	11	<1	160.7	33.6	
2mL AP + 0.4mL CPP	18	12	18	<1	89.8	29.3	
2mL AP + 0.6mL CPP	29	13	24	<1	71.1	25.7	
2mL AP + 0.8mL CPP	32	23	28	1	61.2	24.9	
2mL AP + 1.0mL CPP	41	20	34	1	55.3	23.0	

Note: AP = adsorbed plasma, FFP = fresh frozen plasma, CPP = cryopoor plasma

Discussion

- An adsorption protocol using oral barium sulfate suspension has been confirmed to adsorb plasma of vitamin K dependent coagulation factors.
- Based on preliminary data, the addition of fresh frozen plasma and cryopoor plasma to adsorbed plasma does improve PT and PTT.
- Increasing amounts of volume added of fresh frozen plasma and cryopoor plasma correlates with a greater correction in coagulation times.

Limitations

- *In vitro* study may not correlate to a clinical application due to the complicated physiology of coagulation and rodenticide intoxication.
- In our preliminary data, Factor X appears to continually be adsorbed following removal of barium sulfate by centrifugation and thus may affect PT and PTT.
- While we have analyzed the adsorbed plasma samples for PT, PTT, and concentrations of fibrinogen and coagulation factors II, VII, IX, and X, it is unknown if our protocol thoroughly mimics clinical rodenticide toxicity.

Future Directions

- Six additional mixing trials using different fresh frozen plasma and cryopoor plasma will be added to adsorbed plasma to complete phase 2 to determine the optimal transfusion value of cryopoor plasma in comparison to fresh frozen plasma.
- Then, statistical analysis will be completed to determine the optimal transfusion value and correlate it with clinically relevant transfusion doses for cryopoor plasma.
- In the future, we hope to use this data to design a clinical trial for the use of cryopoor plasma to treat canine anticoagulant rodenticide intoxication.

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