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BROMETHALIN TOXICOSIS-EVALUATION OF AMINOPHYLLINE TREATMENT AND AN EPIDEMIOLOGIC ASSESSMENT

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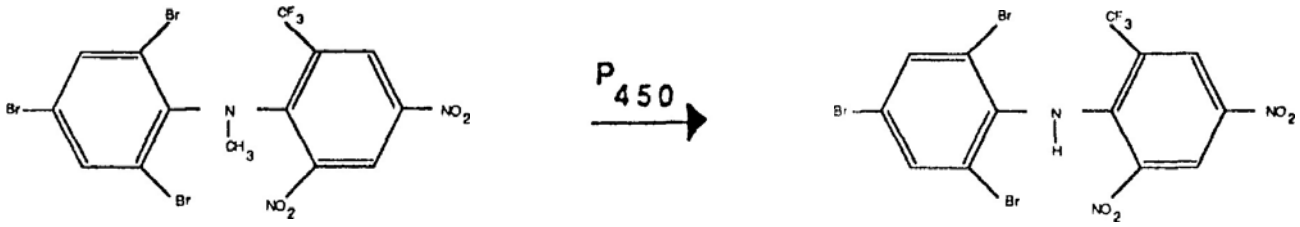
INTRODUCTION

The reported LD_{50} for technical grade bromethalin ranges from 1.8 mg/kg in the cat, 4.7 mg/kg in the dog, and 13 mg/kg in rabbits, and up to >1000 mg/kg in guinea pigs (VanLier and Ottosen 1981, VanLier and Cherry 1988). Mitochondrial electron transport studies using purified rat brain and liver mitochondria have been performed, and have established that bromethalin is an effective uncoupler of oxidative phosphorylation (VanLier and Ottosen 1981, Cherry et al. 1982, VanLier and Cherry 1988). Mitochondrial oxidative phosphorylation is the major mechanism for production of ATP in the brain. Uncoupling of this reaction in bromethalin-poisoned animals may result in a lack of adequate ATP formation and diminished Na^+ - K^+ ion channel pump activity. We are currently investigating alternate mechanisms of action for bromethalin toxicosis in the rat. Regardless of mechanism of action, cerebral edema and elevated cerebrospinal fluid pressure (CSFP) develops. Microscopic examination of neurologic tissues (brain and spinal cord) from lethally dosed animals revealed diffuse spongy degeneration of the white matter and intramyelinic edema (VanLier and Cherry 1988, Dorman et al. 1990).

The toxic syndrome induced by bromethalin is dose-dependent. Rats given high oral doses (in excess of the LD_{50}) of bromethalin develop an acute toxic syndrome characterized by severe muscle tremors, hyperexcitability, and seizures (VanLier and Cherry 1988). Death may result within 8 to 24 hours due to apparent respiratory arrest. Following the ingestion of lower oral doses (less than an LD_{50}) the onset of clinical signs may be delayed (e.g., 24 to 86 hours). This second syndrome is characterized in the rat by the development of hindlimb weakness, paralysis, prostration, and lethargy (VanLier and Cherry 1988).

Bromethalin is N-demethylated by the hepatic mixed function oxidase (mfo) system to its desmethylbromethalin metabolite (Fig. 1). Desmethylbromethalin has been demonstrated to be a 10- to 1000-fold more potent uncoupler of oxidative phosphorylation than is bromethalin (VanLier and Cherry 1988). The apparent lack of toxicity of bromethalin observed in guinea pigs may be partially explained by their relative deficiency in N-demethylase activity. Production of desmethylbromethalin, therefore, appears to be involved in the production of the toxic syndrome. The use of an antimetabolite which inhibits the conversion of bromethalin to its desmethylbromethalin metabolite may represent another potential treatment for bromethalin toxicosis.

An ideal antimetabolite of bromethalin should have the following characteristics: a) it should be metabolized by the same microsomal isozymes which N-demethylates bromethalin, b) it should be low in toxicity, and c) it should be readily available. Based upon these considerations aminophylline (theophylline ethylenediamine) was selected as a potential bromethalin antimetabolite. Theophylline is a 1,3-dimethylated xanthine which is similar in structure to caffeine and theobromine (Fig. 2). Theophylline is metabolized by two N-demethylations and an 8-hydroxylation to form 1-methylxanthine, 3-methylxanthine, and 1,3-dimethyluric acid (Lohman and Meich 1976). Studies of the in vitro metabolism of theophylline by human microsomes indicate that two distinct cytochrome P-450 isozymes are involved in these reactions (Robson et al. 1988). Theophylline is commonly used as a bronchodilator and is readily available. Theophylline is of moderate oral toxicity with an acute oral LD_{50} of 300 mg/kg in the dog and 700 mg/kg in the cat.



Bromethalin

Desmethylbromethalin

Figure 1. Metabolism of bromethalin.

The purpose of this report is to further define the toxicity of bromethalin-based rodenticides in nontarget species by presenting the clinical signs associated with spontaneous bromethalin rodenticide ingestion. Furthermore, we also examined the efficacy of aminophylline administration in the treatment of lethal bromethalin toxicosis in the rat.

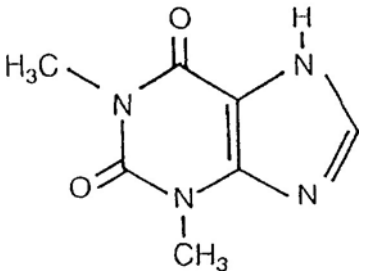


Figure 2. Structure of theophylline.

MATERIALS AND METHODS

A. Retrospective Epidemiologic Study

Epidemiologic and clinical information was compiled from the Illinois Animal Poison Information Center (IAPIC) case record database. The IAPIC is located at the University of Illinois and receives calls from throughout the United States and Canada. The IAPIC protocol collects the following information whenever possible: the animal's age, sex, breed, species, number at risk, number exposed, the number of animals affected, the amount of product involved, source of the animal's exposure, place where the exposure occurred, and the level of assurance of exposure. A clinical history is also obtained whenever possible and includes clinical signs and their severity, clinical chemistry alterations, pathological findings, and the animal's response to therapy. The clinical syndrome is also characterized by the time between the exposure and the onset of clinical signs. The duration of the clinical syndrome is recorded.

On the basis of clinical, temporal, and exposure histories, IAPIC veterinarians categorize each call into one of several subcategories including: "toxicosis," suspected toxicosis," "doubtful toxicosis," "exposure," or "other situation" (syndrome due to a nontoxicologic cause). The IAPIC veterinarians will base these decisions on expected clinical signs from available literature, on temporal and dosage considerations, and previous experience with the agent in question. A case is defined as an "exposure" if clinical signs are not present at the time of the initial call. Clinical illness believed to be due to etiologies other than a known or suspected toxicant (e.g., infection, foreign body) is assessed as "other situation." If the animal has clinical signs at the time of the call, or if the animal subsequently develops clinical signs, a "toxicosis," "suspected toxicosis," or "doubtful toxicosis" assessment is used. For a case to be assessed as a "toxicosis" all temporal, clinical, and exposure data must be consistent with the expected syndrome. When the syndrome described is characteristic for a given agent but some data cannot be obtained, the case generally is assessed as a "suspected toxicosis." Assessment as a "toxicosis" or "suspected toxicosis" generally is unconfirmed by analytical methodologies.

B. Aminophylline Treatment Study

The experimental protocol was reviewed and accepted for animal use and welfare by the Laboratory Animal Use Committee of the University of Illinois. Rats were serially monitored with neurologic and physical examinations for 2 hours following dosing. The rats were then monitored at 2-hour intervals until euthanasia was performed. Euthanasia with an intraperitoneal overdose of pentobarbital was performed if an animal displayed severe or persistent seizures (≥15 minutes of persistent seizure activity) or if paralysis was present for 24 or more hours.

Twenty adult (50-54 days, 200-225 gram), male, colony-bred Sprague-Daley rats were evenly assigned to either a bromethalin and saline or a bromethalin and aminophylline treatment group. Rats were housed in standard plastic rodent cages (2 rats per cage), and were fed commercial rodent chow and water ad libitum. Food was withheld for 4 hours prior to bromethalin administration. Hexane-dissolved bromethalin (analytical grade bromethalin, donated by Lilly Research Laboratory, Greenfield, IN) was given (4.5 mg/kg) to all rats by gavage (total volume <0.5 ml). Aminophylline (Elkins-Sinn, Inc., Cherry Hills NJ) was given (15 mg/kg, IM, every 6 hours). An equivalent volume of saline was given to all bromethalin-no treatment (control) rats. Clinical signs, time to onset of signs, and survival time were recorded. Data concerning mean survival time were analyzed with a one-way ANOVA Significance was determined for all results at $P \leq 0.05$.

RESULTS

A. Epidemiologic Study

Of the 156 cases involving accidental bromethalin exposure received by the Illinois Animal Poison Information Center (IAPIC) between January 1, 1989, and December 31, 1989, 6 (3.8%) were classified as "toxicosis," 18 (11.5%) as "suspected toxicosis," 17 (10.9%) as "doubtful toxicosis," and 112 (71.8%) as "exposure." There were 145 dogs, 41 cats, 6 pheasants, 2 goats, 1 horse, and 1 hamster reported to have ingested a bromethalin-based rodenticide during this time period. An estimate of amount was unavailable in 33% of all calls assessed as "exposure." An ingestion of an amount of bromethalin believed to be consistent with a toxicosis occurred in 21% of all calls assessed as "exposure." The IAPIC was contacted within less than 2 hours of ingestion in 63% of all calls assessed as "exposure." The time duration between time of call and ingestion was unknown for only 6% of all calls assessed as "exposure." No age or breed predispositions were noted for bromethalin-poisoned animals. Most bromethalin ingestions (90%) were considered accidental and 71% occurred in the home. During the same time period (January 1, 1989, to December 31, 1989) the IAPIC received 26,744 calls involving animals exposed to all products.

Clinical signs associated with bromethalin poisoning in 15 dogs and 13 cats were (in decreasing order): depression (61%), ataxia (38%), tremors (32%), paralysis (18%), vomiting (18%), seizures (15%), salivation (15%), and death (11%). Less frequently reported clinical signs included anorexia, diarrhea, vocalization, hyperesthesia, dyspnea, nystagmus, and coma.

B. Aminophylline Treatment Study

One rat in the group given bromethalin and saline died during dosing due to apparent pulmonary aspiration of hexane. Clinical signs observed in rats included tremors, seizures, hyperactivity, ataxia, hindlimb paralysis, anorexia, prostration, and death. Clinical signs of bromethalin toxicosis developed within 1 to 3 hours postdosing. Deaths occurred in untreated rats within 2.5 to 7.5 hours of ingestion. The

mean survival time observed in untreated rats was 4.7 hours (n=9). Deaths occurred in aminophylline-treated rats within 1.5 to 25 hours of ingestion. The mean survival time observed in aminophylline treated rats was 8.0 hours (n=10). No statistically significant difference between mean survival times was noted ($P < 0.05$).

DISCUSSION

Bromethalin-based rodenticides, as other modern rodenticides, may constitute a significant hazard if improperly used and are ingested by nontarget species. Bromethalin-based rodenticides were the sixth most commonly ingested rodenticide by animals as reported to the IAPIC in 1987 (Trammel et al. 1989). During 1987, rodenticides with the greatest IAPIC call volume included brodifacoum (4061 calls), warfarin (488 calls), cholecalciferol (362 calls), bromadiolone (225 calls), diphacinone (165 calls), and bromethalin (148 calls). Although infrequently associated with toxicosis, the majority of bromethalin related calls to the IAPIC during 1989 occurred before clinical signs developed ("exposures"). This finding was not unexpected due to the delay (days) in onset of the development of toxicosis. Of these calls assessed as "exposure," over 20% involved animals which ingested an amount of bromethalin believed to potentially cause toxicosis. The high percentage of calls (61%) in which exposure had occurred less than 2 hours before contacting the IAPIC indicates a high potential for early therapeutic intervention. As with other rodenticides, bromethalin ingestion by animals most commonly involved dogs, occurred in the home environment, and was considered accidental. Therefore, efforts to instruct consumers and pest control applicators in the safe use of this rodenticide in the home (e.g., the use of tamper-proof bait stations) may greatly reduce inadvertent animal exposure.

Clinical signs associated with bromethalin poisoning in dogs and cats reported to the IAPIC in this retrospective study included depression, ataxia, tremors, paralysis, vomiting, seizures, salivation, and death. Less frequently reported clinical signs included anorexia, diarrhea, vocalization, hyperesthesia, dyspnea, nystagmus, and coma. Reported clinical signs of bromethalin toxicosis were similar to those seen in a suspected case of bromethalin toxicosis in a cat and experimental studies in dogs (Martin and Johnson 1989, Dorman et al. 1990).

Currently, treatment of bromethalin toxicosis in animals is primarily directed towards reducing the gastrointestinal absorption of bromethalin and controlling cerebral edema (Carson 1989). Treatment with mannitol and dexamethasone has been reported to reverse the development of increased cerebral spinal fluid pressure (CSFP) in rats which were sublethally dosed with bromethalin (VanLier and Cherry 1988). The efficacy of dexamethasone and mannitol treatment has been studied in lethally dosed dogs (Dorman et al. 1990b). There was no increase in survival in dogs given this treatment. Repeated oral administration of superactivated charcoal/sorbitol (SAC/sorbitol) was associated with a delay in the development of clinical signs and increased survival time when monitored for 8 days. Therefore, activated charcoal administration appears to be a partially effective treatment in lethally-dosed dogs (Dorman et al. 1990b).

Our limited success with treatments designed to minimize exposure and reduce cerebral edema stimulated a search for alternative treatment modalities. Previous studies have indicated that desmethylbromethalin is a more potent uncoupler of oxidative phosphorylation, and may contribute significantly to the toxicity of bromethalin. Further, the relative lack of toxicity observed in a species (guinea pigs), which is deficient in its ability to form desmethylbromethalin, strongly suggested a role for the microsomal activation of bromethalin. The use of a therapeutic agent which might limit the N-demethylation of bromethalin to desmethylbromethalin appeared rational based upon available toxicologic data.

Aminophylline (theophylline ethylenediamine) was selected for its potential as a bromethalin antimetabolite, since it is N-demethylated by cytochrome P_{450}, relatively low in toxicity, and readily available to veterinarians. Aminophylline administration to rats given a uniformly lethal dose of bromethalin was not, however, effective in prolonging rat survival. Bromethalin administration did produce the previously described toxic syndrome in all rats regardless of treatment. Although aminophylline administration was not associated with a statistically significant increase in survival time, its administration was associated with almost a doubling of mean survival time. Whether aminophylline administration would be effective in modifying the more commonly observed delayed syndrome characterized by hindlimb paralysis and central nervous system depression associated with the ingestion of smaller bromethalin doses ($<LD_{50}$) is not known. Further studies with aminophylline or other similar drugs using lower bromethalin doses may identify a suitable treatment for this bromethalin-induced toxic syndrome. Based upon our current knowledge of bromethalin metabolism and toxicity, the use of antimetabolite agents like aminophylline may hold promise as a treatment in the future.

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