CASE REPORT

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Fatal Brodifacoum Rodenticide Poisoning: Autopsy and Toxicologic Findings

REFERENCE: Palmer RB, Alakija P, Cde Baca JE, Nolte KB. Fatal brodifacoum rodenticide poisoning: autopsy and toxicologic findings. J Forensic Sci 1999;44(4):851–855.

ABSTRACT: This report details the pathologic and toxicologic findings in the case of a 15-year-old girl who deliberately and fatally ingested brodifacoum, a commonly used rodenticide. The mechanism of death, massive pulmonary hemorrhage, has not been previously reported. Brodifacoum was quantitated in liver, spleen, lung, brain, bile, vitreous humor, heart blood, and femoral blood using HPLC with fluorescence detection. The highest brodifacoum concentrations were detected in bile (4276 ng/mL) and femoral blood (3919 ng/mL). No brodifacoum was detected in brain or vitreous humor. A brodifacoum concentration of 50 ng/g was observed in frozen liver while formalin fixed liver exhibited a concentration of 820 ng/g. A very high blood:liver brodifacoum concentration ratio suggested acute poisoning but the historical and pathologic findings suggested a longer period of anticoagulation. Though most cases of brodifacoum poisoning in humans are non-fatal, this compound can be deadly because of its very long half-life. Forensic pathologists and toxicologists should suspect superwarfarin rodenticides when confronted with cases of unexplained bleeding. Anticoagulant poisoning can mimic fatal leukemia or infectious diseases such as bacterial sepsis, rickettsioses, plague, and leptospirosis. A thorough death scene investigation may provide clues that a person has ingested these substances.

KEYWORDS: forensic science, brodifacoum, rodenticide, tissue distribution, hemorrhage, anticoagulant, pathology, toxicology, poisoning

Brodifacoum is a member of the second-generation anticoagulant rodenticides known as "superwarfarins" (1). This compound and others like it (e.g., bromadiolone, difenacoum, chlorophacinone, etc.) were developed to combat rodent resistance to warfarin (1). Brodifacoum is readily available over-the-counter in hardware stores and supermarkets and is marketed under numerous trade names in North America, Europe, Australia, and New Zealand. Three common US trade names for products containing brodifacoum are Talon®, d-Con Mouse Prufe-II®, and Havoc®.

Brodifacoum has been used in many parts of the world to control pests such as mice, rats, rabbits, opossums, and wallabies (2); however, it is well established that brodifacoum is not a rodent-selective toxin. Fatal brodifacoum poisoning has been reported in other vertebrates including dogs (2–4), poultry (4), cats (4), sheep (2), cattle (2), llamas (3), and humans (3,5). Non-fatal brodifacoum poisoning in humans has also been reported (1,6–8). More than 13,000 cases of human superwarfarin exposures were reported to poison control centers in 1995 (9). The vast majority of superwarfarin and brodifacoum exposures are nonfatal, accidental ingestions by children. Adult ingestions of these substances are often suicidal and can be fatal (5,10–11).

Brodifacoum, like warfarin, is thought to exhibit its anticoagulant effects through inhibition of vitamin K epoxide reductase (12). This NADH-dependent enzyme is necessary for the reduction of vitamin K epoxide to its hydroquinone. The cyclic interconversion of vitamin K hydroquinone with vitamin K epoxide is coupled with the carboxylation of descarboxyprothrombin to form prothrombin. The epoxide is reduced by the warfarin-sensitive enzyme regenerating the hydroquinone allowing the cycle to start again (12). Reduced vitamin K is also necessary for the synthesis of other biologically active proteins. Despite the similarity in mechanism of action between brodifacoum and warfarin, brodifacoum is at least five times more potent as an anticoagulant rodenticide (1,13).

The pharmacokinetic and pharmacodynamic behavior of brodifacoum has been studied in humans (1), rabbits (14–16), and warfarin-sensitive rats (15). The terminal half-life of brodifacoum is very long and independent of species. In rat studies, a half-life of greater than 6.5 days (156 h) was reported (15). Brodifacoum half-lives in rabbits (14) and humans (1) were 20.3 days and 24.2 days, respectively. This half-life is approximately nine times longer than that of warfarin, which averages 15 to 70 h (15,17). Brodifacoum also has a volume of distribution roughly six times that of warfarin (15). In rabbits, the volume of distribution of brodifacoum is 0.985 L/kg compared to 0.17 L/kg for warfarin (18). The systemic clearance in rats is estimated at 4.45 mL/kg/h for brodifacoum and 8.88 mL/kg/h for warfarin (15). It has been suggested that this slow clearance rate may be partially due to enterohepatic recycling (15). The long half-life, large volume of distribution, and low rate of systemic clearance all contribute significantly to brodifacoum's toxicity.

We report a case of fatal ingestion of the brodifacoum-containing rodenticide d-Con Mouse Prufe-II® in a 15-year-old girl. This case is unique in that anticoagulant ingestion was unsuspected be-

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Received 19 Aug. 1998; and in revised form 28 Oct. 1998; accepted 29 Oct. 1998.

fore autopsy. We believe this to be the first reported case of quantitative tissue distribution of brodifacoum in a single-compound human poisoning.

Case History

A 15-year-old girl was found dead at home. She was last seen the previous evening when she was "upset" over a family argument. One day prior to death she returned home from vacationing in an area with a high incidence of tick bites and associated infections. At that time, she complained of a cough due to a "cold." Relatives reported that for the previous one to two months the decedent had unusually heavy menses, bruised easily, and had small sores on her shins that would bleed for extended periods of time. She had attempted suicide six months earlier by ingesting over-the-counter acetaminophen, iron, and diet tablets. This incident did not require hospitalization and was described by her physician as an "impulsive act" in response to family discord. The decedent was prescribed paroxetine (Paxil®) for obesity but had not taken any for at least two months prior to death. She had no history of anticoagulant ingestion, and no family members were prescribed anticoagulant medications.

Autopsy Findings

The decedent was an obese (114 kg) teenage girl. Multiply colored (green, purple, red) ecchymoses, ranging in size from 2 to 10 cm, were distributed on the neck, shoulders, lower abdomen, mons pubis, buttocks, arms, legs, left heel, and both palms. There were petechial hemorrhages densely concentrated on the shins (Fig. 1) and less concentrated on the face, trunk, arms, and thighs. The hands and feet had petechial hemorrhages with sparing of the soles and palms. Multiple crusted and healing abrasions were on the shins. A section of a crusted abrasion revealed a shallow ulcer (consistent with trauma) with dermal hemorrhage and a mild reactive dermal infiltrate of lymphocytes. A 0.3-cm crusted hemorrhagic lesion was located on the lower lip mucosa. Sections of this lesion had submucosal hemorrhage and secondary obstruction of a minor salivary gland.

Thin films of bilateral liquid subdural hemorrhages covered the parietal and temporal cerebral lobes. Petechial hemorrhages were distributed on the epicardial surfaces, gastric mucosa, and small and large bowel mucosa. A 4-cm hematoma replaced most of the right ovary. The right and left lungs weighed 830 g and 650 g, respectively. The lungs were diffusely hemorrhagic and firm (Fig. 2). Microscopic examination of lung sections revealed acute alveolar hemorrhage with a dense background of alveolar hemosiderin-laden macrophages (old hemorrhage), as well as a slight neutrophilic infiltrate in focal alveoli and bronchioles (early bronchopneumonia). The bone marrow was hypercellular with trilineage hematopoieses and a mild increase in megakaryocytes.

Cultures of blood, cerebrospinal fluid, spleen, and both lungs grew no organisms. Lung cultures and smears for *Yersinia pestis* and *Legionella* species were negative. Serology for *R. rickettsii, R. typhi, C. burnetti* (phase 1 and phase 2) was negative.

Routine toxicologic evaluation by gas chromatography for ethanol, and immunoassay for drugs of abuse (barbiturates, opiates, cocaine, stimulants, benzodiazepines, propoxyphene, methadone, Δ-9-tetrahydrocannabinol [THC] and phencyclidine [PCP]) were negative. Additional drugs were excluded using gas chromatography-mass spectrometry (GC-MS). High performance liquid chromatography (HPLC) assays for the therapeutic anticoagulants warfarin and dicoumarol were also negative. Despite the decedent being prescribed paroxetine, none of this compound was detected in the postmortem toxicologic analysis.

Further death scene investigation revealed the decedent had access to d-Con Mouse Prufe-II® brand rodenticide, which contains 0.005% brodifacoum as the active ingredient. The decedent's history, combined with the autopsy picture, heightened suspicion for anticoagulant rodenticide poisoning. Samples were therefore submitted for analysis for brodifacoum.

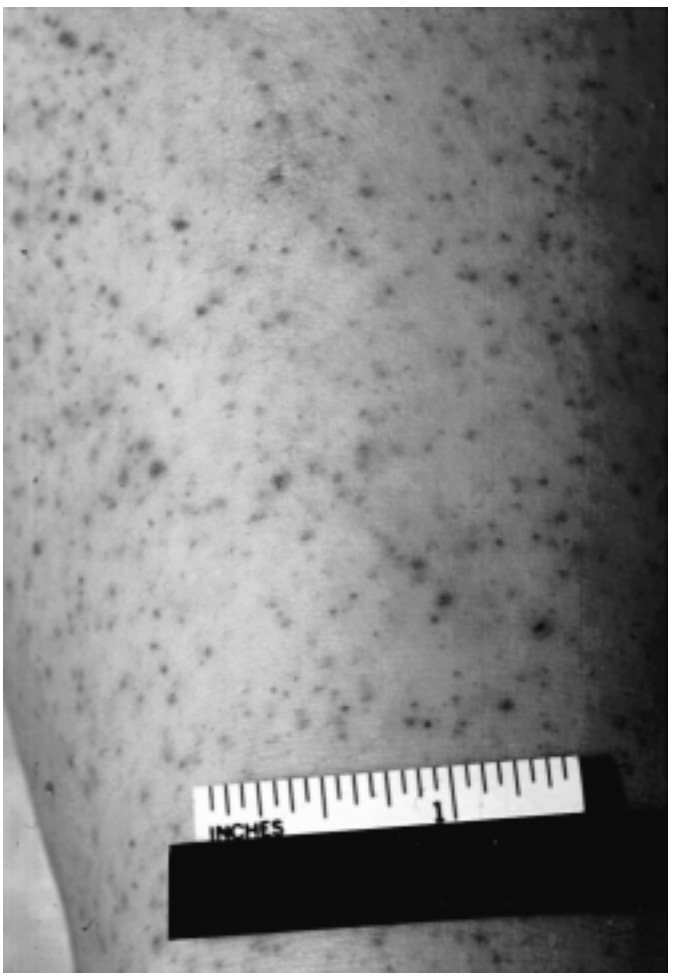

FIG. 1—*Photograph of decedent's shin demonstrating petechial hemorrhages.*

FIG. 2—*Photograph of the decedent's right and left lungs with diffuse alveolar hemorrhage.*

Experimental Procedures

Materials

Brodifacoum was purchased from Chem Service (West Chester, PA). A package of d-Con Mouse Prufe-II® was obtained from the incident scene. Rat liver was obtained from drug-free Sprague-Dawley rats and stored at −20°C until used. All buffer reagents were J.T. Baker Reagent grade and solvents were Fisher Optima grade.

Extraction Methods

Calibration standards and controls were extracted from rat liver for tissue samples and banked human blood or plasma for fluid samples. Samples, calibration standards (10–1000 ng/g) and controls were extracted from 2.0 mL aliquots of body fluid (blood, vitreous humor, bile, or formalin) or from 2.0 g homogenized tissue (liver, spleen, lung, or brain). Concentrations above the linear range were established by dilution. Working standards were prepared from a 1 mg/mL brodifacoum stock solution (in acetone).

Instrumentation

Brodifacoum concentrations were quantitated using HPLC with fluorescence detection using the method of O'Bryan and Constable (19). A Thermoseparations Products (TSP) P200 HPLC pump equipped with a TSP AS3000 autosampler and TSP FL2000 fluorescence detector were used. Excitation and emission wavelengths were 248 and 386 nm, respectively. An Alltech Econosil (10 μm) C_{18} 250 mm by 4.6 mm ID column was maintained at 45°C with a Timberline TL-30 column heater controlled by a Timberline TL-50 temperature controller. The mobile phase (acetonitrile:0.025 *M* ammonium acetate buffer (pH4):*n*-propanol, 62:35:3) was degassed with a TSP membrane degasser and pumped at a flow rate of 1 mL/min. An injection volume of 50 mL was used. The retention times were 12.7 and 16.0 mins for the *cis*- and *trans*- isomers of brodifacoum, respectively.

The limits of detection were 5 ng/mL and 5 ng/g for fluids and tissues, respectively. Limits of quantitation in postmortem cases were established as 10 ng/mL and 10 ng/g. The calibration curves were linear ($r^2 \geq 0.975$) in both cases over the ranges 10–1000 ng/mL and 10–1000 ng/g.

Results

Measured brodifacoum concentrations (fluids in ng/mL and tissues in ng/g) are listed in Table 1.

TABLE 1—*Tissue and fluid concentrations of brodifacoum.*

Tissue	Brodifacoum (ng/g)
Liver	50
Spleen	34
Lung	31
Brain	ND*
Fixed Liver	820
Fluid	**Brodifacoum (ng/mL)**
Heart Blood	2240
Femoral Blood	3919
Vitreous	ND*
Bile	4276
Formalin from Fixed Liver	5440

* None detected.

Severely hemolyzed postmortem blood samples significantly complicated analysis because interfering fluorescent substances co-extracted with the analyte. Reproducible results could only be obtained if the plasma was first centrifuged and filtered and then had the proteins precipitated with trichloroacetic acid.

Discussion

The cause of death in this young woman was brodifacoum poisoning and the manner of death was suicide. This case offered a unique opportunity to document the pathology and determine the tissue distribution of brodifacoum in a human fatality.

Only three other reports of fatal brodifacoum poisoning in humans were found using a MEDLINE search of the medical literature written in English (5,10–11). At least two of the previously reported cases were autopsied (5,10). In contrast to the present case, these patients died of combined intraparenchymal and subarachnoid brain hemorrhage (5,10) and massive "intradural" hemorrhage (11). Although in the present case, small, non-lethal subdural hemorrhages were noted, the mechanism of death was massive pulmonary hemorrhage. We found no reported cases where brodifacoum, or any other superwarfarin, caused death by this means. However, pulmonary hemorrhage was reported in one fatal case though the extent of hemorrhage was not described (5).

Anatomic sites of hemorrhage were tabulated for 36 cases of fatal and non-fatal long-acting anticoagulant ingestion (20). In that report, the most common site of hemorrhage was the urinary tract (hematuria) in 24 cases. Intracranial hemorrhage was noted in only three cases. Additionally, that study listed four individuals with thoracic/pulmonary hemorrhage (hemoptysis or hemothorax) (20). Eighty percent developed bleeding at more than one site (20). The reasons why hemorrhage occurred in different organs in different individuals were not discussed.

In a previous report, pulmonary hemorrhage in a nonfatal, deliberate brodifacoum poisoning was attributed to repeated ingestion (21). The history and autopsy findings in the present case suggest that brodifacoum may have been ingested one or more times within several weeks of death. Heavy menses, easy bruising, and frequently bleeding shin ulcers were likely caused by brodifacoum-induced anticoagulation. The pulmonary siderophages also suggest an anticoagulated state for at least a few days prior to death. The early bronchopneumonia was likely a complication of the underlying pulmonary hemorrhage.

The concentrations of brodifacoum distributed in blood and tissue specimens suggest a high-dose, acute-phase poisoning. The compound is known to specifically bind to hepatic tissues within 24 h of oral ingestion, which is why liver is most often the tissue selected for postmortem analysis (15,22). Analysis of brodifacoum concentration in rat intestine after oral ingestion shows rapid removal of the compound from the gastrointestinal tract (15). However, brodifacoum does not distribute significantly outside of liver, kidney, and blood in animals (3). In poisoned rats, concentrations of brodifacoum in the kidney are similar to those in hepatic tissues, making the kidney also suitable for toxicological analysis (3). However, study animals (rats and dogs) had a rather lengthy average survival time of 5.7 days (range 5 to 11 days) after poisoning perhaps because they were aggressively treated with transfusions and vitamin K (3). Though these animal models provide some guidance, the temporal course for brodifacoum distribution in humans remains conjectural.

Tissue distribution and postmortem redistribution profiles of brodifacoum in humans have not been studied. Liver brodifacoum

concentrations of 100–2000 ng/g (mean 580 ng/g) were found in 18 animal cases of fatal brodifacoum poisoning (4). A rat study reports a liver:serum brodifacoum concentration ratio of 21.2 (15). In another study, more than 90% of a dose of brodifacoum was found in the liver during the later stages of elimination (19). Our study does not demonstrate similar liver:serum partitioning. We observed a brodifacoum concentration of 50 ng/g in unfixed liver while the blood concentration was much higher. These observations are probably due to a relatively short survival interval after ingestion. Only a small difference existed in the brodifacoum concentrations in heart blood (2240 ng/mL) as compared to femoral blood (3919 ng/mL) suggesting minimal release of tissue bound brodifacoum into the vasculature.

Brodifacoum is reported to have strong interactions with biomacromolecules (23). A previous report suggests gross hemolysis does not interfere with HPLC-fluorescence analysis for brodifacoum (19); however, we found it necessary to introduce an additional protein precipitation step in order to obtain reproducible results from grossly hemolyzed blood samples. However, the possible loss of significant amounts of brodifacoum in this step is acknowledged. In the present study, extraction of nonhemolyzed control plasma and serum samples demonstrated that hemolysis was responsible for the analytical difficulties encountered.

The level of brodifacoum in fixed liver (820 ng/g) was approximately 16 times greater than in unfixed frozen liver (50 ng/g). Possible explanations for this observation include: (1) liver tissue was dehydrated by the formalin; (2) metabolism of brodifacoum continued in the frozen sample but the metabolic enzymes were denatured by the formalin fixative; or (3) brodifacoum was released from macromolecules in fixed liver. The very high levels of brodifacoum detected in formalin fixative (5440 ng/mL) support the third explanation. The HPLC assay used will detect only unbound (free) brodifacoum. The most reasonable explanation for such high levels of free brodifacoum in the fixed tissue and formalin fixative is significant brodifacoum release from hepatic tissues. Significant variability in measured drug concentrations between frozen and fixed tissue samples is well documented (24). In the determination of levels of tricyclic antidepressants in frozen and fixed human liver, one set of formalin fixed liver samples had amitriptryline levels nearly 75 times greater than those measured in frozen samples from the same liver (24). Detectable amounts of amitriptyline were also noted in the formalin fixative (24).

Unlike most other cases of brodifacoum poisoning, the initial presentation of this case revealed no suspicion of anticoagulant ingestion. The differential diagnosis for the rapid death of a teenager with cough, multiple bruises, and petechiae includes hematological disorders such as leukemia; infectious diseases such as plague (25), rickettsioses (25), leptospirosis (26), and other causes of bacterial sepsis (25); and ingestion of common prescription anticoagulants such as coumadin and dicoumarol. Discovery of rodenticide packages from a subsequent death scene investigation was therefore critical to the direction of diagnostic toxicologic evaluation.

Conclusions

Anticoagulant poisoning can mimic fatal leukemia or infectious diseases such as bacterial sepsis, rickettsioses, plague, and leptospirosis. Hemorrhages may be widespread and involve multiple organs. The pathologist should suspect anticoagulant poisoning including rodenticides when confronted with fatal cases of unexplained bleeding.

Following fatal poisoning, brodifacoum distributes among several organs within the body. Due to the lack of interfering chromatographic signals and high brodifacoum levels, bile is as reliable a sample as liver and kidney and is far simpler to prepare. Formalin fixed liver may also be assayed though the resultant levels of brodifacoum may be quite high relative to frozen liver samples. These observations demonstrate that in cases of suspected brodifacoum poisoning, toxicologists can take samples from the autopsy stock jars and still obtain confirmatory analytical results.

Acknowledgments

The authors would like to thank Dr. Jerry Born, Mr. Javier Campos, and Mr. Dean Argyres for their assistance with the brodifacoum analyses and Drs. Pamela J. Baxter-Palmer, James L. Luke, Bronwyn E. Wilson, and Ross E. Zumwalt for their critical reviews of this manuscript.

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