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Placental transport of brevetoxin-3 in CD-1 mice

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Abstract

The purpose of this study was to examine the distribution of brevetoxin-3 administered to pregnant dams and to determine the extent of placental transport to fetuses. Twenty-nine pregnant CD-1 mice were administered ^{3}H-brevetoxin-3 (~1.3 μCi/animal; ~2.8 μg compound/kg) by intratracheal instillation on one of gestational days 15–18. Groups of four or five dams were killed at selected times through 48 h post dosing. Four pregnant dams were administered ^{3}H-brevetoxin-3 on gestational day 15 or 16 via osmotic minipump to provide continuous delivery of compound (~0.13 μCi, 7.5 ng compound/day) over a 72-h period after which the dams and fetuses were d. Brevetoxin-associated radioactivity was detected in placentas and fetuses within 0.5 h of intratracheal administration. Concentrations of brevetoxin equivalents in fetuses were approximately 0.3 ng/g throughout the 48-h post dosing, resulting in a calculated dose to fetuses of 19 ng/g-h. Following brevetoxin infusion, concentration of brevetoxin equivalents in fetuses was 0.1 ng/g, lower than that present in most maternal tissues. Results demonstrated placental transport of brevetoxin or its metabolites following maternal acute exposure and repeated low-dose exposure. The consequences of these findings for pregnant women exposed to brevetoxins by inhalation or ingestion remain to be determined.

Keywords

Brevetoxin-3; placental transport; intratracheal instillation; mice

1. Introduction

Brevetoxins are naturally occurring environmental contaminants produced by the several species of dinoflagellates, most notably, *Karenia brevis* (*K. brevis*). *K. brevis* is the organism

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responsible for the red tides occurring primarily in the Gulf of Mexico and sporadically along the Atlantic Coast of Florida. Brevetoxins are lipid soluble neurotoxins. In humans, exposure to brevetoxins produces two pronounced effects, depending on the route of exposures. With ingestion of brevetoxin-contaminated shellfish, neurotoxic shellfish poisoning (NSP) occurs with mild gastroenteritis and neurological symptoms. Inhalation of brevetoxins aerosolized by wind and surf produces irritation of mucous membranes and airways (Baden et al., 1982; Kirpatrick et al., 2004). Brevetoxin-induced bronchoconstriction is well documented in humans following inhalation in occupational and recreational settings (Backer et al., 2003, 2005; Fleming et al., 2005a,b) and in sheep in laboratory experiments (Abraham et al., 2005a,b).

Experiments in laboratory rats and mice have shown that brevetoxins are rapidly absorbed and widely distributed among tissues following a single administration by ingestion (Cattet and Geraci, 1993), intravenous injection (Poli et al., 1990a,b), and intratracheal instillation (Benson et al., 1999; Tibbetts et al., 2006). As expected, relative concentrations of brevetoxin in tissues vary with route of administration and time after dosing. For example, concentrations of brevetoxin equivalents were highest in liver following oral administration in rats (Cattet and Gerachi, 1993), and highest in lung following intratracheal instillation (Benson et al., 1999). Concentrations of brevetoxin equivalents in brain were among the lowest of all tissues examined following both oral and intratracheal administration. Regardless of the exposure route, muscle, GI tract, and liver contained the largest percentages of the administered dose. Muscle appears to serve as a sink for brevetoxins, while liver and GI tract are major organs of metabolism and excretion. Few differences between rats and mice exist in the distribution of intratracheally instilled brevetoxin; however, clearance rates differ somewhat between the species (Benson et al., 1999; Tibbetts et al., 2006).

Brevetoxin-3 undergoes metabolism in rodents (Poli et al., 1990a,b and Benson et al., unpublished observations). However these metabolites remain to be identified. *In vitro* studies indicated brevetoxins 1 and 2 are metabolized by rat hepatocytes and liver microsomal enzymes, suggesting P450 mediated metabolism (Wang et al., 2005). Studies by Poli et al. (2000) evaluating metabolites of brevetoxin in urine of individuals with NSP and in brevetoxin-contaminated shellfish suggest that brevetoxin metabolites may also be toxic. Brevetoxin is eliminated from the body in both feces and urine, with 78% (Cattet and Geraci, 1993) to greater than 90% (Poli et al., 1990a,b) being eliminated within 7 days of exposure.

In addition to being potent neurotoxicants, recent data also suggest that brevetoxin exposure may also adversely affect the immune system. Kirkpatrick et al. (2006) have reported increased frequency of emergency room visits for respiratory related illnesses, including pneumonia, during Florida red tide events. Brevetoxin-induced inhibition of antibody production has been found in rats (Benson et al., 2004, 2005). In addition, Sayer et al. (2005) reported DNA damage in human lymphocytes exposed to nanomolar concentrations of brevetoxins *in vitro*. The latter findings of genotoxicity have implications beyond adverse effects to the immune system. The wide tissue distribution of brevetoxin following exposure suggests the feasibility of placental transport, with possible binding to targets for toxicity in the embryo or fetus.

Placental transfer of environmental contaminants in humans and laboratory animals is well documented for several classes of lipophilic environmental pollutants, including halogenated organic compounds (Guvenius et al., 2003; Mazdai et al., 2003; Meerts et al., 2002; Sinjari and Darnerud, 1998; Roman et al., 1998) and polycyclic aromatic hydrocarbons (Perera et al., 1999, and references cited therein). Further evidence indicates that fetuses are more susceptible than adults to a variety of environmental toxicants (Perera et al., 1999). The developing nervous system is a particularly sensitive target for environmental toxicants (Rodier, 1995; Needleman, 1995). In addition, organogenesis and development of the immune system occurs during the

prenatal, and to a lesser extent, the early postnatal periods of mammalian development. Studies suggest that the fetus may be more prone to genetic damage caused by certain agents because of differences in concentrations of microsomal metabolizing enzymes, DNA repair enzymes, or in extent of body fat and muscle that can serve as a sink for lipid soluble toxicants.

To date, no studies have addressed the placental transfer or developmental toxicity of brevetoxins in mammals. The purpose of this study was to examine the tissue distribution of brevetoxin-3 administered to pregnant dams and to determine the existence and extent of placental transport of the parent compound or its metabolites to fetuses following acute and repeated exposure. Brevetoxin-3 was chosen for these studies because it is a major component of brevetoxin-containing aerosols along red tide affected beaches (Cheng et al., 2005). Further, it is a potent neurotoxin *in vitro* (Baden, 1989) and suppresses humoral immunity in rats following repeated inhalation (Benson et al., 2004, 2005).

2. Materials and methods

2.1. Chemicals

Brevetoxin-3 was isolated and purified from the Wilson clone of *K. brevis* at the Center for Marine Science at the University of North Carolina at Wilmington, NC. ^{3}H-Brevetoxin-3 (C-42; 15.5 Ci/mM) was also prepared by the Center for Marine Science, using the method described by Poli and coworkers (1986). The tritium label is covalently attached at C-42 and is nonexchangeable. The radiochemical purity of the ^{3}H-brevetoxin-3, as determined by high-performance liquid chromatography (HPLC), exceeded 95%. The dosing solution had a specific activity of 7.94 Ci/mM (10 μCi/mL; 1.13 μg brevetoxin-3/mL dosing solution; 8.85 μCi/μg).

Acetone (HPLC grade), methanol (biotech grade), and hexane (HPLC grade) were purchased from Fischer Scientific (Hampton, NJ).

2.2. Animals

Male and female CD-1 mice were purchased from Charles River Laboratories (Wilmington, MA). The mice were 10–11 weeks old when received. Animals were housed in shoebox cages with hardwood chip bedding and filter tops. The animal rooms were maintained at 20–22 °C with a relative humidity of 20–50% and a 12-h light cycle beginning at 0600. Food (Harlan Teklad, Madison, WI) and water were provided *ad libitum*. All housing was in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The study protocol was reviewed and approved by the Lovelace Respiratory Research Institute's Animal Care and Use Committee. For breeding, one male and one female were paired on 3–4 consecutive nights and females were checked for sperm plugs the following mornings. The morning when a plug was present was counted as gestational day 1. Pregnant females were housed separately.

3. Experimental design

3.1. Intratracheal instillation study

Twenty-nine pregnant mice were administered ^{3}H-brevetoxin-3 (1.3 μCi, 0.14 μg/animal, ~2.8 μg brevetoxin-3/kg body weight) by intratracheal instillation on one of gestational days 15–18. The mice were anesthetized using 5% isoflurane in a 50:50 mixture of oxygen and nitrous oxide. A catheter was placed transorally into the trachea for administration of the dose in 0.1 mL saline containing 0.1% Tween 80. Aliquots (0.1 mL) of dosing solution were collected for estimation of the initial doses to the instilled mice (initial body burden).

Dosed mice were killed by intraperitoneal injection of Euthasol® (468 mg/kg sodium pentobarbital; 60 mg/kg phenytoin) at 0.5, 1, 4, 8, 16, 24, and 48 h post-instillation. At sacrifice, maternal blood, liver, kidneys, spleen, lung, brain, ovaries, GI tract with contents, pelt, uterus, placentas (pooled), and fetuses (pooled) were removed and weighed. Fetuses were euthanized by decapitation. All tissue samples were immediately processed for radiochemical analysis. In one instance, milk was obtained from the stomachs from pups born prior to a designated 48-h sacrifice. The dam and pups were killed and tissues processed as for the other mice.

3.2. Repeated exposure by osmotic pump

Four pregnant mice were surgically implanted with Alzet® micro-osmotic pumps (DURECT Corp., Cupertino, CA) while under isoflurane anesthesia on gestational days 15–16. Target daily doses were 1 μCi ^{3}H-brevetoxin-3/day. The daily dose was intended to be equivalent to that provided once as a bolus dose by intratracheal instillation. Continuous compound release by the pump was intended to simulate slow continuous absorption as may occur following inhalation over a prolonged period, and to assess whether tissue accumulation of compound may occur with continuous exposure over several days. Actual daily doses, determined by *in vitro* experimentation, were 0.13 μCi ^{3}H-brevetoxin (containing approximately 14 ng brevetoxin-3). The animals exposed to ^{3}H-brevetoxin-3 were killed and processed as described for tracheally instilled animals.

3.3. Radiochemical analysis

The tissue samples were digested *in toto* using a 35% solution of tetraethylammonium hydroxide (~1 mL/g tissue; Sachem, Inc., Austin, TX), and the weight of the each digestate was recorded. Weighed aliquots of digestate (~1 g) were neutralized with concentrated hydrochloric acid (VWR International, West Chester, PA), decolorized with 30% hydrogen peroxide (ACS grade; Fisher Scientific, Hampton, NH), and mixed with Ultima Gold XR® scintillation cocktail (Packard Instrument Co., Meriden, CT) for quantitation of tritium activity by liquid scintillation counting. Aliquot counts were then used to calculate the total activity for the corresponding digestate.

3.4. Measurement of parent brevetoxin-3 in fetal tissues

In order to determine if parent brevetoxin-3 was present in fetuses following maternal exposure for 72 h, eight pregnant dams were surgically implanted with osmotic pumps as described above. Four dams received daily brevetxoin-3 doses of approximately 0.3 μg/kg/day. The remaining four dams received vehicle alone (phosphate buffered saline containing 0.1% Tween 80). Fetuses from dams exposed to vehicle or to brevetoxin-3 were pooled within their groups, homogenized, and subjected to solvent extractions using previously described methods (Naar et al., 2002). Briefly, pooled fetuses were homogenized in equal volumes of phosphate buffered saline. Nine, 1-g aliquots of each homogenate were extracted twice with 2 mL acetone. The acetone extracts were dried under nitrogen, redissolved in 80% methanol, and defatted by extraction with n-hexane. The defatted extracts were dried under nitrogen and redissolved in 25% methanol for purification on a C-18 separation column (Alltech, Deerfield, IL). The purified fractions were analyzed by liquid chromatography/mass spectrometry (LC/MS) for brevetoxin-3. Aliquots of control fetal homogenates were spiked with known quantities of brevetoxin-3 and subjected to the same extraction procedures. These samples were used to determine the efficiency of the extraction procedure and as positive controls for the LC/MS analyses.

Liquid chromatographic (LC) separation was conducted with a Shimadzu LC-10 ADVP fitted with a C_{18} “Aqua” column (3 micron, 125 A, 75 × 200 mm; Phenomenex #003-4311-B0, Torrance, CA) and C_{18} guard column. The column temperature was set at 32 °C (Eppendorf TC-50, Fisher Scientific). The flow rate was 150–200 μL/min. The injection volume was 50

μL with a 100 μL overfill (auto-injector). The mobile phases for chromatographic separation were purified water (solvent A) and methanol:1 mM ammonium acetate (solvent B) that were operated on the following time cycle: 20% B for 0–3 min, 90% B for 3–10 min, 20% B for 10–13 min.

Triple quadrupole mass spectrometry (MS^3) was performed using an Applied Biosystems (Foster City, CA) 365 instrument. The data station was Analyst Version 1.3.1 (Agilent Technologies, Palo Alto, CA). Analytes were ionized using a heated nebulizer ionization source. The instrument was run in multiple response monitor mode and used the parent/daughter ion pairs of 897.5/725.4 to identify and quantify brevetoxin-3 components.

Quality control measures for each set of samples included analysis of unexposed tissue extracts as “blanks” to assess the potential for background contamination. Fetal homogenates were spiked with 250 ng/g and 500 ng/g tissue prior to extraction and analysis. The extraction efficiencies (mean ± SD; n =3) for fetal tissue spiked with 250 ng/g tissue and 500 ng/g were 63.6 ± 3.3 and 52.6 ± 1.4 percent, respectively.

3.5 Statistical analyses

Means and standard deviations of brevetoxin-3 equivalents in tissues were calculated using Microsoft Excel® (Redmond, WA) software. The kinetics of brevetoxin-equivalents elimination for each tissue were modeled with standard pharmacokinetic models using TableCurve 2D® software (SPSS Science, Chicago, IL). Estimates of total dose to tissue, calculated by integrating the area under the elimination curve from 0.5 h–48 h for each tissue, were also obtained using TableCurve 2®. Doses are expressed as nanogram brevetoxin-3 equivalents-h/g of tissue.

4. Results

4.1. Distribution of brevetoxin equivalents

Intratracheally instilled brevetoxin-3 distributed rapidly throughout the pregnant dam, with the greatest percentages of brevetoxin-associated ^{3}H-activity distributing in carcass (muscle), the tissue sinks for brevetoxin, as well as to the GI tract and liver, the primary organs of metabolism and excretion (Figure 1a). Following these tissues, the fetuses contained the next highest percentage of the administered dose with almost 3% present 0.5 h after dosing (Figure 1b). At 48 h post dosing, fetuses contained the highest percentage of brevetoxin-associated activity (4.76%) of all tissues examined, while approximately 1% of the administered activity was associated with placentas and uterus.

Initial concentrations of brevetoxin equivalents were highest in GI tract, kidney, liver, and lungs (Table 1). Transient increases in concentrations in kidney and GI tract between 1 and 8 h is likely related to clearance of parent compound or metabolites via urine and feces. Interestingly, concentrations in ovaries were greater than in blood, brain, spleen, uterus, placentas, and fetus through 24 h post dosing. While group mean concentrations of brevetoxin-associated activity in uterus, placentas, and fetuses were generally less than 0.5 ng/g tissue throughout the 48-h period post dosing, concentrations in these tissues remained fairly constant over time (Table 1, Figure 2). For example, concentrations of brevetoxin equivalents in carcass, GI tract, liver, lungs, ovaries, and spleen at 48 h were 25% or less than those present 0.5 h post dosing. Forty-eight-hour concentrations in blood, brain, and uterus were 50% or more of the 0.5-h concentration. By contrast, the 48-h concentration in fetuses was 150% of that at 0.5 h and similar to that present 1 to 24 h post dosing.

Because of the short time frame of the evaluations, only limited toxicokinetic evaluations could be performed. Tissue clearance data were fit using single-component negative-exponential

functions. Clearance halftimes for brevetoxin equivalents were greatest for fetuses, uterus, and placentas. The area under the curve evaluations from 0.5–48 h, however, show dose to fetuses of approximately 14 ng/g-h, the second lowest dose next to brain, of any tissue examined.

The concentration of brevetoxin-associated activity in milk obtained from the stomachs of pups born before the designated 48-h sacrifice was 0.26 ng/g milk, approximately half the concentration present in maternal blood at the time of sacrifice (0.43 ng/g).

Concentrations of brevetoxin equivalents in maternal tissues and fetuses from dams administered brevetoxin for 72 h beginning at gestational days 15 or 16 are summarized in Table 2. Blood, GI tract, kidneys, and liver contained the highest concentrations of brevetoxin equivalents. Concentrations in carcass, pelt, lungs, ovaries, spleen, uterus, and placentas were very similar, ranging from 0.14–0.18 ng/g tissue. Fetus and brain had the lowest concentrations, 0.10 and 0.7 ng/g, respectively.

4.2. Quantitation of parent brevetoxin-3 in fetuses

Parent brevetoxin-3 was not detected by mass spectrometry in extracts of pups from dams administered brevetoxin-3 by osmotic minipumps. The limit of quantitation for brevetoxin-3 was 1 ng/g tissue.

5. Discussion

The purpose of this study was to examine the uptake and tissue distribution of brevetoxin-3 administered to pregnant dams and to determine the existence and extent of placental transport to fetuses following a single intratracheal instillation and after 72 h of infusion by osmotic pump.

As has been reported for male mice and rats administered ^{3}H-brevetoxin-3 by intratracheal instillation, the organs in pregnant dams with the highest percentages of the administered dose included the GI tract (with contents), carcass (primarily muscle and fat) and liver (Benson et al., 1999; Tibbetts et al., 2006). Further, as was found for adult male rats and mice, percentages of the initial administered dose distributing to potential target organs for toxicity, including brain and spleen, were less than 1%. Distribution of brevetoxin equivalents to ovaries of pregnant dams and testes of male mice (Tibbetts, et al., 2006) suggests exposure to germ cell populations to the potential genotoxic effects of brevetoxin (Sayer et al., 2005).

This study is the first to identify placental transport of brevetoxin. Clearance of brevetoxin equivalents from the fetuses was relatively slow following intratracheal instillation compared to other tissues, suggesting continuous redistribution of material to the fetus from other tissues. Slow absorption of brevetoxin by pregnant dams from the osmotic pumps, simulating absorption expected to occur following prolonged inhalation exposure, also resulted in distribution of brevetoxin equivalents to fetuses. Concentrations of brevetoxin equivalents in fetuses were slightly lower than concentrations in maternal tissues following 72 h of continuous exposure, but this is likely a consequence of total dose administered over the three days was very much less by minipump than by intratracheal instillation.

The presence of brevetoxin equivalents in fetuses and milk coupled with *in vivo* and *in vitro* evidence indicates that brevetoxins may be immunotoxic, suggesting the potential for adverse consequences resulting from pre- and perinatal exposure to brevetoxins. The perinatal period is a time of high sensitivity to toxicants, including immunotoxicants and neurotoxicants that cross the placenta or enter the neonate via lactation.

Parent brevetoxin-3 was not detected in fetal tissue by mass spectrometry. This is likely because the concentration of brevetoxin-3 was below the limit of detection of the method, but does not preclude the presence of brevetoxin metabolites in fetus. Although brevetoxin-3 is known to undergo mammalian metabolism (Poli et al., 1990a,b, 2000), the structural identity of mammalian metabolites of brevetoxin-3 and their toxicities have not been determined.

Placental transport of brevetoxin from mother to fetus is possible through at least two routes. The first is by diffusion across the placental membrane. Second, transporters, including organic anion or peptide transporters in placenta, may also participate in movement of toxicants across the placenta into fetal circulation (Unadkat et al., 2004; You, 2004) and rat placenta (Leazer and Klaassen, 2003). The mechanism of brevetoxin transport remains to be identified, but is likely to be a result of diffusion due to the high lipophilicity of the compound.

Among amphibians, *K. brevis* exposure has proven toxic to finfish larvae but not eggs (Riley et al., 1989) or sea urchin embryos (Moon and Morrill, 1976). Recently, Kimm-Brinson and Ramsdell (2001) reported neurotoxicity and developmental toxicity among Medaka fish embryos exposed to parts per million concentrations of brevetoxin-1 by microinjection. But as with any toxicant, effect is a function of dose. Measured brevetoxin concentrations in marine aerosols have generally been less than 25 ng/m^3 and particle size distributions range from 6–12 μm mass median aerodynamic diameter, favoring deposition in the human upper respiratory tract (Cheng et al., 2005). Based on these data, a dose rate of approximately 1.2 ng/h per 1 ng brevetoxin/m^3 air concentration has been derived (Cheng et al., 2005). Therefore, a pregnant woman might absorb up to 5 ng brevetoxin during a 4-h visit to a red tide-affected beach. Assuming a 60-kg woman, the dose would be approximately 84 pg/kg body weight. With complete systemic absorption and fairly equal distribution throughout the body tissues (based on results following infusion of brevetoxin via minipump), the estimated maternal and fetal tissue concentration would be quite low, 84 fg of brevetoxin or brevetoxin metabolites/g tissue.

The implications of fetal exposure to brevetoxins remains to be determined. As yet, it is not known if parent compounds or brevetoxin metabolites reach the fetus, whether metabolism occurs within the fetus, or what the relative toxicity of these metabolites is compared to parent compounds. Studies addressing the potential developmental toxicity of inhaled brevetoxins are planned.

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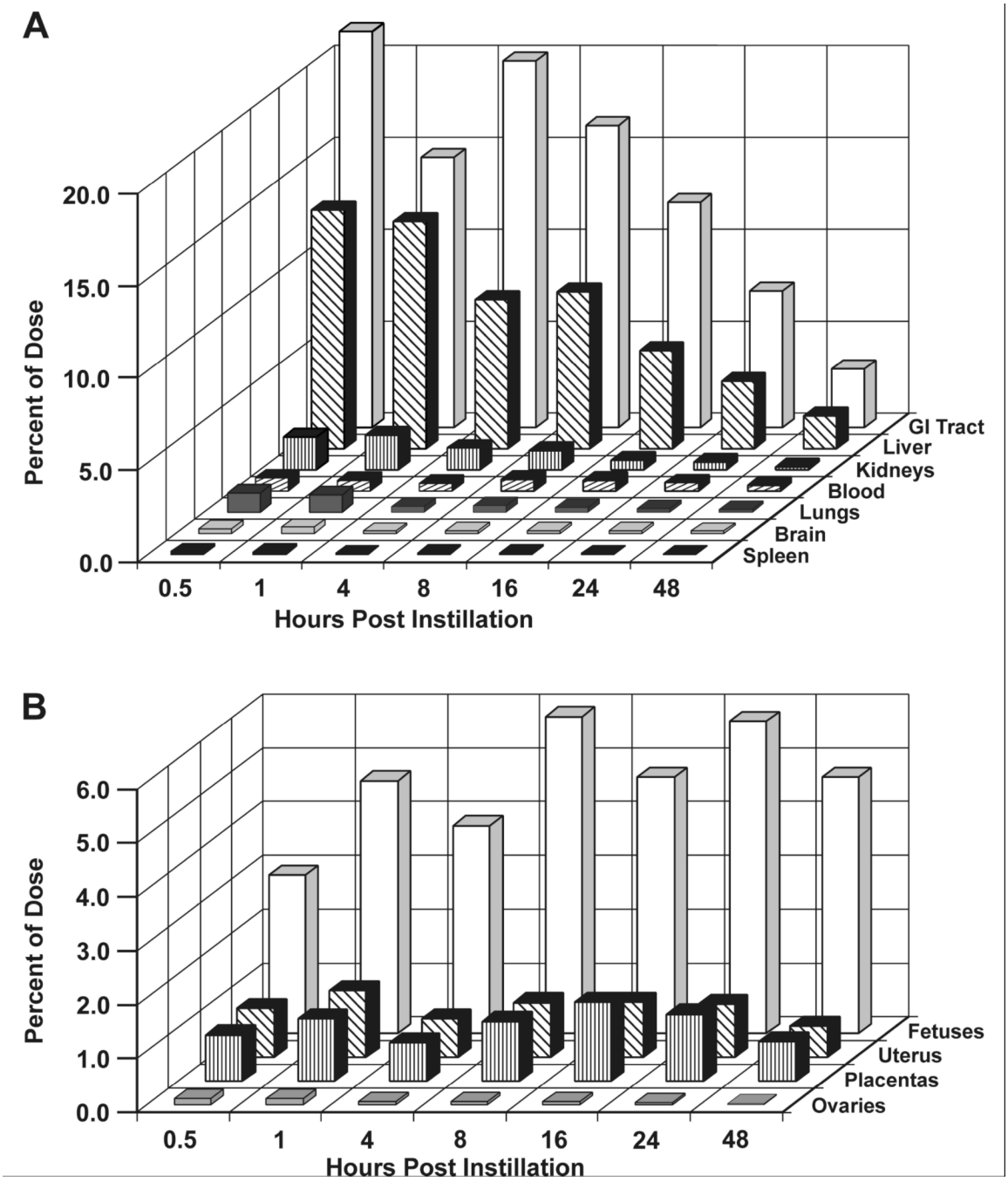

Figure 1.

a. Percentages of the administered brevetoxin dose present in selected maternal tissues up to 48 hours post dosing. Results are the means of 4 values except for all tissues at the 24 hour time point, where n = 5.

b. Percentages of administered brevetoxin dose in maternal blood, reproductive tissues and fetuses as a function of time after dosing. Results are the means of 4 values except for all tissues at the 24 hour time point, where n = 5 and placentas at 48 hr where n = 2.

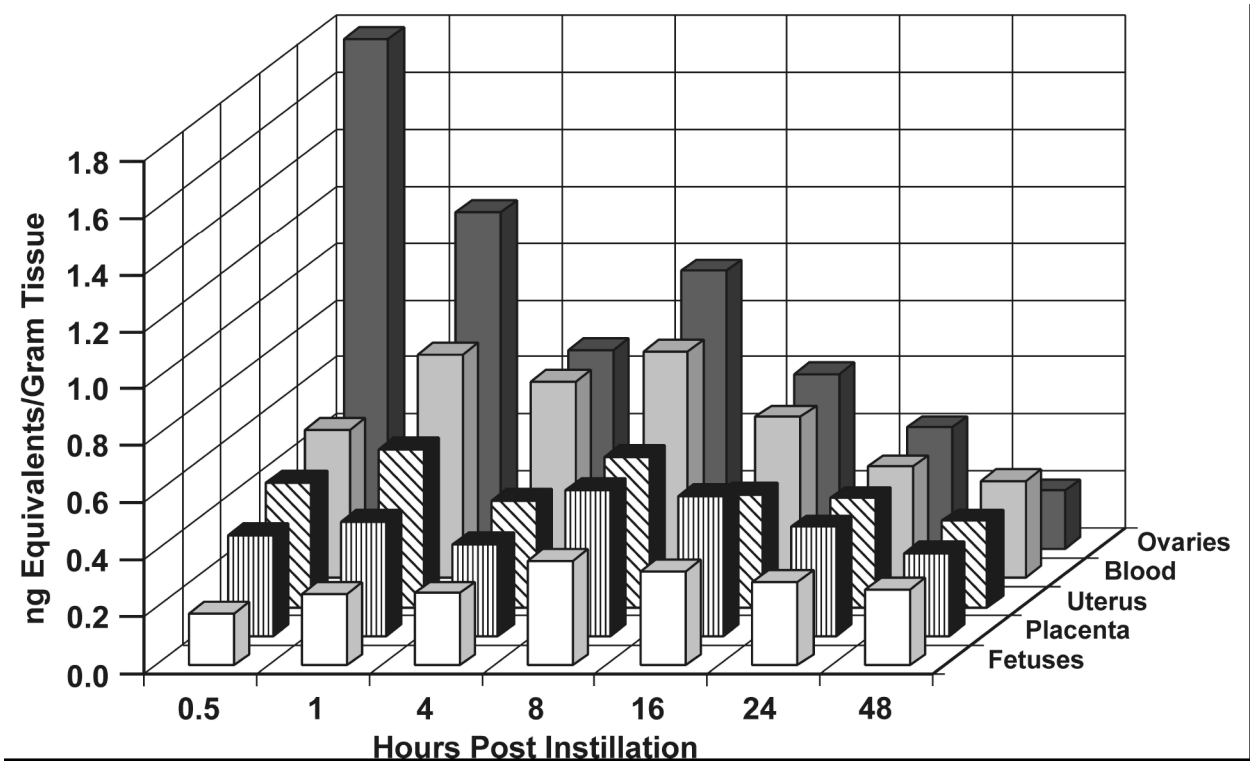

Figure 2.
Concentrations of brevetoxin equivalents present in maternal blood, reproductive tissues and fetuses as a function of time after dosing. Results are the means of 4 values except for all tissues at the 24 hour time point, where n = 5 and placentas at 48 hr where n = 2.

Table 1

Concentrations of brevetoxin equivalents in dams administered ^{3}H-brevetoxin-3 by intratracheal instillation and toxicokinetic parameters[a]

	Ng/g tissue							Limited toxicokinetic parameters		
	Hours post dosing									
	0.5	1	4	8	16	24[b]	48	$t_{1/2}$(h)	Fixed Fraction	Dose to Tissue (ng/g-h)
Blood	0.52 ± 0.25	0.78 ± 0.38	0.69 ± 0.53	0.79 ± 0.44	0.56 ± 0.30	0.39 ± 0.29	0.34 ± 0.12	39.2		23.3
Brain	0.43 ± 0.27	0.53 ± 0.12	0.27 ± 0.10	0.32 ± 0.09	0.29 ± 0.02	0.27 ± 0.08	0.23 ± 0.09	1.7	0.49	13.4
Carcass	1.36 ± 0.69	2.11 ± 0.36	0.83 ± 0.31	1.15 ± 0.78	0.47 ± 0.05	0.37 ± 0.05	0.22 ± 0.09	6.8	0.13	24.8
GI tract and contents	2.75 ± 2.51	1.80 ± 0.26	2.65 ± 0.11	2.39 ± 0.18	1.42 ± 0.46	0.96 ± 0.30	0.43 ± 0.23	14.1		57.4
Kidneys	2.89 ± 1.84	4.14 ± 1.56	2.10 ± 0.62	1.87 ± 0.40	0.91 ± 0.19	0.68 ± 0.25	0.30 ± 0.13	6.4	0.10	45.3
Liver	4.25 ± 2.76	3.45 ± 0.48	2.47 ± 0.95	2.82 ± 0.26	1.40 ± 0.43	1.01 ± 0.22	0.54 ± 0.25	9.0	0.12	64.5
Lungs	2.98 ± 1.95	1.95 ± 0.77	1.09 ± 0.60	1.37 ± 0.30	0.77 ± 0.35	0.63 ± 0.29	0.46 ± 0.20	9.6		30.7
Ovaries	1.79 ± 1.80	1.18 ± 0.22	0.69 ± 0.28	0.97 ± 0.34	0.61 ± 0.18	0.42 ± 0.29	0.20 ± 0.14	20.2		24.2
Pelt	0.77 ± 0.32	0.96 ± 0.1	0.52 ± 0.20	0.65 ± 0.11	0.33 ± 0.13	0.35 ± 0.06	0.28 ± 0.14	6.2	0.33	18.6
Spleen	0.97 ± 0.84	0.80 ± 0.2	0.48 ± 0.16	0.55 ± 0.16	0.40 ± 0.07	0.32 ± 0.13	0.24 ± 0.09	16.9		16.6
Uterus	0.44 ± 0.32	0.55 ± 0.08	0.37 ± 0.15	0.52 ± 0.06	0.40 ± 0.10	0.38 ± 0.07	0.30 ± 0.12	71.4		18.2
Placentas	0.35 ± 0.25	0.40 ± 0.13	0.32 ± 0.12	0.51 ± 0.17	0.49 ± 0.07	0.38 ± 0.11	0.17, 0.41[c]	43.6		19.4
Fetuses	0.18 ± 0.13	0.25 ± 0.06	0.25 ± 0.14	0.37 ± 0.05	0.33 ± 0.03	0.29 ± 0.04	0.27 ± 0.09	83.6		14.1

[a] Mean ± SD, n = 4 unless otherwise noted.

[b] n = 5.

[c] n = 2.

Table 2

Concentrations of brevetoxin equivalents in maternal and fetal tissue following 72 h of continuous administration by osmotic pump[a]

Tissue	ng/g tissue
Blood	0.28 ± 0.23
Brain	0.07 ± 0.02
Carcass	0.13 ± 0.007
GI tract and contents	0.39 ± 0.10
Kidneys	0.24 ± 0.06
Liver	0.36 ± 0.07
Lungs	0.15 ± 0.02
Ovaries	0.17 ± 0.02
Pelt	0.16 ± 0.03
Spleen	0.14 ± 0.02
Uterus	0.15 ± 0.04
Placentas	0.18 ± 0.01
Fetuses	0.10 ± 0.02

[a]Mean ± SD, n = 4.