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The Toxicity of Soman in the African Green Monkey (*Chlorocebus aethiops*)

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In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals* by the Institute of Laboratory Animal Resources, National Research Council, in accordance with the stipulations mandated for an AAALAC International accredited facility.

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ABSTRACT This study determines soman toxicity in African green monkeys (*Chlorocebus aethiops*) and is the first step in exploring the suitability of this species as a model for nerve agent studies. Male African green monkeys were surgically implanted with telemetry devices to monitor electroencephalographic (EEG) and electrocardiographic (ECG) activity. Blood was taken at various times to measure whole blood acetylcholinesterase (AChE) activity and cardiac troponin I (cTnI). Blood AChE activity relative to baseline was 0.0% to 2.5% 6 h after soman exposure and recovered to 31.9% to 72.0% by 30 days after exposure. The 6 h postexposure cTnI levels varied from 0.64 to 6.55 ng/mL, suggesting cardiac damage. Soman was prepared in saline to a concentration of 100 μg/mL. Using an up-down design for small samples, subjects were exposed to 5.01, 6.31, or 7.94 μg/kg soman IM. The first subject was given 5.01 μg/kg soman IM and survived. Three subjects received 6.31 μg/kg soman IM and survived. Three subjects received 7.94 μg/kg soman IM and died within 25 min, 26 min, or 6 h. In all subjects, toxic signs of muscle fasciculation, tremors, chewing, and profuse salivation developed within 2 to 7 min. Tonic-clonic motor convulsions and EEG seizure began between 2 and 18 min after tremor onset. The 48 h IM LD50 of soman in saline in the African green monkey was calculated to be 7.15 μg/kg. The signs and speed of soman intoxication in African green monkeys were consistent with those described in rhesus, cynomolguscynomolgus, and baboons.

KEYWORDS Acetylcholinesterase; African Green Monkey; Cardiac Troponin I; ECG; EEG; Intramuscular; LD50; Nonhuman Primate; Rhesus Macaque; Soman; Telemetry

INTRODUCTION

Phylogenetically, nonhuman primates (NHPs) are the closest animals to man. In biomedical research, they are considered to be the animal that physiologically most closely approximates how a drug or toxin would act in man (Miller 1967; Dixon 1976; Krasovskii 1976). The rhesus monkey (*Macaca mulatta*) has traditionally served as the NHP research species of choice to assess nerve agent toxicity and the effectiveness of various medical countermeasures. The "choice" of the rhesus monkey seems to be a case of historical default. In the late 1940 s, animal research involving toxicology of nerve agents and the development of medical countermeasures was first initiated in the United States and various allied countries. At that time, rhesus monkeys were readily available

and widely used for biomedical research. Since then, research in the nerve agent arena appears to have simply followed this trend. Some investigators examined comparative intraspecies (e. g., monkey vs. dog) differences in toxic mechanisms and/or response to therapies (DeCandole et al. 1953; DeCandole and McPhail 1957; Johnson et al. 1958), while others used rhesus to characterize the effects of medical countermeasures against specific aspects of nerve agent toxicity (Lipp 1968, 1972, 1973; Lipp and Dola 1978; Dirnhuber et al. 1979).

India, the major foreign source of rhesus monkeys, stopped exporting these animals for biomedical research purposes in the late 1970s. Subsequently, increasing demand for these animals, coupled with limited domestic breeding sources, greatly decreased availability while substantially increasing cost. In addition, rhesus monkeys pose a serious health hazard to research and husbandry personnel due to the potential for transmission of *Cercopithecine herpesvirus 1* (monkey B virus), which has exceptional virulence in humans (Artenstein et al. 1991; Davenport et al. 1994; Holmes et al. 1990). Protecting personnel from this virus requires additional personal protective equipment and special medical monitoring, all of which increase the overall husbandry and research costs of using these animals. For these reasons, there is a need to find an alternative monkey species to evaluate nerve agent toxicity and medical countermeasures against chemical warfare nerve agents. Two other NHP species, the cynomolgus monkey (*Macaca fascicularis*) (Carpentier et al. 2001; Krummer et al. 2002; Lenz et al. 2005; Lallement et al. 1997, 1998, 1999, 2000, 2002; von Bredow et al. 1991) and the common marmoset (*Callithrix jacchus*) (D'Mello and Scott 1986; Muggleton et al. 2003; Wetherell and French 1991; van der Schans et al. 2003; van Helden et al. 1992, 2003, 2004a, 2004b; Busker et al. 1996; Philippens et al. 2000), have been used in this regard. Both of these species have drawbacks. The cynomolgus monkey also carries *Cercopithecine herpesvirus 1*, thus posing a health risk equivalent to the rhesus monkey. The marmoset is substantially smaller (body weight 250–450 g), which hinders the ability to take repeated blood samples of significant quantity.

The African green monkey (*Chlorocebus aethiops*) may be an ideal replacement for the rhesus monkey. African green monkeys are old-world monkeys that grow to about 60% the size of a rhesus and are from the same subfamily as baboons and macaques. African green monkeys are similar to rhesus in anatomy, physiology, hematology, blood chemistry, and social organization (Fairbanks 2002). They are considerably less aggressive than rhesus, and well-trained personnel can perform repeated blood sampling from superficial veins. Wild-caught, Caribbean origin, African green monkeys are available from a variety of sources for around 30% of the cost of a rhesus monkey and, most important, they do not carry *Cercopithecine herpesvirus 1*.

African green monkeys are used in biomedical research for behavior, AIDS, diabetes, genetics, infectious disease, neurobiology, and cell biology studies (Fairbanks 2002). However, there are no previous toxicological or pharmacological research studies with nerve agents or any of the standard medical countermeasures using African green monkeys. Before an informed decision can be made about the suitability of the African green monkey for future research, it is necessary to determine the comparability of data obtained with the African green monkey to historical data already available for other NHPs including the rhesus monkey. This study was designed to determine the toxicity of the nerve agent soman in African green monkeys for comparison to other primate species. The IM LD50 dose of this agent was to be established along with a description of the time course and severity of physiological signs of intoxication. An emphasized goal of this work was to use as few animals as possible for this determination.

MATERIALS AND METHODS

Animals

All animals were housed and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1996). Seven adult male, wild-caught African green monkeys (*Chlorocebus aethiops*) from the island of St. Kitts, weighing 4.5 to 7.0 kg, served as subjects. Animals were housed individually in 4.3-square-foot stainless steel squeeze-back cages with built-in perches. They were fed commercial certified primate ration by Harlan/Teklad (15%) (W), fresh fruit, and tap water ad libitum. Animal rooms were maintained at 21 ± 2°C, relative humidity of 50% ± 10%, and a 12-h light (0600–1800):12-h dark (1800–0600) cycle with no twilight. The U.S. Army Medical Research Institute of Chemical Defense is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

The Institutional Animal Care and Use Committee (IACUC) approved the research presented here.

Soman

Soman was obtained from the Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD. It was assayed to be >98% pure by nuclear magnetic resonance analysis. It was diluted in saline to a concentration of 1.95 mg/mL and maintained as frozen stock at -80°C; purity was further verified by gas chromatography to be 98.7%. From this frozen stock a single 1-mL vial was slow thawed and dilutions made to prepare multiple vials containing 1 mL of 100 μg/mL soman in physiological saline. These 1-mL vials were then frozen at -80°C and single vials were removed for the dosing of each animal.

Surgery

Six of the seven animals were implanted with cortical electroencephalographic (EEG) leads and a telemetry device (animal V5342 was not implanted). Animals were fasted overnight prior to surgery. Each animal was anesthetized initially with ketamine IM (10 mg/kg), then intubated. Further anesthesia was maintained using isoflurane (0.8%–2% with nitrous oxide and oxygen in a 2:1 ratio). The animal was then placed in a Kopf (Turlingua, CA) stereotaxic frame and prepared for sterile surgery. Approximately 15 min before the first incision was made, the antibiotic cephazolin was administered IV (25 mg/kg). Approximately 10 min before the first incision, a mixture of epinephrine (1:1000, 0.3 mL), lidocaine (2%, 0.5 mL), and sterile physiologic saline (0.9%, 2.2 mL) was infused around incision sites to provide local anesthesia and promote hemostasis. A midline incision was made in the scalp. Burr holes were drilled (bilaterally for four-channel telemetry device) in the skull over the frontal and central sites, right temporal and right occipital cortices, and a frontal midline for ground, and then a stainless steel screw was inserted into each hole. An incision was then made 5 to 8 cm below the left scapular region of the back and a subcutaneous pouch created to accept the biopotential transmitter device (Models TL10M4-D70-EEEE, four channel or TL10 M3-D70-EEE, three channel; Data Sciences International [DSI], St. Paul, MN). The leads from the transmitter were tunneled subcutaneously from the subscapular region to the skull. The frontal and central screws on the left side served as one EEG channel, the temporal and occipital screws on the right side served as a second EEG channel, and the frontal and central screws on the right side served as a third EEG channel when a four-channel transmitter was used (three animals). The frontal and central screws on the left side served as one EEG channel and the temporal and occipital screws on the right side served as a second EEG channel when the three-channel transmitter was used (three animals). The leads were trimmed to length, a small (~0.5 cm) part of the silastic covering of the lead was removed, and the bare lead wire was wrapped around the shaft of the screw; the screw was then turned into the skull, anchoring the wire in place. The screw head and wire leads were covered with dental acrylic and the incisions closed using absorbable suture. Lead II electrocardiographic (ECG) capability was created when electrodes from the transmitter were tunneled subcutaneously and placed beneath the overlaying musculature using 2–0 Prolene to anchor the wire electrode to the body wall. The negative lead was placed in the upper right chest quadrant near the heart base, and the positive electrode was placed near the heart apex in the lower left chest quadrant. Muscle and fascia were closed in layers using a simple interrupted pattern with absorbable suture; skin was closed with an intradermal continuous pattern using absorbable suture. All skin incisions were reinforced with Vetbond tissue adhesive (3M, St. Paul, MN). Each animal received a systemic antibiotic IM (40,000 units/kg penicillin G benzathine) and an analgesic IM (buprenorphine, 0.01 mg/kg) postoperatively. Approximately 4 to 6 weeks passed between surgery and nerve agent exposure.

EEG and ECG Recording

EEG recordings were obtained by telemetry from freely moving animals in their home cages using DSI Dataquest ART (version 2.3) software and a computer monitor to display the signals. At least three baseline recording sessions, 18 to 24 h in duration, were obtained for each animal prior to nerve agent exposure. Recordings were obtained continuously during and for 48 h after agent exposure. In surviving animals, 24-h EEG records were made on days 3, 10, 15, 30, 45, 60, 75, and 90 throughout the 3-month survival time before euthanasia. ECG recordings were obtained by

telemetry from freely moving animals in their home cages using DSI Dataquest ART (version 2.3) software and a computer monitor to display the signals. ECG recordings were used on the day of soman exposure to assess the clinical condition of subjects during their cholinergic crisis.

Blood Collection

Blood was drawn from the saphenous vein using 25-gauge needles affixed to 1-mL syringes precoated with 1000 USP units/1 mL sodium heparin. The venipuncture site was clipped and swabbed with 70% isopropyl alcohol, and $\leq$1.0 mL of blood was taken for each sample.

Nerve Agent Exposure

On the day of exposure, a blood sample was obtained to determine pre-exposure blood acetylcholinesterase (AChE) and cardiac troponin I (cTnI) baseline values. The soman vial was slow thawed and the appropriate dose drawn up in a syringe within a safety hood, then placed on ice within a sealed container for transport of the syringe to the animal quarters. The animal was then injected with the predetermined dose of soman (0.100 mg/mL in saline) in the calf muscle while being briefly restrained in a squeeze-cage. The injection site was wiped using a gauze sponge wetted with a 1% bleach solution followed by wiping with a water-dampened sponge. The syringe and gauze wipes were placed in full-strength bleach solutions for decontamination. Three soman doses were used in the study: 5.01 μg/kg (N = 1), 6.31 μg/kg (N = 3), and 7.94 μg/kg (N = 3). EEG recording began within 1 min of injection and clinical observations were performed continually for at least 4 h following exposure. Additional blood samples were obtained to measure AChE at 6 h, 3 days, 10 days, and 30 days after exposure in survivors; the 6-h blood samples were also assayed for cTnI.

Acetylcholinesterase Assay

Blood samples were transferred to microfuge tubes containing 30 μL of 1000 USP units/1 mL sodium heparin to prevent clotting. Blood AChE determination was performed using a microtiter plate modification of the WRAIR method (Feaster 2000) that utilizes acetylthiocholine iodide as a substrate. Ten microliters of whole blood was pipetted into a labeled 0.5-mL tube containing 190 μL of sterile water and mixed thoroughly. These tubes were then frozen to −80°C until thawed for AChE measurement. A SpectraMax Plus 384 microtiter spectrophotometer (Sunnyvale, CA) and a PC with associated SoftMax controller software were used to analyze samples.

Troponin Assay

Plasma was obtained from all animals before and after soman exposure. After 10 μL of whole blood was taken for the AChE assay previously described, the blood samples were centrifuged for 10 min at 14,000 rpm. The plasma was transferred to a 3-mL Nalgene screw-top vial and frozen to −80°C until thawed for cTnI measurement. A TOSOH AIA 600 Automated Immunoassay Analyzer (TOSOH MEDICS, INC., South San Francisco, CA) was used to determine cTnI plasma levels. A single measure of cTnI was made for each plasma sample. The AIA-PACK cTnI second generation is a two-site immunoenzymometric assay. This assay is performed entirely in plastic test cups containing lyophilized magnetic beads coated with anti-cTnI mouse monoclonal antibody and mouse monoclonal antibody to cTnI that is conjugated to bovine alkaline phosphatase with 0.1% sodium azide as a preservative. The cTnI present in the test sample binds with the monoclonal antibody immobilized on a magnetic bead and enzyme-labeled monoclonal antibody in the antibody immunoassay test cup. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate. The amount of enzyme-labeled monoclonal antibody bound to the bead is directly proportional to the cTnI concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve (TOSOH Medics, 2001).

Experimental Design

An up-down design for small samples was used (Dixon and Massey 1981). The starting dose was 5.01 μg/kg ($\log_{10}$ = 0.70), which is roughly halfway between the reported soman LD50 for rhesus monkeys

(7.4 μg/kg IM; Adams et al. 1976) and that of cynomolgus monkeys (3.7 μg/kg IM; Adams 1990). Based on the toxic response of a particular test animal (alive vs. dead at 48 h), the next soman dose was increased or decreased in a 0.1 $\log_{10}$ unit increment for the next test animal.

RESULTS

Clinical Toxicity to Soman

Soman doses of 5.01 μg/kg, 6.31 μg/kg, and 7.94 μg/kg elicited severe signs of nerve agent intoxication. Within minutes of soman injection, the animals developed chewing and/or facial automatisms. This was immediately followed by mild and intermittent tremor in the limbs, which shortly progressed to strong and continuous tremor in the whole body accompanied by facial grimacing. This phase was soon followed by uncoordinated thrashing movements that rapidly progressed to tonic-clonic convulsions, EEG seizures, loss of posture, and unresponsiveness to external stimuli. A profuse, thick, ropy salivation developed and persisted throughout the period of seizure activity. EEG seizure activity and motor convulsions were prominent features of intoxication. Table 1 summarizes the times for seizure onset and their duration. Figure 1 provides an example of the EEG record of a seizure. There was a notable cycling of seizure/convulsive activity; it would primarily be characterized as clonic with intermittent intense episodes of tonic activity. After the first 15 to 30 min of seizure, there was notable waxing and waning of seizure/convulsive activity, with periods of 2 to 4 min quiescence in epileptiform EEG activity and convulsive movements between episodes of seizure/convulsive movements. This waxing and waning would become more prominent as the seizure duration grew longer (>1 h).

Each challenge dose elicited a distinct progression and duration of toxic signs. The animal (V564) dosed with 5.01 μg/kg of soman had the longest latency for seizure onset, and the seizures terminated after almost 1 h. Shortly after termination of the seizures, the animal slowly regained consciousness, although tremor, fasciculations, and salivation were still evident for 4 to 8 h as coordination and normal behavior returned. Within 2 days, this animal appeared normal. Three animals (V471, V576, V584) were dosed with 6.31 μg/kg soman IM. These animals experienced seizure durations of 2.5 to 3.5 h, and it was 2 to 8 h after the seizure ended before evidence of consciousness (response to sound or touch, voluntary movement) returned. One animal (V471) initially appeared to recover, but displayed at least three spontaneous seizures (2- to 4-min duration) several days after the intoxication. This animal then failed to eat or drink and was given subcutaneous fluids. Even with these measures his physical condition deteriorated to the point where a decision was made, 6 days postexposure, to euthanize him for humane reasons. In contrast, the other two animals displayed uncoordinated behavior that slowly resolved over 1 to 3 days postexposure, after which they appeared to fully recover. Three animals (V331, V361, V5342) were intoxicated with the 7.94-μg/kg dose of soman. After the initial progression of signs, two animals (V361, V5342) developed chaotic (Cheyne-Stokes) respiratory efforts 10 to 15 min after exposure; cyanosis developed, seizure/convulsive activity rapidly declined, and then periods of apnea developed, accompanied by depressed heart rates. Both animals died about 25 min after exposure. The third animal (V331) displayed seizures for 1 h 50 min. The seizure spontaneously terminated, after which the animal continued to salivate profusely over the next 4 h; there was a slow, steady decline in body temperature and heart rate until the animal died 6 h after exposure.

TABLE 1 Onset of tremors, seizure onset, seizure duration, and outcome

Subject	Soman dose	Tremor onset	Seizure onset	Seizure duration	Outcome
V564	5.01 μg/kg	7 min	17 min 29 sec	57 min	Lived 90 days
V471	6.31 μg/kg	3 min	6 min 38 sec	3 h 23 min	Euthanized 6 days postexposure
V576	6.31 μg/kg	6 min	14 min 11 sec	2 h 54 min	Lived 90 days
V584	6.31 μg/kg	2 min	4 min 46 sec	2 h 29 min	Lived 90 days
V331	7.94 μg/kg	2 min	7 min 5 sec	1 h 50 min	Died 6 h
V361	7.94 μg/kg	2 min	2 min 21 sec	21 min	Died 26 min
V5342	7.94 μg/kg	3 min	5 min 13 sec	18 min	Died 25 min

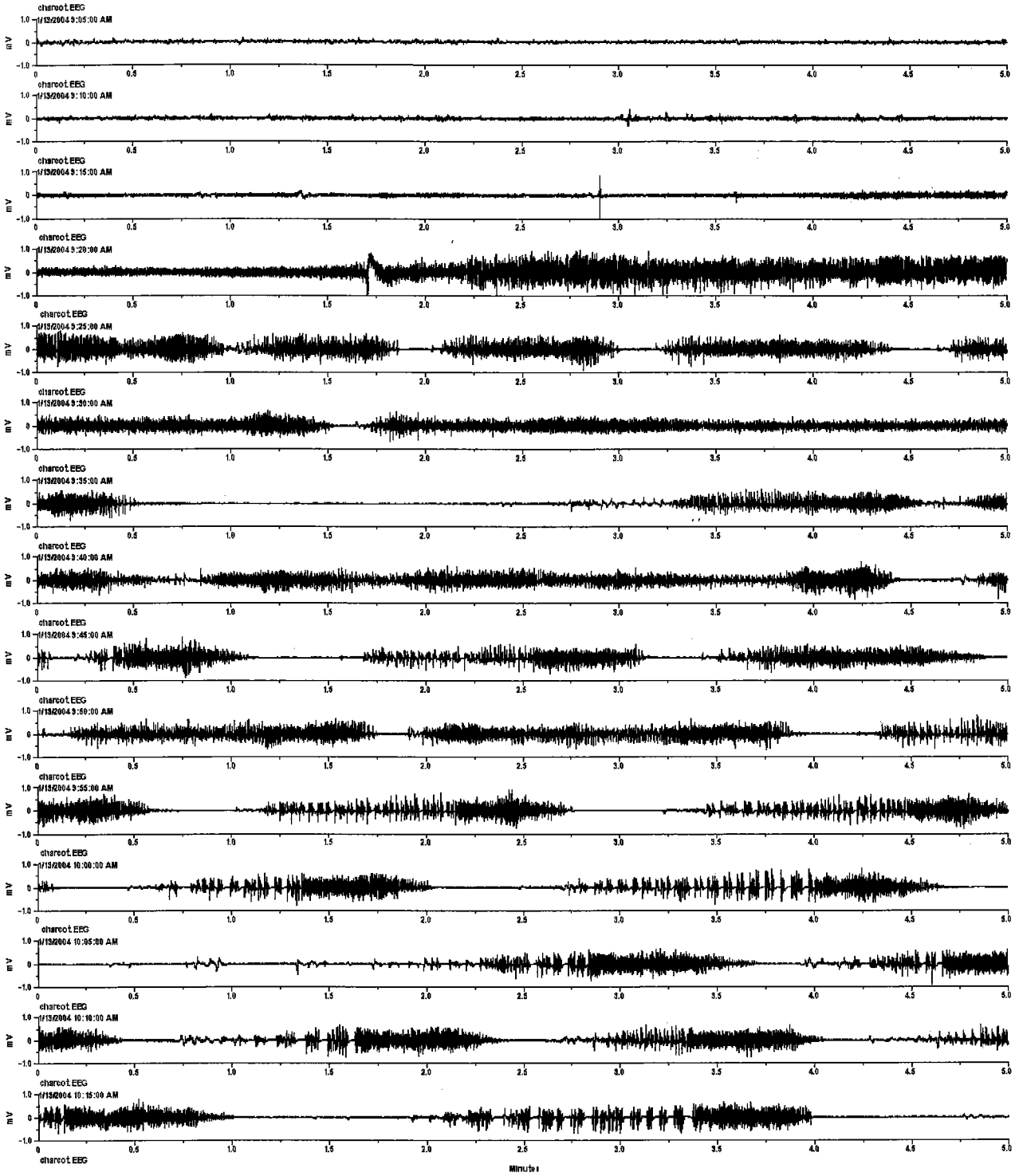

FIGURE 1 A continuous 75-min record of EEG following the administration of 5.01 μg/kg soman to subject V564. Each x-axis line is 5 min and y-axis is millivolt amplitude; the left frontal-central screws are the EEG leads recorded. Soman was given at 9:04 AM; the record starts 1 min later at 9:05 AM. Approximately 14 min after the start of the recording, there is a notable and sustained increase in EEG amplitude that culminates in seizure onset at 17.5 min after injection. The seizure continues uninterrupted at very high amplitudes for almost 5 min and then shifts into periods of waxing and waning of different durations for the next 52 min, where, at the end of a burst of rapid high-amplitude spiking, the seizure abruptly ends and does not reoccur for the rest of the 24-h record. By 10 min after seizure termination the animal behaviorally demonstrated signs of alertness.

TABLE 2 Percent acetylcholinesterase (AChE) activity after soman exposure relative to baseline

Subject	Soman dose	6 h AChE activity	3 days AChE activity	10 days AChE activity	30 days AChE activity
V564	5.01 μg/kg IM	1.5%	3.5%	6.6%	72.0%
V471	6.31 μg/kg IM	Undetectable activity	0.3%	Died	Died
V576	6.31 μg/kg IM	Undetectable activity	1.1%	22.5%	31.9%
V584	6.31 μg/kg IM	2.5%	2.0%	7.7%	45.2%
V331	7.94 μg/kg IM	0.3%	Died	Died	Died

Blood Acetylcholinesterase and Plasma Cardiac Troponin I Levels

Relative to baseline values, whole blood AChE activity was severely depressed by soman exposure, with no significant difference between doses. However, there was a notable recovery of enzyme activity by 30 days after exposure. Table 2 summarizes the blood AChE activity relative to baseline for subjects surviving 6 h, 3 days, 10 days, and 30 days after exposure to soman.

Blood samples taken 6 h after exposure for the three monkeys that received the 6.31-μg/kg dose of soman had elevated cTnI levels. Table 3 summarizes the baseline and 6-h cTnI levels for these three animals.

Animal Dose Sequencing and 48-h Survival

Table 4 displays the sequence in which the animals were exposed, the response, and the detailed calculations recommended by Dixon and Massey (1981) to estimate the IM LD50 of soman. Note that the variance (σ) in this calculation is assumed to be equivalent to the step size (the increment between doses). Based on these calculations, the estimated 48-h IM LD50 of soman is 7.15 μg/kg (6.28–8.13 μg/kg = ± 1 SEM).

DISCUSSION

Soman is a lethal organophosphorus nerve agent that irreversibly binds to acetylcholinesterase at central and peripheral sites causing a buildup of the neurotransmitter acetylcholine and sustained activation of acetylcholine receptors. Excess acetylcholine initiates rapid progression of miosis, hypersecretions, muscular fasciculation, tremors, seizures, convulsions, and death in laboratory rodents, NHPs, and man (Sidell 1997; Tryphonas and Clement 1996; Baze 1993).

The rate of soman intoxication and observed clinical signs in African green monkeys were consistent with descriptions in the literature for the rhesus monkey, cynomolgus monkey, and baboon (Adams et al. 1976; Adams 1990; Anzueto et al. 1986). As Lipp (1968) had indicated, electrographic tonic-clonic seizures and motor convulsions were prominent aspects of the toxic symptomatology of soman intoxication in all animals at the doses studied, although it must be emphasized that only one animal was exposed at the lowest dose.

Cardiac damage following soman exposure has been reported in rhesus and cynomolgus monkeys, as well as in baboons (Britt et al. 2000; Baze 1993; Anzueto et al. 1986). The data in Table 3, obtained from single samples tested with no replicates, demonstrate

TABLE 3 African green cTnI plasma levels at baseline and postexposure to soman

Subject	Soman dose	Baseline cTnI	6 h Postexposure cTnI
V471	6.31 μg/kg IM	0.00 ng/mL	0.99 ng/mL
V576	6.31 μg/kg IM	0.00 ng/mL	6.55 ng/mL
V584	6.31 μg/kg IM	0.00 ng/mL	0.64 ng/mL

TABLE 4 Summary of animal responses and LD50 calculations for the up-down method

Dose (μg/kg)	Log_{10} Dose	Response (Animal Tattoo)		
7.94	0.9	$X_{(V331)}$	$X_{(V361)}$	$X_{(V5342)}$
6.31	0.8	$0_{(V471)}$	$0_{(V576)}$	$0_{(V584)}$
5.01	0.7	$0_{(V564)}$		

0 = 48-h survival; X = fatality.
LD50 = χ_f + (K × d).
LD50 = 0.9 + (−0.458 × 0.1).
LD50 = 0.9−0.0458.
LD50 = $0.8542_{(log10)}$.
LD50 = 7.15 μg/kg (6.28–8.13 μg/kg = ± 1 SEM).
χ_f = last dose tested = 0.9 (log_{10}).
K = Dixon & Massey table 19.2 = −0.458.
d = interval between test doses = 0.1(log_{10}).
N′ = total number of subjects tested = 7.
N = sample size = 6.
σ (variance) = d; SE = 0.56 σ.

TABLE 5 Soman LD50 values in nonhuman primate species

Species	LD_{50} (Survival Times)	Route	Diluent	Reference
Rhesus	12.9 μg/kg*	SC	Not stated	Fukuyama and Askwich 1963
Rhesus	9.5 μg/kg*	IM	Saline	Lipp 1968
Rhesus	7.4 μg/kg (24 h) 6.65 μg/kg (5 day)	IM	PEG-DH_2O	Adams et al. 1976
Rhesus	12.3 μg/kg*	SC	Not stated	Dirnhuber et al. 1979
Rhesus	7.3 μg/kg (48 h)	IM	Saline	Olson et al. 1997
Cynomolgus	3.77 μg/kg (24 h)	IM	PEG-DH_2O	Adams 1990
Baboon	6.65 μg/kg (24 h)	IV	Saline	Anzueto et al. 1986

*Survival times not stated.

that cTnI increases to clinically significant levels in the African green monkey after soman exposure, indicating that cardiac damage has occurred. According to assay specifications, within 4 to 8 h following acute myocardial infarction, cTnI will increase above levels that indicate that myocardial infarction has occurred (TOSOH Medics 2001). The 6-h time point selected for cTnI determination is the median of 4 to 8 h and we hypothesized that cTnI levels would be elevated by this time if cardiac damage had occurred.

None of the African green monkeys in this study had gross or histopathologic cardiac lesions at 90 days after soman exposure. However, 48 to 72 h is the optimal time to assess histological changes associated with myocardial degeneration and necrosis (Schoen 2005). By 90 days after injury, small, multifocal cardiac lesions characteristic of soman toxicity are healed, leaving little or no evidence that they ever existed.

Three isoforms of troponin I identified are skeletal slow twitch, skeletal fast twitch, and cardiac. These three are products of different genes with unique amino acid sequences (Adams et al. 1993). Of the three, cTnI is found exclusively in cardiac tissue. Though the three isoforms share some homologous structure, cTnI has a sequence of 31 amino acid residues at the amino terminus of the molecule, which renders cTnI antigenically distinct from the other two isoforms of troponin I, as well as from troponin C and troponin T (Katrukha 2003). Monoclonal antibodies developed to react with the epitope in this unique portion of the cTnI molecule have made possible the development of in vitro diagnostic assays with great specificity for cTnI. Monoclonal antibodies against human cTnI will cross-react with cTnI of the rabbit, baboon, rhesus macaque, and other species (Cummins and Perry 1978; Walker 2006). Our findings support the premise that cTnI is well conserved across a variety of species to include the African green monkey. A highly sensitive, very specific biochemical marker for soman-induced cardiac injury in the African green monkey would be a valuable tool in nerve agent toxicity and medical countermeasure studies. There is a possibility that cTnI is just such a biomarker, and analytical validation of the cTnI assay for the African green monkey is warranted.

Table 5 displays reported LD50 of soman in three large NHP species. In the present study, the 48-h IM LD50 of soman in African green monkeys was 7.15 μg/kg. This dose is almost identical to the 24-h LD50 s reported for soman in rhesus monkeys and baboons by other investigators (Adams et al. 1976; Olson et al. 1997; Anzueto et al. 1986). However, the 3.77-μg/kg 48-h IM LD50 of soman in cynomolgus monkeys reported by Adams (1990) appears to be excessively low based on the consistent LD50 values obtained in the other NHP species. Also, from the literature, it appears that SC injections of agent in this species result in a higher LD50 value than do IM injections. The 24- or 48-h survival period for LD50 determinations with nerve agents is somewhat arbitrary. Although most deaths from nerve agent intoxication occur within the first hours following exposure, deaths can continue for several days even after apparent recovery, as was seen with subject V471 in this study. This has been seen in other NHP studies with nerve agents (von Bredow et al. 1991; Koplovitz et al. 1992; Murphy et al. 1993) as well as rodent studies (McDonough et al. 1989; Shih et al. 1990). Thus, the LD50 estimates at shorter survival times will be higher than those based on longer survival end-points.

The primary advantage of the up-down method is that it concentrates testing near the mean, which increases the accuracy with which the mean can be estimated (Dixon and Massey 1981). This advantage of concentrating testing at the medial lethal dose proves

valuable when determining the LD50 for toxic agents such as soman. It is noteworthy that the LD50 value established in this study using the up-down method was accomplished with less than half the number of animals used in previous studies utilizing more traditional probit methods (Adams et al. 1976; Adams 1990). Since our results are comparable to and of the same level of precision as results obtained using traditional probit design, it is highly recommended that this up-down approach be used in all such future studies that involve NHP species as a means of reducing the number of animals used to obtain valid results.

Based on toxic response and LD50 value, the African green monkey responds to the lethal effects of the nerve agent soman in an almost identical fashion to the rhesus monkey and appears to be an acceptable NHP model to assess mechanisms of nerve agent toxicity and medical countermeasures.

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