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Baseline Serum Clinical Chemistry Values in African Green Monkeys Before and After Sulfur Mustard

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14. ABSTRACT

Clinical blood chemistry constituents were examined in African green monkeys (AGMs) exposed iv to sulfur mustard (HD). Normal cell count and blood chemistry parameters were established in male AGMs (N=49), with a weight of 4-7 kg, via 2.0-ml blood samples collected twice a week for three weeks. Seven randomly chosen background AGMs were challenged with a 1.0-mg/kg iv injection of HD. A 1.5-ml blood sample was obtained preexposure and analyzed; subsequent 1.5-m1 blood draws were performed 3-4 times a week for 30 days after HD exposure. Blood chemistry analyses showed significant ($P<0.05$) increases in alanine transaminase (93 %), aspartate transaminase (189 %), blood urea nitrogen (75 %), creatine kinase (721 %), and lactate dehydrogenase (114 %) one day after HD exposure. These same chemistry parameters, between days 7 and 16, exhibited a biphasic pattern with a second peak occurring at 32, 101, 75, 330, and 94 %, respectively. Analysis also showed significant decreases in albumin, total protein, and calcium levels. Clinically, the overall changes are indicative of cellular injury to smooth muscle, which was supported by gross findings. Overall, this baseline data broadens the available information on AGM models and can serve as an index for future HD AGM studies.

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ABSTRACT

In the present study, we examined clinical blood chemistry constituents in African green monkeys (AGMs) exposed intravenously to sulfur mustard. Normal cell count and blood chemistry parameters were established in male AGMs (N=49), with a weight range of 4-7 kg, via 2.0-ml blood samples collected from the saphenous vein twice a week for three weeks. Seven of the background AGMs were randomly selected and challenged with a 1.0-mg/kg intravenous injection of sulfur mustard. Just prior to exposure, a 1.5-ml blood sample was obtained and analyzed; subsequent 1.5-m1 blood draws were performed 3-4 times a week for 30 days after sulfur mustard exposure. Blood chemistry analyses showed significant ($P<0.05$) increases above control levels in alanine transaminase (ALT, 93 %), aspartate transaminase (AST, 189 %), blood urea nitrogen (BUN, 75 %), creatine kinase (CK, 721 %), and lactate dehydrogenase (LDH, 114 %) one day after sulfur mustard exposure. After an initial fall in levels back to near control values, these same chemistry parameters, between days 7 and 16, exhibited a biphasic pattern with a second peak occurring at 32, 101, 75, 330, and 94 % above controls, respectively. Analysis also showed significant decreases in albumin (ALB), total protein (TP), and calcium (Ca^{2+}) levels. Clinically, increases in ALT, AST, BUN, CK, and LDH and decreases in ALB, TP, and Ca^{2+} are indicative of cellular injury to smooth muscle. We had gross findings of damage to the smooth muscles of the gastrointestinal tract. Overall, this baseline data broadens the available information on AGM models and can serve as an index for future HD AGM studies.

1. INTRODUCTION

Sulfur mustard (HD; Bis [2-chloroethyl] sulfide) is a lipophilic bifunctional alkylating chemical that is rapidly absorbed through the dermal, respiratory, and ocular (corneal and conjunctiva) epithelium. Although best known as a vesicating or blistering agent, HD-induced blister formation is only a local, latent characteristic of HD toxicity. However, systemic intoxication of sulfur mustard is life threatening and may arise before local effects are recognized. Systemic intoxication occurs from the absorption of un-reacted HD at the site of exposure into the circulatory system where it is then widely disseminated to other tissues and organs (Papirmeister et al., 1991). Bone marrow, lymphatic tissue, and the epithelial lining of the gastrointestinal tract have been found to be most sensitive to HD toxicity, which can lead to immune suppression, dehydration, and malnutrition of exposed individuals (Papirmeister et al., 1991; Graef et al., 1948).

HD was first utilized in World War I and has since been deployed in 11 other conflicts, inflicting mass casualties. To date there is no medical antidote that protects or treats HD-induced toxicity other than early (<5 min) decontamination of the epidermis (Papirmeister et al., 1991; Naghii, 2002). In recent years terrorist groups have begun to produce and utilize many of the same chemical and biological weapons that were once only associated with the military powers of the world. In the mid-1990s the terror group Aum Shinrikyo turned to the use of the chemical warfare agent sarin to inflict mass casualties on the civilian population in the subway of Tokyo, Japan. In the United States the biological weapons anthrax and ricin were mailed to Congressional offices and news agencies in 2001 and the White House in 2003, respectively. HD may not have the same appeal to terrorist groups as other chemical and biological weapons because exposure results in only a small percentage of death. However, other properties of HD, such as ease and cost of production, stability of storage and transport, persistence, and the latency of symptoms, elevate HD as a possible terror agent. The continued use of chemical and biological weapons in war and by terrorist organizations around the world has reiterated the need for viable medical countermeasures for the protection of Soldiers and civilian populations.

In conducting such research, many animal models have been developed, with the rhesus monkey (*Maccaca mulatta*) as the non-human primate (NHP) model of choice. However, in recent years there has been a shift from rhesus to alternative NHP models, one of which is the African green monkey (AGMs; *Chlorocebus aethiops*). This switch has evolved due to the limited availability, extreme cost, and the increased health risk involved with using rhesus monkeys. Among NHP species, there are significant differences in clinical parameters (Altshuler et al., 1971). Therefore, the transition from rhesus to AGMs in HD medical research requires new and species-specific documentation of baseline serum clinical data of both exposed and control animals. Biochemical clinical chemistries can be vital in determining/evaluating disease and toxicological damage to tissues and organs in living animals. For this reason, this paper provides baseline clinical chemistry data for AGMs, as well as documents HD-induced changes in these parameters for 30 days postexposure.

The data presented herein were observed and collected as part of a larger drug efficacy study (Anderson et al., 2006) in which granulocyte colony stimulating factor was evaluated as a treatment compound for HD-induced neutropenia.

2. MATERIALS AND METHODS

2.1 Animals

Baseline blood cell differential counts and serum chemistry values were obtained from 49 male AGMs with a weight range of 4.0 to 7.75 kg. Seven of these AGMs were randomly chosen for HD challenge. In conducting the research described in this report, the investigators complied with the regulations and standards of the Animal Welfare Act and adhered to the principles of the Guide for Care and Use of Laboratory Animals (NRC, 1996), in accordance with the stipulations mandated for an AAALAC accredited facility.

2.2 Baseline Clinical Chemistry Values

Clinical chemistries were determined using an Olympus AU 400E clinical analyzer (Dallas, TX). The following serum clinical chemistries were determined for each sample using commercially available kits from Olympus (Melville, NY) per the manufacturer's instructions: albumin (ALB), alkaline phosphate (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TBIL), calcium (Ca^{2+}), creatine kinase (CK), creatinine (CREA), gamma glutamyl transpeptidase (GGT), glucose (GLU), inorganic phosphorous (IP), lactate dehydrogenase (LDH), magnesium (Mg^{2+}), total protein (TP), and blood urea nitrogen (BUN). Sodium (Na^{+}), potassium (K^{+}), and chloride (Cl^{-}) levels were determined by using ion specific electrodes from the Olympus AU 400E clinical analyzer.

2.3 Blood Sampling and Processing

Chemical sedation was deemed necessary since Altshuler and Stowell (1972) observed that AGMs become highly excited and do not calm under restraint like most other species of monkeys. Therefore, animals were anesthetized with Ketamine HCl (10 mg/kg, im) and weighed; blood samples (2.0 ml) were drawn from the saphenous vein. An aliquot (0.5 ml) was immediately placed into an EDTA-treated tube, mixed, and placed on a rocker to prevent coagulation. The remainder (~1.5 ml) of the blood was placed into a serum separator tube and later centrifuged for serum collection. From the EDTA-treated aliquot, a cell differential count was determined using a Cell Dyn 3500 hematology analyzer (Abbott Diagnostics, Santa Clara, CA). Clinical chemistries and cell differentials were run on all subjects two days per week for three weeks to establish baseline values prior to HD exposure (a total of 221 baseline samples), and then 3-4 days per week for 30 days after HD exposure.

2.4 Sulfur Mustard Exposure

Animals were anesthetized with Ketamine HCl (15 mg/kg, im) and weighed; a catheter was introduced into the saphenous vein for HD administration. The HD stock solution concentration was 9.5 mg/ml in absolute ethanol. Immediately before injection, the HD stock solution was further diluted in saline to 1.0 mg/ml and injected at a volume of 1.0 ml/kg. HD was administered as two half-dose injections, each infused over 2.5 min with a microprocessor-controlled infusion pump (Stoelting, Model 200P). The half-life for HD hydrolysis in saline is 30.3 min, so each injection was made from a fresh dilution. The average time from start of a

dilution to completion of an HD injection was 6 min. Each HD injection was followed with a 1.0-ml saline flush to ensure a complete dose. The HD dose was selected by study design to produce leukopenia. According to Willems' (1989) clinical observations of human casualties, leukopenia is indicative of severe HD toxicity.

2.5 Data Analysis

Data were analyzed with a one-way ANOVA followed by Dunnett's multiple comparison test with statistical significance determined at $P<0.05$.

3. RESULTS

3.1 *Clinical Chemistry*

Blood serum chemistry data were collected and analyzed as part of a larger study conducted by Anderson et al. (2006) and utilized as a diagnostic tool to monitor individual animal health. A comparison of mean baseline clinical chemistry values to mean values from HD-exposed AGMs for each day sampled postexposure is listed in Appendix A.

Blood biochemistry analysis showed significant increases in ALT, AST, BUN, CK, and LDH one day after HD exposure, with levels rising 93, 189, 75, 721, and 114 % above control levels, respectively. These constituents showed a biphasic pattern with levels decreasing toward normal levels before a second peak formed at 32, 101, 75, 330, and 94 % above control levels, respectively, which occurred between days 7 and 16 (Figs. 1 & 2). TBIL levels showed a similar biphasic pattern with a significant increase at day 4 and a secondary maxima on day 14 (Fig. 3). ALP levels increased on day 7 to 33 % over controls with a maximum at day 21 of 70 % above control levels (Fig. 3). Analysis also showed significant decreases in ALB, TP, and Ca^{2+} levels ($P<0.05$; Fig. 4). ALB levels decreased significantly starting at day 7 and lasting through day 23, reaching a nadir of 3.67 g/dL (-20 %) at day 11 compared with a control level of 4.58 g/dL. TP levels revealed a similar pattern, with significant decreases observed from day 9 through day 21, and a nadir of 5.92 g/dL (-14 %) compared with a control level of 6.93 g/dL. Ca^{2+} levels also decreased significantly 1 day after HD exposure and at days 4 through day 16. The significant HD-induced serum chemistry alterations, in comparison to control values, are summarized in Table 1.

3.2 *Blood Hematology*

Cell differential blood count analysis showed a reduction in lymphocytes (~70 %) typically observed at 1 day postexposure and reaching a nadir of 460 cells/uL (- 78 %) approximately 4 days postexposure. In contrast, the absolute neutrophil counts (ANC) generally rose 1 day postexposure and then began to drop with a nadir of 143 cells/uL (%) reached on day 7 (Fig. 5).

4. DISCUSSION

The goal of this study was to document baseline clinical chemistry data in AGMs and to evaluate HD-induced changes in these parameters. Our results indicate that HD exposure has an effect on several important blood chemistries in the AGM. They also corroborate clinical observations of HD-exposed casualties during the Iran-Iraq War in which Willems (1989) reported increases in SGOT (AST), SGPT (ALT), and LDH and decreases in ALB, Ca^{2+} and TP by days 5-14 postexposure. The changes in these chemistry parameters for the Iranian casualties were attributed by Willems (1989) to "a general state of illness" and "reduced caloric intake," respectively. Both of these conditions were also observed in the HD-exposed AGMs during a similar timeframe postexposure. Although there were significant changes in many of the tested chemistries one day after exposure, during the 7- to 14-day postexposure period the AGMs appeared to be in their worst physical condition. During this same period of time both neutrophil and platelet levels reached their respective nadir (Anderson et al., 2006).

The deteriorating state of health was reflected by several clinical chemistry parameters. This 7- to 14-day period corresponded with the second peak of the biphasic pattern seen in the parameters mentioned above and can be attributed to the decreased caloric intake and general sickness. The first peak observed one day after exposure may be related to the animals' primary stress response to acute HD toxicity. In support of this interpretation, neutrophil counts were elevated one day after exposure (Fig. 5; Anderson et al., 2006).

Clinically, increases in ALT, AST, TBIL, BUN, CK, and LDH and decreases in ALB, TP, and Ca^{2+} can also be indicative of cellular injury in the liver and GI tract. Histopathologic analysis from the only euthanized HD-exposed AGM were consistent with these results. Histopathologic analysis showed mild, multifocal, periportal and random histiocytic hepatitis in the liver as well as moderate, diffuse mucosal atrophy and dilated lymphatics with moderate diffuse submucosal edema in the small and large intestine. While the elevations in the level of liver enzymes were found to be statistically significant, clinically they are only considered mildly elevated with limited clinical significance. One of the major routes of HD excretion is through bile (Papirmeister et al., 1991). This can possibly lead to latent HD-induced intestinal damage and biliary flow impairment. Along with elevations in ALT, AST, and TBIL, hepatobillary disease is most often associated with parallel increases in ALP and GGT levels. Evidence of this was seen with significantly elevated ALP levels from days 7 to 21, which can be an indicator of such impairment (Loeb, 1986). However, GGT levels did not increase over the same 30-day period. In addition, histopathologic analysis was unable to confirm hepatobiliary impairment. While these data seem to indicate that hepatobillary damage was not induced by HD exposure in this group of AGMs, biliary impairment cannot be dismissed because of the lack of a statistically relevant number of animals subjected to histopathology. The significant increase in BUN levels that occurred for the first two days postexposure may be indicative of renal malfunction and or damage. However, there was no concurrent elevation in serum CREA levels during this same timeframe or throughout the 30-day period postexposure. Therefore, these data do not imply that intravenous HD administered at 1.0 ml/kg induces kidney damage. CK, LDH, and AST and decreased K+ levels can be associated with striated or smooth muscle damage and/or hemolysis. However, histopathologic evaluation showed no evidence of skeletal muscle damage or hemolysis. In addition to histopathological findings, the gross observation of bloody stool

indicates the most likely cause of the CK, LDH, and AST spikes and related K+ decreases are due to intestinal smooth muscle damage that can be attributed to HD exposure.

Baseline, clinical chemistry values were also measured by Altshuler and Stowell (1972), who found variability between not only primate species but also genders within AGMs. Also noted by Altshuler et al. (1971) in a previous primate study was the variability in glucose values depending on the method of restraint used for blood collection. Loeb (1986) also mentions that in male AGMs glucose results are more variable and significantly different when taken in manually restrained animals vs chemically restrained animals. In comparing male physically restrained AGM values published by Altshuler and Stowell (1972) to the current study of chemically restrained baseline values, there are slight differences in chemistry values across the board with no single pattern apparent (Table 2). However, physically restrained animals in the Altshuler and Stowell (1972) study did have higher GLU (104.4 ± 26.6) and BUN (23.1 ± 6.0) baseline values as well as lower ALT (28.9 ± 18.3) and ALP (31.5 ±36.9) levels. It is not possible to determine whether these differences are due to stress in physically restrained animals or due to drug-induced effects of chemically restrained animals.

The data presented provides valuable clinical chemistry information on the AGM. This is critical since the AGM is being used increasingly in research due to problems of availability, cost and safety concerns associated with rhesus monkeys. First, this study includes a large volume of baseline data collected from a relatively large number of AGMs, thereby broadening the available information on this species. Secondly, it provides clinical chemistry data on HD-exposed AGMs for 30 days postexposure, information that has been lacking in the literature and will contribute to our understanding of the HD injury. Overall this baseline data broadens the available information on AGM models and can serve as an index for future HD AGM studies.

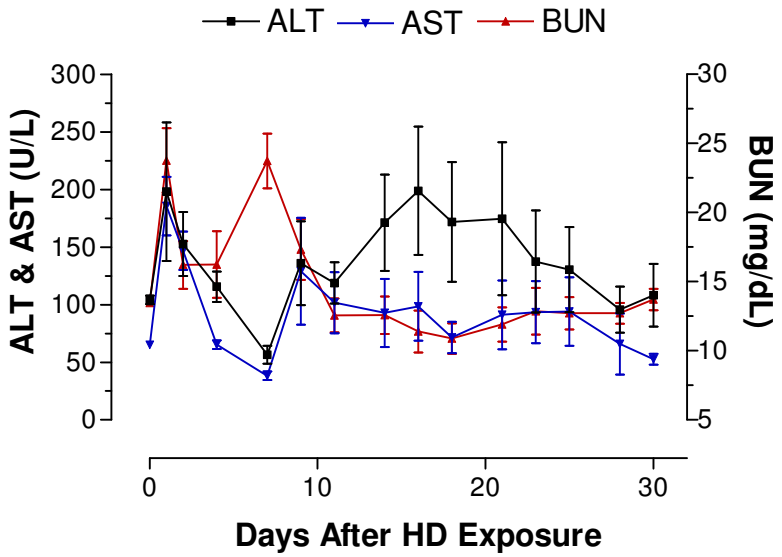

Fig. 1. HD-induced changes in ALT, AST, and BUN levels (mean ± S.E.) in AGMs for 30 days postexposure. Note the immediate increase in levels that occurs on day 1 and then recovers to normal levels followed by a second, and in the case of ALT a more sustained, increase starting at 5-9 days postexposure. ALT (alanine transaminsae), AST (aspartate transaminase), BUN (blood urea nitrogen).

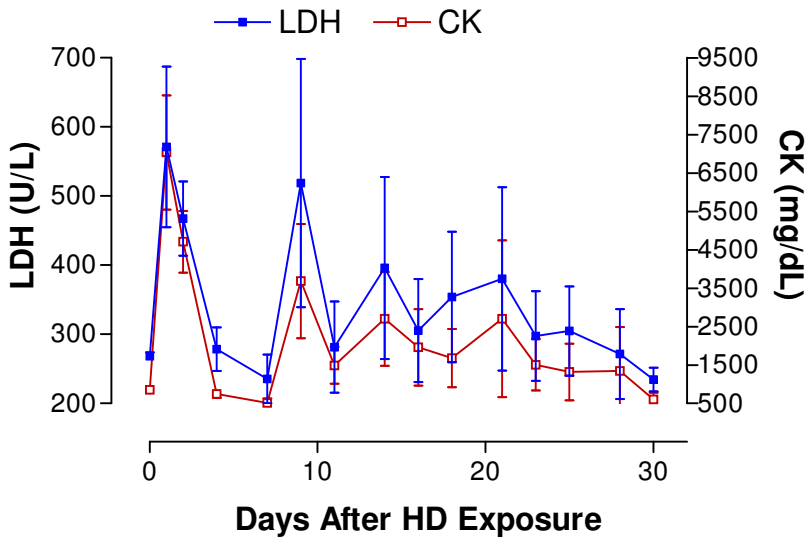

Fig. 2. HD-induced changes in LDH and CK levels (mean ± S.E.) in AGMs for a 30-day period postexposure. Note the immediate increase in levels that occurs on day 1 and then recovers to normal levels followed by a second increase starting at day 9 postexposure. LDH (lactate dehydrogenase), CK (creatine kinase).

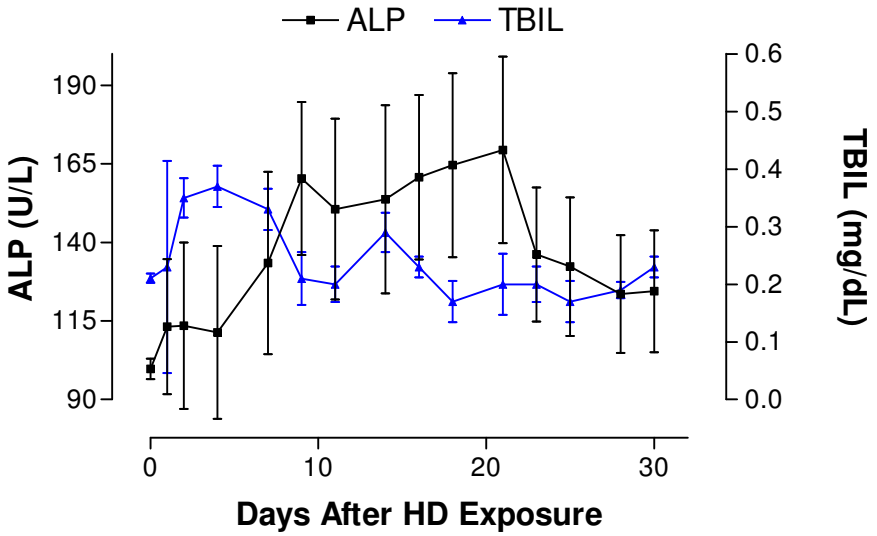

Fig. 3. HD-induced changes in ALP and TP levels (mean ± S.E.) in AGMs for a 30-day period postexposure. TBIL levels began to rise slightly on day 1 postexposure and more sharply on day 2 postexposure. ALP (alkaline phosphatase), TBIL (total bilirubin).

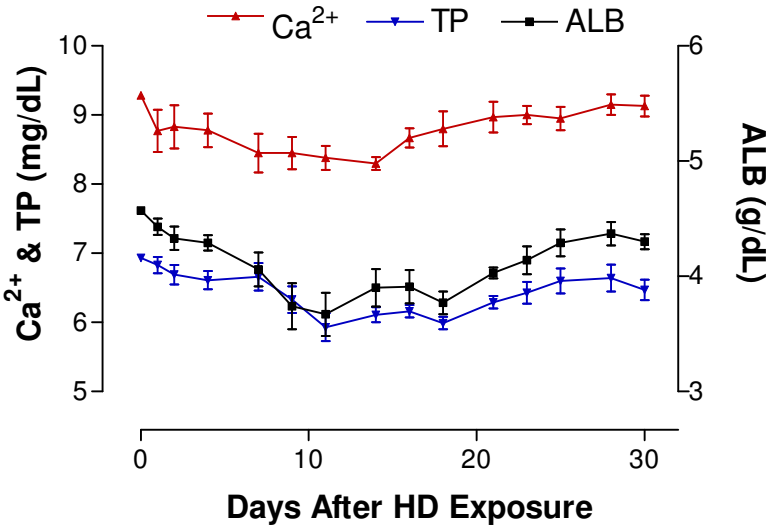

Fig. 4. HD-induced changes in ALB, Ca^{2+}, and TP levels (mean ± S.E.) in AGMs for a 30-day period postexposure. ALB, Ca^{2+}, and TP levels decreased starting at day 1 and continued until day 11 (ALB and TP) and day 14 (Ca^{2+}) before rising toward normal levels out to day 28. This was followed by a slight decrease at day 30 for all three parameters. ALB (albumin), Ca^{2+} (calcium), TP (total protein).

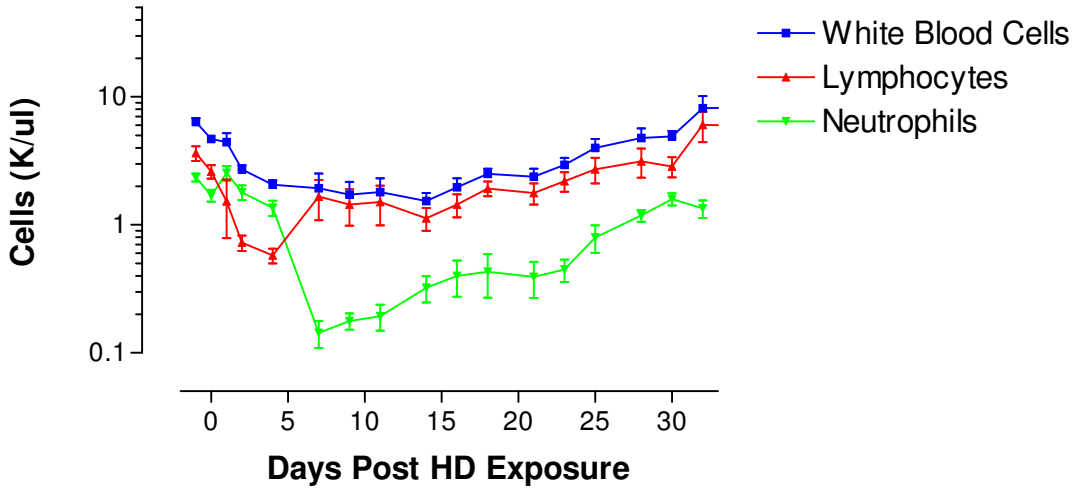

Fig. 5. Leukocyte counts (mean ± S.E.) out to 30 days in AGMs exposed to HD. Lymphocyte and total white blood counts steadily decreased within one day following HD exposure and continued to decline out to days 5 and 15, respectively, before returning to normal values by day 30 postexposure. A transient increase in neutrophil counts was observed on day 2 followed by a dramatic decrease in counts, reaching a nadir at day 7, before increasing toward control levels out to day 28.

Clinical Chemistry	Days After HD Exposure													
	1	2	4	7	9	11	14	16	18	21	23	25	28	30
Alkaline Phosphatase									+	+				
Alanine Transaminse	+							+						
Aspartate Transaminase	+				+									
Blood Urea Nitrogen	+			+										
Creatine Kinase	+	+			+		+			+				
Lactate Dehydrogenase	+	+			+									
Total Bilirubin			+											
Albumin				-	-	-	-	-	-	-	-			
Calcium	-		-	-	-	-	-	-						
Total Protein					-	-	-	-	-	-				

Table 1. Significant (P<0.05) increases (+) or decreases (-) of AGMs clinical chemistry parameters following an HD exposure over a 30-day period.

		Baseline values			
		Altshuler and Stowell		Holmes et al	
Chemistry	Units	Mean	Std. Dev ±	Mean	Std. Dev. ±
Sodium	mEq/L	154.5	4.7	145.8	5.4
Potassium	mEq/L	4.9	0.7	4.4	0.7
Calcium	mg/dL	10.5	0.7	9.3	0.4
Phosphorous	mg/dL	5.5	1.4	4	0.9
Total Protein	g/dL	7.8	0.9	6.9	0.6
Albumin	g/dL	4.1	1.0	4.6	0.02
Glucose	mg/dL	104.4	26.6	83.1	17.3
Blood Urea Nitrogen	mg/dL	23.1	6.0	13.6	3.2
Total Biliruben	mg/dL	0.3	0.15	0.2	0.1
Alanine transaminase	U/L	60.2	22.7	64	24.7
Aspartase transaminase	U/L	28.9	18.3	103	56.7
Alkaline phosphatase	U/L	31.5	36.9	100	57.5
Chloride	mEq/L	106.8	4.4	105.1	4.4

Table 2. Baseline serum chemistry values (mean ± S.E.) obtained from Altshuler and Stowel (1972) and the current study. With a few exceptions (alanine transaminase and aspartase transaminase) all values and standard deviations are extremely similar to the 1972 baseline values.

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Appendix A.

Chemistry		ALB	ALP	ALT	AST	TBIL	Ca^{2+}	CK	CREA	GGT
Units		g/dL	U/L	U/L	U/L	mg/dL	mg/dL	Units/L	mg/dL	Units/L
Basline AGM N=49		4.57 ± .02	100 ± 3.23	103 ± 3.75	64 ± 1.64	0.21 ± .008	9.35 ± .025	856 ± 53	0.87 ± .008	43 ± .886
Days After SM Exposure										
Exposed AGM N=7	1	4.43 ± .071	113 ± 21.5	198 ± 60.2	186 ± 25.5	0.23 ± .018	8.77 ± .306	7037 ± 1487	0.87 ± .084	44 ± 7.35
	2	4.33 ± .102	113 ± 26.5	153 ± 27.8	147 ± 16.7	0.35 ± .034	8.83 ± .312	4710 ± 806	0.86 ± .075	41 ± 7.47
	4	4.29 ± .067	111 ± 27.6	116 ± 13.3	65 ± 3.8	0.37 ± .036	8.78 ± .243	742 ± 95	0.83 ± .078	46 ± 7.64
	7	4.06 ± .148	133 ± 29.0	57 ± 7.7	38 ± 3.7	0.33 ± .036	8.45 ± .280	516 ± 110	1.00 ± .069	37 ± 5.56
	9	3.74 ± .199	160 ± 24.3	136 ± 36.5	129 ± 46.3	0.21 ± .046	8.45 ± .235	3684 ± 1488	0.90 ± .053	39 ± 7.72
	11	3.67 ± .187	151 ± 28.8	119 ± 17.9	102 ± 26.5	0.20 ± .031	8.38 ± .174	1491 ± 480	0.77 ± .081	33 ± 5.80
	14	3.90 ± .163	154 ± 29.9	171 ± 41.9	93 ± 29.6	0.29 ± .034	8.30 ± .093	2700 ± 1221	0.80 ± .069	35 ± 5.90
	16	3.91 ± .145	161 ± 26.2	199 ± 55.8	99 ± 29.9	0.23 ± .018	8.67 ± .138	1960 ± 993	0.74 ± .048	35 ± 5.06
	18	3.77 ± .099	165 ± 29.3	172 ± 52.0	72 ± 13.7	0.17 ± .036	8.80 ± .253	1676 ± 758	0.73 ± .057	33 ± 4.43
	21	4.03 ± .047	169 ± 29.7	175 ± 66.4	91 ± 29.8	0.20 ± .053	8.97 ± .222	2705 ± 2045	0.76 ± .053	37 ± 5.48
	23	4.14 ± .121	136 ± 21.3	137 ± 44.3	93 ± 26.8	0.20 ± .031	9.00 ± .134	1508 ± 671	0.73 ± .042	35 ± 4.85
	25	4.29 ± .116	132 ± 22.0	130 ± 36.8	94 ± 29.6	0.17 ± .036	8.95 ± .169	1317 ± 740	0.74 ± .030	36 ± 4.75
	28	4.37 ± .102	124 ± 18.7	96 ± 20.0	66 ± 26.6	0.19 ± .014	9.15 ± .148	1350 ± 1142	0.80 ± .031	36 ± 5.33
	30	4.30 ± .065	124 ± 19.4	108 ± 27.2	53 ± 4.7	0.23 ± .018	9.13 ± .150	606 ± 169	0.76 ± .049	36 ± 5.37

Chemistry		GLU	IP	LDH	Mg^{2+}	TP	BUN	Na^{+}	K^{+}	Cl^{-}
Units		mg/dL	mg/dL	Units/L	mg/dL	g/dL	mg/dL	mEq/L	mEq/L	mEq/L
Basline AGM N=49		83 ± .974	3.98 ± .059	267 ± 5.32	1.81 ± .014	6.93 ± .032	13.7 ± .180	146 ± .306	4.44 ± .038	104 ± .249
Days After SM Exposure										
Exposed AGM N=7	1	86 ± 5.26	4.92 ± .286	571 ± 116	1.77 ± .096	6.83 ± .119	23.8 ± 2.32	146 ± .896	3.93 ± .127	103 ± 2.34
	2	78 ± 5.82	4.35 ± .332	467 ± 54	1.65 ± .076	6.69 ± .140	16.2 ± 1.72	146 ± .837	4.07 ± .222	103 ± 2.35
	4	77 ± 4.42	4.48 ± .247	278 ± 32	1.63 ± .071	6.61 ± .132	16.2 ± 2.41	146 ± 1.52	4.24 ± .154	105 ± .841
	7	81 ± 7.54	4.08 ± .435	235 ± 35	1.85 ± .106	6.66 ± .201	23.7 ± 1.98	143 ± 1.67	4.51 ± .265	104 ± .944
	9	91 ± 5.25	3.45 ± .430	519 ± 180	1.78 ± .119	6.33 ± .192	17.3 ± 2.20	145 ± 1.63	4.33 ± .234	103 ± 1.15
	11	84 ± 3.73	2.92 ± .483	281 ± 66	1.70 ± .097	5.93 ± .202	12.6 ± 1.23	145 ± 1.69	4.14 ± .194	104 ± 1.07
	14	77 ± 4.35	2.90 ± .529	396 ± 132	1.68 ± .079	6.11 ± .108	12.6 ± 1.36	146 ± 1.72	4.17 ± .149	107 ± .782
	16	87 ± 5.16	3.38 ± .550	305 ± 75	1.65 ± .050	6.16 ± .090	11.4 ± 1.52	146 ± 2.30	3.97 ± .138	106 ± 1.14
	18	83 ± 5.78	4.08 ± .521	354 ± 94	1.75 ± .062	5.99 ± .086	10.9 ± 1.10	146 ± 1.61	4.09 ± .147	106 ± .649
	21	77 ± 4.06	4.87 ± .292	380 ± 133	1.77 ± .061	6.29 ± .088	11.9 ± 1.24	148 ± 1.25	4.03 ± .115	106 ± .800
	23	93 ± 6.30	4.10 ± .193	297 ± 65	1.77 ± .061	6.43 ± .157	12.9 ± 1.68	146 ± 1.03	3.70 ± .105	106 ± 1.04
	25	80 ± 6.58	4.18 ± .255	305 ± 65	1.80 ± .086	6.60 ± .181	12.7 ± 1.17	145 ± .680	3.99 ± .130	106 ± 1.11
	28	89 ± 4.01	4.22 ± .466	271 ± 65	1.78 ± .083	6.64 ± .194	12.7 ± .747	144 ± 1.00	3.94 ± .46	105 ± 1.22
	30	79 ± 4.51	4.38 ± .190	234 ± 17	1.65 ± .072	6.47 ± .149	13.7 ± .779	146 ± .865	3.89 ± .099	106 ± 1.04

Mean (± S.E.) clinical chemistry values of baseline AGMs compared with mean (± S.E.) values of HD-exposed AGMs (1.0mg/kg, iv) for each recorded day postexposure. Recorded clinical chemistries for albumin (ALB), alkaline phophatase (ALP), alanine transaminase (ALT), aspartate transaminase, total bilirubin (TBIL), calcium (CA^{2+}), creatine (CREA), glutamyl transpeptidase (GGT), glucose (GLU), inorganic phosphourous (IP), lactate dehydrogenase (LDH), magnesium (Mg^{2+}), total protein (TP), blood urea nitrogen (BUN), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}).