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14. ABSTRACT In previous studies, hematocrit has been consistently increased in an anesthetized animal model after exposures to TASER® conducted energy weapons (CEWs). In the present study, we analyzed changes in blood cell counts and red blood cell membrane proteins following two 30-s applications of a TASER C2 device (which is designed for civilian use). Hematocrit increased significantly from 33.2 ± 2.4 (mean ± SD) to 42.8 ± 4.6 %, immediately after CEW exposure of eleven pigs (*Sus scrofa*). Red blood cell count increased significantly from 6.10 ± 0.55 X 10^{12}/L to 7.45 ± 0.94 X 10^{12}/L, and mean corpuscular volume increased significantly from 54.5 ± 2.4 fl to 57.8 ± 2.6 fl. Mean corpuscular hemoglobin concentration decreased significantly from 20.5 ± 0.7 to 18.5 ± 0.6 mM. Thirty protein spots (from two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis, selected for detailed comparison) exhibited greater densities 30-min post-exposure compared with pre-exposure values. A greater number of echinocytes were observed following CEW exposure. On the basis of the results, it appears that, during the strong muscle contractions produced by TASER CEWs, a specific population of red blood cells may be released from the spleen or other reservoirs within the body. Whether this phenomenon is applicable to exposures of human subjects, either experimentally or in law-enforcement situations, is unknown. The total time (one min) of CEW exposure in the present study was extremely long compared with exposures in common law-enforcement scenarios. Despite statistically significant changes in red blood cell counts (and other measures directly related to red blood cells), the alterations were transient, with values beginning to return toward baseline levels within 30 min.

15. SUBJECT TERMS
TASER; Conducted energy weapon; Electronic control device; Electro-muscular disruption; Erythrocytes; red blood cells

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ORIGINAL ARTICLE

Effects of a TASER® conducted energy weapon on the circulating red-blood-cell population and other factors in *Sus scrofa*

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Abstract In previous studies hematocrit has been consistently increased in an anesthetized animal model after exposures to TASER® conducted energy weapons (CEWs). In the present study we analyzed changes in blood cell counts and red blood cell membrane proteins following two 30-s applications of a TASER C2 device (which is designed for civilian use). Hematocrit increased significantly from 33.2 ± 2.4 (mean ± SD) to 42.8 ± 4.6 % immediately after CEW exposure of eleven pigs (*Sus scrofa*). Red blood cell count increased significantly from 6.10 ± 0.55 × 10^{12}/L to 7.45 ± 0.94 × 10^{12}/L, and mean corpuscular volume increased significantly from 54.5 ± 2.4 fl to 57.8 ± 2.6 fl. Mean corpuscular hemoglobin concentration decreased significantly from 20.5 ± 0.7 to 18.5 ± 0.6 mM. Thirty protein spots (from two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis, selected for detailed comparison) exhibited greater densities 30-min post-exposure compared with pre-exposure values. A greater number of echinocytes were observed following CEW exposure. On the basis of these results it appears that, during the strong muscle contractions produced by TASER CEWs, a specific population of red blood cells (RBCs) may be released from the spleen or other reservoirs within the body. The total time of CEW exposure in the present study was relatively long compared with exposures in common law-enforcement scenarios. Despite statistically significant changes in red blood cell counts (and other measures directly related to RBCs), the alterations were short-lived. The transient nature of the changes would be likely to counteract any potentially detrimental effects.

Keywords TASER · Conducted energy weapon · Electronic control device · Electro-muscular disruption · Muscle contraction · Erythrocytes · Red blood cells

Introduction

Medical examiners have listed conducted energy weapons (CEWs) (sometimes referred to as "electronic control devices"), including those manufactured by TASER International, as a primary or contributory cause of death, despite the lack of a clear-cut unifying pathophysiological hypothesis [1] (TASER® is a registered trademark of the company in Scottsdale, AZ, USA.). CEW-induced incapacitation is due to muscle contraction resulting in "gravitational dysreflexia (i.e. fall to the ground), and loss of ability to perform coordinated action for the duration of the impulse" [2]. Several potentially-detrimental physiological changes have been noted in animal-model studies, including acidosis and a substantially-increased hematocrit (Hct) [3–10]. The majority of CEW users have been employed in law-enforcement agencies. In 2008 however, a private-citizen version of a CEW, known as the ''C2'' [11], became available (C2 is a trademark of TASER

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International, Inc.). Unlike previous models of CEWs, which deliver a series of electric pulses over a 5 s period after one pull of the trigger, the C2 CEW delivers pulses for the majority of a 30 s period.

The strong muscle contractions caused by CEW exposures may result in findings similar to those from previous studies of muscular exercise [12]. The present study was designed to investigate changes in the population of circulating erythrocytes (red blood cells (RBCs)) that may occur concurrently with increased Hct in the period immediately after C2 CEW exposure in an anesthetized pig model. Techniques to measure such changes had not been used in previous CEW studies. In the present studies, in addition to standard measurements (including heart and respiratory rates, blood cell counts, and blood factors), RBC membranes were prepared for analysis of potential protein changes. This pilot study of proteomic analysis was included since changes in blood proteins may occur concurrently with an increased Hct due to CEW applications. Muscle contraction due to acute physical exercise causes changes in RBC membrane properties [13], including expression of proteins such as spectrin and actin [14].

Mild and moderate exercise-induced hypoxemia can cause increased numbers of echinocytes in blood samples [15]. Along with hemoconcentration, acute strenuous muscle contraction can also cause increases in plasma fibrinogen [16], C-reactive protein, and interleukin-6 [17]. For these reasons, we performed measurements of the degree of echinocytosis (from peripheral blood smears) and of plasma levels of these respective proteins.

Materials and methods

Animal model, anesthesia, and experimental set-up

Eleven male domestic pigs (*Sus scrofa*), ranging in weight from 49.9 to 67.1 kg (mean ± standard error of the mean, 58.4 ± 1.8 kg), were used for these studies. All experiments and animal care procedures were approved by the Institutional Animal Care and Use Committee of the Air Force Research Laboratory, Brooks City-Base, TX, USA, and were conducted according to the US National Institutes of Health's ''Guide for the Care and Use of Laboratory Animals,'' prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources—National Research Council.

For each experiment, a pig was given pre-anesthetic (atropine, 0.05–0.5 mg/kg body weight, subcutaneously) and analgesic (meloxicam [Metacam®; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA], 1 mg/kg body weight, intramuscularly) 10–15 min prior to induction of anesthesia. The animals were anesthetized with an intramuscular injection of tiletamine HCl and zolazepam HCl (Telazol®, 6 mg/kg), followed by oral endotracheal intubation, with the tube secured to the maxilla or mandible. An aural intravenous catheter (3/4–1 in, 20–22 ga.) was placed and secured with a cyanoacrylate adhesive and tape. Anesthesia was maintained with 100–125 μg/kg/min (or to anesthetic effect) of propofol (PropoFlow®, Abbott Laboratories, North Chicago, IL, USA) delivered by a Baxter syringe pump. Aspects of propofol relevant to these experiments, compared with other anesthetics, have been discussed previously [4–6, 18].

Depth of anesthesia was verified by nasal septum pinch, coronary band hoof pressure, and jaw tone. Absence of both reflexes and lack of jaw tone were taken to indicate the animal was at a suitable anesthesia plane. A jugular venous catheter was placed for subsequent central-venous blood sampling. Each pig was delivered to the laboratory anesthetized and placed on its dorsal surface in a canvas sling. Heart rate, respiration rate, and oxygen saturation were monitored continuously using a pulse oximeter (VetOx® G2 Digital, Heska Corporation, Fort Collins, CO, USA), with the probe placed on an ear. At the conclusion of the day's experiment, each animal was euthanized with pentobarbital sodium (Nembutal®), 100 mg kg^{-1} intravenously, without regaining consciousness.

Conducted energy weapon exposures

The darts of the C2 CEW were inserted into each pig at a standardized position through the skin [4, 6, 10]. One dart was placed ~5 cm to the right of the midline (~13 cm cranially from the xiphoid process); the other was placed ~7 cm left of the umbilicus (resulting in ~30 cm separation between darts diagonally). Based on previous studies the degree of muscle contraction caused by a TASER CEW does not increase appreciably with any wider dart separation [19]. Pigs were then exposed twice to the standard 30 s output of the C2 CEW with a 5 s period between each exposure. The same device was used for all experiments, with a new cartridge inserted for each individual exposure (i.e. two cartridges were used for each animal). The total duration of CEW exposure in this study was greater than exposures in common law-enforcement scenarios.

Blood sampling and measurements

Central-venous blood samples (2 cc each) were taken from the jugular vein within one min before and one min after each CEW exposure, and at 30 min post-exposure,

for measurement of Hct and of whole blood pH, pCO_2, pO_2, sodium, potassium, calcium, glucose, and lactate ($N = 11$ animals). Levels of these blood factors were measured immediately with a Model GEM Premier 3000 blood gas/electrolyte analyzer (Instrumentation Laboratory, Lexington, MA, USA). An additional 3 cc of blood was drawn and transferred to BD Vacutainer® collection tubes sprayed with 7.2 mg of potassium EDTA, for analysis of RBC membrane proteins (see "Pilot study: Proteomic analysis" below) and plasma factors. Blood was spun for plasma (centrifugation was performed at $400\times g$ for 40 min at room temperature), and some plasma samples were refrigerated until assay for total protein (analyzed by AniLytics® Incorporated, Gaithersburg, MD, USA). Other plasma samples were stored at −80 °C for subsequent analyses of fibrinogen, C-reactive protein, and interleukin-6 in our lab. Plasma protein levels were measured using enzyme-linked immunosorbent assays (ELISAs) specific for pig fibrinogen and C-reactive protein (Kamiya Biomedical Company, Seattle, WA, USA) and for pig interleukin-6 (R&D Systems, Minneapolis, MN, USA).

For six of the animals, an additional 3 cc of blood was drawn and transferred to collection tubes with sodium heparin, for analysis of other whole blood factors. Blood cell counts and volumes, and other factors (including red cell distribution width, and hemoglobin) were measured with an automated hematology analyzer Micros 60 (Horiba ABX, Montpelier, France). Samples were refrigerated and analyzed within 3–5 days. Ihedioha et al. [20] reported only minor changes in these factors for porcine blood after such a period of storage.

Peripheral blood smears were performed and stained with Wright-Giema stain. Degree of echinocytosis (i.e. number of echinocytes [burr cells] per field) was graded on a scale from 1 to 4, as described by Bell [21].

General data analysis

Normality of blood cell counts, blood chemistry factors, and physiological variables was assessed using the Shapiro–Wilk test on each group of data. Statistical analyses were performed on data for each variable to compare immediate-post-exposure and 30-min post-exposure conditions with the baseline pre-exposure condition. Repeated measures for each animal were used, with a linear mixed model (if data were not different from normal) for all conditions tested, followed by Dunnett's test for multiple comparisons when appropriate. If data were different from normal, Friedman's test was applied across all conditions, followed with Friedman's test on pairs of conditions when appropriate. $P < 0.05$ was considered to be statistically significant.

Pilot study: Proteomic analysis

Preparation of RBC ghosts

Whole blood (from collection tubes sprayed with 7.2 mg of potassium EDTA) was diluted 1:1 with Dulbecco's phosphate buffered saline (DPBS), pH 7.4, and then transferred to a tube containing Ficoll-Paque™ PREMIUM. Centrifugation was performed at $400\times g$ for 40 min at room temperature. The RBC pellet was washed once with DPBS and centrifuged at $20{,}000\times g$ for 15 min.

RBCs were osmotically lysed to dissociate hemoglobin and centrifuged at $20{,}000\times g$ for 15 min at 4 °C. The supernatant was discarded and the RBC pellet was washed twice more with ice-cold DPBS. The resultant pellet of fluffy white ghosts was then lysed in Qiagen QProteome™ PM protein lysis buffer with protease inhibitors (since RBC membranes retain the "ghostly" outline of the original cell, such preparations are referred to as "ghosts."). As the majority of the proteins remained insoluble, a non-ionic detergent was added as described below.

Detergent extraction of membrane proteins

The methods of Mouro-Chanteloup et al. [22] and Liu et al. [23] were followed. An equivalent volume of 1 % Triton X-100 in DPBS, pH 7.4, was added to the ghost samples. Additional protease inhibitors (10 µL of protease inhibitor cocktail set III (Calbiochem, Billerica, MA, USA)) were added to each sample. The samples were incubated on ice for 1 h, followed by centrifugation at $20{,}800\times g$ at 4 °C for 30 min. The soluble fractions were transferred to clean microcentrifuge tubes. The detergent insoluble pellets were stored at −80 °C.

Protein concentration was determined spectrophotometrically using the Quick Start™ Bradford Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). Raw data were obtained using BioTek® (Winooski, VT, USA) KC4 software.

Electrophoresis, image acquisition, and analysis of RBC membrane proteins

Two-dimensional (2-D) electrophoresis involves separation of proteins by both (a) molecular weight and (b) isoelectric point (the pH at which the molecule carries no net electrical charge). 2-D sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed as follows: 300 µg of protein per sample was concentrated and prepared for isoelectric focusing (IEF) using ReadyPrep.™

Clarified protein samples were directly applied to 11 cm ReadyStrip™ immobilized-pH-gradient (IPG) strips pH 3–10. Rehydration and IEF were performed with minor

modifications according to Bio-Rad's "2-D Gel in a Day" protocol. Equilibration of the IPG strips was performed according to the manufacturer's instructions. After equilibration, the protein-bound IPG strips alongside Bio-Rad's Precision Plus Protein™ standards (All Blue and unstained plug) were then separated according to mass on 8–16 % Tris–HCl polyacrylamide gels. Electrophoresis conditions were as follows: 150 V for approximately 2 h and 20 min using the Criterion Dodeca™ cell attached to a chiller recirculator set at 4 °C. Protein detection was accomplished using Coomassie Brilliant Blue R-250 (Fisher Scientific, Pittsburgh, PA, USA).

For 2-D gel imaging, a gel was used for each time point for each animal, providing 33 gels for analysis. After 24 h, a 2-D array of protein spots separated by molecular weight and electrical charge resulted. Images of the gels were made using Molecular Imager FX Pro® (Bio-Rad Laboratories). Images were normalized and their common spots were aligned using PDQuest™ Advanced 2-D Analysis Software (v.8.0.1, Bio-Rad Laboratories) interactively. Spot densities were automatically recorded in Excel 2007 (v.12 SP3, Microsoft Corporation, Redmond, WA, USA).

Proteomic data analysis

Statistical analysis of 2-D gel spot images was performed with programs written in the R language (v. 2.14.0) [24]. In addition to the base statistical and plotting capabilities of the language, functions from packages limma [25] and imputation [26] were used. Linear model methods developed for microarray analysis [27] in the limma package were used to analyze spot densities on the gels. In a second analysis, values for missing data in the dataset were imputed with the k = 10 nearest neighbor method using the kNNimpute() function in the imputation package. A linear model was fit to the log density values of each spot using function lmFit() in limma, which was originally developed for application to gene expression values on microarrays. The functions contrasts.fit() and eBayes() were used to derive moderated F-statistics for each spot. The function topTable() ranked spots on the basis of *P* values adjusted by the method of Benjamini and Hochberg [28], which controls false discovery rate.

Additional verification of use of antibodies and immunoanalysis

Since commercially-available swine antibodies are scarce, antibodies for erythrocytic cytoplasmic and membrane proteins chosen were based on BLAST (Basic Local Alignment Search Tool, Human Proteome Organization) comparability results. The following antibodies from Abcam® (Cambridge, MA, USA) were screened, using Western blots, for reactivity to swine RBCs: α-1 spectrin (mouse monoclonal [AF10]), also specific for β-1 spectrin against ghost proteins of human RBCs); Band 3 (rabbit polyclonal); EPB42 (mouse monoclonal); dematin (mouse monoclonal); MPP1 (rabbit polyclonal); β actin (mouse monoclonal [AC-15]); tropomodulin 1 (mouse monoclonal); GAPDH (mouse monoclonal [6C5]); SM22α (rabbit polyclonal); and glycophorin A (rabbit polyclonal). These proteins have been fully characterized for human RBCs [29], but not for swine RBCs. Secondary antibodies were peroxidase-conjugated to rabbit and mouse IgG.

Swine RBCs were lysed using the nonionic detergent Triton X-100 as described earlier. Proteins were separated on 10 % polyacrylamide gels then transferred to polyvinylidene fluoride (PVDF) membranes using the Trans-Blot® SD Semi-Dry Cell (Bio-Rad Laboratories). Transfer conditions were optimized to obtain both high and low molecular weight proteins. PVDF membranes were blocked for 1 h in blocking reagent then incubated overnight in primary antibody(s) at 4 °C. On the next day, the membranes were washed in buffer, and then incubated for 1 h at room temperature with the appropriate secondary antibody conjugated to horse radish peroxidase (HRP). Immunoreactive protein bands were visualized using HRP chromagenic substrate 3′,5,5′-tetramethylbenzidine.

Results

Blood cell counts and other general findings

Heart rate increased and central-venous blood oxygen saturation decreased significantly after CEW exposure (Table 1). Apnea was noted during each CEW exposure except for one breath during the 0.5 s pause in pulsing (a pause characteristic of the C2 device; the pulse rate of the C2 is: 17 pulses per second [PPS] for 5 s, 12 PPS for 15 s, a 0.5 s pause, 17 PPS for 1.5 s, and 8 PPS for the remaining 8 s, as shown in Ref. [7]).

Blood pH decreased significantly, while blood pCO_2, sodium, potassium, calcium, glucose, and lactate increased significantly (Table 2). Hct increased significantly (an increase of 29 % above baseline value) following CEW exposure (Fig. 1). This occurred in parallel with significant increases in white blood cell count, RBC count, hemoglobin, mean corpuscular volume, and mean platelet volume; and significant decreases in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (Fig. 1; Table 2). Thirty min after CEW exposure, values of these factors had started to return toward pre-exposure baseline levels.

Table 1 Physiological variables measured with the VetOx

Variable	Units	Pre				Post				30 min			
		Mean	SD	Median	Range	Mean	SD	Median	Range	Mean	SD	Median	Range
Oxygen saturation	%	93.7	2.1	93.7	91–97	63.2*	17.4	66.5	36.7–91.3	92.2	2.7	92.0	85.5–95
Heart rate	Beats/min	104.2	21.4	106.0	70.5–127	132.0*	23.7	126.0	104.5–170	109.8	20.9	102.7	78–151
Respiration rate	Breaths/min	24.0	6.8	25.3	11.5–32	21.5	16.7	19.2	7–65	22.7	7.8	21.8	11–35.7

* Significantly different from pre-exposure value

Table 2 Blood cell and chemistry variables (mean ± SD) for pre, post, and 30-min measurement times

Variable	Units	Mean ± SD			Pre–post	Pre-30 m
		Pre	Post	30 min		
White blood cell count	10^9/L	16.85 ± 3.31	22.03 ± 3.90	19.92 ± 3.93	<0.001	0.001
Red blood cell count	10^{12}/L	6.10 ± 0.55	7.45 ± 0.94	7.35 ± 1.08	<0.001	<0.001
Hemoglobin	mM	6.82 ± 0.44	7.92 ± 0.74	7.80 ± 0.80	<0.001	0.001
Hematocrit	%	33.23 ± 2.41	42.83 ± 4.58	41.65 ± 5.20	<0.001	<0.001
Platelet count	10^9/L	191.3 ± 61.8	236.2 ± 80.1	228.7 ± 72.9	0.013	0.033
Mean corpuscular volume	fl	54.5 ± 2.4	57.8 ± 2.6	56.7 ± 2.1	0.014	0.001
Mean corpuscular hemoglobin	fM	1.12 ± 0.06	1.07 ± 0.06	1.07 ± 0.06	<0.001	<0.001
Mean corpuscular Hb concentration	mM	20.5 ± 0.7	18.5 ± 0.6	18.8 ± 0.6	<0.001	<0.001
Red cell distribution width	%	14.8 ± 0.2	14.6 ± 0.2	14.5 ± 0.3	NS	NS
Mean platelet volume	fl	9.97 ± 0.43	12.35 ± 1.28	10.77 ± 0.38	<0.001	NS
Whole blood						
pH	pH units	7.37 ± 0.03	7.05 ± 0.07	7.12 ± 0.04	<0.001	<0.001
pCO_2	mmHg	62.5 ± 6.8	112.1 ± 10	67 ± 9.5	0.002	NS
pO_2	mmHg	46.3 ± 11.4	38.3 ± 12.8	49.8 ± 9.8	0.024	NS
Sodium	mM	140.3 ± 3.1	150 ± 4.8	144.1 ± 3.5	<0.001	0.002
Potassium	mM	3.98 ± 0.17	5.34 ± 0.51	3.72 ± 0.27	<0.001	NS
Calcium	mM	1.31 ± 0.03	1.45 ± 0.06	1.34 ± 0.05	<0.001	NS
Glucose	mg/dl	120.1 ± 25.9	135.9 ± 19.3	141 ± 28.4	NS	0.016
Lactate	mM	1.18 ± 0.61	12.15 ± 3.07	14.37 ± 1.5	<0.001	0.002
Plasma						
Total protein	g/L	4.11 ± 0.68	4.2 ± 0.54	4.4 ± 0.35	NS	NS
Fibrinogen	μg/mL	2700 ± 658	3376 ± 775	3040 ± 630	NS	NS
C-reactive protein	μg/mL	10.5 ± 6.8	11.3 ± 9.92	17.4 ± 21.3	NS	NS
Interleukin-6	pg/mL	10.58 ± 5.23	10.36 ± 4.12	10.49 ± 4.04	NS	NS

Results of a linear mixed model and subsequent Dunnett's test for significant differences for each variable, or Friedman's test for significant differences

NS not significant

Echinocytes in blood smears

The echinocyte grades for pre-exposure, immediate post-exposure, and 30-min post-exposure for each animal analyzed, are presented in Table 3. Examples of microscope fields related to one animal are shown in Fig. 2.

Pilot study: Proteomic analysis

In this study, we verified the feasibility of preparation of swine RBC ghosts, extraction of proteins, electrophoresis, image acquisition, and analysis of RBC membrane proteins from swine. One sample of a SDS-PAGE gel from an RBC ghost, post-CEW-exposure, is shown in Fig. 3. A total of

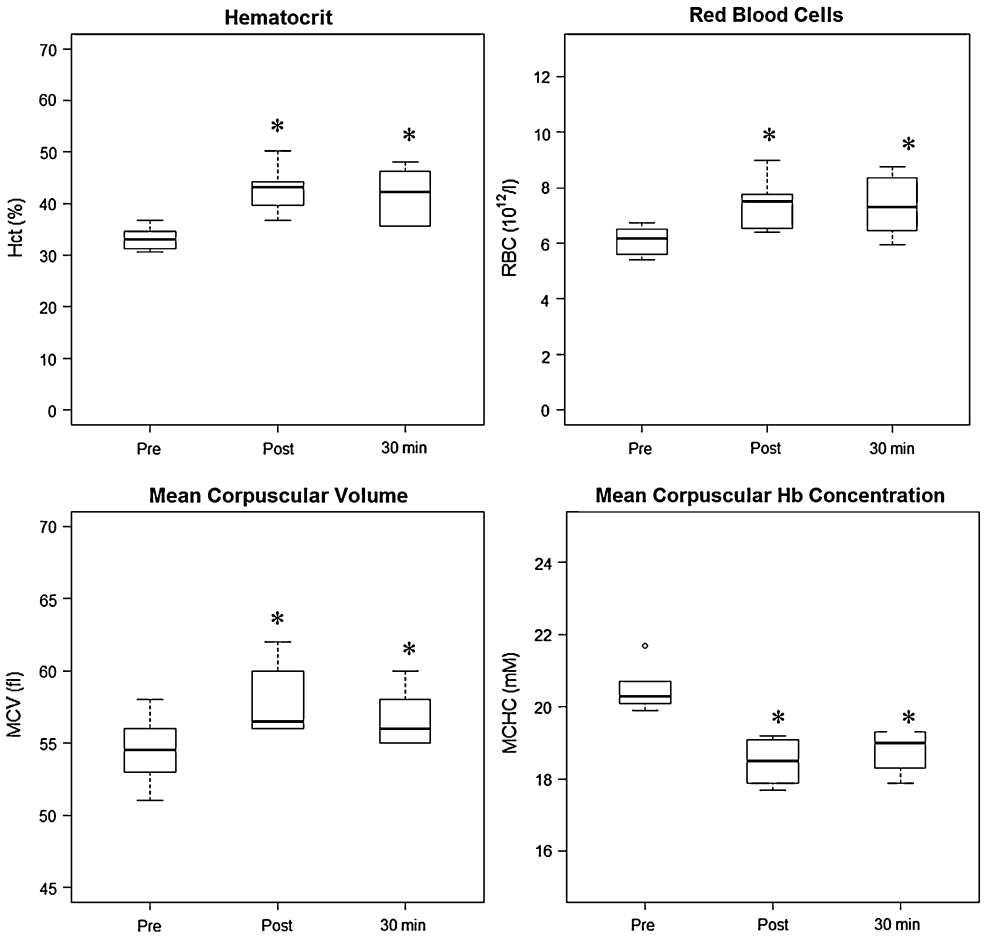

Fig. 1 *Box* plots with medians and quartiles for hematocrit, red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin concentration, pre-exposure, immediate-post, and 30-min post-CEW-exposure ($N = 11$ for hematocrit; $N = 6$ in each other case). * is indication of $P < 0.05$, ° is indication of an outlier

Table 3 Enchinocyte grades

Animal no.	Pre-exposure	Immediate post-exposure	30-min post-exposure
1	1+	4+	3+
2	2+	4+	4+
3	1+	4+	2+
4	1+	4+	4+
5	1+	4+	3+
6	1+	4+	4+

376 spots representing membrane proteins were identified in the 33 different gels, but not every spot appeared in all gels. The 74 spots seen in more than 70 % (23 of 33) of gels were used for further analysis. This dataset contained 2,442 entries, 74 rows (spots) by 33 columns (gels). The base-2 logarithm of the 2,442 density values was used in subsequent analysis. The adjusted P value was less than 0.05 for 30 spots. Comparisons for the 30 spots with the smallest adjusted P values were selected from results of function decideTests(), which indicated whether a pairwise comparison between time point means was an increase, a decrease, or not significant at the 0.05 level. There was no decrease in log density across time; this was the case for all 74 spots. Only two spots showed an increase in log density between immediate-post and pre-exposure time points. Six spots showed an increase between 30 min and immediate-post time points. All 30 spots showed an increase in log density between 30 min and pre-exposure time points.

Although specific details of molecular weights and isoelectric points of proteins have not been quantitated specifically for porcine RBC membranes, on the basis of previous information regarding human spectrin characteristics [29] one may conclude that the location corresponding to spectrin (in both molecular weight and isoelectric pH) on our gels was approximately as shown (in square brackets) in Fig. 3. Examples of the likely RBC-membrane spectrin-specific areas of 2-D gel electrophoresis, for pre-exposure, immediate-post, and 30-min post-CEW exposure, in one animal, are shown in Fig. 4. This conclusion is dependent on an assumption that species differences between swine and humans are minor. Whitfield et al. [30] noted that mobilities of pig RBC membrane skeleton spectrin chains are similar to those of the human. The isoelectric-point shifts of spectrin detected in our

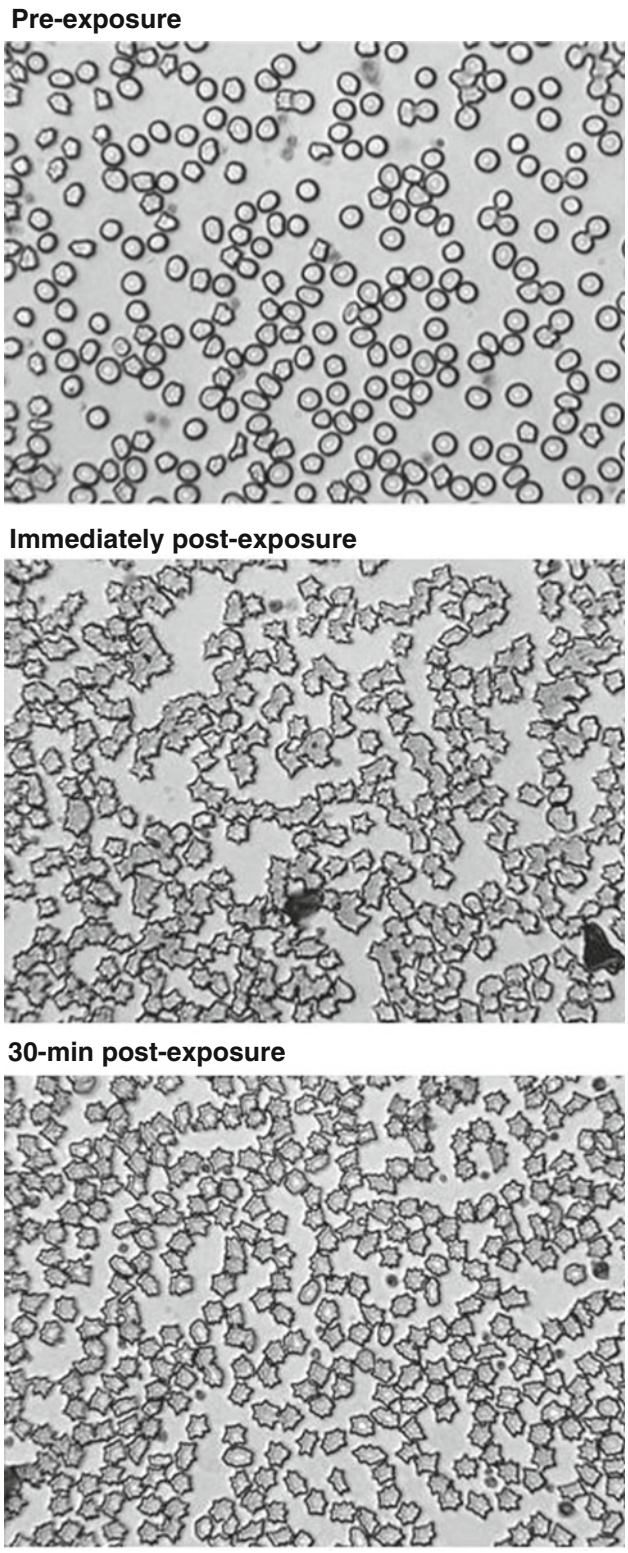

Fig. 2 Representative examples of peripheral blood-smears for analyzing degree of echinocytosis. Oil immersion; magnification ×50

experiments (represented by different spots along horizontal lines in Figs. 3, 4) could have been due to phosphorylation, proteolysis of the parent peptide, minor non-phosphate-related charge shifts [31], various oligomers of spectrin [32], or sub-complexes of different stoichiometry [33], as seen in human RBC membranes.

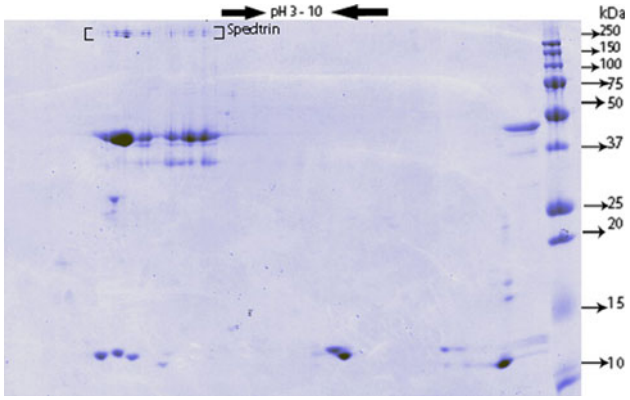

Fig. 3 Representative example of 2-D gel electrophoresis of RBC membranes. Relative pH is shown on the *x* axis; size of proteins is shown on the *y* axis

Large acidic protein bands in the range of 40 kDa in our study (example in Fig. 3) were consistent with similar bands identified as actin in human RBC membranes [29] and human neutrophils [34].

Discussion

General findings

Changes in heart rate, venous-blood oxygen saturation, lactate, pH, and electrolytes were similar to those in our previous studies [3–8, 10]. The discussion below will be focused on newer findings from the present study.

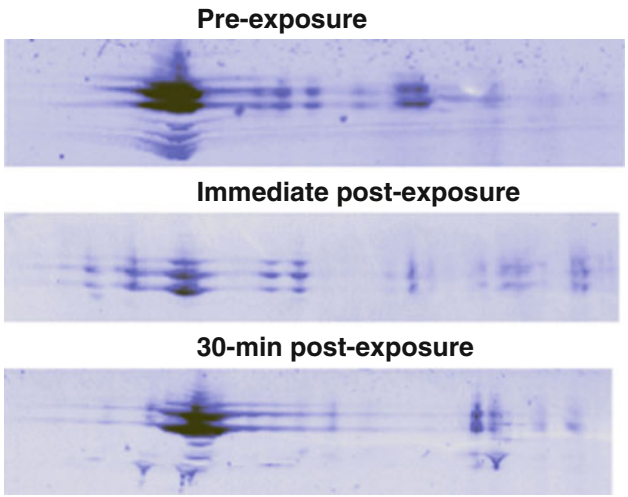

Fig. 4 Examples of RBC-membrane spectrin-specific areas of 2-D gel electrophoresis, for pre-exposure, immediate-post, and 30-min post-CEW exposure in one animal

Hct, blood cell counts, corpuscular volume, and hemoglobin

The increased Hct after CEW exposure (Fig. 1) was similar to that in our previous studies [9]. Changes in RBC count, mean corpuscular volume, and mean corpuscular hemoglobin were similar to those in experiments of endurance exercise in humans by Oostenbrug et al. [35]. Some potential detrimental effects associated with increased Hct are dependent on the intensity and duration of muscle contraction [36]. Any decrease in plasma volume resulting from dehydration is thought to play a relatively minor role in the hemoconcentration that may occur during short-term exercise [37]. The CEW exposures in the present experiments would also not be expected to result in enough dehydration to play a major role in hemoconcentration.

Potential mechanisms of changes in Hct during muscle contraction due to CEW applications have been reviewed previously [9]. One mechanism may be splenic contraction. Splenic contraction in pigs can cause an increased Hct with no change in total blood protein content [38]. Plasma total protein content did not change significantly in our current study. Since most information regarding the reservoir function of the spleen has been obtained from animal studies, the contribution of the organ to a human's circulating blood volume has often been considered to be unimportant. During exhaustive exercise, however, contraction of the spleen in humans can account for approximately ¼ of any increased Hct [39]. During isometric handgrip exercise in humans, splenic volume has been reduced by as much as 18 % during the first minute of exercise [40]. During strenuous exercise, as much as 50 % of blood in the human spleen can be dynamically ejected into the systemic circulation [41]. Pinkus et al. [42] noted that the spleen of humans contains an extensive arrangement of smooth muscle components throughout the organ, and suggested the possibility of an active mechanical mechanism for splenic contraction similar to that in animals.

The percentages of increased hemoglobin in our experiments were within the range reported by Duncker and Bache [43] for swine exhibiting α-adrenergic-mediated contraction of the spleen during exercise. Dogs, horses, and sheep, show more prominent increases in hemoglobin concentration than humans do during exercise [44], with swine being more comparable to humans in this respect. Other aspects that are similar between swine and humans, regarding RBCs, have been reviewed previously [9].

Although mean corpuscular volume and hemoglobin are normally much greater in humans than in swine, the ratios of mean corpuscular hemoglobin to mean corpuscular weight are similar in the two species [45]. In our experiments, post-CEW-exposure RBCs exhibited significantly greater mean corpuscular volume and lower mean corpuscular hemoglobin concentration. Werre et al. [46] concluded that, in humans, RBCs with the highest volume and lowest mean corpuscular hemoglobin concentration are relatively young cells. Snow and Martin reported that RBCs released from the spleen of exercising horses were relatively young [47]. Conversely, Boucher et al. [37] reported a decreased mean corpuscular volume in equine blood after endurance exercise. The authors speculate that the changes were consistent with the release of older smaller RBCs from the spleen during exercise. Whether our finding of increased mean corpuscular volume is related to a differently aged population of RBCs is unknown (also see discussion of "Echinocytes in blood smears" below). Katiukhin et al. [48] suggested that treadmill exercise could result in release of RBCs of younger age and larger volume from bone marrow in humans. The liver of humans may also serve as a reservoir of RBCs during exercise [49].

Artificially increasing plasma sodium and potassium in horses has resulted in increased mean corpuscular volume [50]. In our experiments, significant increases in blood sodium and potassium also occurred along with the increased mean corpuscular volume.

White blood cell count increased 31 % after CEW exposure (Table 2). For comparative purposes, one may note that in previous experiments of endurance exercise testing [35] and of exhausting exercise [51] of human subjects, white blood cells increased 26 and 23 %, respectively after exercise. Although the percentage increase in our present study was greater than those in the previous reports, the change would not have been expected to be detrimental in terms of immediate effects. Simonson and Jackson noted that, during both resistance exercises and aerobic activity, changes in populations of immune cells were transient and inconsequential [52].

Echinocytes in blood smears

RBCs, which appear as discocytes in a test tube, may become echinoctyes simply when being prepared in a dried, fixed, stained blood smear on a glass slide (particularly swine RBCs [53]). In addition, propofol may induce a slight, dose-dependent echinocytic shape transformation of RBCs in vitro [54]. More recently, Kim et al. [55] reported no direct effects on propofol, at clinical doses, on RBC deformability, aggregation, or morphology over a 4-h incubation period. Even if blood-smear fixing or propofol played a role in our experiments, there was a large increase in echinocytes *post*-CEW-exposure. Boucher et al. [37] previously found an increased number of spiculated RBCs (including echinocytes) after endurance exercise in horses. The authors noted that the increased number was consistent

with mobilization of spiculated cells from the spleen. Whether the spleen played any major role in our results cannot be determined from our data. The increase in echinocytes was transient in our study, with values beginning to return toward baseline levels within 30 min.

Brecher and Bessis [56] noted that normal aging of RBCs in the circulation may include transformation into echinocytes. The increased number of echinocytes in our present experiments, however, may have simply been due to a release of a population (possibly from the spleen) of cells more predominately of the echinocyte type. The hypoxic and acidotic environment of the spleen (which could be exacerbated by external factors such as CEW exposures) would favor development of echinocytes [37]. Thus transformation of RBCs already in the peripheral circulation would not be necessary to explain our results.

In addition to storing normally functional RBCs, the spleen of both humans and swine serves as a site for breakdown of older RBCs. The selective retention of abnormal RBCs ("culling") by the spleen has been demonstrated by Levesque and Groom [57].

The red pulp of the spleen, which can actively remove old and damaged RBCs [58], is packed with RBCs in all species studied [59]. The extent to which the spleen culls normally senescent RBCs is unclear [59]. Groom et al. [59] noted, "The spleen has a remarkable ability to remove, selectively from the blood, those RBCs that are so slightly damaged that they would escape retention elsewhere." One may suspect that during CEW exposure, the spleen could release aging or damaged cells (ready for and awaiting final destruction) that would normally not have been released back into the active peripheral circulation. Buffet et al. [60] noted that the spleen can preferentially contain less-deformable RBCs, and echinocytes can exhibit impaired deformability [61]. The extent to which different processes would have occurred in our study favoring either young or old RBCs being released from the spleen is unknown.

Since alkalosis (rather than acidosis) tends to result in echinocytosis (at least in vitro) [62], the decreased pH in our present study was unlikely to have been related to the higher number of echinocytes observed post-CEW-exposure.

Platelets

In one study of humans, propofol administration was associated with a slight decrease in platelet counts [63]. In addition, increased storage time can result in a significant decrease in platelet count [64]. Despite the use of propofol and the storage time in our experiments, however, pre-exposure platelet counts were within the range of other investigators using swine [65]. Pre-exposure, immediate post-exposure, and 30 min post-exposure samples from each animal were analyzed at the same time.

The increased platelet count post-CEW exposure was consistent with other researchers' findings related to strenuous exercise, which can increase platelet count significantly [66]. Mean platelet volume increased in our study, as it does in humans after strenuous exercise [67]. Watson and Ludlam reported that young platelets are preferentially retained in the spleen of humans [68]. Chamberlain et al. [67] concluded that platelets released from the spleen due to exercise have a larger volume, but are of similar age and density compared with a basal circulating population in humans. Whether these aspects are true for swine is unknown.

Plasma proteins

Plasma concentrations of total protein, fibrinogen, and other factors can influence RBC aggregation during an increase in Hct [69]. In the present study, however, there were no significant changes in plasma total protein, fibrinogen, C-reactive protein, or interleukin-6. It may be necessary for muscle contraction to be sustained for longer periods to observe any such changes. For example, serum interleukin-6 and C-reactive protein were elevated 30-fold and 20-fold, respectively, in 24-h ultra-marathon runners [70]. Pedersen and Febbraio found that different modes of acute exercise were associated with variable increases in plasma interleukin-6 [71].

RBC membrane proteins

Although thorough 2-D map analyses of proteins from human RBC membranes have been accomplished (reviewed by D'Alessandro et al. [72]), complete characterizations of RBC membrane proteins from *S. scrofa* have not been attempted to date. Only a few previous studies have included analyses of human RBC membrane protein changes due to conditions that may be somewhat similar to CEW applications, such as acute exercise [13, 14], hypoxia [73], or decreased pH [74].

All RBC-membrane protein spots that were examined in detail in our present experiments exhibited greater densities 30-min post-exposure compared with pre-exposure values. Although normal cell aging is associated with many significant changes in RBC membrane proteins [75], it is unknown whether the changes in protein spot densities in our current experiments were associated with cell populations of different ages.

Acute exercise can result in the disappearance of spectrin bands shown by electrophoresis [76]. Hypoxic exercise leads to decreased spectrin and actin contents in RBCs of human subjects [77]. Although the intensity of spectrin

bands appeared to be decreased in some of the animals after CEW exposure in our experiments, the change was not consistent in all animals. It is unknown whether changes in spectrin expression may have prognostic or predictive importance, as in hereditary elliptocytosis and spherocytosis [78], for muscle-contraction-related conditions.

Brecher and Bessis postulated that spiny processes in echinocytes were formed by a contracting sub-membraneous network of spectrin filaments [56]. Ziparo et al. [79] noted, although formation of echinocytes may partly be due to some action of spectrin, the exact mechanism by which spectrin would cause these changes is unknown.

Since density values of RBC-membrane protein spots were significantly greater 30 min after CEW exposure, future studies may focus on determining the identity of such proteins. Although the majority of human antibodies may be unreactive with porcine blood cells [80], the finding in our study of cross-species reactivity to antibodies for α-1 spectrin and β-actin (in both whole RBCs and ghosts) may also be useful in upcoming investigations.

Overall biological significance of the current findings

In contrast with the results of our present experiments, Dawes et al. [81] found no change in Hct after 30-s exposures of human subjects to the C2 device. There were also no changes after 5–15 s exposures to other specific CEW devices [82, 83]. In all of our previous studies of CEWs, apnea was present during the exposures [8]. In contrast, the human subjects in the studies of Dawes et al. [81–83] were able to consciously force themselves to breathe adequately. The spleen may expel RBCs due to apnea [84] (including apnea of very short time periods, e.g. only 15 s [85]), thereby raising Hct. If Hct increases only during periods of apnea, this phenomenon could be an explanation of why Hct increased in our experiments but not in the previous experiments of Dawes et al. [81–83]. It is also possible that humans simply will not react to CEW-induced muscular activity with any measurable splenic contraction.

Ahmadizad and El-Sayed concluded that, in normal healthy individuals, a single period of heavy resistance exercise alters blood rheological factors, but that any changes are transient and could be due simply to exercise-induced hemoconcentration [16]. Whether the two 30 s CEW exposures, as performed in the present experiments, would cause such changes in humans is unknown.

It would be difficult to perform controlled experimental studies of humans to reproduce all potential conditions during law-enforcement situations (including use of CEWs), which may also involve co-morbidities such as physical restraint, intense emotional stress, psychiatric illness, and use of illicit drugs. Future controlled studies using animal models, however, may enable researchers to focus on pathophysiologic variables that may play roles in effects of long-duration CEW exposures.

The total time (one min) of CEW exposure in the present study was relatively long compared with exposures in common law-enforcement scenarios. Despite statistically significant changes in red blood cells and many blood factors, the alterations were transient, with values beginning to return toward baseline levels within 30 min. Even if long-duration CEW applications were to be partially responsible for any detrimental effects, the usual short-term applications for law-enforcement purposes would not be likely to have similar consequences.

Key Points

1. This is the first published study regarding effects of conducted energy weapons on the circulating red-blood-cell population and other related factors in an animal model of exposure.
2. Transient increases in hematocrit and red blood cell count due to conducted-energy-weapon applications were consistent with previous reports in the literature dealing with studies of muscle stimulation or exercise.
3. A significant increase in mean corpuscular volume and significant decrease in mean corpuscular hemoglobin concentration, and an increase in numbers of echinocytes, may have been related to a sub-population of red blood cells released from the spleen.
4. Despite statistically significant changes in red blood cells and other factors, after the specific type of CEW exposures used in the present study (two 30-s applications of a TASER C2 device), the alterations were short-lived. The transient nature of the changes would be likely to counteract any potentially detrimental effects.

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Conflict of interest The authors have not had any relationship with any manufacturers of conducted energy weapons, including employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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