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Sulfanegen Sodium Treatment in a Rabbit Model of Sub-Lethal Cyanide Toxicity

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Abstract

The aim of this study is to investigate the ability of intramuscular and intravenous sulfanegen sodium treatment to reverse cyanide effects in a rabbit model as a potential treatment for mass casualty resulting from cyanide exposure. Cyanide poisoning is a serious chemical threat from accidental or intentional exposures. Current cyanide exposure treatments, including direct binding agents, methemoglobin donors, and sulfur donors, have several limitations. Non-rhodanese mediated sulfur transferase pathways, including 3-mercaptopyruvate sulfurtransferase (3-MPST) catalyze the transfer of sulfur from 3-MP to cyanide, forming pyruvate and less toxic thiocyanate. We developed a water soluble 3-MP prodrug, 3-mercaptopyruvatedithiane (sulfanegen sodium), with the potential to provide a continuous supply of substrate for CN detoxification. In addition to developing a mass casualty cyanide reversal agent, methods are needed to rapidly and reliably diagnose and monitor cyanide poisoning and reversal. We use non-invasive technology, diffuse optical spectroscopy (DOS) and continuous wave near infrared spectroscopy (CWNIRS) to monitor physiologic changes associated with cyanide exposure and reversal. A total of 35 animals were studied. Sulfanegen sodium was shown to reverse the effects of cyanide exposure on oxyhemoglobin and deoxyhemoglobin rapidly, significantly faster than control animals when administered by intravenous or intramuscular routes. RBC cyanide levels also returned to normal faster following both intramuscular and intravenous sulfanegen sodium treatment than controls. These studies demonstrate the clinical potential for the novel approach of supplying substrate for non-rhodanese mediated sulfur transferase pathways for cyanide detoxification. DOS and CWNIRS demonstrated their usefulness in optimizing the dose of sulfanegen sodium treatment.

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Keywords

Chemical and biological weapons; cyanide toxicity reversal; optical hemodynamic monitoring

Introduction

Cyanide poisoning is a serious chemical threat worldwide from accidental as well as intentional exposures (Baskin and Brewer, 1997; Martin and Adams, 2003; Cummings, 2004; Irwin and Rippe, 2007a; Irwin and Rippe, 2007b). Clinical cyanide toxicity can occur within seconds to minutes, making cyanide one of the fastest-acting toxic chemicals. Industrial accidents, fires, or acts of terrorism and war may simultaneously expose multiple victims to cyanide, either directly from the use of cyanide or cyanogenic compounds, or indirectly from combustibles and explosives (Baskin and Brewer, 1997; Martin and Adams, 2003; Cummings, 2004; Eckstein and Maniscalco, 2006; Keim, 2006; Maniscalco, 2006; Hall *et al.*, 2009).

Current treatments for cyanide exposure include three general classes of agents: methemoglobin generators (sodium nitrite, amyl nitrite, and dimethyl aminophenol), sulfur donors (sodium thiosulfate and glutathione), and direct binding agents (hydroxocobalamin and dicobalt edetate) (Baskin and Brewer, 1997; Cummings, 2004). Nitrites reverse cyanide poisoning by inducing methemoglobinemia (with increased cyanide binding affinity), which must be carefully controlled since oxygen transport is reduced by methemoglobin formation (Baskin *et al.*, 1992; Gracia and Shepherd, 2004; Morocco, 2005). Consequently, nitrite therapy is relatively contraindicated in smoke inhalation victims with concurrent carboxyhemoglobinemia (Cummings, 2004; Gracia and Shepherd, 2004). Hydroxocobalamin, a direct cyanide binding agent is relatively safe and effective, but must be administered intravenously in large volumes (Martin and Adams, 2003; Cummings, 2004), and thus is not a treatment agent that can be used in mass exposure scenarios. Given the ongoing threat of mass casualty cyanide exposure (Gracia and Shepherd, 2004; Associated-Press, 2009), we seek to develop treatments that can be rapidly, safely, and effectively given to large numbers of potential cyanide exposure victims, such as agents that can be administered by IM injection.

Thiosulfate based antidotes predominately rely on rhodanese (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1) to detoxify cyanide. Rhodanese, however, has many limitations—it is concentrated in the mitochondrial matrix of the liver and kidneys (Hol *et al.*, 1983), leaving the heart (Alexander and Baskin, 1987) and central nervous system relatively unprotected (Jarabak and Westley, 1980; Bhatt and Linnell, 1987; Sylvester and Sander, 1990), and the total amount of rhodanese activity may not be sufficient to rapidly reverse cyanide toxicity even in the presence of adequate thiosulfate substrate. Other sulfurtransferases, such as 3-mercaptopyruvate sulfurtransferase (3-MPST), (3-mercaptopyruvate:cyanide sulfurtransferase, EC 2.8.1.2), may be able to provide an alternative route for cyanide detoxification. 3-MPST catalyzes the transfer of sulfur from 3-MP to cyanide, forming pyruvate and less toxic thiocyanate, which is excreted in the urine (Baskin *et al.*, 1992; Nagasawa *et al.*, 2007). However, 3-MP rapidly decomposes in the blood, making it ineffective when administered intravenously. This deficiency could potentially be counteracted by making the 3-MPST natural substrate, 3-mercaptopyruvate (3-MP), continuously available. Consequently, we have developed a water soluble (158 mg/ml) 3-MP prodrug, 3-mercaptopyruvatedithiane (sulfanegen sodium: 2, 5-dihydroxy-1,4-dithiane-2,5-dicarboxylic acid disodium salt) (Figure 1) (Nagasawa *et al.*, 2007) (Crankshaw *et al.*, 2007).

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In addition to developing a mass casualty cyanide reversal agent, methods are needed to rapidly and reliably diagnose and monitor cyanide poisoning and reversal (Lee *et al.*, 1988; Panda and Robinson, 1995; Borron and Baud, 1996; Borron, 2006). We use non-invasive technology, diffuse optical spectroscopy (DOS) and continuous wave near infrared spectroscopy (CWNIRS) to rapidly and noninvasively monitor physiologic changes associated with cyanide exposure and reversal (Lee *et al.*, 2007a; Lee *et al.*, 2009). These changes, including tissue oxy- and deoxyhemoglobin levels, can be identified through continuous measurement of simultaneous near infrared tissue optical scattering and absorption (Merritt *et al.*, 2003; Lee *et al.*, 2007a; Lee *et al.*, 2007b).

In this study, we investigate the ability of intramuscular and intravenous sulfanegen sodium treatment to reverse cyanide exposure effects in an animal model as a potential treatment for mass casualty resulting from exposure to cyanide.

Materials and Methods

General preparation

Pathogen-free New Zealand White rabbits weighing 3.5-4.5 kg (Western Oregon Rabbit Supply), were used. There are number of reasons that we selected the rabbit model for these studies {Lee, 2009 #821;Brenner, 2010 #819;Brenner, 2009 #818;Lee, 2007 #317}. Rabbits are large enough to be readily intubated, and instrumented with arterial and venous catheters as well as cardiac output monitors, and have sufficient muscle mass that is suitable for DOS and CWNIRS. This enables assessment of the rate of development of the effects of CN toxicity and of the CN treatment agents {Lee, 2009 #821;Brenner, 2010 #819;Brenner, 2009 #818;Lee, 2007 #317}. Rabbits are more facile to work with than pig, dog, or other larger vertebrate animals, making them more practical for moderate scale studies. All procedures were reviewed and approved by the University of California, Irvine, Institutional Animal Care and Use Committee (IACUC). The methods for cyanide induction and DOS monitoring have been described previously (Lee *et al.*, 2007a) and are summarized here:

Animals were anesthetized with an intramuscular injection of a 2:1 ratio of ketamine HCl (100 mg/ml, Ketaject, Phoenix Pharmaceutical Inc., St. Joseph, MI): Xylazine (20 mg/ml, Anased, Lloyd Laboratories, Shenandoah, IA) at a dose of 0.75 cc/kg, using a 23 gauge 5/8 inch needle. After the intramuscular injection, a 23 gauge 1 inch catheter was placed in the animals' marginal ear vein to administer continuous IV anesthesia. Torbutrol, 0.1-0.5 mg/kg , was given subcutaneously. The animals were intubated with a 3.0 cuffed endotracheal tube secured by a gauze tie and were mechanically ventilated (dual phase control respirator, model 32A4BEPM-5R, Harvard Apparatus, Chicago, IL) at a respiratory rate of 32/min, a tidal volume of 50 cc, and FiO_2 of 100%. A pulse oximeter (Biox 3700 Pulse Oximeter, Ohmeda, Boulder, CO) with a probe placed on the tongue was used to measure SpO_2 and heart rate. Blunt dissection was performed to isolate the femoral artery and vein on the left thigh for blood sampling, cyanide infusion, and systemic pressure monitoring. Sodium cyanide (10 mg) dissolved in 60 ml of 0.9% saline was given intravenously over 60 min; this dose of cyanide (~2.5 mg/kg) is sub-lethal to rabbits, but does cause marked changes in tissue oxy- and deoxyhemoglobin concentrations (Lee *et al.*, 2007a). On completion of the experiment, the animals were euthanized with an intravenous injection of 1.0 cc Euthasol (390 mg pentobarbital sodium, 50 mg phenytoin sodium; Vibrac AH, Inc, Fort Worth, Texas) administered through the marginal ear vein.

Venous blood cyanide levels were measured at baseline, 5 min prior to completion of the cyanide infusion, at the end of the cyanide infusion, and at 2.5, 5, 7.5, 10, 15, 30, 45, 60 and 90 minutes after injecting the sulfanegen sodium. During this time, DOS and CWNIR

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measurements were taken continuously, with each measurement set requiring an average of 30 seconds and 1 second to complete, respectively.

Study and control groups

The sulfanegen sodium dose was calculated to achieve a molar equivalent for cyanide neutralization, and the injections were performed intramuscularly in the left latissimus dorsi muscle or intravenously in the left femoral vein. Trials were conducted in such a way as to achieve a dose response curve with 2 animals per dose used to test for the effective dose and 6 animals used at the effective dose. A total of 35 animals were studied. As controls, 7 animals were used and received intramuscular saline injection. The 7 control animals consisted of data from 2 new animals, added to 5 previously completed and published animal data sets (Brenner et al., in press). For the IM trials 16 animals were used and they received an intramuscular injection of a dose range from 0.05 to 0.21mmol of sulfanegen sodium dissolved into 1.5cc 0.9% NaCl solution. For the IV trials a total of 12 animals were used and they received an intravenous dose range from 0.0125 to 0.05mmol of sulfanegen sodium dissolved into 5.0cc 0.9% NaCl. The dose and number of animals for each dose are shown in table 1.

Non-invasive measurements using diffuse optical spectroscopy (DOS)

Diffuse optical spectroscopy (DOS) measurements were obtained through a fiberoptic probe with a light diode emitter and detector at a fixed distance (10 mm) from the source fiber, which was placed on the shaved surface of the right inner thigh of the animal. The broadband DOS system we constructed (Lee *et al.*, 2007a) combines multi-frequency domain photon migration with time-independent near infrared spectroscopy to accurately measure bulk tissue absorption and scattering spectra. It employs six laser diodes at discrete wavelengths (661,681,783, 805, 823, and 850 nm), and a fiber coupled avalanche photo diode (APD) detector (Hamamatsu high-speed APD module C5658, Bridgewater, NJ) for the frequency domain measurements. The APD detects the intensity-modulated diffuse reflectance signal at modulation frequencies between 50 to 550 MHz after propagation through the tissue. Absorption and reduced scattering coefficients are measured directly at each of the six laser diode wavelengths using frequency-dependent phase and amplitude data. Reduced scattering coefficients are calculated as a function of wavelength throughout the NIR region by fitting a power-law to six reduced scattering coefficients. Steady-state acquisition is accomplished using a broadband reflectance measurement from 650 to 1000 nm that follows frequency domain measurements using a tungsten-halogen light source (Ocean Optics HL-2000, Dunedin, Fl) and a spectrometer (BWTEK BTC611E, Newark, DE). Intensity of the steady-state reflectance measurements are calibrated to the frequency domain values of absorption and scattering to establish the *absolute* reflectance intensity (Lee *et al.*, 2007a; Lee *et al.*, 2007b; Pan *et al.*, 2007). Tissue concentrations of oxy- and deoxyhemoglobin are calculated by a linear least squares fit of the wavelength-dependent extinction coefficient spectra of each chromophore. We used oxy- and deoxyhemoglobin absorption spectra reported by Zijlstra et al. (Zijlistra *et al.*, 2000) for subsequent fitting and analysis.

Continuous wave near infrared spectroscopy

CWNIRS was used to assess oxy and deoxyhemoglobin effects of cyanide toxicity and reversal in the forearm muscle region. CWNIRS provides rapid, real time measures of tissue oxy- and deoxyhemoglobin concentration changes and penetrates more deeply into tissues than DOS (Kim and Liu, 2008); it can, therefore, be used to assess regions such as the CNS and can collect data more frequently which is important in the study of pharmacokinetics. CWNIRS, however, does not account for scattering affects, and provides only relative

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information on changes in the concentrations of molecular species (as opposed to absolute concentrations obtained by DOS).

The CWNIRS system consists of a light source (HL 2000, Ocean Optics, FL), a CCD spectrometer (USB4000, Ocean Optics, FL), and customized optical fiber guides (Kim *et al.*, 2009). Continuous wave near infrared light was delivered to the rabbit forearm using a fiber optic probe (9mm source-detector separation), and transmitted light intensities at five wavelengths (732, 758, 805, 840, 880 nm) were measured using the CCD spectrometer every second. We quantified changes in oxy- and deoxyhemoglobin concentrations throughout the experiment using a modified Beer-Lamberts' law and those changes are displayed in real time using Labview software (Labview 7.1, National Instrument, TX) (Kim *et al.*, 2009).

Measurement of Red Blood Cell (RBC) Cyanide Concentration

Cyanide in blood is bound almost exclusively to ferric (met) hemoglobin in RBCs; thus, blood cyanide can be measured by separating RBCs from plasma, and acidifying the RBCs to release cyanide as HCN gas (Lundquist *et al.*, 1985). Immediately after drawing blood from the rabbits, the samples were cooled to 4°C, centrifuged, and the plasma and RBC fractions separated. All samples were sealed and kept at 4°C for analysis. For analysis, the RBCs were lysed in ice-cold water. The lysates were placed into the outer chamber of Conway diffusion cells (Bel-Art Products, Pequannock, NJ) adjacent to an equal volume of 10% trichloroacetic acid. The inner chambers contained collecting solution of 0.01 mM cobinamide in 0.1 M NaOH. The trichloroaetic acid and lysate were mixed in the outer cell chamber after sealing the polypropylene covers to the cells with vacuum grease (Dow Corning). Time of diffusion was 25 min at 24 °C. Cyanide bound to cobinamide in 0.1 M NaOH was measured by spectrophotometric assay, using a ratio method of 366 nm / 505 nm recently developed. (Blackledge et al., 2010). Concentrations were determined from standard curves using freshly prepared KCN dissolved in 0.1 M NaOH. Duplicate samples showed < 15% variation.

Statistical Methods

Baseline statistics across groups were compared using analysis of variance (ANOVA). Response to treatment across the groups was compared using repeated measures ANOVA for cyanide level comparisons. Time constants for changes in tissue hemoglobin oxygenation parameters obtained from DOS and CWNIRS were compared using ANOVA. A p value <0.05 was considered significant. All data were analyzed using a standard statistical package (Systat-12, Systat Software, Inc., Chicago, IL 60606).

Results

DOS monitoring of peripheral muscle region cyanide toxicity and recovery

The deoxygenated hemoglobin concentration in peripheral muscle fell progressively during the 60 minutes of cyanide infusion [Fig. 2a; fractional changes in deoxyhemoglobin (deoxyhemoglobin) concentration from the baseline (pre cyanide infusion) concentration are shown for a representative animal]. A fall in deoxygenated hemoglobin would be expected, because of cyanide inhibition of cellular respiration and the concomitant decrease in tissue oxygen consumption. When the cyanide infusion was stopped at 60 minutes, the deoxyhemoglobin concentration slowly returned over >60 minutes towards baseline values in control (non-sulfanegen sodium-treated) animals (Fig. 2a, grey line). The rate of return of deoxyhemoglobin levels in the sulfanegen sodium-treated animals was much faster than in control animals, particularly in the first 10 minutes after the intramuscular injection, which would be a critical time period for recovery (Fig. 2a, black line).

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Changes in deoxyhemoglobin concentrations for all animals during the recovery/treatment phase are shown in Fig. 2b, which illustrates the progression of fractional change in deoxyhemoglobin after completion of the cyanide infusion, during the 60 minutes following injection of normal saline (control) or sulfanegen sodium. Post cyanide infusion, immediately prior to treatment administration, the absolute fractional deoxyhemoglobin values of the control and treatment animals were similar (mean −23.7 versus −28.7 %; difference in means 5; p = .56). The deoxyhemoglobin concentration at the completion of the cyanide infusion and start of the treatment period is designated as time zero, and is the starting point for the recovery period data analysis for calculation of % return to pre-cyanide baseline for recovery time constant analysis. From the data in Fig. 2b, the time constant for recovery of deoxygenated hemoglobin to baseline, pre-cyanide poisoning levels is calculated as (~ (1-exp(-t/τ), where τ is a time constant). τ from fittings are listed below in Table 2 to determine the effective "time constant" to recovery where τ is the classic time to recovery of an exponential decay fit curve to 63.2% of baseline (Kim and Liu, 2008). Using this formula, the time constant for recovery of deoxygenated hemoglobin was a mean of 100 minutes in control animals, compared to 38 minutes for sulfanegen sodium-treated animals (0.05 mmol IM); a difference of 62 minutes ($p < 0.01$).

CWNIRS monitoring of peripheral muscle region cyanide toxicity and recovery

CWNIRS monitoring over the forearm muscle region yielded analogous results to DOS monitoring over muscle. Specifically, sulfanegen sodium markedly increased the rate of recovery of muscle region oxy- and deoxyhemoglobin toward baseline values (Fig. 3a and 3b). The time constant for recovery of deoxyhemoglobin concentrations was a mean > 350 minutes in control animals and 13 minutes in sulfanegen sodium treated animals (IV_0.05 mmol) with a difference of > 300 minutes (p = 0.01) (Table 2). In the critical first 10 minutes after the end of the cyanide infusion when sulfanegen sodium was injected (IV_0.05 mmol), the slope of recovery of the deoxyhemoglobin concentration was ten times greater in the sulfanegen sodium-treated animals than in controls (Table 2). The time constant for returning oxyhemoglobin concentrations was in a mean >70 minutes in control animals and 9 minutes for sulfanegen sodium-treated animals (IV_0.05mmol) with a difference of > 60 minutes (p = 0.02) (Table 2). Again, the rate of return of the oxyhemoglobin concentration during the first 10 minutes after stopping the cyanide infusion was almost eight times greater in the sulfanegen sodium-treated animals (IV_0.05mmol) than in control animals (Table 2).

Red blood cell cyanide concentrations

The difference in rate of decrease in concentration of cyanide in red blood cells was statistically significant following intramuscular or intravenous sulfanegen sodium injection when compared to control animals (Fig. 4). At 30 minutes, cyanide concentrations had fallen to a mean 48 % of peak values in sulfanegen sodium-treated animals (IV 0.05mmol) compared to 83 % of peak levels remaining in control animals with a difference of 35 %.

Dose effects on hemodynamic recovery rate

DOS and CWNIRS were used to non invasively monitor the recovery rate of oxy- and deoxyhemoglobin concentration with 4 different doses of sulfanegen sodium as shown in Table 1 in order to determine the effectiveness of sulfanegen sodium treatment via IV and IM adminstration. Figure 5 shows the change of 10 min slope values from oxy- and deoxyhemoglobin concentration during recovery with four different doses of sulfanegen sodium administration. For the sulfanegen sodium IV treatment groups, both oxy and deoxyhemoglobin recovery rates are reaching plateau in effectiveness at 0.025 mmol dose, with little further improvement in recovery rate with the 0.05 mmol dose in this model. Compared to IV administration groups, IM administration groups show a more linear improvement of recovery rate with dose escalation (Fig. 5c). This implies that

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administration of even greater than 0.21 mmol sulfanegen sodium via IM might possibly improve recovery from cyanide toxicity.

Comparison between IV and IM

We compared the recovery rate of oxy and deoxyhemoglobin concentration between IV and IM administration of sulfanegen sodium. The highest dose we used for IV administration was 0.05 mmol and the same dose was applied to IM administration of sulfanegen sodium. However, as can be seen in Table 2, the recovery rates of oxy and deoxyhemoglobin from 0.05 mmol IM group were slower than those from 0.05 mmol IV group. Therefore, we increased the dose for IM administration as shown in table 1, and found that 0.21 mmol of IM administration results in similar recovery rates of oxy- and deoxyhemoglobin as found in 0.05 mmol IV group ($p>0.05$). Compared to the control group, all sulfanegen sodium treated groups (IV_0.05mmol, IM_0.05mmol, and IM_0.21mmol) showed significantly faster recovery of oxy and deoxyhemoglobin (except for oxyhemoglobin recovery between the control and 0.05 mmol IM sulfanegen sodium groups).

Total Tissue Hemoglobin Levels

Total tissue hemoglobin levels measured by DOS did not change significantly during the post sulfanegen sodium administration (0.05mmol IV) recovery time or in control animals during the recovery period ($p > 0.10$).

Blood pressure

There was no difference in systolic, diastolic, or mean blood pressure in the sulfanegen sodium treated animals compared to control animals at any time during the study. Prior to initiation of cyanide infusion, systolic blood pressures were in a mean of 91 mmHg and 94 mmHg in the sulfanegen sodium-treated (0.05 mmol IV) and control groups, respectively. At 5 and 45 minutes following intravenous injection of 0.05 mmol sulfanegen sodium, systolic blood pressure was 79 mmHg and 75 mm Hg versus 82 mmHg and 73 mmHg in sulfanegen sodium-treated versus control animals, respectively ($p > 0.20$ for differences between control and sulfanegen sodium-treated animals).

Preliminary sulfanagen toxicity investigations in mice

Preliminary studies were performed at the University of Minnesota (SP, U Minn ARC approved) to screen for possible toxicities. Sulfanegen was dissolved in sterile saline and the dose administered in two intramuscular injections per animal, one into each leg. There were four mice per group in three groups (n=12). A high dose of 8.9 mmoles/kg (4789 mg/kg), was given first, followed by a low dose of 2.5 mmoles/kg (1320 mg/kg). The mice were watched closely for two hrs following the injections and then checked daily for one week, and surviving animals were sacrificed at 2 weeks post exposure. At sacrifice (or death) blood samples were taken by cardiac puncture.

Our range finding plan dictated administration of increasing doses from the lowest dose until we reached LD50. Our first increased dose of 3.6 mmoles/kg (1939 mg/kg) achieved that goal. Death, when it occurred, was within 5 min of the injections. ALT and creatinine levels were measured and in all cases were normal. Full toxicity studies will be performed when the final drug formulations are determined.

Discussion

In this study, sulfanegen sodium was shown to reverse the effects of cyanide exposure on oxyhemoglobin and deoxyhemoglobin rapidly, significantly faster than control animals when administered by either intravenous or intramuscular routes. Intravenous administration

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resulted in faster return to baseline than intramuscular administration at an equal dose, as expected. In addition, RBC cyanide levels also returned to normal more quickly with both intramuscular and intravenous sulfanegen sodium treatment than controls. These studies demonstrate the clinical potential for the novel approach of supplying substrate for non-rhodanase mediated sulfur transferase pathways for cyanide detoxification. Whether mechanisms in addition to 3- mercaptopyruvate sulfur transferase pathways are involved in the sulfanegen sodium cyanide physiologic effect reversal process seen in these animals is not known (Crankshaw *et al.*, 2007; Nagasawa *et al.*, 2007).

Potential advantages of sulfanegen sodium-based treatment approaches for cyanide toxicity include the ability to administer treatment in patients with combined carbon monoxide and cyanide poisoning, since no reduction in oxygen carrying capacity is expected. In addition, sulfanegen sodium could likely be administered with other antidotes such as hydroxocobalamin (if no unforeseen drug interactions occur), since their mechanisms of action involve different biochemical pathways. It is possible that combination therapies with more than one antidote may enable reversal of more serious toxic exposures than single agent treatments can provide.

Previous studies have discussed pathways for reversing cyanide toxicity and proposed sulfanegen sodium as a candidate substrate for enhancing sulfur transferase detoxification of cyanide (Westley *et al.*, 1983; Crankshaw *et al.*, 2007; Nagasawa *et al.*, 2007). This is the first reported study in animals to investigate the rate of reversal of the physiologic effects of cyanide exposure using sulfanegen sodium with non-invasive optical technologies to demonstrate more rapid return to baseline of RBC cyanide levels. Although there are a number of effective treatments for cyanide toxicity, none of the currently available treatments can be administered intramuscularly, and are therefore not applicable to mass exposure treatment settings. There are highly effective intravenous agents available, including FDA approved hydroxycobalamin. A prior manuscript that demonstrated the effectiveness of hydroxycobalamin in reversing cyanide poisoning in the rabbit model using the same method used in this study {Brenner, 2010 #819}. Our study with sulfanegen indicates it is highly effective by both intravenous and intramuscular routes of administration compared to hydroxycobalamin treatment. For these reasons, further development of sulfanegen sodium related formulations may provide a significant advance in cyanide toxicity treatment.

There are a number of limitations to this study. Some are inherent to the model system and optical monitoring technology as previously discussed (Lee *et al.*, 2007a; Brenner *et al.*, 2009; Lee *et al.*, 2009). A major goal of this program is to begin the development of an intramuscular antidote for treatment of mass casualty cyanide poisoning. While an intramuscular injection may be ideal for mass casualty settings, intravenous administration of a cyanide antidote may be preferable for individual exposures because of potentially more rapid systemic distribution. However, the time required to establish intravenous access could offset the advantage of faster distribution of intravenous compared to intramuscular drug administration. Further studies will be needed to address these questions.

As was shown in this manuscript, there is a relationship between the physiologic disturbances detected by near infrared spectroscopy methods and blood cyanide levels. However, there is substantial variability in sensitivity to cyanide in animals. Similar variability in sensitivity of human beings to cyanide exposure is also well documented. Blood cyanide levels also vary widely in relationship with the extent of cyanide toxicity in animals as well as patients. Thus, blood or red blood cell cyanide levels may not be “gold standard” for determining the degree of cyanide toxicity and the effectiveness of antidote therapies. Sulfanegen sodium intramuscular injection was demonstrated to rapidly reverse

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the physiologic effects of cyanide exposure following a sublethal cyanide dose in this animal model study. However, due to the limitations of solubility of sulfanegen sodium in aqueous media, we calculate that a volume of greater than 20 ml of maximally concentrated sulfanegen sodium solution would be required to neutralize one lethal dose of cyanide in a 70 kg person. In order to be able to reverse a twice lethal level exposure, more than 40 ml would have to be injected intramuscularly. This large volume is not practical for auto injectors or intramuscular administration. Therefore, other analogues or formulations of sulfanegen sodium with higher aqueous solubility, enabling more concentrated antidote with lower injection volumes should be investigated as alternative approaches. A number of potential prodrug core structures have been proposed (Crankshaw *et al.*, 2007; Nagasawa *et al.*, 2007). Although a systematic investigation of the leading candidate agents will be needed in order to develop an optimal mass casualty cyanide treatment, this study demonstrates the potential of the alternative sulfertransferase enzyme system approach for detoxificiation of cyanide.

The potential role for near infrared spectroscopy-based diagnostics including DOS and CWNIRS in mass casualty cyanide poisoning may involve model and treatment research and development, field diagnostics, and therapeutic monitoring. The hardware and software utilized in these studies could be miniaturized and developed into field deployable microchip and laser diode-based monitors. However, there may be limitations in being able to definitively diagnose CN poisoning in individuals when baseline measurements prior to toxicity are not available. In mass casualty exposures, once one victim has been diagnosed with cyanide poisoning, it may be of little value to diagnose the cause in the remaining victims, since the cause will likely be the same. Thus, the role in mass casualty diagnostics may be limited by both need and capabilities. However, for therapeutic monitoring, a potential role for DOS and CWNIRS portable monitoring may be envisioned where small, inexpensive monitors could be used to titrate antidote agent effects in real-time until adequate physiologic response is seen.

Utilizing DOS and CWNIRS based hemoglobin oxygenation as the major outcome indicator for cyanide toxicity reversal does not prove efficacy in lethal cyanide poisoning, since this is not a lethal model. Therefore, we cannot draw conclusions regarding effectiveness of sulfanegen sodium in treating lethal cyanide poisoning from this study. Animals were anesthetized in compliance with animal welfare regulations; the number of animals studied was limited, as was the duration of follow-up, preventing subtle toxicities from cyanide or sulfanegen sodium from being detected. As this is a sub-lethal model, determining whether similar beneficial effects and complete toxicity reversal will be seen in a higher dose lethal model will also require subsequent investigations. Many of the physiologic changes we monitored during these studies are not specific to cyanide poisoning, and in the face of additional pathologic conditions, may be limited in their capabilities to accurately assess the CN poisoning and reversal. This may be most particularly important in the case of combined cyanide toxicity and carbon monoxide poisoning, which is commonly clinically encountered. Further studies will be needed to define the role in such situations.

DOS and CWNIRS measure average tissue constituents to a depth of approximately 4 mm (at the source detector separations used in these studies) (Lee *et al.*, 2007a). Deeper tissue effect and organ specific toxicities cannot be assessed using the current study design. Furthermore, as with all near-infrared optical absorption technologies, other potential optically interfering agents, or medical conditions that might alter blood oxygenation levels could affect the ability to diagnose and monitor cyanide toxicity, including concurrent carbon monoxide and cyanide toxicity states that may be encountered in smoke inhalation victims.

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Even with the limitations mentioned above, however, DOS and CWNIRS demonstrated their usefulness in optimizing the dose of sulfanegen sodium treatment. The recovery rate of oxy- and deoxyhemoglobin were shown to be faster with the increase of sulfanegen sodium dose from both IV and IM administration. We found that there may not be further improvement in recovery rate at doses higher than 0.05mmol of sulfanegen sodium via IV administration in this model, while the recovery through IM administration can be possibly improved by increasing the dose to greater than 0.21 mmol. As mentioned in the introduction, IM administration is a preferred administration approach for treatment of mass casualty cyanide exposure victims.

In summary, we have demonstrated the ability of sulfanegen sodium to rapidly reverse the physiologic effects of cyanide exposure in a rabbit model using noninvasive optical monitoring and confirmed by conventional red blood cell cyanide levels and arterial blood gas analysis. This novel approach exploits sulfur transferase enzyme systems that may be more abundant and better distributed than thiosulfate rhodanase–based substrate systems (Nagasawa *et al.*, 2007; Brenner *et al.*, 2009). Obviously, no single species or single model system can definitively define all of the treatment capabilities, limitations, and toxicities of a cyanide treatment agent. Only through a combination of investigations of lethal, and sublethal models, various injuries and doses, and timing of administration of antidotes will confident understanding of treatment be provided. Since the FDA requires a minimum of two distinct animal species to be studied extensively for GLP efficacy, pharmacokinetics, pharmacodynamics, and toxicity studies before the approval consideration, future studies with other animal models are needed.

We have demonstrated that intramuscular injection is feasible with potential for development of mass casualty treatment approaches. Future studies utilizing more soluble analogues to enable increased intramuscular dose delivery and animal models using higher, lethal level cyanide exposures will be needed to fully develop a mass casualty cyanide poisoning treatment.

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References

Alexander K, Baskin SI. The inhibition of cytochrome oxidase by diaminomaleonitrile. Biochim Biophys Acta. 1987; 912:41–47. [PubMed: 3030429]

Associated-Press. The Orange County Register. Santa Ana, CA: 2009. Al-Qaida suspect used Hotmail in planning; p. 1

Baskin, SI.; Brewer, TG. Medical aspects of chemical and biological warfare. Chapter 10, Cyanide Poisoning. In: Sidell, FR.; Takafuji, ET.; Franz, DR.; Borden Institute (U.S.). , editors. Textbook of military medicine. Part I, Warfare, weaponry, and the casualty. Borden Institute, Walter Reed Army Medical Center ; Office of the Surgeon General, U.S. Army ; U.S. Army Medical Dept. Center and School ; U.S. Army Medical Research and Material Command ;Uniformed Services University of the Health Sciences; Washington, D.C.: 1997. p. 272-286.

Baskin, SI.; Horowitz, AM.; Nealley, EW. J Clin Pharmacol. Vol. 32. 1992. The antidotal action of sodium nitrite and sodium thiosulfate against cyanide poisoning; p. 368-375.

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Bhatt HR, Linnell JC. Vitamin B12 homoeostasis after haemorrhage in the rat: the importance of skeletal muscle. Clin Sci (Lond). 1987; 73:581–587. [PubMed: 3690974]

Blackledge WC, Blackledge CW, Griesel A, Mahon SB, Brenner M, Pilz RB, Boss GR. New Facile Method to Measure Cyanide in Blood. Anal. Chem. 2010; 2010(82):4216–4221. [PubMed: 20420400]

Borron SW. Recognition and treatment of acute cyanide poisoning. J Emerg Nurs. 2006; 32:S12–18. [PubMed: 16860672]

Borron SW, Baud FJ. Acute cyanide poisoning: clinical spectrum, diagnosis, and treatment. Arh Hig Rada Toksikol. 1996; 47:307–322. [PubMed: 8989894]

Brenner M, Kim JG, Mahon SB, Lee J, Kreuter KA, Blackledge W, Mukai D, Patterson S, Mohammad O, Sharma VS, Boss GR. Intramuscular Cobinamide Sulfite in a Rabbit Model of Sublethal Cyanide Toxicity. Ann Emerg Med. 2009

Crankshaw DL, Goon DJ, Briggs JE, DeLong D, Kuskowski M, Patterson SE, Nagasawa HT. A novel paradigm for assessing efficacies of potential antidotes against neurotoxins in mice. Toxicol Lett. 2007; 175:111–117. [PubMed: 18024011]

Cummings TF. The treatment of cyanide poisoning. Occup Med (Lond). 2004; 54:82–85. [PubMed: 15020725]

Eckstein M, Maniscalco PM. Focus on smoke inhalation--the most common cause of acute cyanide poisoning. Prehosp Disaster Med. 2006; 21:s49–55. [PubMed: 16771012]

Gracia R, Shepherd G. Cyanide poisoning and its treatment. Pharmacotherapy. 2004; 24:1358–1365. [PubMed: 15628833]

Guilbault G, Kramer D. Ultrasensitive, specific method for cyanide using p-notrobenzaldehyde and o-dintrobenzene. Anal Chem. 1966; 28:834–836.

Hall AH, Saiers J, Baud F, ric. Which cyanide antidote? Critical Reviews in Toxicology. 2009; 39:541–552. [PubMed: 19650716]

Hol WG, Lijk LJ, Kalk KH. The high resolution three-dimensional structure of bovine liver rhodanese. Fundam Appl Toxicol. 1983; 3:370–376. [PubMed: 6357922]

Irwin, RS.; Rippe, JM. Irwin and Rippe's intensive care medicine. Lippincott Williams & Wilkins; Philadelphia, PA: 2007a. Section 3: Cardiovascular Problems and Coronary Care.

Irwin, RS.; Rippe, JM. Irwin and Rippe's intensive care medicine. Lippincott Williams & Wilkins; Philadelphia, PA: 2007b. Section 4: Pulmonary Problems in the intensive care unit.

Jarabak R, Westley J. 3-Mercaptopyruvate sulfurtransferase: rapid equilibrium-ordered mechanism with cyanide as the acceptor substrate. Biochemistry. 1980; 19:900–904. [PubMed: 6928374]

Keim, M. Terrorism involving cyanide: the prospcet of improving preparedness in the prehospital settting. Prehospital and Disaster Medicine. 2006. Web Article: http://pdm.medicine.wisc.edu/Volume_21/issue_22/keim.pdf

Kim JG, Lee J, Roe J, Tromberg BJ, Brenner M, Walters TJ. Hemodynamic changes in rat leg muscles during tourniquet-induced ischemia-reperfusion injury observed by near-infrared spectroscopy. Physiol Meas. 2009; 30:529–540. [PubMed: 19436084]

Kim JG, Liu H. Investigation of biphasic tumor oxygen dynamics induced by hyperoxic gas intervention: the dynamic phantom approach. Appl. Opt. 2008; 47:242–252. [PubMed: 18188206]

Lee J, Armstrong J, Kreuter K, Tromberg BJ, Brenner M. Non-invasive in vivo diffuse optical spectroscopy monitoring of cyanide poisoning in a rabbit model. Physiol Meas. 2007a; 28:1057–1066. [PubMed: 17827653]

Lee J, Cerussi AE, Saltzman D, Waddington T, Tromberg BJ, Brenner M. Hemoglobin measurement patterns during noninvasive diffuse optical spectroscopy monitoring of hypovolemic shock and fluid replacement. J Biomed Opt. 2007b; 12:024001. [PubMed: 17477716]

Lee J, Keuter K, Kim J, Tran A, Uppal A, Mukai D, Mahon SB, Cancio L, Batchinsky A, Tromberg B, Brenner M. Noninvasive In Vivo Monitoring of Cyanide Toxicity and Treatment Using Diffuse Optical Spectroscopy in a Rabbit Model. Military Medicine. 2009; 174:615–621. [PubMed: 19585775]

Lee PA, Sylvia AL, Piantadosi CA. Cyanide-related changes in cerebral O2 delivery and metabolism in fluorocarbon-circulated rats. Toxicol Appl Pharmacol. 1988; 94:34–44. [PubMed: 2836966]

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Lundquist P, Rosling H, Sorbo B. Determination of cyanide in whole blood, erythrocytes, and plasma. Clin Chem. 1985; 31:591–595. [PubMed: 3978792]

Maniscalco, PM. From Smoke Inhalation to Chemical Attacks: Acute Cyanide Poisoning in the Prehospital Setting. Prehospital and Disaster Medicine. 2006. Web Article: http://pdm.medicine.wisc.edu/Volume_21/issue_22/maniscalco.pdf

Martin CO, Adams HP Jr. Neurological aspects of biological and chemical terrorism: a review for neurologists. Arch Neurol. 2003; 60:21–25. [PubMed: 12533084]

Merritt S, Gulsen G, Chiou G, Chu Y, Deng C, Cerussi AE, Durkin AJ, Tromberg BJ, Nalcioglu O. Comparison of water and lipid content measurements using diffuse optical spectroscopy and MRI in emulsion phantoms. Technol Cancer Res Treat. 2003; 2:563–569. [PubMed: 14640767]

Morocco AP. Cyanides. Crit Care Clin. 2005; 21:691–705. vi. [PubMed: 16168309]

Nagasawa HT, Goon DJ, Crankshaw DL, Vince R, Patterson SE. Novel, orally effective cyanide antidotes. J Med Chem. 2007; 50:6462–6464. [PubMed: 18038966]

Pan T, Rasmussen JC, Lee JH, Sevick-Muraca EM. Monte Carlo simulation of time-dependent, transport-limited fluorescent boundary measurements in frequency domain. Med Phys. 2007; 34:1298–1311. [PubMed: 17500461]

Panda M, Robinson NC. Kinetics and mechanism for the binding of HCN to cytochrome c oxidase. Biochemistry. 1995; 34:10009–10018. [PubMed: 7632673]

Sylvester M, Sander C. Immunohistochemical localization of rhodanese. Histochem J. 1990; 22:197–200. [PubMed: 2387754]

Westley J, Adler H, Westley L, Nishida C. The sulfurtransferases. Fundam Appl Toxicol. 1983; 3:377–382. [PubMed: 6357923]

Zijlistra, WG.; Buursma, A.; Assendelft, OWV. Visible and Near Infrared Absorption Spectra of Human and Animal Haemoglobin: Determination and Application. VSP; 2000.

Figure 1.

Structure of sulfanegen sodium and enzymatic transfer of sulfur from sulfanegen sodium by 3-mercaptopurine sulfur transferase to pyruvate with conversion of cyanide to thioscyanate (Westley *et al.*, 1983).

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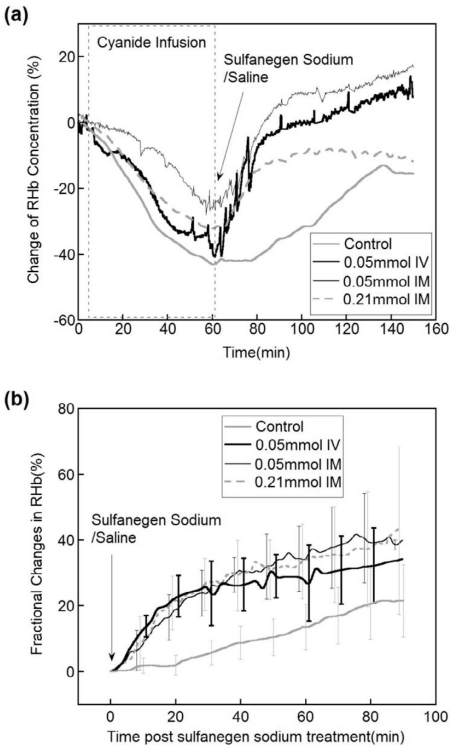

Figure 2.
(a) Percent changes of deoxyhemoglobin during CN infusion and post sulfanegen sodium treatment from the representative animals and (b) the averaged fractional changes during sulfanegen sodium or saline administration.

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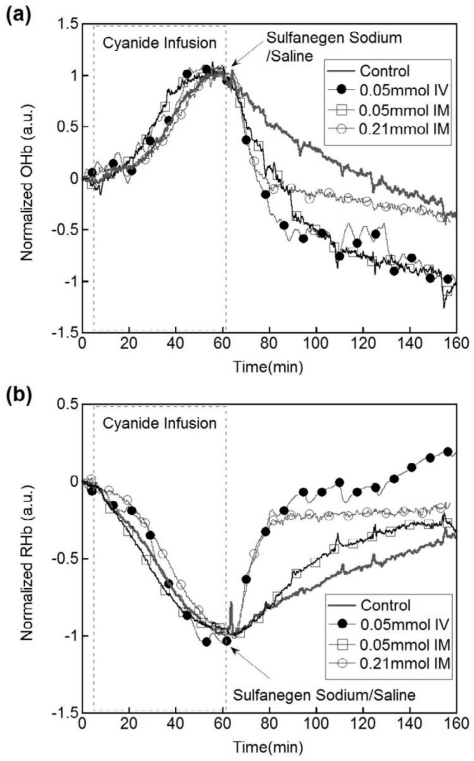

Figure 3.

Normalized changes of (a) oxyhemoglobin (OHb) and (b) deoxyhemoglobin (RHb) during cyanide infusion and saline/sulfanegen sodium treatment measured by CWNIRS. Both OHb and RHb from all sulfanegen sodium treated groups showed a faster recovery compared to control group. There was no significant difference of recovery rate between 0.05 mmol IV group and 0.21 mmol IM group.

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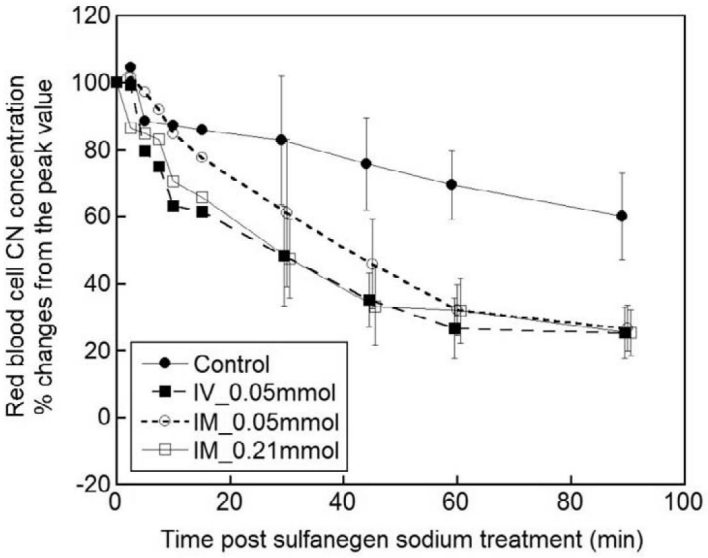

Figure 4.

Figure 4 shows cyanide levels in red blood cell (RBC) post administration of sulfanegen sodium or saline. For clarity, the error bars up to 20min post sulfanegen sodium administration are not shown. All groups that were treated with sulfanegen sodium showed significantly faster reduction of RBC cyanide level compared to the control. sulfanegen sodium 0.05mmol IM group showed a faster recovery from cyanide poisoning than the control, but was slower in recovery compared to 0.05mmol IV and 0.21mmol IM groups.

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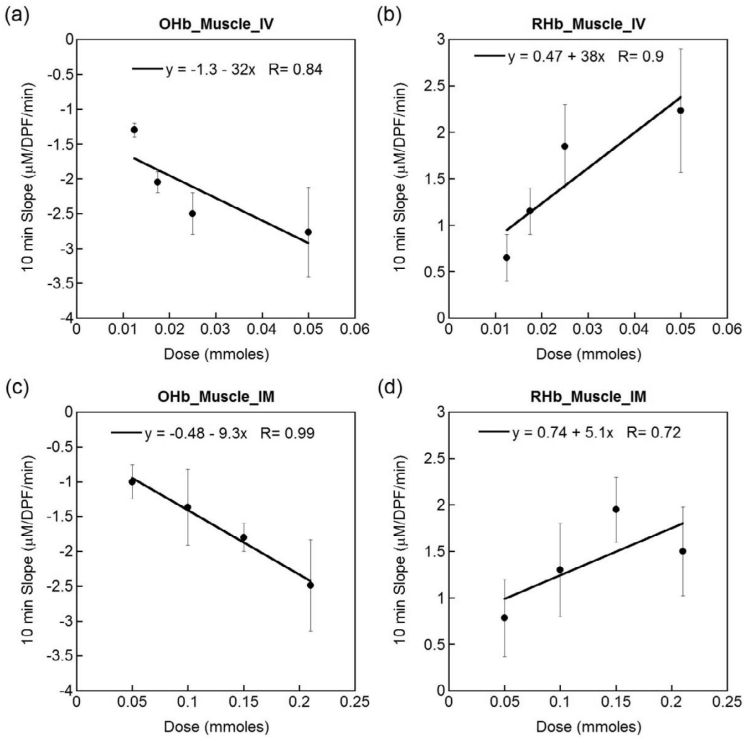

Figure 5.

Correlation between OHb or RHb slope during first 10min post sulfanegen sodium administration and sulfanegen sodium dose. Top two figures (and b) are from sulfanegen sodium IV administration and bottom two figures (c and d) are from sulfanegen sodium IM administration.

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Table 1

Sulfanegen sodium dose and number of animals used for this study.

IM Trials

Sulfanegen Sodium (mg)	Sulfanegen Sodium (mmol)	Solution	Number of animal(s)
17.8	0.05	1cc 0.9%NaCl	6
35.6	0.10	1cc 0.9%NaCl	2
53.4	0.15	1.5cc 0.9%NaCl	2
74.8	0.21	1.5cc 0.9%NaCl	6

IV Trials

Sulfanegen Sodium (mg)	Sulfanegen Sodium (mmol)	Solution	Number of animal(s)
4.45	0.0125	5cc 0.9%NaCl	2
6.23	0.0175	5cc 0.9%NaCl	2
8.9	0.025	5cc 0.9%NaCl	2
17.8	0.05	5cc 0.9%NaCl	6

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Table 2

Time constant and 10 min slope values from control and sulfanegen sodium treated groups obtained from DOS (top) and CWNIRS (bottom). A "*" represents that there is no significant difference between IV_0.05mmol and IM_0.21mmol groups ($p>0.05$).

DOS

Time Constant (min)	Control	IV_0.05mmol	IM_0.05mmol	IM_0.21mmol
Deoxyhemoglobin	100 ± 24	19.0 ± 6.9*	38.8 ± 9.5	18.7 ± 10.7*

CWNIRS

Time Constant (min)	Control	IV_0.05mmol	IM_0.05mmol	IM_0.21mmol
Deoxyhemoglobin	357 ± 270	12.7 ± 4.8*	37.1 ± 16.6	12.4 ± 3.3*
Oxyhemoglobin	73.0 ± 43.8	9.1 ± 4.5*	28.9 ± 8.3	14.1 ± 5.8*

10 min Slope (μM/DPF/min)	Control	IV_0.05mmol	IM_0.05mmol	IM_0.21mmol
Deoxyhemoglobin	0.20 ± 0.13	2.23 ± 0.66*	0.80 ± 0.42	1.50 ± 0.48*
Oxyhemoglobin	−0.98 ± 0.51	−2.77 ± 0.64*	−0.99 ± 0.24	−2.49 ± 0.66*