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**Abstract.** Noninvasive near infrared spectroscopy measurements were performed to monitor cyanide (CN) poisoning and recovery in the brain region and in foreleg muscle simultaneously, and the effects of a novel CN antidote, sulfanegen sodium, on tissue hemoglobin oxygenation changes were compared using a sub-lethal rabbit model. The results demonstrated that the brain region is more susceptible to CN poisoning and slower in endogenous CN detoxification following exposure than peripheral muscles. However, sulfanegen sodium rapidly reversed CN toxicity, with brain region effects reversing more quickly than muscle. *In vivo* monitoring of multiple organs may provide important clinical information regarding the extent of CN toxicity and subsequent recovery, and facilitate antidote drug development. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.10.105005]

Keywords: cyanide poisoning; metabolism; diffuse optical spectroscopy; sulfanegen sodium; brain; muscle; cyanide antidote.

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## 1 Introduction

Accidental exposure to cyanide (CN) in fires and industrial accidents is a continual concern, and intentional CN poisoning by terrorists could lead to mass casualties. CN poisoning induces lethal histotoxic anoxia by a number of mechanisms, including inhibition of aerobic cell metabolism by disabling the function of cytochrome c oxidase in mitochondria.<sup>1,2</sup> As a result, cells consume less oxygen. Therefore, more oxygen remains in the circulating blood within the tissues. We have shown that continuous-wave near-infrared spectroscopy (CWNIRS) can be used to indirectly monitor CN poisoning and recovery *in vivo* by measuring the changes in tissue hemoglobin oxygenation,<sup>3-9</sup> even though (CWNIRS) cannot directly measure the level of CN in tissues. We now utilize these capabilities *in vivo* to demonstrate (1) differences in the sensitivity of a vital organ (central nervous system/brain region) to CN toxicity, (2) differences in rate and extent of response to a novel CN antidote treatment agent in the brain region compared to periphery, and (3) that CWNIRS technology can be used as an enabling tool for novel CN antidote drug development.

It has been known that CN toxicity sensitivity varies amongst organs.<sup>1,10</sup> Brain and heart are particularly susceptible to CN toxicity. Nagahara et al., showed the ratios of specific sulfur transferase activities of two CN detoxifying enzymes, rhodanese and 3-mercaptopyruvate sulfurtransferase (3-MPST) in different

organs of rats.<sup>10,11</sup> The low concentrations of these CN detoxifying enzymes may explain some of the increased susceptibility of brain tissue to CN toxicity.<sup>1,10</sup> The levels of 3-MPST sulfur transferase activities were shown to be twice as high as rhodanese levels in cerebellum and 30% higher in cerebral tissue. However, there are few reports comparing different rates and extent of CN toxicity and recovery between brain and other organs *in vivo*.

In contrast to rhodanese which is localized within mitochondria, 3-MPST exists in both cytoplasm and mitochondria.<sup>10,12,13</sup> MPST assays on rat tissues showed that 70% of the MPST activity is found in the cytosol, while the rest is found within mitochondria.<sup>12</sup> Since sulfur transferase detoxification may be substrate-limited under severe CN loads, we propose that it may be possible to try to develop a CN antidote based on 3 methyl pyruvate (3-MP) which is a substrate of 3-MPST. 3-MPST catalyzes the transfer of sulfur from 3-MP to CN, forming pyruvate and less toxic thiocyanate, which is subsequently excreted in the urine.<sup>14,15</sup> However, 3-MP rapidly decomposes in the blood, making it ineffective when administered intravenously. This instability could potentially be counteracted by making the 3-MPST natural substrate, 3-mercaptopyruvate (3-MP), continuously available. To accomplish this, we have developed a water soluble (158 mg/ml) 3-MP prodrug, 3-mercaptopyruvate-dithiane (sulfanegen sodium: 2, 5-dihydroxy-1,4-dithiane-2, 5-dicarboxylic acid disodium salt).<sup>15,16</sup> We hypothesize that sulfanegen could thus overcome the sulfur donor substrate limitations for the sulfur transferase 3-MPST, leading to CN

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detoxification, with particular benefit in the vital CNS region where rhodanese is less prevalent than 3-MPST.

In this study, we utilized simultaneous noninvasive *in vivo* CWNIRS measurements of both the brain region and foreleg muscle regions during CN poisoning to monitor rate and extent of toxicity effects and responses to sulfanegen sodium treatment. The differential effects between the brain region and muscle were compared as manifested by dynamics of circulating tissue hemoglobin oxygenation changes.

## 2 Materials and Methods

### 2.1 Continuous-Wave Near-Infrared Spectroscopy

The CWNIRS system we constructed consists of a 20-W tungsten-halogen white light source (HL2000-HP, Ocean Optics Inc., Dunedin, FL), 16-bit CCD spectrometer (BTC111E, B&W TEK, Inc., Newark, DE, or USB4000, Ocean Optics) as a light detector, and customized 1.25-mm-diameter fiber bundles (RoMack, Williamsburg, VA).<sup>8,9,17</sup> We utilized two CWNIRS systems in this study to simultaneously monitor CN poisoning and sulfanegen sodium detoxification effects on both brain region and foreleg muscle. The power of light measured at the tip of the probes was 8.5 W for the brain region probe, and 1.8 W for muscle region probe at 690 nm.

### 2.2 Algorithm

If we assume that oxygenated hemoglobin (OHb) and deoxygenated (RHb) hemoglobin are the optically dominant near infrared (NIR) absorbing major molecules in tissues with changing concentrations during the study period that absorb light in the wavelength range from 700 to 900 nm; then Eq. (1), based on Beer Lambert's law, can be used to estimate their concentration changes:

$$(\Delta OD)^\lambda = (\epsilon_{RHb}^\lambda \epsilon_{OHb}^\lambda) \begin{pmatrix} \Delta [RHb] \\ \Delta [OHb] \end{pmatrix} L, \quad (1)$$

where  $\Delta OD^\lambda$  is the change of optical density at wavelength  $\lambda$ ,  $\epsilon_{RHb}(\lambda)$  and  $\epsilon_{OHb}(\lambda)$  are the extinction coefficients at wavelength  $\lambda$  for molar concentrations of [RHb] and [OHb], respectively, and  $L$  is the length of light path through the tissues which is  $d \times DPF$ . DPF represents differential pathlength factor<sup>18</sup> and  $d$  is the source-detector separation. A detailed description of this approach can be found in prior publications.<sup>19,20</sup> For this study, our system acquired reflected light intensity at five wavelengths (730, 785, 805, 830, and 880 nm) and estimated changes of [OHb] and [RHb] in real time using Eq. (2).

$$\begin{pmatrix} \Delta [RHb] \\ \Delta [OHb] \end{pmatrix} = \frac{1}{d \times DPF} \begin{pmatrix} \epsilon_{RHb}^{730} & \epsilon_{OHb}^{730} \\ \epsilon_{RHb}^{785} & \epsilon_{OHb}^{785} \\ \epsilon_{RHb}^{805} & \epsilon_{OHb}^{805} \\ \epsilon_{RHb}^{830} & \epsilon_{OHb}^{830} \\ \epsilon_{RHb}^{880} & \epsilon_{OHb}^{880} \end{pmatrix}^{-1} \begin{pmatrix} \Delta OD^{730} \\ \Delta OD^{785} \\ \Delta OD^{805} \\ \Delta OD^{830} \\ \Delta OD^{880} \end{pmatrix}, \quad (2)$$

However, since the matrix of  $\epsilon$  is not a square matrix, the Moore-Penrose inverse method can be applied to obtain the pseudoinverse matrix of  $\epsilon$  as shown in Eq. (3).<sup>21</sup>

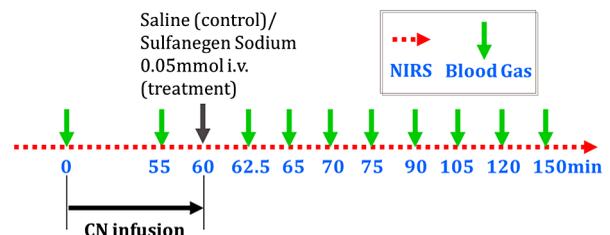
$$\Delta C = \frac{1}{d \times DPF} (\epsilon^T \cdot \epsilon)^{-1} \cdot \epsilon^T \cdot \Delta OD. \quad (3)$$

### 2.3 Animals and Procedures

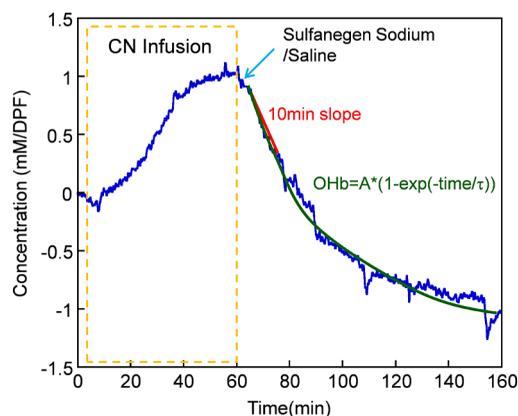
The animal use protocol was reviewed and approved by the University of California Irvine Institutional Animal Care and Use Committee. New Zealand white rabbits (3.5 to 4.5 kg) were divided into two groups, control ( $n = 7$ ) and sulfanegen sodium treatment ( $n = 6$ ) group. Animals were anesthetized 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-in. needle. After the intramuscular injection, a 23-gauge 1-in. catheter was placed in the animals' marginal ear vein to administer continuous IV anesthesia. Torbugesic-SA (butorphanol tartrate, Fort Dodge Animal Health, Fort Dodge, IA), 0.1 to 0.5 mg/kg, was given subcutaneously and then the animals were intubated with a 3.0 mm 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 breaths/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. Femoral venous and arterial lines were placed to provide central venous and arterial access. Blood gas sampling, CN infusion and continuous blood pressure monitoring were accomplished via these lines. Both control and treatment groups were administered 10 mg of NaCN in 60 cc normal saline, infused intravenously at a rate of 1 ml/min for 60 min. After NaCN infusion stopped, the treatment group was administered 0.05 mmol of sulfanegen sodium (5 ml) IV while the control group received 5 ml of saline and animals were monitored for an additional 90 min during recovery (Fig. 1). A CWNIRS probe (1.65-cm source detector separation) was placed on the forehead, and another probe (0.95-cm source detector separation) was placed on the right foreleg muscle. Changes in [OHb] and [RHb] during CN infusion and recovery were simultaneously measured from both brain region and foreleg muscles every second and were compared to each other.

### 2.4 Cyanide Poisoning Recovery Rate

The recovery of [OHb] and [RHb] after ceasing NaCN infusion were fitted using Eq. (4) and time constant,  $\tau$  values were



**Fig. 1** Experimental design timeline for sulfanegen sodium treated animals. CWNIRS measurements were recorded continuously from brain and muscle regions, and blood gas measurements were taken at intervals indicated by the green arrows. Sulfanegen sodium (or saline in controls) was administered IV at the end of the 60 min NaCN infusion.



**Fig. 2** Illustration of the manner in which 10-min slope and time constant values were calculated from a typical CWNIRS plot of [OHb] versus time from forearm muscle in a sulfanegen sodium treated subject.

obtained to compare between brain region and muscle and between control and sulfanegen sodium treated group.<sup>8,9</sup>

$$\Delta[\text{OHb}] \quad \text{or} \quad \Delta[\text{RHb}] = A \left\{ 1 - \exp\left(-\frac{t}{\tau}\right) \right\} \quad (4)$$

where  $A$  is an amplitude of [OHb] or [RHb] changes and  $t$  is time.

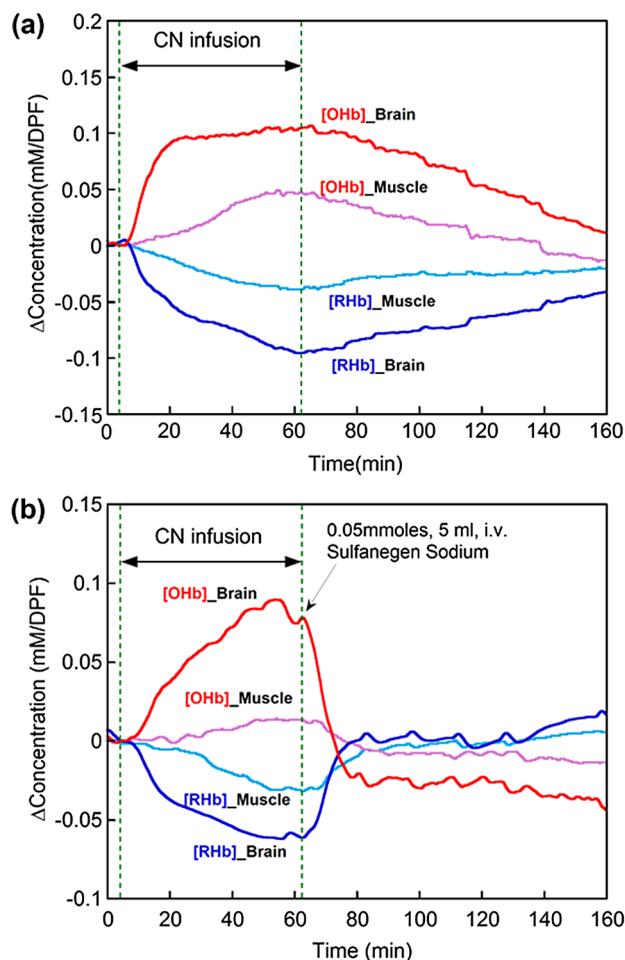
We also obtained the slope value from the first 10 min of the recovery to compare the initial recovery rate between brain region and muscle and also between control and treatment groups.<sup>8,9</sup> Figure 2 illustrates how 10-min slope values and time constant,  $\tau$  values were obtained, in this case from the forearm muscle region.

### 2.5 Statistical Analysis

The obtained time constant values and 10-min slope values during CN recovery were compared between groups and measurement sites by using ANOVA.

## 3 Results

Figure 3(a) shows representative changes of [OHb] and [RHb] during CN poisoning and recovery from the control group (without any CN antidote treatment). [OHb] from both the brain region and foreleg muscle increased, while [RHb] decreased gradually during the CN infusion, as a result of reduced tissue oxygen utilization from inhibition of cytochrome oxidative metabolic pathways and resultant increase in blood oxygen. Conversely, during the recovery period, [OHb] falls and [RHb] rises as the animal undergoes endogenous recovery from CN poisoning. However, a greater magnitude of brain [OHb] and [RHb] change and faster kinetics indicate that brain tissue appears more affected by CN toxicity than the foreleg muscle region. For the quantitative comparison of [OHb] and [RHb] amplitudes, we used differential path length factors (DPF) values of 6.26 for the CNS and 4.16 for the muscle<sup>22</sup> and found that the changes of [OHb] and [RHb] from CNS are much greater than those from muscle during CN poisoning ( $p < 0.01$ , see Table 1). Conversely, during the recovery period, [OHb] and [RHb] changes in brain region were initially faster than those from foreleg muscle as shown by 10-min slope values (see Table 2), but were slower than those from muscle during the



**Fig. 3** Representative [OHb] and [RHb] changes in brain and muscle regions during CN poisoning and recovery from (a) control group subject without any CN antidote treatment, and (b) sulfanegen sodium treatment group subject.

full 90-min recovery period (see Table 3). This may be indicative of the comparably smaller native CN detoxification capacity of brain.

We compared the changes in [OHb] and [RHb] post CN infusion between control and sulfanegen sodium treated groups. Figure 3(b) shows the representative changes of [OHb] and [RHb] from brain and foreleg muscle regions of a sulfanegen sodium treated subject. During NaCN infusion, treated animals showed an increase of [OHb] and a decrease of [RHb] which is consistent with the results from controls. After cessation of NaCN infusion, however, the sulfanegen sodium administered animals showed a rapid reversal of both [OHb] and [RHb]

**Table 1** The maximum amplitudes of oxyhemoglobin and deoxyhemoglobin changes during CN poisoning.

Amplitude ( $\mu\text{M}$ )	CNS	Muscle
Deoxyhemoglobin	$-9.1 \pm 2.7$	$-6.6 \pm 2.1^a$
Oxyhemoglobin	$10.5 \pm 4.2$	$6.2 \pm 2.8^a$

Note: The values are shown as mean  $\pm$  standard deviation.  
<sup>a</sup>( $p < 0.01$  compared to CNS values).

**Table 2** Fitted 10-min slope values during the initial recovery period from CN poisoning.

10-min slope ( $\mu\text{M}/\text{DPF}/\text{min}$ )	Control		Sulfanegen sodium	
	CNS	Muscle	CNS	Muscle
Deoxyhemoglobin	$0.8 \pm 0.4$	$0.2 \pm 0.1^a$	$4.7 \pm 2.1^b$	$2.2 \pm 0.7^{a,b}$
Oxyhemoglobin	$-1.5 \pm 1.1$	$-1.0 \pm 0.5$	$-6.5 \pm 2.1^b$	$-2.8 \pm 0.6^{a,b}$

Note: The values are shown as mean  $\pm$  standard deviation.

<sup>a</sup> $p < 0.05$  compared to CNS values.

<sup>b</sup> $p < 0.01$  compared to the same site in control group).

**Table 3** Fitted time constant values during the 90-min recovery period following CN poisoning.

Time constant (min)	Control		Sulfanegen Sodium	
	CNS	Muscle	CNS	Muscle
Deoxyhemoglobin	$1327 \pm 749$	$357 \pm 270^a$	$4.0 \pm 1.0^b$	$12.7 \pm 4.8^{a,b}$
Oxyhemoglobin	$258 \pm 143$	$73.0 \pm 43.8^a$	$4.3 \pm 1.3^b$	$9.1 \pm 4.5^b$

Note: The values are shown as mean  $\pm$  standard deviation.

<sup>a</sup> $p < 0.05$  compared to CNS values.

<sup>b</sup> $p < 0.01$  compared to the same site in control group).

changes, while the control group showed a much slower recovery of [OHb] and [RHb]. Unlike the control group, the brain region showed a faster recovery time during both initial and 90-min recovery than did muscles after sulfanegen sodium administration (see Tables 2 and 3).

The mean and standard deviation values from fitted time constant and 10-min slope are summarized in Tables 2 and 3. The higher values of 10-min slope and the lower values of time constant imply faster recovery from CN poisoning. In control group animals, the brain region showed a faster initial recovery compared to that of muscle seen by 10-min slope values (RHb: 0.8 versus 0.2  $\mu\text{M}/\text{DPF}/\text{min}$  and OHb:  $-1.5$  versus  $-1.0$   $\mu\text{M}/\text{DPF}/\text{min}$ ). However, time constant values at the later times show different results from early 10-min slope values. Brain region showed a much slower recovery than that of muscle for the long term CN detoxification (RHb: 1327 versus 357 min and OHb: 258 versus 73 min). Compared to control animals, sulfanegen sodium treated animals showed much faster recovery from CN poisoning evidenced by higher 10-min slope values and smaller time constant values of both [OHb] and [RHb]. Interestingly, the brain region recovers from CN poisoning faster than muscles when animals were treated with sulfanegen sodium. This may be due to the higher perfusion in brain so that the delivery of CN antidote to the brain is much faster and greater than muscles. Comparison between control and sulfanegen sodium treated groups showed that [OHb] and [RHb] recovery rates from both CNS and muscle are significantly faster in the treatment group than those from the control group ( $p < 0.01$ ).

#### 4 Discussion

This study was designed to demonstrate differences in sensitivity of a vital organ (central nervous system/brain region) to CN toxicity, and evaluate differences in rate and extent of response to novel CN antidote treatment agent in the brain region

compared to periphery using noninvasive optical technologies. There are studies that show differences in rhodanese and 3-MPST enzyme activity amongst organs from animals and humans. Aminlari et al. reported rhodanese activity differences across organs from sheep and cattle in 1989,<sup>23</sup> sheep, cattle, camel, horse, donkey, dog in 1991,<sup>24</sup> chicken in 1994<sup>25</sup> and 1997,<sup>26</sup> pig in 2002,<sup>27</sup> and cat in 2007.<sup>28</sup> In 2007, Aminlari et al. also compared human rhodanese activity with domestic animals and reported that muscle has 1.4 times higher rhodanese activity than that of brain in humans.<sup>29</sup>

The tissue distribution of 3-MPST has been studied in rats,<sup>13,30</sup> guinea pigs,<sup>30</sup> sheep,<sup>23</sup> and cattle.<sup>23</sup> Wrobel et al. showed that there is difference in 3-MPST activity amongst liver, kidney and brain in mice.<sup>31,32</sup> However, there are no published reports showing the 3-MPST activity in different organs from human or rabbit. One of us (SP) at University of Minnesota measured the specific activity of 3-MPST from liver, kidney, and blood in human and rabbit and found that the 3-MPST activity in rabbit is close to that in humans (unpublished).

Although there is no reference showing the 3-MPST activity difference between brain and muscle in rabbit, brain is one of the most susceptible organs to CN toxicity, indicated by the evidence of much lower levels of rhodanese and 3-MPST compared to liver and kidney from other animals. As mentioned in the introduction, CWNIRS does not measure CN toxicity directly, but measures the changes in the hemoglobin oxygenation in the blood perfusing the tissue in the interrogated region. There may be confounding factors that could affect tissue hemoglobin oxygenation assessed in these studies other than CN toxicity itself. We tried to minimize systemic and regional hemodynamic fluctuations that can affect tissue hemoglobin oxygenation by using a continuous infusion of anesthesia (rather than boluses) and to assess the contribution of such effects by monitoring blood pressure and performing blood gas analysis. Therefore, we believe that the change of tissue oxygenation monitored by CWNIRS

appears to reflect the CN toxicity and recovery effects since the experiments were performed in this controlled manner and compared to controls. Furthermore, the changes were rapid and fairly dramatic, consistent with severe CN poisoning and recovery.

The central nervous system may be more susceptible to CN toxicity for many reasons other than intrinsic sulfurtransferase activity. The central nervous system has high metabolic requirements, high levels of aerobic metabolism, and extremely high blood flow. These characteristics would be expected to increase susceptibility of the CNS to metabolic toxins such as CN. As expected, tissue oxygenation increased as CN infusion started, but the brain region showed a much faster and more profound magnitude of response to CN infusion than that seen from muscle. These faster and greater changes of tissue oxygenation in the brain region imply that brain is more susceptible to CN poisoning than muscle. Compared to CN poisoning, the natural recovery from CN poisoning is slower in the brain region than in muscle as shown by longer time constant values, even though the initial recovery rate is faster for the brain. We speculate that the faster initial recovery in brain region may be due to the high perfusion rate, while the slower later overall recovery rate may be due to the low availability of natural CN detoxifying enzymes such as rhodanese and 3-MPST. In contrast, sulfanegen sodium administration caused both faster initial and overall recovery rate from the brain region than from muscle. The improved overall recovery rate in the brain appears likely to be due to the increased supply of 3-MP to 3-MPST in brain by administration of sulfanegen sodium.

Whether a significant component of nonbrain/CNS optical signals may be present in our "brain region" measurements cannot be determined from this study. However, the source/detector separation used in this study provides a depth of penetration and volume in the area of the brain, and was selected based on stability and characteristics of signal detection suggestive of predominantly brain activity, distinct from skin and muscle.

Cytochrome oxidase, especially cytochrome c oxidase measurements would be ideal direct measures of tissue oxidation inhibition by CN. Cytochrome c oxidase (CcO) contains four redox active metal centers (two heme groups and two copper groups,  $CU_A$  and  $CU_B$ ). In the wavelength region between 600 and 1000 nm, the oxidized states have broad absorptions centered at 830 nm.<sup>33</sup> The reduced state of CcO has a strong absorption peak at 605 nm.<sup>34,35</sup> However, the key challenges for *in vivo* optical monitoring of CcO redox changes come from the fact that there is a significant overlap in the NIR spectra of hemoglobin and the respiratory chain. In order to overcome the spectral overlap and potential crosstalk from hemoglobin NIRS signal, it is imperative to utilize the unique absorption characteristics of CcO redox states. Due to potential interference by the absorption from hemoglobin (in CNS) and myoglobin (in CNS and muscle regions), future studies will be needed with extended range spectra (550 ~ 650 nm) to determine the ability of CWNIRS to detect cytochrome oxidase changes during CN poisoning and reversal.

## 5 Conclusion

We applied noninvasive CWNIRS for monitoring CN poisoning and recovery by a novel CN antidote, sulfanegen sodium. Compared to controls, sulfanegen sodium administration caused a rapid reversal of [OHb] and [RHb] perturbations, which implies a greater effectiveness of sulfanegen sodium for CN

detoxification. Hemoglobin oxygenation change patterns from the control group demonstrated that the brain region is more susceptible to CN poisoning and less capable of CN detoxification compared to peripheral muscles. However, sulfanegen sodium administration caused faster recovery of [OHb] and [RHb] changes from CN poisoning in the brain than in muscle. These results demonstrate that multi-organ monitoring during CN poisoning and recovery may provide important clinical information regarding extent of toxicity, as well as effectiveness and rates of recovery. In addition, CWNIRS may be a useful tool for developing and optimizing novel drugs such as sulfanegen in CN detoxification research.

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