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Whole Blood Redox Potential Correlates with Progressive Accumulation of Oxygen Debt and Acts as a Marker of Resuscitation in a Swine Hemorrhagic Shock Model

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Abstract

Introduction—Oxidation-reduction reactions involve electron exchanges that require optimal balance for proper cell function. This balance is measured via redox potential and reflects oxidative stress. Despite the critical role of oxidative stress in critical illness and injury, little is known regarding redox potential. We hypothesize redox potential measurements will correlate with accumulation of O₂ debt produced by hemorrhage over time.

Methods—Ten swine were studied using a polytrauma hemorrhagic shock model. Whole blood and plasma redox potential measures were obtained at defined stages of O₂ debt (20, 40, 60, 80 mL/kg), and through resuscitation. Redox potential was determined by measuring open circuit potential using novel gold nanoporous electrodes with Ag/AgCl reference.

Results—Whole blood redox potential showed negative change as O₂ debt accumulated, exhibiting positive response during resuscitation, and correlated with O₂ debt across all animals ($p < 0.001$). Redox potential changes throughout O₂ debt accrual were significant compared to baseline ($p = 0.05$), and at end resuscitation compared to O₂ debt 60 mL/kg ($p = 0.05$) and 80 mL/kg ($p = 0.02$). Whole blood redox potential measures also correlated with oxygen extraction ratio, ScvO₂, and lactic acid, appearing very sensitive to acute changes. Plasma RP showed no correlation with O₂ debt.

Conclusions—Whole blood redox potential demonstrates significant correlation to O₂ debt at all stages in this model. These results set the stage for further study of redox potential as a direct

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measure of oxidative stress and potential clinical tool. Given redox potential plasma performance, these measures should be made in whole blood versus plasma.

Keywords

Redox; Oxidation-Reduction; Redox Potential; Oxidative Stress; Shock; Hemorrhagic Shock; Resuscitation; Swine; Whole Blood; Plasma

Introduction

Oxidation-Reduction (Redox) reactions, including those involving reactive oxygen species (ROS), lie at the heart of nearly every physiologic stress response in illness and injury. These redox reactions reflect the overall oxidative stress of the system, and involve the transfer of electrons between oxidants and reductants. The continuous measurement of these combined ubiquitous reactions in blood can be termed as ambient blood redox potential. As redox potential is the balance of oxidants and reductants present, it is a measure of electron pressure in the system much in the same way as pH is the measure of proton pressure (balance of acid and base) in a system. Despite the seeming importance of the measure, it is not widely understood and there are currently few means by which to make the measurement. As opposed to the measurement of pH, measurements of redox potential involve the quantification of electron exchange, and the ultimate balance of these exchanges, which presents unique challenges, especially when testing redox potential in complex biologic fluids.

The primary reason for the difficulty in making redox potential measurements in complex biologic fluids such as whole blood involves electrode biofouling, or passivation, caused by protein adsorption on the electrode surface that can lead to decreased sensor response and bias of measurement (1, 2). While some investigators have attempted to chemically modify the electrode's surface or utilize polymer coatings on the surface to circumvent this problem (3–6), these methods may impede electron exchange at the electrode interface and/or produce measurement bias by selective partitioning or exclusion of analytes and redox species given the chemically modified surfaces. As such, little is known regarding the changes in redox potential during dynamic states of critical illness and injury. While various redox pairs such as lactate-pyruvate or oxidative stress indicators such as thiobarbituric acid reactive substances have been studied in an attempt to measure oxidative stress as an indicator of redox potential (7, 8), such individual markers cannot reflect the system as a whole since there are a large numbers of redox pairs contributing to overall ambient redox potential similar to the many acid-base pairs contributing to pH. In addition, these markers also have their limitations with regard to fully assessing systemic redox state, as they can be affected by the origin of the species measured (e.g. chemical vs enzymatic) and by the analysis techniques utilized for their measurement (9, 10).

Developing means to rapidly measure redox potential in complex biologic fluids such as plasma and whole blood could produce a powerful new point-of-care testing method leading to new diagnostic and therapeutic options in critical illness and injury, as well as other pathologic states where inflammation or ischemia and reperfusion significantly alters the

state of oxidative stress in the host (11). In addition, new therapies could be designed to optimize host redox potential, including providing precision endpoints for the administration of antioxidant therapies. Therefore, while evaluating the performance of redox potential measures in controlled states of oxidative stress could lay the groundwork for establishing these measures as a valuable clinical tool, we must also identify any significant differences between the redox potential of whole blood versus plasma so that the appropriate source is used in future measurements. Further, we must also begin to characterize redox potential changes in clinically relevant states of illness and injury.

We report on the evaluation of redox potential in both whole blood and plasma in a swine model of traumatic hemorrhagic shock utilizing a new nanoporous gold electrode, described previously by our group (12), that resists biofouling and allows free exchange of redox electron species throughout the testing surface without chemical modification, thus maintaining sensitivity of measurement in biologic media that has rendered traditional flat electrodes unresponsive. In addition, in order to establish redox potential as a rapid, direct measure of oxidative stress in states of shock, we compare redox potential measures directly with defined stages of progressive oxygen debt during hemorrhage, and throughout 60 minutes of resuscitation. We hypothesize that redox potential measurements will not only correlate with the progressive accumulation of oxygen debt, but will also serve as a marker of resuscitation in this model reflecting dynamic changes in the accumulation and reaction balance between reductants and oxidants. Because significant differences may exist in the redox potential between whole blood and plasma, a secondary goal is to compare whole blood and plasma measures throughout the experiment and evaluate which source (whole blood vs plasma) most accurately reflects documented changes in oxygen debt and oxidative stress, as this may underscore the importance of technology differences and make the case for utilizing one source over the other for redox potential measurements in further study and in clinical applications.

Materials and Methods

Nanoporous Gold Electrode Fabrication

The nanoporous (np) gold electrode was fabricated by overlaying a nanoporous gold structure onto gold coated slides, precut to an approximate dimension of 1 inch $\times$ 6mm. The nanoporous gold structure was obtained by dealloying gold leaf (Manetti 12 karat white gold) in nitric acid and rinsing with deionized water, which produces a complex matrix of nanopores with diameters of approximately 20–50 nanometers each (Figure 1). The combined gold slide with overlaid np structure was then treated under ultra-violet light for 4 hours and the resultant np gold coated electrode was then covered with Teflon tape containing a 1/8 inch diameter hole punch in the center to provide a defined area and region for redox testing. We have previously demonstrated this electrode to be resistant to biofouling (12).

Swine Hemorrhagic Shock Model

The University (of Michigan) Committee on Use and Care of Animals approved this study, which adhered to the *Guide for the Care and Use of Laboratory Animals* (National Research

Council, revised 2011). Details of this swine model of traumatic shock have been previously described which demonstrate the stability and consistency of this model (13). In brief, this model of controlled hemorrhagic shock is performed in the setting of tissue trauma targeting oxygen debt as a primary endpoint for hemorrhage. Oxygen debt, in mL/kg, was obtained by calculating the sum of multiple measures of oxygen deficit obtained at a sampling rate of 200 time points/sec throughout the experiment using indirect calorimetry (Biopac Systems Inc., Goleta, CA). Oxygen deficit (mL/kg/min) was calculated as the difference between baseline and current oxygen consumption (VO_2), after an initial drop in end-tidal partial pressure of carbon dioxide (PetCO_2) of 4 mmHg. This drop in PetCO_2 was used as a criterion for critical oxygen delivery (DO_2) to minimize variability around critical DO_2 and prevent any overestimation of oxygen debt (13).

In this protocol, animals were fasted overnight with free access to water. On the day of the experiment, anesthesia was induced with ketamine/xylazine (20/2 mg/kg) and maintained with 1–2% inhalant isoflurane for the duration of the experiment. Animals were intubated using a 7.5mm cuffed endotracheal tube and mechanically ventilated to maintain a baseline PetCO_2 (Biopac Systems Inc., Goleta, CA) levels between 35–45mmHg. FiO_2 was maintained at 0.21 throughout the experiment. Core temperature was maintained between 37.5° and 39.0 °C with a temperature blanket using closed loop temperature feedback based on rectal temperature, Blanketrol (Cincinnati Sub-Zero Medical, Cincinnati, OH). ECG was monitored continuously using a standard 3-lead configuration (Biopac Systems Inc., Goleta, CA). The carotid artery was cannulated for continuous arterial pressure monitoring (MAP) and arterial blood sampling. A pulmonary artery catheter (746F8, Edwards Lifesciences, Irvine, CA) was inserted into the pulmonary artery via the right external jugular vein for sampling of central venous hemoglobin oxygen saturation (ScvO_2). Cardiac output was measured using vigilance I (Edwards Life Sciences, Irvine, CA). The left external jugular vein was cannulated for fluid administration while the left femoral artery was cannulated for removal of blood during controlled hemorrhage. All blood pressure monitoring was performed using Biopac Systems Inc (Goleta, CA), transducers and amplifiers.

Prior to hemorrhage, animals underwent soft tissue injury of the bilateral hind-limb skeletal muscle as well as comminuted femur fracture using captive bolt discharge (Accles & Shelvoke, Ltd., Birmingham, England). Skeletal muscle injury is included as a representative soft tissue injury, which has significant effects on oxygen transport by causing greater reductions in splanchnic blood flow and the attainment of dysoxia at higher levels of O_2 Debt than with hemorrhage alone (13, 14). Animals were then hemorrhaged by removing 45–60% of their blood volume while maintaining a mean arterial pressure (MAP) of 30–35 mmHg, ultimately targeting an oxygen debt of 80mL/kg. Eight (8) central venous blood samples from the proximal port of the pulmonary artery catheter were taken at regular intervals of oxygen debt and separated into whole blood and plasma aliquots, with measurements of central venous oxygen saturation (ScvO_2) recorded as well (ABL800, Radiometer America). These intervals of oxygen debt were: baseline (0), 20, 40, 60, and 80 mL/kg. Once 80mL/kg of oxygen debt was achieved, animals were resuscitated with approximately 15mL/kg of whole blood (shed blood) and, if needed, normal saline was administered during resuscitation to maintain MAP above 65 mmHg (mean = 1690mL +/- 1068, N=10). Additional blood samples were obtained at end of resuscitation and at both

30 minutes and 60 minutes post resuscitation. At the end of the experiment, animals were euthanized using 40meq/mL potassium chloride under anesthesia.

Redox potential measurements were obtained and processed immediately at time of blood draw. Approximately 4mL of whole blood was obtained at each time point during the experiment and immediately collected into sodium-heparin tubes. One aliquot of approximately 1 mL of whole blood was retained and immediately used for direct redox potential testing at the point of sample collection, while the remainder was then immediately spun down to collect plasma through separation by centrifugation at 3000 rpm for 5 min. Direct measurement of redox potential was then performed by measuring open circuit potential (OCP) of whole blood and plasma via the np gold electrode, with Ag/AgCL reference, using a ParstatMC™ multi-potentiostat (Princeton Applied Research). Redox potential measurements for both whole blood and plasma were then recorded. Redox potential measurements were not adjusted for pH.

Initial redox potential measurements were made in triplicate from both whole blood and plasma samples to enhance inter-rater reliability. After demonstrating excellent concordance among the values obtained with multiple np electrodes (± 1 mV difference demonstrated between electrodes at each measurement), subsequent samples were measured with one electrode at each sample collection point.

RP Statistical Analysis

A fixed effects mixed regression model was used to evaluate the relationship between redox potential and oxygen debt, as well as other oxygenation parameters over time, including oxygen extraction ratio (O₂ER) and ScvO₂, among all animals studied (N=10). The level of significance was set at $\alpha = 0.05$. Data was analyzed using SAS9.4 statistical programming software (SAS Institute Inc.).

Results

Ten animals were tested in total. Initially, triplicate measurements were obtained and excellent concordance of measurement and inter-rater reliability was noted among all three electrodes from both sources (whole blood and plasma) at each time point tested (± 1 mV), with a standard deviation at all measurement points of ± 0.058 across the three electrodes used for that time point. This held true for all animals studied in which triplicate measures were obtained (3 animals, including 24 time points utilizing 72 electrodes). Given the excellent concordance of measurement and previous work demonstrating electrode reliability (12), one fresh np electrode was used for each redox potential measure from both whole blood and plasma at each time point throughout the shock model for all remaining animals.

Whole blood redox potential demonstrated an overall negative change from baseline during O₂ debt accumulation and also exhibited positive response during resuscitation (Figure 2) reflecting an increase in overall systemic oxidation, or stated another way, a more oxidated state. Redox potential changes throughout shock and resuscitation are represented as an overall mean and standard error of all animals throughout the experimental protocol (N=10).

Analysis of the data via a mixed effects regression model demonstrated a strong relationship between redox potential and O₂ debt over time for all animals ($p=0.002$). Redox potential changes throughout O₂ debt accrual (at O₂ Debt 20, 40, 60, and 80mL/kg) were also found to be significantly different from baseline measurements ($p=0.002$ at all points). Further, redox potential at the end of resuscitation (ER) time point showed a significant positive response compared to redox potential at O₂ debt 80 mL/kg ($p=0.02$) and 60 mL/kg ($p=0.05$), again reflecting an increase in overall oxidative state. Although whole blood redox potential measures demonstrated dynamic changes during shock and resuscitation, plasma redox potential showed no significant change through shock and resuscitation, whether in individual animals or when evaluated collectively ($p=0.74$), as noted in Figure 3.

As an individual example of these findings, and to better visualize the differences in simultaneous measures of whole blood and plasma redox potential throughout the protocol, a representative swine from the cohort is shown in Figure 4. In this animal, time-matched, triplicate measures, from 3 separate electrodes were taken from whole blood and plasma at each time point demonstrating excellent redox electrode reliability with little to no variation in measurement at any time point (SD of triplicate measures at each time point demonstrated less than 0.58 mV variation). As plotted, whole blood redox potential measures ranged from -52mV at baseline to -98mV at O₂ debt 80 and showed ongoing negative progression throughout hemorrhage and progressive shock, which is characteristic of the cohort when evaluated collectively, and demonstrated subsequent positive change during resuscitation, increasing to -42mV at end of resuscitation (ER) and ultimately ending at -53mV at 60 minutes post-resuscitation (PR60). In contrast, plasma redox potential measures ranged from -71mV to -85mV, but showed no apparent progression or response to either progressive shock or to subsequent resuscitation.

In addition to O₂ Debt, we also evaluated whole blood redox potential in comparison with oxygen extraction ratio (O₂ER), ScvO₂, and lactic acid (LA) measures at matched time points across all ten animals. In this analysis, whole blood redox potential demonstrated a significant relationship with oxygen extraction ratio (O₂ER), defined as oxygen consumed (VO₂, in mL/kg/min) divided by oxygen delivered (DO₂, in mL/kg/min), among all animals studied via a mixed effects regression model ($p=0.012$), with whole blood redox potential mirroring O₂ER throughout shock and resuscitation as demonstrated in Figure 5. While whole blood redox potential was also found to correlate with ScvO₂ throughout shock and resuscitation in univariate analysis ($p=0.001$) as well, we noted that in some individual animals whole blood redox potential appeared more sensitive to changes in oxidative stress than ScvO₂ at critical time points, especially during periods of high O₂ Debt (e.g. O₂ Debt 60 and 80) and as resuscitation progressed.

Given lactic acid (LA) is a common clinical parameter used in the evaluation of shock and resuscitation, we also measured LA at each time point and compared LA with O₂ Debt and whole blood redox potential throughout shock and resuscitation among all swine ($N=10$). Results showed that while LA demonstrated association with O₂ debt over the course of shock progression and O₂ debt accrual in our mixed effects regression analysis (i.e. increasing LA with increasing O₂ Debt, $p < 0.001$), LA continued to climb throughout the end resuscitation (ER) time point before beginning to eventually clear and decline as

resuscitation proceeded. Although whole blood redox potential was also found to have a significant overall correlation with LA in this cohort ($p=0.005$), whole blood redox potential demonstrated an immediate positive response with resuscitation, following the overall improvement in O_2 Debt and O_2ER noted between O_2 Debt 80 and ER among all swine studied (Figure 6). Plasma redox potential had no significant relationship with O_2 debt, O_2ER , $ScvO_2$, or LA.

Discussion

Redox reactions lie at the heart of physiologic processes in critical illness and injury. While metabolically active biologic systems rely on orderly conduction of electrons through interconnected redox reactions, alterations in redox balance have been linked to the generation of harmful free radicals(15), cellular apoptotic signaling (16), and the disruption of extracellular redox balance (15, 17), subsequently producing systemic redox shifts and oxidative stress. These redox shifts and oxidative stress also play a central role in driving the systemic inflammatory response in critical illness and injury and contribute to the development of multiple organ dysfunction (18, 19). In fact, mitochondrial ROS have been found to have the ability to drive the initiation of the inflammatory cascade via inflammasome-dependent and independent pathways, as well as through their interaction with Damage Associated- and Pathogen Associated-Molecular Pattern Molecules (DAMPs and PAMPs) (20). In addition, while restoring redox balance is of vital importance to improving the metabolic derangements in shock (21, 22), a strong association has also been demonstrated between individual markers of cellular redox balance and the onset of organ failure after injury, and these measurements are able to differentiate survivors from non-survivors during critical illness in one study (23). Without the necessary technology to make high fidelity redox potential measures in whole blood or plasma, previous investigators have relied on the measurement of specific redox pairs (lactate/pyruvate) or levels of antioxidants (vitamin C, etc) as indicators of oxidative stress. However, since the number of specific oxidants and reductants are so large, the need to know the ambient redox potential of the system would seem to be very important. Indeed, entities such as albumin and bilirubin serve as antioxidants but are not widely viewed as such (24, 25). Therefore, while cellular determinants of redox potential are important, we must better understand the ambient, or systemic, redox state as well in order to truly understand the complexities of oxidative (or reductive) stress in critical illness and injury, and we may now be able to evaluate redox potential in biologic fluids, including whole blood and plasma in a true point of care fashion by using novel nanoporous redox electrodes that resist biofouling.

Since redox potential is a measure of the relative concentrations of oxidants and reductants in a system and the degree to which a solution will gain or lose electrons, a low potential (more negative) indicates a larger concentration of reductants in the system (gain of electrons and/or decrease in oxidation state) with a negative redox trend indicating ongoing systemic reduction. Conversely, a high potential indicates a larger concentration of oxidants (loss of electrons and/or increase in oxidation state), with a positive redox trend indicating an accumulating oxidation. As noted in Figures 2 and 4, an overall decline in whole blood redox potential occurs from baseline, through successive levels of O_2 debt in the swine traumatic shock model. This corresponds to increasing tissue hypoxia and increasing O_2ER ,

with an accumulation of reduced redox species and thus the potential for the blood to be subsequently oxidized on reperfusion. This systemic reduction (gain of electrons and/or decreased oxidation state) is likely multifactorial, and may be influenced by decreased oxygen delivery and progressive mitochondrial dysfunction occurring during hemorrhagic shock, with an associated loss of mitochondrial membrane potential that significantly reduces the ability to utilize NADH, increasing its concentration, which subsequently decreases the NAD⁺/NADH ratio (26, 27). Through transhydrogenation, NADPH levels increase as well, and although NADPH may not leave the cell, other reduced species carrying excess hydrogen, such as sulfhydryls and ascorbic acid, may cross the cell membrane and decrease the overall redox potential as measured in whole blood (28).

As resuscitation progresses, whole blood redox potential then demonstrates a significant positive change, with end resuscitation values significantly more positive than the redox potential taken at the height of O₂ debt accumulation. This represents the reperfusion injury state in which an oxidant load is expected as there is an increase in oxygen delivery and oxygen influx that drives ROS production by multiple mechanisms, including xanthine oxidase mediated pathways and increases in ROS production via NADPH oxidase activity in the presence of already elevated concentrations of NADH and NADPH (29). While these mechanisms may be responsible for the increasing reductive state during shock and increasing oxidative state during resuscitation, much more work remains in characterizing this response.

In contrast to redox potential measures in whole blood, plasma values throughout the experimental period show very little variation of measurement and demonstrate no correlation with oxygen debt, O₂ER, ScvO₂, or LA. While reasons for this observation may be multifactorial, these measurements were obtained from the same blood draw at each time point, based on O₂ Debt, and suggest this observation is likely due to the loss of critical redox species and cell components resulting from the separation of plasma from whole blood. Indeed, cellular based influences on redox potential like those described above likely account for the loss of sensitivity for accurate measurements of redox potential in plasma as these cellular components and their contributions to the overall redox state of whole blood are removed, including cellular redox species such as NADPH Oxidases (Nox), Nitrogen Oxide Synthases (NOS) and Myeloperoxidases (MPO), among others. This data also suggests that whole blood may be superior to plasma as a source for measuring redox balance in conditions that rapidly alter oxidative stress.

Given this evidence, redox potential measurements in whole blood may provide a direct measure of the activity and balance of active redox species in real time, and may serve as a helpful measurement for the clinical evaluation of oxidative stress in shock and resuscitation. Although there was significant variation in baseline redox potential among the swine, and thus some variation in the redox potential measures to follow, all were housed under similar conditions, and it is not surprising that such variation could exist based on individual differences in activity, stress, oxidant stores, and other variables, such as epigenetic profiles and individual response to oxidative stress, further emphasizing the need for a precision tool to make these measurements. While additional measures of oxidative stress and specific redox pairs were not measured in this study, concurrent measures of

cellular redox state and oxidative stress, such as thiobarbituric acid reactive substances (TBARS) and nuclear factor erythroid 2-related factor (Nrf2) activity, along with systemic redox potential measures would be helpful in future studies to correlate ambient and cellular redox states in critical illness and injury and these studies are planned for the near future.

Finally, in order to compare whole blood redox potential with a current clinical measure utilized in evaluating shock and resuscitation, we also performed lactic acid (LA) measures at each time point throughout the experiment and compared LA with both O₂ Debt and redox potential measures among all swine studied. The resulting analysis demonstrated that while LA showed a steady rise over the course of increasing O₂ Debt, LA continued to rise through resuscitation, peaking after the end resuscitation (ER) time point and then following a gradual decline after this point. There was no immediate response to initial resuscitation or to changing O₂ Debt during this period, especially between O₂ Debt 80 and ER. This follows a similar pattern we see clinically, as LA continues to rise throughout the course of shock and during initial phases of resuscitation, generally reaching a plateau at some point, and then follows a subsequent gradual decline. However, in our model, whole blood redox potential demonstrates immediate response to resuscitation and changing O₂ Debt in real time. This supports our hypothesis that whole blood redox potential measures may serve as a valuable, direct measure of oxidative and metabolic stress and can allow for a rapid, real-time evaluation of shock and resuscitation. This data further confirms what is often seen clinically - although LA shows reliable trends over time, as a secondary marker of oxidative and metabolic stress, LA reflects the underlying state of shock and resuscitation in a delayed fashion and may not be an optimal single measure for guiding real-time therapy. In addition, while there was an overall correlation between whole blood redox potential measures and time matched values of ScvO₂ throughout the swine shock experiment in univariate analysis, potentially significant differences were noted at critical time points in some individual swine. As an example, in one animal ScvO₂ reached its nadir at O₂ Debt 60, with little change to O₂ Debt 80, while whole blood redox potential continued to decline over this period. These periods of discrepancy may demonstrate that although there is relatively little change in ScvO₂, there is continued accumulation of reductive species driven by mechanisms described previously, that result in ongoing redox imbalance as measured by whole blood redox potential. While further investigation will need to be performed in additional shock models, this may indicate that whole blood redox potential may provide a better measure of real time and progressive oxidative stress when compared with ScvO₂, as ScvO₂ is also a secondary marker of these changes, measuring the end sum of venous oxygen saturation, but without the ability to directly reflect the underlying pathways and reactions contributing to ScvO₂.

If these findings are consistent in future studies, this data could provide evidence for the use of whole blood redox potential in not only providing diagnostic information regarding the state of oxidative stress, but may also play a role in guiding therapeutic intervention. In this way, these measures may serve as a unique marker for the use of antioxidant and/or antireductant therapies as well. Although there is much debate regarding antioxidant therapies in critical illness, there have been multiple studies demonstrating the potential benefits of these therapies, including the use of ascorbic acid to attenuate the development of organ injury (30, 31) and to reduce the proinflammatory and procoagulant states that induce

lung vascular injury in sepsis (32). In addition, the use of resuscitation fluids with targeted anti-oxidant therapy, such as MitoQ, an antioxidant targeted to the mitochondria to counteract mitochondrial oxidative damage, and others that act as mitochondrial antioxidants and reactive oxygen species (ROS) scavengers, have been reported to improve organ function, recovery, and survival times in experimental models of sepsis (33, 34). Therefore, the ability to monitor whole blood redox potential may aid in optimizing the use of such therapies.

Limitations

This is a preliminary study of redox potential measurements in whole blood and plasma using a novel nanoporous electrode that is resistant to biofouling. One limitation is the number of swine (10) studied. While it appears that redox potential measures in plasma show little overall variation in both individual animals and when evaluated collectively, it would be beneficial to obtain additional measures in a larger cohort of swine, and ultimately in a patient population to determine a range of normal baseline redox measures in both swine and humans. Although we utilized a well established model of swine shock with controlled hemorrhage and shock progression, further studies must be performed in additional animals in order to evaluate redox potential measurements in hemorrhagic shock and in other shock states, particularly septic shock, to further investigate how these redox potential measures compare with established clinical measures of oxidative stress, such as LA and ScvO₂ in these models. We also did not resuscitate animals with supplemental oxygen. This could have had an effect of increasing redox potential during the resuscitation phase. We also did not measure simultaneous arterial and venous redox potential so we cannot say which may be a better indicator of actionable data for decision-making, and we did not make concomitant measures of known redox pairs or other indicators of oxidative stress, although future studies will address these issues. Lastly, more population based data will need to be collected to understand ranges of redox potential in patients with various conditions and in response to various therapies. This will be essential to understand what values would prompt actionable diagnoses and therapies.

Conclusions

In this swine model of hemorrhagic shock, whole blood redox potential demonstrates a significant correlation with oxygen debt measurements and oxygen extraction ratio over the course of shock and resuscitation and shows consistent and immediate response with resuscitation initiation. While LA measures continue to rise over the initial phase of resuscitation, whole blood redox potential measures respond in real-time. Further, whole blood redox potential may serve as more specific, direct measure of oxidative and metabolic stress at critical points of shock and resuscitation, while serving as a marker of resuscitation. In addition, plasma redox potential measurements demonstrated no significant response to shock or resuscitation in this model and suggests that redox potential measurements should be taken from whole blood in future studies and for clinical use.

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Figure 1. Scanning Electron Microscopy of the Nanoporous Gold matrix produced after dealloying gold leaf.

Redox Potential Measured in Whole Blood from All Swine Throughout Shock and Resuscitation

Figure 2.

Plot of mean redox potential $\pm$ SEM measured in whole blood at matched time points across 10 animals throughout swine traumatic hemorrhagic shock and resuscitation. Samples were tested from each animal at baseline, through O₂ Debt accrual (at O₂ Debt 20, 40, 60, and 80), at end of resuscitation (ER), and post resuscitation at 30 and 60 minutes (PR30 and PR60).

Whole blood redox potential was found to have significant overall correlation with O₂ Debt throughout shock and resuscitation when evaluated among all animals in a mixed effects regression analysis ($p=0.0016$).

In addition, redox potential changes throughout O₂ debt accrual (at O₂ Debt 20, 40, 60, and 80mL/kg) were found to be significant (*) compared to baseline measures ($p=0.012$) with redox potential at end resuscitation (ER) showing a significant positive response (#) compared to redox potentials at O₂ Debt 60 and O₂ Debt 80 ($p=0.05$ and $p=0.02$).

Redox Potential as Measured in Plasma Throughout Shock and Resuscitation

Figure 3.

Mean redox potential $\pm$ SEM measured in plasma at matched time points across 5 animals during swine hemorrhagic/traumatic shock for each point of collection among the animals. Plasma redox potential was found to have no significant correlation with O₂ Debt when compared among all animals in a mixed effects regression analysis ($p=0.74$). In addition, redox potential changes throughout O₂ debt accrual (at O₂ Debt 20, 40, 60, and 80mL/kg) were found to have no significant change compared to baseline RP measurement ($p=0.48$).

Whole Blood vs Plasma Redox Potential Measured in an Individual Swine Throughout Shock and Resuscitation

Figure 4.

Redox potential measured in triplicate (3 separate electrodes, SD = 0.58mV) at matched time points in whole blood (solid line) vs plasma (dashed) in a single representative animal from the swine traumatic shock cohort. Measurements were taken at progressive stages of shock (Baseline; O₂ Debt 20, 40, 60, and 80), at End of Resuscitation (ER) and post resuscitation (PR) at 30 and 60 minutes. Note appropriate redox trends in whole blood through shock and with resuscitation, while there is lack of overall variability and no apparent reflection of O₂ debt changes or resuscitation in plasma redox potential.

Whole Blood Redox Potential (RP) and Oxygen Extraction Ratio (O₂ER) Measured Throughout Shock and Resuscitation

Figure 5.

Whole blood redox potential plotted with Oxygen Extraction Ratio (O₂ER) as measured throughout shock and resuscitation among all animals studied (n=10). Whole blood redox potential shows a near mirror image response to O₂ER among animals with significant correlation between whole blood redox potential measures and calculated O₂ER in mixed effects regression analysis (p=0.012). Data shown as mean ± SEM at each time point.

Whole Blood Redox Potential (RP) and Lactic Acid (LA) Measured Throughout Shock and Resuscitation

Figure 6.

Whole blood redox potential plotted with Lactic Acid (LA) as measured throughout shock and resuscitation among all animals studied ($n=10$). While redox potential was found to correlate with LA ($p=0.005$) in our mixed effects regression analysis, redox potential demonstrated real-time response to resuscitation, with positive change over this period (mid resuscitation point is noted by vertical dotted line), while LA continues to rise, peaking sometime after resuscitation before beginning to decline. Data shown as mean $\pm$ SEM at each time point.