Temporal Variation In Tissue Oxygen Tension And Perfusion Response To Hypoxia And Ischaemia: The Importance Of Local Control Of The Microcirculation

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Citation

Abstract
Introduction: The relationship between tissue oxygenation and perfusion is important since hypoxia remains a core component of many acute and chronic diseases, particularly those leading to admission to the intensive care unit. We hypothesised that skeletal muscle perfusion and oxygenation would behave similarly regardless of whether tissue hypoxia was caused by reducing blood flow or arterial oxygen content.

Methods: We performed a prospective laboratory study in 18 Sprague-Dawley rats. We measured (simultaneously) tissue oxygen tension and perfusion in hind limb skeletal muscle. Rats were allocated to three groups: control (n=6), ischaemia-anoxia (n=6) and anoxia-ischaemia (n=6). Ischaemia was induced by aortic occlusion for 30 minutes and anoxia by reducing the inspired oxygen concentration to zero for 90 seconds.

Results: Tissue perfusion and PtO2 changed according to an exponential mathematical model during ischaemia, anoxia, reperfusion and reoxygenation. Irrespective of whether ischaemia was the initial or subsequent insult, both LDF and PtO2 decreased. This was followed by a hyperaemic LDF response with partial recovery of PtO2. In contrast, LDF increased when anoxia was the first intervention but not when it was preceded by ischaemia; this combination resulted in reduced perfusion. Following reoxygenation, PtO2 and LDF generally recovered to baseline values.

Conclusions: We conclude that changes in tissue oxygen tension and perfusion during ischaemia and anoxia depend on the order in which the insults occur, and that they may be described by an exponential model. These data also confirm the presence of exhaustible local hypoxic control mechanisms in the microcirculation.

KEY MESSAGES:
Microcirculatory vasomotor tone is regulated by a complex balance of local and central reflex mechanisms. Both may be affected by disease processes that result in reduced tissue oxygen delivery.

Fibreoptic systems are now readily available for oxygen tension measurement in biological tissues; they are able to detect accurately those changes induced by clinically meaningful reductions in local and global oxygen delivery.

Increases and decreases in tissue oxygen tension and perfusion may be accurately described by an exponential mathematical model, whether they occur as a result of ischaemia or hypoxia.

ABBREVIATIONS:
CaO2: Arterial oxygen content DO2: Oxygen delivery FiO2: Inspired oxygen tension LDF: Laser Doppler flowmetry NO: Nitric oxide PtO2: Tissue oxygen tension

INTRODUCTION
The measurement of tissue oxygen flux has been of interest to those involved in critical care medicine research and practice for some time [1,2]. Numerous techniques and devices have been described for this purpose [3,4]. However, clinical practice is currently restricted to measuring tissue oxygenation in terms of whole body oxygen transport using oxygen delivery derived from oxygen content of arterial and mixed venous blood and cardiac output [5]. As measurement systems become more advanced it is anticipated that monitoring of oxygenation will occur at the organ and tissue level, and possibly ultimately at the level of the cell [6].
We recently described and validated the use of a new technique that accurately measures oxygen tension at the tissue level \[10\]. From the clinical observations that both reduced tissue blood flow (for example compression of an artery following line removal) and hypoxia (for example in respiratory failure, during bronchoscopy or following intubation) occur commonly, we hypothesised that skeletal muscle perfusion and oxygenation would change similarly regardless of whether tissue hypoxia was caused by reducing blood flow or arterial oxygen content. Using the technique described previously \[10\] we have measured oxygen tension (PtO$_2$) in an animal model of skeletal (gastrocnemius) muscle ischaemia and anoxia, and in this paper we report the relationship between tissue oxygen tension and perfusion (measured using laser Doppler flowmetry (LDF)) in skeletal muscle following various combinations of ischaemia and anoxia.

METHODS

All experiments were carried out according to guidelines laid down by the National Institute of Health \[11\] and were approved by the University of Texas M.D. Anderson Cancer Center institutional animal care and use committee. Experiments were performed after an initial period of model development.

ANIMAL MODEL

Eighteen adult, male Sprague-Dawley rats weighing 350-400g were housed in a conventional facility with 12 hour light cycling and allowed free access to food and water until the morning of the experiment. Animals were anaesthetised using an inhaled mixture of 3-5% isoflurane in oxygen. When the animal was sufficiently anaesthetised (judged by loss of ear pinch reflex) a tracheostomy was performed and the animal's lungs mechanically ventilated (Harvard Inspira ASV ventilator, Harvard Apparatus Inc, Holliston, MA). Anaesthesia was maintained using 1-2% inhaled isoflurane in a mixture of oxygen and nitrogen. The inspired oxygen tension (FiO$_2$) was maintained at 35% throughout except when the experimental protocol dictated otherwise.

Cannulae (PE-20, Harvard Apparatus Inc, Holliston, MA) were inserted into the left femoral vein and artery to facilitate infusion of fluid and measurement of arterial blood pressure (and heart rate as a depth of anaesthesia monitor.) To prevent reflex movements associated with profound systemic hypoxia, muscle relaxation was achieved with pancuronium bromide (IV bolus of 0.02mg 100g-1 and IV infusion of 0.02mg 100g-1 hr-1). 0.9% saline was administered (1ml 100g-1 IV bolus and 1ml 100g-1 hr-1 IV infusion) to prevent dehydration during the experiment.

A small midline laparotomy was performed and a 3:0 silk sling placed around the aorta, immediately inferior to the renal arteries. This sling was then externalised to allow subsequent aortic occlusion by gentle tightening. The laparotomy incision was then closed. The tissue oxygen probe (FOXY, Ocean Optics Inc, Dunedin, FL) was calibrated as described previously \[10\] and then placed percutaneously into the belly of the right gastrocnemius muscle. Finally a single fibre laser Doppler flowmetry probe (Moor Instruments Inc, Wilmington, DE) was placed alongside it. Both probes lay within 3 mm of each other in the skeletal muscle bed and were maintained in position by micromanipulators.

Core temperature was maintained between 37.5 and 38ºC by an automated rodent homeothermic blanket warming system (Harvard Apparatus Inc, Holliston, MA). Animals were allowed to stabilise for 20 minutes after surgery before the experimental protocol began.

EXPERIMENTAL PROTOCOL

After a 20-minute stabilisation period followed by 5 minutes of baseline data recording, animals were allocated to one of the following groups:

Control (n=6): no ischaemia or anoxia. Data was recorded at the same intervals as the other groups.

Ischaemia-anoxia (n=6): these animals underwent a 30-minute period of aortic occlusion achieved by tightening the aortic sling until the arterial pressure trace became flat. This was intended to reproduce the clinical effect of severe vascular compromise. The sling was then released for a period of 20 minutes to allow reperfusion of the hind limb. Thereafter a 90-second period of anoxia was introduced by ventilating with 100% nitrogen, after which the FiO$_2$ was returned to 35%. Data was collected for a subsequent 30 minutes following reoxygenation after which the animal was killed by overdose of isoflurane and exsanguination via the arterial line. We previously determined that periods of anoxia in excess of 90 seconds were rapidly fatal in this model.

Anoxia-ischaemia (n=6): these animals underwent exactly the same interventions as group (b) except that the anoxic
period preceded the ischaemic period.

**STATISTICAL ANALYSIS**

Continuous variables were compared within and between groups using 2-way repeated measures analysis of variance, with Bonferroni post-tests if statistical significance was detected at the 5% level. LDF data were normalised to percent change from baseline because the raw data generated from this device has arbitrary units.

To assess applicability of a mathematical model to the results, data were divided into groups representing each intervention; namely ischaemia, reperfusion, anoxia and re-oxygenation. An exponential (attack or decay) model was applied to each of these groups and the relevance of the model determined by application of a runs test. This test assesses whether or not the data fit the model by calculating the likelihood of observing as many (or as few) consecutive data points (runs) above or below the fitted curve.

To assess whether or not the two physiological signals were associated with each other, product-moment correlation coefficients were calculated for each intervention period. For all statistical tests a p value of less than 0.05 was considered significant. Analyses were carried out using Prism v3.0 (GraphPad Software Inc, San Diego, CA) and Microsoft Excel 2000 with the Analyse-It v1.61 software add-in (Analyse-It Ltd, Leeds, UK).

**RESULTS**

PtO$_2$ and LDF signals were both readily recordable and are plotted against the control group for the ischaemia-anoxia group (Figure 1) and anoxia-ischaemia group (Figure 2), respectively.

**Figure 1**

Figure 1: Tissue oxygen tension (A) and laser Doppler flowmetry (B) during ischaemic-anoxic hypoxia. (?) Intervention group, (•) Control group. * p
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Figure 2
Figure 2: Tissue oxygen tension (A) and laser Doppler flowmetry (B) during anoxic - ischaemic hypoxia. (†) Intervention group, (•) Control group. * p

ISCHAEMIA-ANOXIA GROUP
Both the PtO\textsubscript{2} and LDF signals fell precipitously during ischaemia. On reperfusion, a marked hyperaemic response (120% above baseline LDF) occurred. The PtO\textsubscript{2} signal, however, failed to recover (69% recovery of baseline PtO\textsubscript{2}, p<0.01) during reperfusion. With the subsequent anoxic period both LDF and PtO\textsubscript{2} signals fell precipitously and this was in stark contrast to the anoxia-ischaemia group, where anoxia resulted in hyperaemia. On reoxygenation, only 50% of the initial ischaemia-induced hyperaemic response reoccurred. PtO\textsubscript{2} did return to its pre-anoxic (but not original baseline, p<0.01) value, however.

ANOXIA-ISCHAEMIA GROUP
The PtO\textsubscript{2} signal fell precipitously during anoxia. In contrast, LDF increased (70% above baseline, p<0.01) markedly, in keeping with a hyperaemic response. On reoxygenation, the hyperaemia response abated and baseline flow recovered. A comparable recovery (to 90% of baseline, p=NS) was seen in the PtO\textsubscript{2} signal. During the subsequent ischaemic period, both LDF and PtO\textsubscript{2} signals fell precipitously. On reperfusion there was a lesser hyperaemic response (25% above baseline, p=NS) and PtO\textsubscript{2} recovery was incomplete (80% recovery of baseline PtO\textsubscript{2}), although this was not statistically significant.

For both the ischaemia-anoxia and the anoxia-ischaemia groups, analysis of the individual intervention periods (ischaemia, reperfusion, anoxia and reoxygenation) revealed striking consistency in the way that the LDF and PtO\textsubscript{2} signals changed; example curves from the reperfusion period in the ischaemia-anoxic animals are shown in Figure 3. The PtO\textsubscript{2} and LDF data for these intervention periods did not differ significantly from the (attack or decay) exponential model that was applied to them. Table 1 shows the correlation coefficients and 95% confidence intervals for the two signals for these intervention periods, together with the p values of the runs tests used to assess divergence from the model. Figure 4 shows mean arterial pressure data for the animals in both groups. The femoral monitoring site reflects the perfusion pressure into the skeletal muscle during both intervention periods.
Figure 3
Figure 3: Exponential signal recovery during reperfusion period in ischaemic-anoxic animals. (? LDF, (?) PtO2. Curves are computer-fitted lines of best fit according to the exponential model that best described the observed data. Correlation coefficients together with their 95% confidence intervals are shown in Table 1.

Figure 4
Figure 4: Mean arterial pressure (MAP) during ischaemia-anoxia (top) and anoxia-ischaemia (bottom). Signal recorded from femoral arterial line distal to site of cross clamp. (?) Intervention group, (•) Control group. * p<.05; † p<.01. Data shown are mean ± SEM.
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Figure 5
Table 1: Relationships between LDF and PtO₂, and goodness of fit data for each signal when applied to an exponential model; a correlation between LDF and PtO₂ for each intervention period within each experimental group; b runs test for divergence from exponential model. Neither signal deviated significantly from the exponential model during any intervention. The runs test looks for divergence from a model, and thus if there is no significant difference then the conclusion is that the data set does not differ from the model - i.e. it agrees with, or fits. There is no suggestion that a high p value means better agreement, tempting though it is to conclude thus.

<table>
<thead>
<tr>
<th>Intervention period</th>
<th>Correlation coefficient (95% CI)</th>
<th>p value</th>
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<tr>
<td></td>
<td>ICHAemic-anoxia</td>
<td>Anoxia-ischaemic</td>
</tr>
<tr>
<td>Ischaemia</td>
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<td>0.51 (0.02 to 0.80)</td>
</tr>
<tr>
<td>Reperofusion</td>
<td>0.97 (0.87 to 0.95)</td>
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<tr>
<td>Anoxia</td>
<td>0.94 (0.65 to 0.96)</td>
<td>0.95 (0.09 to 0.99)</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>0.33 (0.61 to 0.96)</td>
<td>0.74 (0.02 to 0.32)</td>
</tr>
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</table>

DISCUSSION

As long ago as 1925, it was suggested that vasodilatation occurred in response to a reduction in cellular PO₂ [11]. More recently, it has been suggested that it is the arterial oxygen content (CaO₂) rather than oxygen tension that is the important determinant of vasomotor tone [12,13]. Additionally, other mediators have been implicated in the regulation of vasomotor tone. Skinner and Marshall [14] demonstrated that hypoxia induced muscle vasodilatation is nitric oxide (NO) dependent. The same group subsequently showed that adenosine is involved in the process, suggesting that the A1 subgroup of this receptor type mediates this aspect of hypoxia-induced vasodilatation [15]. These investigators also demonstrated that adenosine acts via those same receptors to increase the synthesis of nitric oxide via an ATP-sensitive potassium channel [16]. Their observation that pharmacological antagonists of adenosine only block approximately 50% of hypoxia-induced muscle vasodilatory activity lead to the suggestion that other mediators are involved. There is in vitro evidence suggesting involvement of the prostaglandin superfamily, [17] and this is supported by further evidence linking prostaglandin formation and NO production [18]. It is certainly possible that these mechanisms are interdependent, [19] and our data appear to suggest that they are endothelium dependent too, since ischaemia-reperfusion is itself known to cause significant endothelial injury [20]. Anning et al [21] studied tissue oxygenation in endotoxaemic rats; they concluded that nitric oxide synthase inhibition had a deleterious effect on muscle PtO₂ and further that volume resuscitation could reverse these changes. We only allowed our animals a 20-minute period of reperfusion and it is possible that this is not long enough either to regenerate stores of NO or to allow up regulation of the inducible form of NO synthase.

We did not measure arterial haematocrit (serial blood draws in small animals can rapidly lead to hypovolaemia and anemia which would themselves affect oxygen flux) or cardiac output in our animals - and thus cannot comment on whether a relationship exists between CaO₂ or oxygen delivery (DO₂) and skeletal muscle blood flow or not - our data support the idea that vasomotor tone depends on a dynamic interaction between sympathetic tone and local vasoactive mediator release. In our experiments anoxia had differing effects, dependent on whether it occurred before (vasodilatation) or following (vasoconstriction) an ischaemic insult. This raises the possibility that profound drops in tissue oxygenation occurring before a period of hypoperfusion may be less deleterious than if they occur afterwards, for example following surgical anastomosis. The effect of pancuronium on muscle oxygenation is important too. It is known to reduce skeletal muscle oxygen consumption and to increase resting heart rate. It is not thought to have directly mediated effects on vasomotor tone other than those reflex responses to the mild haemodynamic changes mentioned above. We suggest that our use of pancuronium may also have contributed to our high resting tissue PO₂ levels.

After a period of hypoperfusion, the reduction in flow we observed during subsequent anoxia might be explained by one of two processes. Firstly, it is possible that depletion of endogenous vasodilatory mediators during the prior ischaemic insult, associated with increased sympathetic tone (known to occur during anoxia) might lead to a paradoxical vasoconstriction response. This depletion of endogenous vasodilatory mediators may also explain the impaired hyperaemic response that occurs when ischaemia is the secondary insult, as opposed to the extensive hyperaemic response when hypoperfusion is the primary insult. Secondly, ischaemic (anoxic) preconditioning may occur with a prior insult. This phenomenon, where a smaller insult protects against a subsequent larger one, is well described in the ischaemia-reperfusion literature [22,23]. Preconditioning is usually associated with absolute ischaemia for a period of 10 minutes or so followed by a more prolonged reperfusion period. Our animals were only anoxic for 90 seconds and there was little in the way of post anoxic change that...
persisted for any significant period of time. As anesthesiologists we are aware of the protective effect of isoflurane, but feel that this is not responsible for the differences between the groups since all were anesthetized in exactly the same way.

Despite improved blood flow (hyperaemia) following ischaemia, PtO$_2$ only partially recovered to baseline levels. This was less marked following anoxic insults, when we observed almost full recovery of PtO$_2$ to pre-anoxic baseline levels. This suggests that oxygen diffusion was impaired at the interstitial level, and may implicate an ischaemia-reperfusion injury of the endothelium. When anoxia preceded ischaemia we observed an exponential increase in LDF (hyperaemia) that accompanied the decrease in PtO$_2$ signal. With reperfusion there was a prompt recovery in both the LDF and PtO$_2$ signals to near baseline values. This may merely reflect that the duration of the insult was insufficient to cause an injury that resulted in a sustained reduction in the PtO$_2$ signal. Reperfusion following the longer ischaemic period was characterised by a PtO$_2$ signal that did not recover to its baseline value.

The PtO$_2$ values reported here are higher than those reported by other groups [25,26]. We suggest that this is because our animals were ventilated with a baseline FiO$_2$ of 35% (in a deliberate attempt to prevent inadvertent hypoxia during surgery), as opposed to the more typical 21% oxygen in room air. When our (model development) animals were ventilated with room air the measured PtO$_2$ did reduce to the levels reported by other groups [25,26] which are typically 20 - 30 torr (2.5 - 4.0 kPa) lower than those reported here. We did not deliberately ventilate our protocol animals with an FiO$_2$ of 0.21 during the experiments because we did not wish them to become even mildly hypoxic during their surgery. For comparative reasons this would have had to have been done at the end of the experiment, after periods of both ischaemia and anoxia. Under these circumstances we felt it would be extremely difficult to interpret the tissue PO$_2$ measurements.

Additionally, this is only the second report of the use of this new oxygen sensor as an experimental tool and our data are consistent with those previously reported by us [27]. It is possible that trauma may have caused an artificially high tissue PO$_2$ in our model. However, we feel that this is unlikely to have occurred in all of the animals we studied. Additionally we would have expected a much dampened signal in response to the experimental changes imposed. We did not observe such a change.

The significance of the exponential model that describes our data remains unclear. It is possible that this merely reflects a tendency of biological signals to follow this type of pattern [28] or there may be a deeper significance. One possibility is that it is an intrinsic feature of the device, except that the response time of the device is in the order of 60 seconds with a silicone coat, as used here. Figure 3 shows that the time-frame of changes following reperfusion is of the order of 15 minutes. In addition there are two separate instruments, the oxygen sensor and the laser Doppler flowmeter, yet both show similar patterns. Soller et al [29] fitted data obtained with a fibre-optic sensor from their swine hepatic dysoxia model to both exponential and linear models. They compared tissue PO$_2$ with tissue pH (as opposed to time) and concluded that the exponential equation was more sensitive in terms of predicting critical values for the tissue parameters. Venkatesh et al [30] measured subcutaneous oxygen tensions in a rat model of hemorrhagic shock and demonstrated good correlation with ileal luminal CO$_2$ tension. Although they did not fit their data to a mathematical time-PO$_2$ model the curves presented in their work do appear to follow a similar pattern to that observed by us. This could only be confirmed from the original data however. For practical purposes the point is moot: the fact that each signal changes within a matter of seconds of the intervention means that either may possibly prove useful in detecting tissue compromise. The PO$_2$ signal is much easier to obtain and interpret than the LDF signal and is more reproducible within and between subjects (the error bars in the figures attest to this). In addition the data is quantitative and is easily interpretable, although the precise PO$_2$ at which tissue compromise begins is still unclear [31,32]. It is likely that this value is different for different tissues, and probably also for different disease states.

**CONCLUSIONS**

We conclude that changes in tissue oxygen tension and perfusion during ischaemia and anoxia depend on the order in which the insults occur, and that they may be described by an exponential model. The data presented here confirm the presence of exhaustible local hypoxic control mechanisms in the microcirculation, and provide further insight about the relationship between general and local tissue responses to a given ischaemic insult.
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