A technique for the measurements of renal ATP in a large animal model of septic shock

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Introduction Acute renal failure (ARF) is a common condition in critically ill patients and is commonly associated with sepsis [1] and high mortality [2]. Understanding of the pathogenesis of septic ARF is poor. Small-animal models of ARF have led to the belief that renal ischemia might be responsible for ARF [1]. However, models of hyperdynamic sepsis fail to show either decreased global renal blood flow [3] or decreased medullary flow [4] suggesting that during hyperdynamic sepsis, global renal ischemia may not occur. Cellular ischemia might occur even in the presence of continuing global blood flow or regional blood flow. Thus, the in-vivo measurement of ATP during hyperdynamic sepsis could provide information on the role of cellular ischemia/hypoxia. ^{31}P magnetic resonance spectroscopy (MRS) in small animals is limited by the need for frequent haemodynamic monitoring, to confirm the adequacy of resuscitation and a systemic hemodynamic state that reproduces human hyperdynamic sepsis. In large animals long-term monitoring is possible, once limitations due to organ movement during ventilation and maintenance within the magnet of an anaesthetised, paralysed and ventilated animal are overcome.

We describe a technique for the detection of sequential ^{31}P spectra over a prolonged period of time in a large mammal receiving continuous invasive blood pressure monitoring during experimental septic shock and induced circulatory arrest.

Methods An implantable coil was placed around the left kidney of three adult Merino ewes. The coil consisted of a single-tuned ^{31}P match+tune+tune-balance variable capacitor network using a Helmholtz-shaped transmit-receive coil, designed to fit snugly around the main body of the kidney without causing superficial ischaemia. Two flexible leads of ~200 mm length connected the coil to the external capacitor network. Isoflurane anaesthesia was maintained with an oxygen/air mix. Fractional inspired oxygen was altered to maintain PaO_2 ~100 mmHg and ventilation controlled to maintain $PaCO_2$ ~40 mmHg. Haemodynamic parameters were recorded continuously. Following a 2-hour observation period, sepsis was induced by intravenous bolus injection of 1.2 ml of 3 x 10^9 colony forming units of live *E. coli* in 50ml normal saline.

^{31}P phosphorus magnetic resonance spectra were recorded at 51.705 MHz in a 3.0 T MR scanner (LX horizon, GE Medical Systems, WI) using a flip angle of 90° and a relaxation delay of 2 sec. Spectra were recorded from a 10 cm thick slice using a spin-echo sequence and represent an average of 450 transients of 2048 data points acquired over a spectral width of 16000 Hz. Spectra were recorded every 15 min for the duration of the study. Respiratory gating was not used.

Results Sustained septic shock was achieved after the administration of *E. coli* with the onset of significant hypotension. The sheep became hypotensive within 30 min (mean arterial pressure dropped from 75 mmHg to 45 mmHg).

The ^{31}P spectra obtained at baseline, after 2 hours of sustained septic shock and following euthanasia demonstrate a relatively limited (18%) change in ATP_β signal during septic shock and contrasts with a prominent fall in the phosphomonoester/phophodiester (PME/PDE) peak ratio (~50%). Preservation of ATP levels occurred despite a significant decrease in kidney perfusion pressure. The changes over time for ^{31}P signals are summarized in figures 1 and 2.

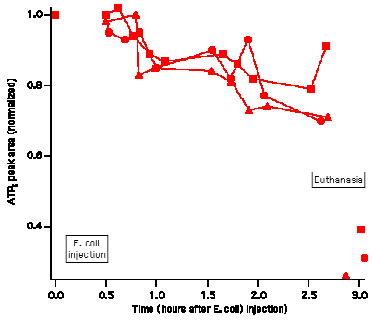
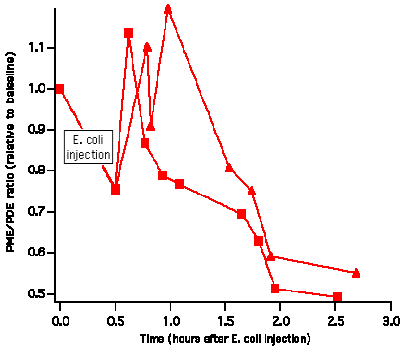


Figure 1 (Left) The changes in ATP peak area during sepsis and after euthanasia for each of the 3 sheep studied. There is a mild decrease in ATP during sepsis and a dramatic reduction immediately after circulatory arrest (euthanasia).

Figure 2 (Right) The changes in ratio of phosphomonoester (PME) to phosphodiester (PDE) peak areas during sepsis (results for 2 sheep).



Discussion The pathogenesis of ARF during sepsis remains unknown but observations in hyperdynamic sepsis do not support ischemia as a possible mechanism [3, 4]. Bioenergetic failure (a decrease in ATP) might occur despite adequate blood flow, however, this has not yet been studied in large animals.

We now report the measurement of ATP over a prolonged period of time in kidneys *in vivo*, using a custom made implanted coil. The results provide provocative insights by showing unexpected changes in membrane related phosphomonoesters/diesters and very limited changes in ATP concentration, a different pattern to that induced by ischemia. These observations suggest that altered cell membrane turnover/cell degradation might be more important than bioenergetic failure.

References

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