The effect of selenium on mitochondrial function and biogenesis in endothelial cells under conditions mimicking sepsis

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Introduction

Sepsis is a major cause of mortality in intensive care units (ICU) and affects about 18 million people every year worldwide [1]. Sepsis is characterised by inflammation and oxidative stress, resulting in massive dysregulated cytokine release and mitochondrial damage.

Mitochondria produce cellular energy in the form of ATP. During this process electrons can leak and initiate oxidative damage via production of superoxide. Although this molecule cannot cross the mitochondrial membrane the cell converts superoxide to hydrogen peroxide in the mitochondria using manganese superoxide dismutase (MnSOD2). The hydrogen peroxide can permeate through the mitochondrial membrane to the cytosol where it can contribute to cellular damage. Cells therefore have several mechanisms for its removal. The glutathione system plays a major role in removing hydrogen peroxide: mitochondrial glutathione peroxidase (GPx) catalyses conversion of hydrogen peroxide to water via cycling of reduced glutathione (GSH) to its oxidised form (GSSG). The GSSG is then converted back to GSH using the enzyme glutathione reductase.

Selenium is a trace element which is necessary for normal immune cell function and several cell signalling pathways. It is essential for the action of selenoproteins including glutathione peroxidase [2]. Trace metals, including selenium, are known to be depleted during sepsis and supplementation with selenium has been suggested to be of potential benefit in patients with sepsis [3,4]. In a study using murine hippocampal neuronal cells, selenium supplementation was shown to enhance mitochondrial biogenesis and improve mitochondrial function even in the absence of oxidative stress [5]. Thus selenium may have benefical effects during sepsis including potentitation of endogenous antioxidant activity via GPx, restoration of mitochondrial function and stimulation of biogenesis, and may also reduce oxidative stress driven inflammation. However the effects of supplementary selenium on sepsis-induced oxidative stress, mitochondrial dysfunction and biogenesis have not been described.

Methods

Human endothelial cells were cultured in the presence of lipopolysaccharide (LPS) and peptidoglycan G (PepG) to mimic sepsis. Cells were exposed to a range of concentrations of sodium selenite to cover physiologically achievable levels. The effects of selenium on aspects of mitochondrial function, mitochondrial mass as a marker of biogenesis, interleukin-6 (IL-6) as a marker of inflammation, and GPx activity were studied.

Experimental treatments

Human endothelial cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% FCS (v/v), 250 μg/ml amphotericin β and 50μg/ml gentamicin

sulphate at 37°C in a humidified incubator in 95% air/5% CO_2. Cells were pre incubated with selenium selenite for five days at 0 – 100nM prior to experimentation in 96 well or 6 well culture plates. Following selenium pre-treatment, cells were then exposed to 2µg/ml LPS and 20µg/ml PepG for up to 7d.

Cell viability
Following experimentation, conditioned medium was removed and the cells were gently washed twice with phosphate buffered saline (PBS). Acid phosphatase buffer, 100µl containing: 0.1% triton X-100, 0.1 M sodium acetate, pH5 and 5 mM p-nitrophenyl phosphate was then added to each well. Cells were then incubated at 37 °C for 1 hour in the dark. Following incubation, the reaction was stopped by the addition of 50µl 0.25 M sodium hydroxide to each well. The absorbance was measured immediately at 405 nm using a spectrophotometer.

Mitochondrial membrane potential
Following experimentation, conditioned medium was removed and the wells were washed twice gently with PBS. JC-1, 7µM in PBS, 100µl was added to each well and plates were incubated for 30min in the dark at 37°C. Following incubation, the cells were washed twice gently with PBS. Finally, 100 µl PBS was added to each well and the plate read immediately using a spectrofluorimeter with excitation at 485 nm and emission at 520/590 nm.

Metabolic activity
Following experimentation, 15µl of Alamar Blue™ dye was added directly to each 96-well plate and mixed gently. The plate was immediately read in a spectrofluorimeter at 37°C, excitation 530 nm and emission 620 nm. Readings were taken every 10min 2h.

Mitochondrial mass
Following experimentation, cells were washed gently with PBS. Mitotracker green FM, 0.5µM was added to PBS and then 100 µl added to each well. Cells were incubated for 30 minutes in the dark at 37°C. Fluorescence was red immediately (excitation 485nm/ emission 520 nm).

Total glutathione peroxidase (GPX) activity
Standard acrylamide gel, 8%, 1.5mm thick were prepared and pre-electrophoresed at 40 mA at 4°C overnight to remove residual APS, TEMED, and incomplete polymerization products. Protein, 200µg was loaded to each well in loading gel buffer. The protein was electrophoresed for 3h at 40 mA at 4°C. Following, this the pre-electrophoresis buffer was removed and replaced with electrophoresis buffer. The gel was run for a further 3h at 4°C and 40mA. Following electrophoresis, the gel was washed x 3 in a 1mM GSH solution for 10min at room temperature. After washing, the gel was incubated with 1% ferric chloride, 1% potassium ferricyanide and 1mM GSH until bands appeared on the gel before rinsing extensively with water. Achromatic bands demonstrate the presence of GPx activity. Gels were imaged and band density calculated using a Syngene Image system.

Interleukin-6
Cells were cultured for 24h as above and conditioned medium was removed. IL-6 was determined using a commercially available enzyme immunoassay kit (R&D Systems, Paisley, UK).

Results

Exposure of cells to selenium for up to 7d had no effect on cell viability (data not shown).

Mitochondrial membrane potential was significantly lower than solvent control cells in cells treated with LPS/PepG without selenium for 7d, but membrane potential was maintained at all concentrations of selenium (Figure 1A). A similar results was found for metabolic activity- LPS/PepG treatment cause decreased metabolic activity and selenium prevented this fall at all concentrations (Figure 1B). However, neither LPS/PepG nor selenium treatment had any effect on mitochondrial mass (data not shown).

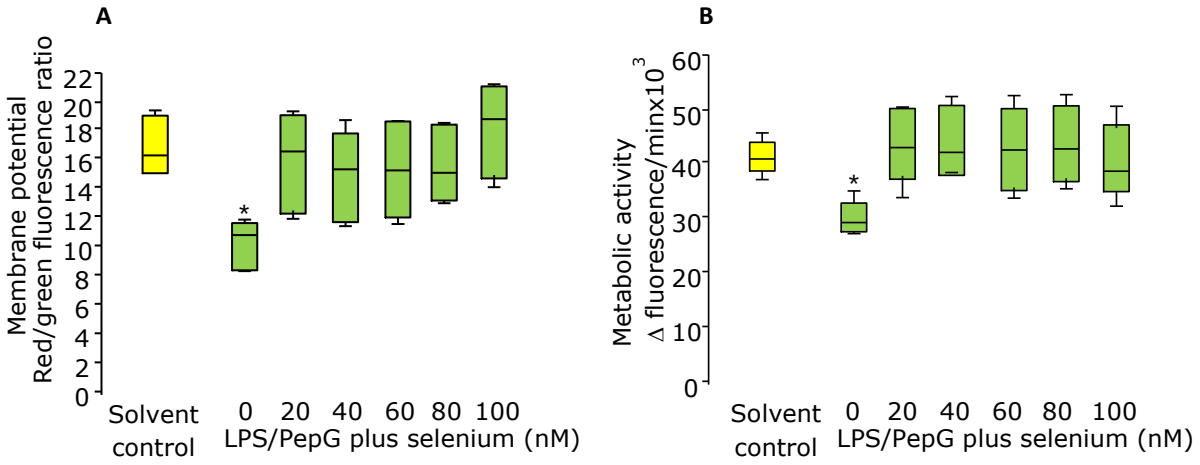
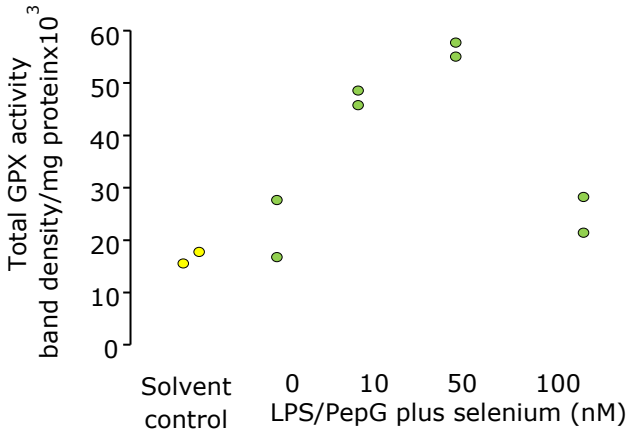


Figure 1
Mitochondrial membrane potential (A) and mitochondrial metabolic actvity (B) in endothelial cells cultured for 7d. Box and whisker plots show median, interquartile and full range (n=6). *=significantly lower than solvent control ($p<0.05$, Mann Whitney U test).

Figure 2
GPx activity in cells cultured for 7d. Individual data points shown (n=2)



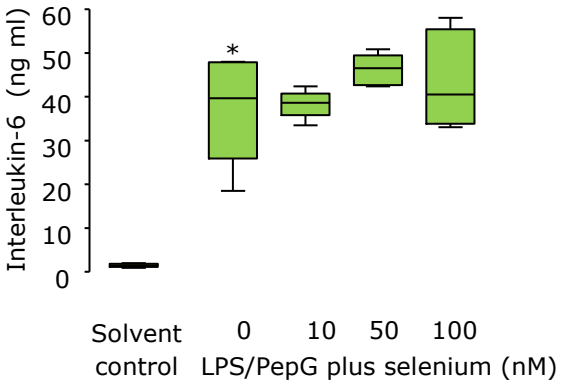


Figure 3
IL-6 in cells cultured for 24h Box and whisker plots show median, interquartile and full range (n=6). *=significantly higher than solvent control ($p<0.05$, Mann Whitney U test).

No conclusions can be drawn from the GPx activity data due to small numbers (Figure 2). However the data do suggest an increase in activity in cells exposed to LPs/PepG plus 20 or 50nM selenium. The lower activity seen in cells exposed to 100nM selenium under conditions of sepsis is unexpected. IL-6 was markedly higher in cells exposed to LPS/PepG as expected but there was no significant effect of selenium (Figure 3).

Discussion

This study showed that LPS/PepG caused changes in mitochondrial function and IL-6 responses without affecting mitochondrial mass in human endothelial cells cultured under conditions similar to sepsis. Exposure of cells to selenium prevented the loss of mitochondrial membrane potential and metabolic activity, but had no effect on IL-6. Selenium may potentiate GPx activity.

Selenoproteins are proteins which contain selenium as selenocysteine. The first identification of a selenoprotein was in 1973 when the role of selenium in GPx was reported; several other functional selenoproteins have since been identified. There are now 17 identified selenoproteins including GPx. In addition to the glutathione system, mitochondrial thioredoxin-2 (TRX-2) has a role in neutralising hydrogen peroxide via peroxiredoxin-3 and subsequent recycling via thioredoxin reductase-2, which is also a selenoprotein. The TRX-2 system is better resistant to the effects of oxidative stress during sepsis and maybe more important in protecting against mitochondrial dysfunction than the GSH system [6].

During sepsis, enhanced mitochondrial generation of the superoxide anion radical is the starting point of mitochondrial oxidative stress [7]. Superoxide does not readily cross the mitochondrial membrane and is catalysed to hydrogen peroxide within the mitochondria by MnSOD. Hydrogen peroxide can diffuse out of the mitochondrion and is metabolised by catalase in the cytosol, but hydrogen peroxide is mainly removed inside mitochondria by oxidation of reduced glutathione using GPx and recycling back to reduced glutathione with glutathione reductase . Cellular GPx activity has been used as a marker of selenium status and indeed the recommended dietary allowance for selenium is based on its effects on cellular GPx. Supplementation with selenium has been shown to lead in increased plasma GPx activity in patients with sepsis [3,4].

Mitochondrial dysfunction is thought to be linked to the development of sepsis induced organ dysfunction [7]. Many studies have shown that derangements in cellular oxygen utilization result from damage to the electron transport chain, and damage to the inner mitochondrial membrane results in impairment of ATP generation and mitochondrial swelling. We assessed the effect of increasing selenium concentrations in protecting against mitochondrial dysfunction in endothelial cells in an environment mimicking sepsis. Mitochondrial dysfunction is associated with collapse of mitochondrial membrane potential, which may further impact on the oxidative phosphorylation pathway (metabolic rate) for ATP generation and may be linked with a state known as the mitochondrial permeability transition. Disruption of the electron transport chain, reduction of ATP synthesis and opening of the transition pore may trigger apoptotic cell death pathways.

Loss of mitochondrial membrane potential and decreased metabolic activity, was seen in LPS/PepG treated cells and selenium protected against this regardless of concentrations. Other studies have reported that selenium can promote biogenesis [5]; in our study we used mitochondrial mass as a measure of biogenesis and found no effect of LPS/PepG exposure and no effect of selenium. The reasons for this are not clear but may reflect different cell types, relative insensitivity of our assay or the type of oxidative insult. In addition the sepsis insult used in our study may not have been severe enough to initiate biogenesis pathways.

Although we showed that LPs/PepG increased IL-6 release from the endothelial cells, selenium exposure had no effect on IL-6 levels. This is in contrast to our previous work using other antioxidants [8] and despite the trend towards increased GPx activity.

In summary we showed that selenium can protect against mitochondrial damage initiated by a sepsis-like insult in human endothelial cells, but failed to have any effect on IL-6 or biogenesis. Further studies are needed to clarify these findings.

References

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