Exposure of mammalian cells to oxygen radicals formed by the reduction of molecular oxygen may result in cell dysfunction and death. In endothelial cells, one of the earliest and most consistent features of oxidant attack is a dramatic depletion in intracellular ATP content. The mechanisms involved in this depletion include inhibition of ADP phosphorylation, inactivation of the glycolytic pathway and activation of poly (ADP-ribose) polymerase due to DNA damage which leads to a consumption of NAD and culminates in a decrease in ATP levels [1,2]. Depletion of ATP, by whichever pathway, will ultimately lead to a perturbation in cellular energy requiring processes. Effective treatment against oxidant damage, in addition to protecting cellular targets from attack, must be able to preserve cellular function. Maintenance of cellular ATP levels may be a good indicator of antioxidant efficacy, however, numerous reports [3,4] have demonstrated that depletion of ATP in itself, is not sufficient to cause cell death and also agents which do not maintain ATP levels have been shown to prevent cell death [3].

In this study we investigated the relationship between early ATP depletion and eventual cell death in human endothelial cells. The ability of various agents to preserve both ATP levels and cell viability, in response to hydrogen peroxide (H$_2$O$_2$), over a short treatment time was compared with their ability to sustain these effects over a longer 'recovery' period. The agents used included catalase, the classic H$_2$O$_2$ scavenger, dimethylthiourea (DMTU) which scavenges both the hydroxyl radical (•OH) and H$_2$O$_2$, phenanthroline, an iron chelator, and 3-aminobenzamide (3-AB), an inhibitor of poly (ADP-ribose) polymerase.

Human endothelial cells were isolated and cultured as previously described [5]. Confluent cell monolayers were washed with a Hank's balanced salt solution containing 0.5% bovine serum albumin (HBSS + A) and treated with H$_2$O$_2$ ± scavenger in 1ml of HBSS + A. After a 5h exposure, HBSS + A was replaced with complete growth media and cells were incubated for a further 24h. ATP was determined by a lucin-luciferase assay [6] and cell viability by the lactate dehydrogenase (LD) leakage assay [7].

After a 5h exposure, H$_2$O$_2$ caused a depletion in ATP levels to 5% of control values (Fig 1). Catalase and DMTU completely prevented this depletion (Fig 1) with a concomitant prevention of cell lysis (results not shown). Similar effects were observed with 3-AB. In contrast, phenanthroline prevented cell lysis but only partially preserved ATP (40% of control values). After 24h, no recovery of ATP was observed in cells exposed to H$_2$O$_2$ alone. Catalase and DMTU treatment maintained ATP content and viability at control levels. In contrast, the short term protective effects of 3-AB were not sustained. Cells treated with phenanthroline did however recover ATP to control levels after 24h.

The different effects of the agents examined illustrate the number of pathways involved in oxidant induced ATP depletion. Scavenging of H$_2$O$_2$ completely abolished ATP depletion whilst inhibiting •OH formation, which is thought to be the major DNA damaging species, by preventing iron-dependent Fenton reactions only partially prevented ATP depletion. However the ability of phenanthroline treated cells to recover ATP levels indicates that such treatment maintained the ATP synthetic capacity. Preventing the metabolic consequences of DNA damage with 3-AB was only effective in the short term and deleterious effects of the agent on the DNA repair process were presumably responsible for the resultant cell death and ATP depletion.

In summary, whilst ATP depletion may be a prerequisite for cell death it is not the sole determinant. The results indicate that long term (24h) maintenance of either ATP levels or ability to recover from depletion by retaining ATP synthetic activity may be a better indicator of the lethality of the original insult and also the efficacy of antioxidant strategies.

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