TRPM2 mediated zinc redistribution mediates H2O2 induced endothelial cell death

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Oxidative stress plays a central role in the pathogenesis of atherosclerosis. By increasing the production of reactive oxygen species, such as H_2O_2, oxidative stress causes apoptosis of endothelial cells. Mechanisms by which H_2O_2 leads to apoptosis, however, are controversial. One study reported that Zn^{2+} released from H_2O_2 oxidation of metallothioneins is the cause of cell death [1], while the other suggests that H_2O_2 activates the TRPM2 channel, resulting in Ca^{2+} influx and cell death [2].

To address the controversy, we have examined the effect of H_2O_2 (1 mM, 6h) on human umbilical vascular endothelial cells using live cell imaging. We monitored changes in the intracellular distribution of free Ca^{2+} and Zn^{2+} using Fluo-4 and FluoZin-3 respectively. We stained lysosomes and mitochondria with LysoTracker and MitoTracker respectively, and dead cells with propidium iodide. Data recorded from N number of cells and n number of independent experiments are expressed as mean±SEM. *P* value of <0.05 (Student's *t*-test) was considered statistically significant.

We found that both Ca^{2+} and Zn^{2+} are highly enriched in lysosomes. Mitochondria showed little stain for either ion. H_2O_2 treatment increased the cytosolic levels of both ions in most cells. In some cells, however, we found striking redistribution of Zn^{2+} from lysosomes to mitochondria: There was a decrease in the number of lysosomes with Zn^{2+} (untreated: 61±6%, n/N=3/75; H_2O_2 treated: 21±1%, n/N=3/49; $P<0.01$), with a concomitant rise in mitochondria with Zn^{2+} (untreated: 11±1%, n/N=3/49; H_2O_2 treated: 41±4%, n/N=3/34; $P<0.01$). These effects were found in the absence of extracellular Zn^{2+}, indicating redistribution of intracellular Zn^{2+}.Inhibition of TRPM2 with PJ34 and 2-aminoethoxydiphenyl borate (2-APB) reduced the H_2O_2 induced release of Zn^{2+} into the cytoplasm (PJ34: 45±2%, n/N=3/43, $P<0.01$; 2-APB: 76±1 %, n/N=3/38, $P<0.05$), as well as its translocation from lysosomes to mitochondria (PJ34: 11±3%, n/N=3/46, $P<0.05$; 2-APB: 13±2%, n/N=3/35, $P<0.05$). Transfected HA-tagged TRPM2 channels showed co-localisation with the lysosomal markers, LysoTracker and CD63, suggesting TRPM2 mediates release of Zn^{2+} from lysosomes.

We next determined the relevance of TRPM2 and Zn^{2+} redistribution to H_2O_2-induced endothelial cell death. Blockers of TRPM2 (PJ34 and 2-APB), as well as chelation of Zn^{2+} with TPEN (N,N,N',N'-tetrakis(2-pyridinylmethyl)-1,2-

ethanediamine), completely inhibited H_2O_2-induced cell death, indicating TRPM2 mediated changes in the redistribution of Zn^{2+} contribute to endothelial cell death.

In conclusion, we discovered a novel mechanism where H_2O_2 activation of TRPM2 causes a redistribution of Zn^{2+} from lysosomes to mitochondria and cytoplasm, resulting in endothelial cell death.

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References:

1. Wiseman, D.A., et al., *Alterations in zinc homeostasis underlie endothelial cell death induced by oxidative stress from acute exposure to hydrogen peroxide.* American Journal of Physiology-Lung Cellular and Molecular Physiology, 2007. **292**(1).

2. Sun, L., et al., *Role of TRPM2 in H2O2-Induced Cell Apoptosis in Endothelial Cells.* Plos One, 2012. **7**(8).

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TO WHOM IT MAY CONCERN

I am writing to confirm that ***Nada Abuarab*** from University of Leeds, registered and attended the 37th Congress of the International Union of Physiological Sciences recently held in Birmingham UK from 21 to 26 July 2013.

I am also able to confirm that the aforementioned individual submitted and presented the poster communication **PCA402** entitled **TRPM2 mediated zinc redistribution mediates H2O2 induced endothelial cell death** at the Congress on Monday, July 22, 2013 and that this abstract is due to be published as part of the online proceedings of IUPS 2013 in early September 2013.

Yours sincerely,

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