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Calcium-independent disruption of microtubule growth following nanosecond pulsed electric field exposure in U87 human glioblastoma cells

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INTRODUCTION

High powered, nanosecond duration pulsed electric fields (nsPEF) have been proposed as a minimal side-effect, electrical cancer therapy, unlikely to result in resistance. They induce apoptotic death in cancerous cells and reduce tumour size by a currently unknown mechanism [1,2].

Microtubule depolymerisation is an intrinsic, early event in normally occurring apoptosis and electric fields have been shown to disrupt microtubule polymerisation [3,4]. Given this, it is therefore possible that nsPEF cause microtubule depolymerisation, which initiates apoptosis.

METHODS

U87 EB3-GFP cells were observed by epifluorescence using a Leica DMI6000 microscope with a 100x objective. Changes in intracellular calcium were measured in U87 cells loaded with 1.25 μ M Fura Red, AM (Life Technologies). Standard HBSS containing either 1.8 mM of calcium or 4 mM EGTA (calcium free) were used as imaging buffers. Intracellular calcium stores were depleted by incubating cells for 30 minutes in calcium free imaging buffer containing 1 μ M thapsigargin. 100, 10 ns, pulses with an electric field strength of 44 kV/cm were applied to cells at a frequency of 10 Hz using an nsPEF generator with 50 Ω output impedance and an electrode delivery system comprising of two steel electrodes, separated by a gap of 1.2 mm and with a 50 Ω impedance in parallel.

RESULTS

Live cell imaging of the microtubule plus end tracking protein EB3-GFP in U87 cells showed that 100, 10 ns pulses delivered at 10 Hz caused a rapid disruption of microtubule growth (figure 1). This disruption occurred within 3 minutes of pulse application, shown by a statistically significant decrease in EB3 fluorescence in nsPEF treated samples, as compared to non-pulsed controls ($t=2.64$, $p=0.02$, $n=11$). A post-pulse increase in intracellular calcium was measured in Fura Red, AM loaded U87 cells. This increase was due to both an influx of calcium from the imaging buffer and release from internal stores.

As calcium is known to depolymerise microtubules we sought to identify if it was the source of the observed microtubule disruption. U87 EB3-GFP cells, with depleted internal calcium stores, were pulsed in calcium free imaging buffer and a post-pulse decrease in microtubule growth, similar to that seen with calcium, was observed (figure 1). Cellular swelling, as a result of nsPEF induced membrane poration, is another possible cause of the microtubule disruption. However, cells pulsed in an isotonic sucrose buffer, to prevent swelling, showed a

similar post-pulse decrease in EB3 fluorescence to those pulsed in normal buffer (figure 1).

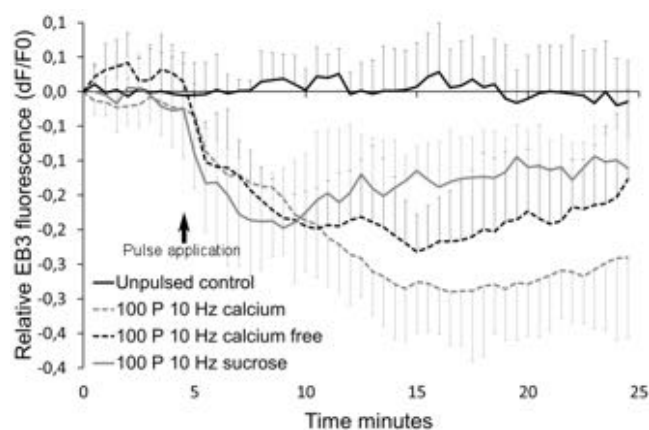


Figure 1: Disruption of microtubule growth, in U87 cells, following nsPEF treatment is independent of increases in intracellular calcium and cellular swelling.

CONCLUSIONS

We show that nsPEF induced microtubule disruption is independent of both changes in intracellular calcium levels and swelling. This suggests the involvement of an alternative mechanism, such as a direct destabilizing action of nsPEF on microtubules or modulation of one of the many microtubule-associated proteins that are linked to microtubule stability. Given the essential role of microtubules in apoptosis execution we propose that their depolymerisation could be an important factor in nsPEF-induced apoptosis.

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