

Impact of Clostridium botulinum C2 Toxin on the ultrastructure of cells

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Background:

The C2 toxin of Clostridium botulinum is a prototypical representative of the binary actin ADP-ribosylating toxin family. It belongs to the AB toxin class, which comprises two components: the enzymatic unit A and the binding and transport unit B. In C2 toxin, C2I represents the enzymatic unit A, and C2II represents the binding component B. To gain its biological activity, C2II must be activated by proteolytic cleavage, converting it to the active component C2IIa. Upon activation, C2IIa binds to the cell surface as a homoheptameric structure and is subsequently endocytosed together with C2I into the cell. In the cytosol, the enzymatically active component, C2I, ADP-ribosylates G-actin. By this, G-actin acts as a capping protein that blocks the polymerisation of actin filaments. This alteration of the actin cytoskeleton eventually leads to cell rounding and ultimately, to cell death¹.

The effect of the C2 toxin on cells was investigated with various assays including fluorescence microscopy of labelled actin². Nevertheless, to date, the effect of C2 toxin on the cellular ultrastructure was not studied. Therefore, we performed transmission electron microscopy (TEM), scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) tomography of C2 intoxicated cells. Furthermore, to better understand the mechanism of C2 intoxication, we also investigated the effect of the individual subunits on the cell architecture.

Methods:

Analysis of the ultrastructure of C2 intoxicated cells was performed using TEM and STEM tomography. For this, cells were high-pressure frozen, freeze-substituted, and embedded in Epon³. The impact of the C2 toxin on the cell surface was investigated using SEM of critical point-dried cells. To test whether the observed effects are mediated by actin, Cytochalasin, an actin polymerisation inhibitor, was used as a control⁴.

Results:

TEM analysis demonstrated that cells exposed to either C2I or C2IIa did not exhibit any ultrastructural changes compared to non-intoxicated cells. However, cells intoxicated with complete C2 toxin displayed many intracellular vesicles and a reduction in cell size. Additionally, blebs were observed along the cell membrane. Control cells, treated with Cytochalasin B, exhibited similar effects as the C2-intoxicated cells, albeit to a lesser degree. SEM analysis revealed that C2 treated cells rounded up and confirmed plasma membrane blebbing. Furthermore, a lack of filopodia on the cell surface was observed. The combination of SEM and TEM analysis revealed that the filopodia were endocytosed by the cell. Cytochalasin B control cells seemed to be close to rounding up, with fewer filopodia on the surface compared to non-treated cells. However, the uptake of filopodia, as seen upon C2 intoxication, was not observed.

STEM tomography of C2-intoxicated cells revealed large vesicles containing cellular debris, as well as stress granules in mitochondria and swollen endoplasmic reticula. In contrast, STEM tomography of untreated cells did not exhibit any of these observations.

Conclusion: The three electron microscopic methods provided a deeper understanding of the impact of C2 toxin on cellular architecture. It was found that only the complete C2 toxin acts as a stressor and affects filopodia, causing them to be engulfed into the cell. Further examination is necessary to gain a better insight into the mechanism behind filopodia engulfment.

Keywords:

AB-Toxin, TEM, SEM, STEM tomography

Reference:

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