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Review

Cationic carriers of genetic material and cell death: A mitochondrial tale

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ABSTRACT

Central to gene therapy technology has been the use of cationic polymers as vectors for DNA and RNA (polyfectins). These have been presumed to be safer than viral systems which, for example, have been found to switch on oncogenes. Two key polycations that have been intensively researched for use as synthetic vectors are poly(ethylenimine) and poly(L-lysine). A frequent stumbling block with these polyfectins is that long-term gene expression in cell lines has not been achieved. Recently it has transpired that both of these polycations can induce mitochondrially mediated apoptosis. It is the aim of this review to discuss the mechanisms behind the observed polycation toxicity including roles for little studied cellular organelles in the process such as the lysosome and endoplasmic reticulum.

1. Introduction

Gene therapy has offered a potential panacea to many heritable genetic conditions and as such has generated intense research interest over more than a decade [1–9]. Key to the success of this technology is high gene transfection rates coupled with low toxicity. Viral vectors have been the vehicles of choice, with some notable success, such as in the treatment of patients with severe combined immunodeficiency (SCID) X1 disease [10,11] who were adenosine deaminase deficient. Advancement in the field has been seriously hampered by induction of severe toxicity and in some cases fatality [11–13]. In order to counteract the deleterious effects incumbent to current viral based vectors, a wide range of synthetic vectors have been developed [14–19]. These have included the polyfectins (cationic polymer–DNA complex), which are based on cationic macromolecules that condense DNA or RNA into stable nanostructures for gene delivery. Two of the most intensely studied polycations are poly(ethylenimine) (PEI) and poly(L-lysine) (PLL). Branched PEI 25 kDa is perhaps among the most efficient synthetic transfectants in experimental gene therapy. PEI condenses DNA into torroidal and globular nanostructures, these can undergo cellular internalization by endocytic and phagocytic routes [20]. Although a range of mechanisms have been suggested, it is generally thought that once internalized into the cell, the polycationic vehicles destabilize endosomal membranes or act as proton sponges resulting in buffering the low endosomal pH and subsequent membrane rupture [21–23], which releases the PEI–DNA complexes into the cytoplasm. Following internalization, poly(L-lysine)s also localize in early vesicles. However, the PLL–DNA complexes do not efficiently escape from endosomes, but curiously gene expression still occurs. A consistent problem in the use of these polyfectin technologies has been a lack of sustained gene expression [24]. Mechanistic studies have shown that both of these polycations condensed with DNA can induce apoptosis through mitochondrially mediated pathways as evidenced by release of cytochrome *c* (Cyt-c) and subsequent induction of executioner caspases (cysteinyl aspartate-specific proteases) (Fig. 1) [25,26]. It is the purpose of this review to discuss these underlying mechanisms of mitochondrially mediated apoptosis induced by the polycations by PEI and PLL and to explore possible roles for organelles such as the lysosome and endoplasmic reticulum [27], that following interaction with polycations can trigger cell suicide [28] via the mitochondrion.

2. PEI-mediated phase 1 cytotoxicity

An in depth study [25] into the cytotoxicity of PEI employed three clinically relevant cell lines (lymphoid, endothelia and hepatic) to

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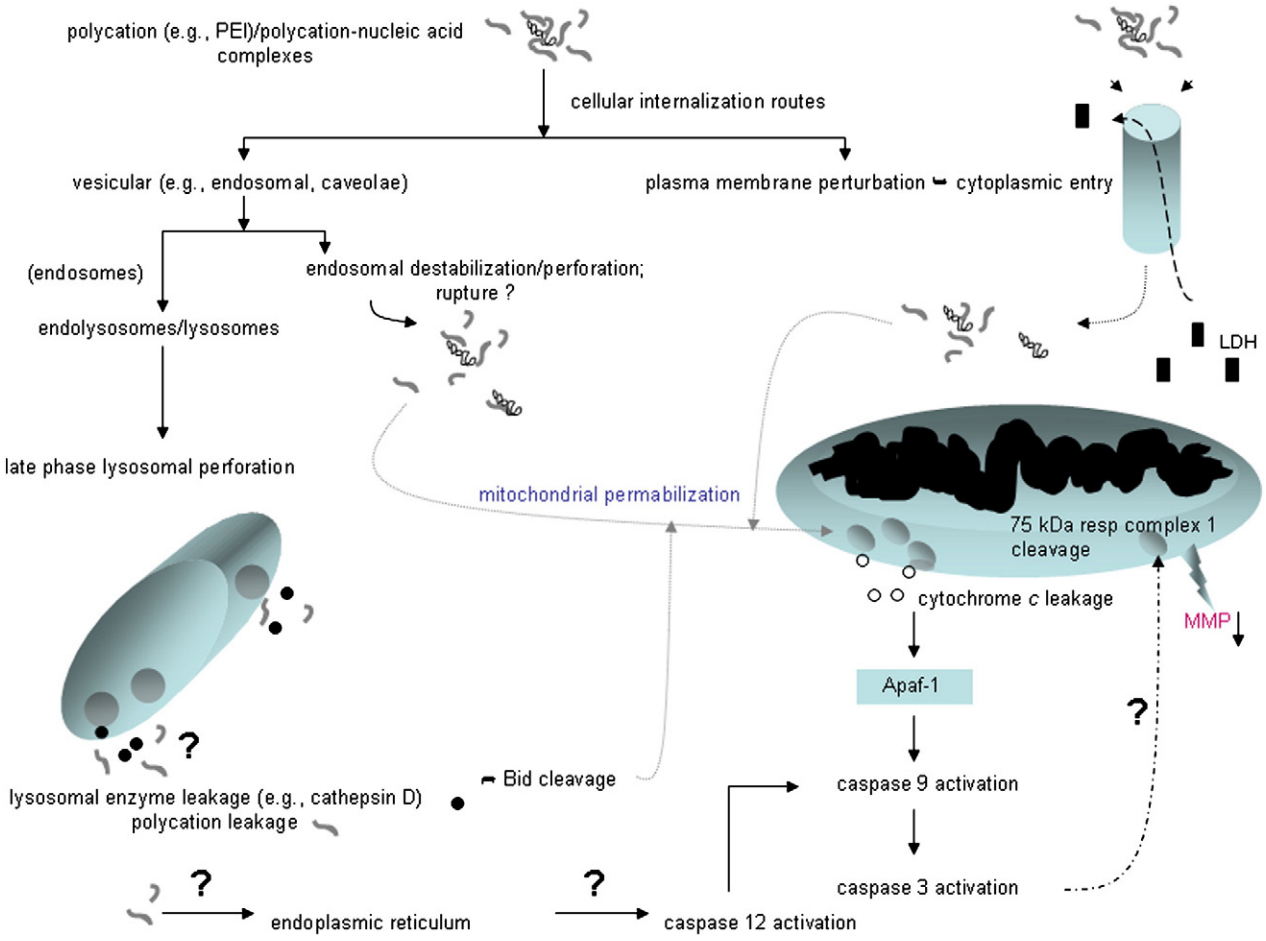

Fig. 1. Schematic diagram showing possible polycation-mediated cell damage/death processes and pathways.

determine the effect of branched (25 kDa) and linear (750 kDa) macromolecular analogues on them. The branched PEI was tested in both free (10 and 30 μg/mL) and complexed with DNA (regardless of PEI:DNA) and was found to generate significant release of lactate dehydrogenase (LDH) within the first hour; this continued in a time-dependent manner. Concomitantly rapid redistribution (30 min) of phosphotidylserine (PS) from the inner plasma membrane to the outer cell surface was observed. The PS externalization was found to be rapid at concentrations of 20 μg/mL and above, in contrast at lower PEI concentrations (10 μg/mL) significant translocation was observed after 24 hours. Although PS translocation is a hallmark of apoptosis [29], it can also occur during early necrotic-like cell damage. Thus, the initial conclusion was that binding of PEI molecules to the plasma membrane proteoglycans [30] results in membrane destabilization and rapid PS exposure. The time-dependent increase in PS translocation supported the activation of apoptotic pathway(s). The mechanics of the apoptotic process result in activated caspases which induce PS "scrambling" followed by a loss of membrane integrity [29,31]. It is difficult to form an exact opinion on the role of the DNA–PEI complex in this interaction due to the fact that the DNA–PEI complex preparations similar to those used in this study may contain as much as 85% free PEI molecules [23] and that even by repeated ultrafiltration procedures not more than 30% of the free PEI cannot be removed. Although, paradoxically this decreases the degree of transcription, presumably through a decrease in available free PEI for membrane rupture [32]. A role for the DNA–PEI complex alone on membrane destabilization and PS exposure cannot be ruled out, as in DNA exchange for anionic proteins in the cytoskeleton, e.g., F-actin [33]. Importantly dissociation in the presence of negatively charged macromolecules (e.g., heparin) has been observed with PEI–DNA complexes [23,34].

The abovementioned observations were consistent with the timeline of interaction observed in a previous study involving intracellular tracking of fluorescently labelled branched PEI–DNA complexes [21]. Fluorescent clumps were seen to attach at discreet features of the plasma membrane in human endothelial-like cells after 30 min incubation. These increased in number over the following 1–2 hours. By 3 hours post-incubation, fluorescence was generated in the cytoplasmic vesicles (endosomes and endolysosomes), which were consistent with complex uptake. Experiments utilizing doubly labelled PEI–DNA demonstrated disruption of some of the endosomes initiating at 4 hours post-incubation with evidence of PEI–DNA separation in the cytoplasm. Nuclear localization (PEI or PEI–DNA) was frequently observed by 3.5–4 hours after administration to the cells. The earliest localization of the complexes within the nuclei preceded the earliest observed transgene expression at 1 hour.

3. PEI-mediated phase 2 cytotoxicity: the role of mitochondria

Significant activation of the effector caspase-3 occurred at around 24 hours post-PEI treatment in all three cell lines, and this was coupled with loss of plasma membrane integrity resulting in apoptotic body formation. Thus, apoptosis represents phase II cytotoxicity. Inclusion of the caspase-3 inhibitor Ac-DEVD-CHO resulted in inhibition of apoptosis. The significance of the degree of apoptosis was highlighted by comparison of that produced by PEI to the established proapoptotic compound doxorubicin hydrochloride (50 μg/mL final concentration). It was found in the case of the Jurkat T-cells that over 90% were in an apoptotic state following 24 hours challenge with doxorubicin. The results of caspase-3 activation with PEI at 10 and 20 μg/mL were approximately 20% and 45%,