EpitopeMap – Exchange Grant Scientific Report

Project: Investigating the effect of cationic nanoparticles on Endoplasmic Reticulum homeostasis

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Introduction

Nanoparticles are present in our daily lives as a result of applications in various areas such as nanotechnology and industry, and also are also found in pollution. As a consequence of their small size, large surface area, chemical composition, and other physicochemical properties, nanoparticles are able to interact with biological systems in ways not yet known. Many of these nanoparticles, such as liposomes and metallic nanoparticulates, display a positive charge on their surface and are known to induce cytotoxicity, but the molecular mechanism by which this occurs has never been described in detail.

We have previously reported that 50 nm amine-modified polystyrene (NH_2-PS) nanoparticles, which display a positive surface charge, induce apoptosis in 1321N1 cells with cleavage of caspases 3, 7 and 9. Damage to the lysosomes and mitochondria was also observed, indicating that the nanoparticles were inducing apoptosis via the intrinsic pathway (Bexiga et al., 2010). The use of this biological system is relevant because it has been reported that nanoparticles present in air pollution not only affect the respiratory tract, but may also cross to the brain causing inflammation, deposition of Amyloid-β, and other pathological events, which are in turn characteristic of neurological diseases such as Alzheimer's and Parkinson's Disease. In addition to the changes we have observed in lysosomes and mitochondria, we have also visualised effects on the morphology of the endoplasmic reticulum (ER), in particular the presence of swollen ER elements several hours after nanoparticle treatment. This suggested an alteration of ER function caused by exposure to the nanoparticles and raised the question of whether the ER was involved in the cytotoxicity mechanism induced by NH_2-PS nanoparticles in 1321N1 cells. Indeed ER stress signaling which is caused by disruption of ER homeostasis has been shown to trigger pro-apoptotic signaling pathways (Szegezdi et al., 2006).

Therefore, the major goal of the visit to the laboratory of Dr. Eric Chevet in Université Bordeaux 2, France, was to evaluate if the ER function was impaired in cells treated with NH_2-PS nanoparticles and if the ER stress response was involved in the cytotoxicity observed in 1321N1 cells upon exposure to NH_2-PS nanoparticles. It was also intended to expand this knowledge to other cell lines. A final aim was to start dissecting the molecular mechanisms of the ER stress response that was observed.

Results

The ER is the site of synthesis and folding for membrane or secreted proteins (Vembar and Brodsky, 2008). In the ER lumen, a complex network of chaperones, foldases and cofactors ensure the proper folding of proteins. However, if ER homeostasis is impaired, protein folding becomes unbalanced and misfolded proteins accumulate in the ER lumen, a condition referred to as ER stress develops. To study the impact of NH_2-PS nanoparticles in cellular function and, more specifically in ER function, 1321N1 cells which had been extensively studied before were once again used. In addition to this cell line, we also used two other cellular models represented by the human glioblastoma cell line U87 and the human hepatoblastoma cell line HepG2 which are commonly used in the Chevet laboratory. In order to validate the use of these cell lines for these studies, the cells were tested for cell death and apoptosis upon exposure to NH_2-PS nanoparticles. It was shown that that incubation with different concentrations of nanoparticles induces cell death with caspases 3 and 7 activation in both cell lines.

1. Impact of NH_2-PS nanoparticles on ER function

To validate our hypothesis that cell exposure to NH_2-PS nanoparticles might affect ER function, we first tested if ER retained its normal function and if secretion had been affected after treatment with the NH_2-PS nanoparticles. This was carried out using ^{35}S-methionine pulse-chase studies in our 1321N1 and U87 cell models. These experiments showed that protein secretion is affected after incubation with NH_2-PS nanoparticles, suggesting that the normal function of the ER is impaired. These results

were confirmed in HepG2 cells, in which treatment with NH_2-PS nanoparticles consistently attenuated the secretion of the secretory protein alpha-1 antitrypsin.

2. Cell exposure to NH_2-PS nanoparticles induces ER stress

As NH_2-PS nanoparticles perturb ER function and lead to an alteration of protein secretion, we first tested whether they also contributed to the induction of ER stress. ER stress leads to the induction of several intracellular signalling pathways collectively known as the UPR (Woehlbier and Hetz, 2011). The UPR is an adaptive response of the cell to try to deal with the stress. If the stress is not resolved, the cell can be driven into apoptosis.

One of the ways in which the UPR acts on the cells is by modulation of transcription of different genes involved in protein synthesis and folding, and also transcription factors. One such factor is CHOP that is activated in response to different signalling pathways of the UPR (Sato et al., 2000). Analysis of CHOP expression in 1321N1 cells revealed an upregulation of this gene up to 12-fold at 9 hours of treatment compared to the untreated cells. Enhanced expression of CHOP occurred relatively early during the incubation with NH_2-PS nanoparticles. Other genes also see their expression upregulated in response to ER stress. Well studied examples include EDEM1, ERDJ4 and HERPUD1, which are all involved in ERAD, which is the cytosolic degradation of misfolded proteins expelled from the ER. HERPUD and ERDJ4 were found to have similar kinetics of expression, which started to increase at 3 hours after the beginning of incubation with NH_2-PS nanoparticles, reaching 3 and 3.5 fold, respectively, after 6 hours before stabilising their expression level. EDEM1 also displayed an increase in its expression, which was confirmed at the protein level by Western blotting for all three cell lines under study. The expression of other genes (GADD34 and GRP94) was also investigated in 1321N1 cells by real-time quantitative PCR, but showed no change when compared to the untreated controls.

Preliminary experiments done in 1321N1, U87 and U87DN cells indicate that eIF2α is phosphorylated upon exposure to NH_2-PS nanoparticles, thereby suggesting that the PERK pathway of the UPR, which has a pro-death role, is being activated.

3. IRE1 signaling contributes to NH_2-PS nanoparticles-mediated cell death

Having found that NH_2-PS nanoparticles perturb ER homeostasis and contribute to the induction of ER stress pathways as assessed by the induction of the expression of ER stress target genes, we next investigated the contribution of each ER stress proximal sensors, namely IRE1, PERK and ATF6. At first we investigated the role of IRE1. To this end we used U87 cells and a stably transfected U87 cell line expressing a dominant negative construct of IRE1α (U87DN (Auf et al., 2010; Drogat et al., 2007)) in which IRE1 activity is impaired. These cells were treated with NH_2-PS nanoparticles and their survival as well as the activation of the caspase pathway was monitored. These results indicated that cells deficient for IRE1 signaling were more resistant to NH_2-PS nanoparticles-induced cell death, thus leading to the conclusion that IRE1 signaling contributed to this pathway.

Conclusions and Future Perspectives

Together with the results previously obtained, these results strongly indicate that ER stress is induced by NH_2-PS nanoparticles in different cells of the neuronal lineage. The cells respond to this by activating the expression of different genes involved in the ER stress response (EDEM1, ERDj4 and HERPUD1) which, in turn, lead to the expression of pro-apoptotic proteins (PUMA and NOXA) with consequent cell death by apoptosis. Autophagy has also been implicated in the cellular mechanism of toxicity. Moreover, the IRE1α and the PERK pathways of the UPR appear to be involved in the ER stress response observed, but this still needs further confirmation. Indeed, the results obtained with the U87DN cell line, strongly suggest that this pathway is involved in the death process observed upon exposure to nanoparticles.

However, despite having clarified that the ER and the ER stress response are involved in the cytotoxicity mechanism induced by NH_2-PS nanoparticles in 1321N1 cells and having expanded that knowledge to other cell lines of the neuronal lineage, the work developed so far in Dr. Eric Chevet's laboratory, has not yet identified the signalling pathways which are activated upon incubation of the cells with NH_2-PS nanoparticles and their contribution to the cytotoxicity mechanism. For that, a new visit to this laboratory in Université Bordeaux 2 has been planned in order to perform

experiments that will allow the clarification of these questions and potentially lead to the publication of an original research article. RNA interference and pharmacological methods will be applied to selectively downregulate the different pathways involved in UPR and measure its influence in cell death. Moreover, the contribution of the individual components of each pathway will also be evaluated in depth.

References

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