Journal of Research and Development

Extended Abstract

Pharma Biotech Congress 2018: Transient loading of CD34+ hematopoietic progenitor cells with polystyrene nanoparticles- Bart Wathiong- Flemish Institute for Technological Research

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Abstract

Introduction: Hematopoietic stem cells and hematopoietic progenitor cells offers the great opportunities to develop the new treatments for numerous malignant and non-malignant diseases.

Recent studies have been focused on the directing NPs towards HSCs; however, the use of NPs in combination with HSCs is not yet fully exploited. HSCs have been labelled with super-paramagnetic iron oxide NPs and gadolinium oxide NPs for magnetic resonance imaging contrast enhancement and cell tracking.

In the present study, we investigated the interaction, which includes the association with the cell membrane and uptake, and kinetics of human cord blood-derived $CD34^+$ HPCs with well-defined, 40 nm sized yellow–green fluorescently labelled carboxylated polystyrene NPs. PS NPs are the medical interest as these are used as multifunctional carriers for therapy. 'These are also commonly used as model NPs to study their interaction with biological systems because of their commercial availability, high quality, and wide range of sizes and surface chemistries. A unique transient loading behaviour was observed, to suggest the active loading and release of the YG-PS NPs by HPCs. This behaviour was compared during the interaction of YG-PS NPs with myeloid-type dendritic cells. DCs are derived by the differentiation from $CD34^+$ HPCs and plays a key role in initiating; directing and controlling both the innate and adaptive immune responses. The CD34-DCs did not exhibit the transient loading behaviour.

Materials & Methods

- Isolation and culture of $CD34^+$ HPCs and CD34-DCs
- Characterization of NPs
- Dialysis of NPs
- Flow cytometry
- Confocal microscopy
- Cell viability and proliferation

Results:

NP characterization

Fluorescently labeled, carboxylated YG-PS NPs with the nominal diameter of 40 nm were used in present study. Before interaction with the cells, proteins and electrolytes present with in the cell culture medium may influence the intrinsic physicochemical parameters of the NP's used. Therefore, YG-PS NP's dispersions in both the water and CCM after different incubation periods were characterized by means of NTA. The resulting YG-PS NP's dispersion's remained to be stable over the duration of the experiment.

Transient NP loading in HPCs

The interaction of HPC's with NP's was evaluated by exposing the cells to fluorescently labelled with the concentration range. After 24 hours of the continuous exposure, the cellular NP load was evaluated by measuring the GMFI by using the flow cytometry. However, compared to the differentiated CD34-DCs, HPCs had a smaller NP loading capacity. As HPC's are non-phagocytic cells and have a lower cytoplasmic-to-nuclear ratio, they are expected to be less efficient in engulfing the large amounts of extracellular

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material, such as NPs, than DCs with a prominent phagocytic activity. In order to confirm the processing of NPs is different by both cell types, the interaction of HPC's and CD34-DCs with NPs was studied as a function of time. The NP load was to be measured at after 1, 2, 3, 4, 5, 6 and 24 hours of exposure to 50 μg mL^{-1} of YG-PS NPs

Energy-dependent loading of HPCs and CD34-DCs with NPs

The NP load of the cells could be accumulating in several ways. Discrimination between passive and active processes can be made by performing the NP exposure of the cells at 4°C. This is the common way to assess the involved mechanisms as cooling down the cells inhibits all energy-dependent cellular processes. Pre-incubation at 4°C for 1 hour and exposure of HPC's, CD34-DCs to 50 μg mL^{-1} of YG-PS NPs also at 4°C strongly abolished the loading with YG-PS NP's. This was even more obvious when compared to acquire load with that accumulated by their cellular counterparts obtained from the same donors but exposed at 37°C.

The observed transient loading cannot be ascribed to the fluorescent staining of the NPs

NP's are recognized to be enter the mammalian cells through the endocytosis mechanism. NPs' journey passes through the endocytic compartment corresponds to an acidification of the NPs' environment. The endosomal acidification was mimicked here by dispersing the YG-PS NPs in citric acid – phosphate buffer having a pH of 5, 6 or 7, which corresponds to the pH within the endolysosomal compartment. Thereafter, YG-PS NPs dispersions were dialyzed against citric acid – phosphate buffers with different acidity. YG-PS NPs stock dispersions were used as purchased or pre-dialyzed for 48 hours to remove all labile dye already present in the NPs dispersions .The time kinetics of the dialyses was measured by fluorescence spectroscopy.

Discussion

In the present study, the interaction kinetics of carboxylate YG-PS NPs in HPCs and CD34-DCs were observed to respond differently & to identical NP's, under identical conditions. HPC's was showed a transient association with these YG-PS NPs, whereas the CD34-DCs displayed a monotonic increase of the NP load over time.

It can partially be explained by the difference in their cell physiology. CD34-DCs are more confined to and involved in antigen presentation and immune responses, whereas the main ability of HPCs is to self-renew or multiply. The differences in cell physiology are coupled with the morphological differences. HPC's was derived from the cord blood having a high nucleus–cytoplasm ratio, with the cytoplasm poor in organelles, although a few mitochondria and endoplasmic reticulum cisternae can be seen. In this contrast, CD34-DCs have been relatively lower nucleus–cytoplasm ratio, with the cytoplasm containing significantly more organelles, including endosomal vesicles.

Conclusion

Therapeutic strategies and transplantations were using the HSCs and HPCs may benefit from their remarkable response to YG-PS NPs. The observed transient loading mechanisms can open the new opportunities for the safe delivery of drugs or molecules of interest with limited bio-accumulation. However, the further investigation was required to be explore whether the observed phenomenon is specific for the NPs used. Effects of NP species, size, functionalization, administered dose, incubation time and intracellular fate should be the carefully investigated. Moreover, further research is needed to reveal how HPC's are capable of mediating the release and which cellular functions and associated dynamics are involved in this process. The resulting knowledge will allow us to exploit the full potential of NP applications in medicine.

This work is partly presented at Annual Pharmaceutical Biotechnology Congress on May 16-17 at 2018 Singapore.