Macedonian pharmaceutical bulletin, 68 (Suppl 1) 21 - 22 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.007
Short communication

Nanotechnology in medicine – our experiences

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Introduction

The success of design criteria for long-circulating drug delivery systems to support membrane receptor-ligand interaction and internalization has been limited and there is a need to develop smarter approaches for efficient drug tumor targeting. In order to overcome some of the current limitations, stimulus-responsive targeting has been combined with passive and active targeting strategies. More innovative nanocarriers that hold promise to optimize targeted drug delivery are systems with the ability for transformation from the stealth long-circulating form to cell interactive form in the complex tumor environment, exposing ligands at their surface for improved ligand-receptor interaction and cell internalization.

This short review will be an overview of several nanomedicines designed and characterized by our research group, and the interplay between their physicochemical characteristics and biological fate.

Discussion

Most commonly used systems to achieve enhanced permeability and retention effect are hydrophilic shell-hydrophobic core nanocarriers, so called polymer micelles, produced with self-assembly of block, grafted or branched copolymers consisting of a hydrophilic backbone and multiple hydrophobic polymer side chains. Smart synthetic linear aliphatic polyesters based on poly(lactic acid), poly(glycolic acid) and poly(ethylene glycol) play a crucial role in the development of a variety of safe, biocompatible, biodegradable therapeutic drug systems, however, their use is limited due to a low degree of functionality (hydroxyl groups). Dimchevska et al. (2017) prepared SN-38 loaded Poly(D,L-lactide-co-glycolide)-b-poly(ethyleneoxide)-b-poly(D,L-lactide-co-glycolide) (PLGA-PEG-PLGA) NPs by nanoprecipitation technique using copolymers with different Mw (LMw NPs 6,000:10,000:6,000Da and HMw NPs 70000:8000: 70000Da), chain length, PLGA/PEG or DLLA/GA ratio, and evaluated their physicochemical properties, nanobiointeractions, toxicity, efficacy as well as biodistribution of radiolabeled NPs in a healthy Wistar rat model. Various polymer properties led to diverse nucleation and growth during nanoprecipitation resulting in difference in the particle size and drug loading between LMw and HMw NPs. Another distinct feature that might also influence the biological fate, protein binding and internalization is the packing density and conformation of the PEG shell which is in control of steric hindrance, hydrophobicity and the zeta potential. Zeta potential was less negative for LMw NPs due to higher density and longer PEG chains. Cytotoxicity of empty NPs in human colon adenocarcinoma SW480 cell culture model was negligible. During internalization studies in SW480 cells, serum proteins showed profound influence in diminishing the differences in the internalization rates among LMw and HMw NPs, highlighting the fact that the protein corona is the nanobiointerface important for cell-NP interaction. In vivo biodistribution studies of radiolabeled NPs verified the efficient protection role of PEG loops directly attached to the NPs surface against immediate sequestration. Blood circulation time was significantly prolonged to several hours for LMw and HMw NPs, though for the nanoparticles with a denser hydrophilic corona (LMw NPs) the condensed packing of the loops could be clearly correlated with more efficient inhibition of phagocytic

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uptake, 30% increased blood circulation time and improved stealth effect. The long circulation time of LMw PLGA-PEG-PLGA NPs loaded with tyrosine kinase inhibitor Ponatinib was confirmed in a zebra fish model. Due to increased tumor distribution in zebrafish xenograft model developed by transplantation of K652 CML cell line into 72 hpf zebrafish embryo, the drug loaded NPs showed significantly lower cardiotoxicity compared to free drug molecules (Al-Thani et al., 2022). Similar effect of contribution of the dense "brush" conformation of PEG hydrophilic corona chains to the stealth effect was noticed for SN-38 loaded PEO-PPO-PEO/Poly(DL-lactide-co-caprolactone) NPs (Geskovski 2015; Koliqi et al. 2016). Two samples of PEO-PPO-PEO/Poly(DL-lactide-co-caprolactone) NPs, prepared by nanoprecipitation procedure with a slightly modified purification step were tested for their biodistribution in Wistar rat model. One of the samples was regularly washed in three cycles and the other was repeatedly purified by centrifugation using ultrafiltration, until no free PEO-PPO-PEO was detected in the supernatants. FTIR evaluation showed two distinctive conformations, brush and loop, of the PEO-PPO-PEO hydrophilic layer for the purified and completely purified sample. Striking similarity of the circulation time of the "brush" corona conformation of PEO-PPO-PEO/ Poly(DL-lactide-co-caprolactone) NPs to PLGA-PEG-PLGA NPs with dense hydrophilic corona was found during the biodistribution studies of radiolabeled samples. Having in mind the close values of hydrodynamic diameters and the zeta potential of the compared NPs we may assume that the Mw, density and surface conformation of PEG corona determines protein corona patterns and the abundance of different proteins in primary and secondary corona, affecting the process of phagocytosis (Djurdjic, 2015; Geskovski, 2015). Compared to the "brush" conformation the "trans" or train/loop conformation of PEG chains of the NPs resulted in almost immediate sequestration and clearance. Therefore, PEG Mw, density or ethylene glycol units per surface area to achieve efficient surface coverage and "brush" conformation should be carefully optimized during the design process if long circulation time has to be achieved. Novel studies indicate to abundance of serum albumin and apolipoprotein E in the protein corona of low-density PEG hydrophilic shell PLGA NPs and higher level of clusterin for shells with increasing density and chain length, confirming that PEG conformation mediates a specific protein adsorption and shapes the protein corona profile. Low degree of functionality of polymers like P(DLLA-CL), PLGA and PCL may be overcome by copolymerization with polyacrylic acid or polyethylene imine. Poly-ε-caprolactone-branched-polyethylene imine (PCL-b-PEI, Mw ~ 40.000-800 Da) was used for design of hyaluronic acid coated paclitaxel loaded PCL-b-PEI multifunctional smart nanocarrier capable of addressing multiple barriers (Moustafa et al. CESPT 2022). Hyaluronic acid structured into charge reversal PCL-b-PEI NP shell improves the EPR effect and tumor localization, decreases the toxicity masking the positive PEI charge and improves specific targeting for CD44 overexpressing tumors. Hyaluronidase enzyme overexpression in tumors amplifies the degradation of HA, exposing the smaller sized positively charged particles in the tumor environment which improves the diffusion through the tumor matrix, membrane interaction, internalization as well as endosomal escape. Stable charge reversal of PCL-PEI with HA and improved biocompatibility was confirmed in A549 cell lines using LDH and MTT tests. Also, higher internalization of HA decorated NPs was verified in A549 cancer cell line overexpressing the CD44 receptor. The anticancer efficacy was also significantly improved in cell lines overexpressing the cluster of differentiation receptor.

Conclusion

Multimodal characteristics of the nanoparticles such as size-tunable properties and charge reversibility induced by pH or enzyme responsive hydrolysis coupled with structural destabilization for rapid drug release, and fused with an efficacious technique for avoiding protein adsorption to minimize or mitigate the negative influence of protein corona on the internalization and sequestration should be future priorities in nanomedicine development.

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