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Ferritin-Based Nanoformulation of Charged Gd-Labelled Acetylcholinesterase Reactivator for Enhanced Bioavailability in CNS

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Extended Abstract

This study focuses on the development of biocompatible delivery systems for acetylcholinesterase reactivators, specifically K2359, which serves as a causal antidote in cases of intoxication caused by organophosphorus compounds [1]. These compounds are commonly found in chemical warfare agents or insecticides, leading to severe and life-threatening poisonings [2]. The primary challenge in treating such poisonings lies in the limited penetration of oximes (reactivators) into the central nervous system, where the intoxication and irreversible nerve tissue changes occur [3]. To address this challenge and enhance the delivery of reactivators to the central nervous system and prevent irreversible nerve damage, a ferritin nanotransporter is proposed. This nanotransporter exhibits a specific binding affinity to the TfR1 receptor, highly expressed in endothelial cells on the blood-brain barrier. Ferritin, a protein responsible for storing and transporting iron in almost all living organisms, shows promise as a drug carrier due to its exceptional biocompatibility and biodegradability. Mammalian ferritins consist of 24 subunits that have the ability to self-assemble into highly organized nanocages with an outer diameter of 12 nm and an inner diameter of 8 nm. Under unfavorable conditions (pH changes), ferritins structure is disrupted, and after adjusting the conditions to favourable ones, it self-reorganizes into nanocages. This process allows an encapsulation of drugs within its internal cavity and allows producing promising nanoformulations useful in targeted anticancer therapy [4]. The presented nanoformulation, when applied after organophosphorus compound intoxication, has substantial potential to increase the penetration of the acetylcholinesterase reactivators through the blood-brain barrier, thereby preventing the consequences of neurological damage [5].

In this study, we encapsulated Gd-labeled charged oxime hereinafter referred to as K2359 within ferritin (to construct K2359@FRT), utilizing an active, pH-dependent, reversible assembly of ferritins quaternary structure. The resulting nanoformulation was thoroughly characterized using atomic force microscopy demonstrating no evidence of ferritins structure deformation. Correct reassembly of ferritins was also confirmed using gel electrophoresis. Further, UV/Vis spectra of K2359@FRT were recorded to determine loading efficiencies (41.0 %, 213.9 μM per 1.0 mg of protein). Fourier transform infrared and Raman spectra further confirm correct protein patterns comparable to native ferritin. The release kinetics of K2359 from K2359@FRT in an acidic endosomal environment was investigated, confirming pH-responsiveness of ferritin. In addition, human plasma hard protein corona analysis revealed practically no binding of plasma proteins on surface of K2359@FRT suggesting good biocompatibility of nanoformulation. Furthermore, examinations of hemocompatibility using human red blood cells revealed no hemotoxicity caused by K2359@FRT. Ongoing follow-up experiments are focused on investigating the *in vivo* real-time monitoring of biodistribution of K2359@FRT using magnetic resonance imaging through Gd-label covalently bound to the K2359 molecule acting as contrast agent.

The findings presented in this contribution establish a robust foundation for future research into the active pH-dependent encapsulation of small hydrophobic compounds to prepare nanoformulations for precise nanomedicine. Noteworthy, ferritins offer a possibility of additional targeting through facile functionalization of ferritin's surface allowing for shifting the nanotransporter affinity to introduce a selectivity towards variety of disease- or tissue-specific proteins.

Acknowledgments

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