Advanced Drug Delivery Reviews 90 (2015) 24–39

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Enzyme therapeutics for systemic detoxification☆

Yang Liu [1], Jie Li [1], Yunfeng Lu *

Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, CA 90095, USA

ARTICLE INFO

Available online 13 May 2015

Keywords:
Detoxification
Protein delivery
Nanocarrier
PEGylation
Protein nanocapsule

ABSTRACT

Life relies on numerous biochemical processes working synergistically and correctly. Certain substances disrupt these processes, inducing living organism into an abnormal state termed intoxication. Managing intoxication usually requires interventions, which is referred as detoxification. Decades of development on detoxification reveals the potential of enzymes as ideal therapeutics and antidotes, because their high substrate specificity and catalytic efficiency are essential for clearing intoxicating substances without adverse effects. However, intrinsic shortcomings of enzymes including low stability and high immunogenicity are major hurdles, which could be overcome by delivering enzymes with specially designed nanocarriers. Extensive investigations on protein delivery indicate three types of enzyme-nanocarrier architectures that show more promise than others for systemic detoxification, including liposome-wrapped enzymes, polymer–enzyme conjugates, and polymer-encapsulated enzymes. This review highlights recent advances in these nano-architectures and discusses their applications in systemic detoxifications. Therapeutic potential of various enzymes as well as associated challenges in achieving effective delivery of therapeutic enzymes will also be discussed.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Current and Forthcoming Approaches for Systemic Detoxification".

* Corresponding author at: Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, Los Angeles, CA 90095, USA.
E-mail address: luucla@ucla.edu (Y. Lu).

[1] Jie Li and Yang Liu contributed equally to this work.

http://dx.doi.org/10.1016/j.addr.2015.05.005
0169-409X/

1. Introduction

In living organisms, numerous biochemical reactions occur synergistically, allowing the organisms to grow and reproduce, convert food to energy, maintain the structure, and response to the environment — all activities that we called "life" [1]. Certain substances, however, could disrupt the processes and induce the organisms to abnormal state, which is termed intoxication [2–7]. Generally, intoxication may originate from toxicants introduced externally or biochemicals produced internally by the metabolic process [8–10]. For example, accumulation or overproduction of certain biochemicals due to metabolic disorder could induce intoxication [11–13], which may even cause life-threatening syndromes in severe situations. Although mechanisms of removal or neutralization of such substances commonly exist in most species, managing intoxication usually requires interventions, generally referred as detoxification, which helps to clear the toxic substances, corrects their levels and minimizes the associated damages [2,14–17].

To date, various methods of detoxification have been developed such as dialysis process and administration of antidote. According to the detoxifying mechanisms, antidote-based detoxification functions mainly through four routes: (1) blocking receptors to reverse the adverse effects caused by the toxicants [15], (2) neutralizing toxicants with high affinity scavengers [18–27], (3) absorbing toxicants physically with carriers [2,28], and (4) converting toxicants to nontoxic substances [16,24,29–32]. Among these strategies, the last one is particularly preferable with fewer side effects [27,33–35]. Enzymes are exquisite biocatalysts that can decompose substrate molecules specifically and efficiently [36]. In this context, enzymes are excellent antidotes against intoxications. Compared to traditional detoxification agents, enzyme-based detoxification agents possess several advantages: (1) high substrate selectivity — enzymes can decompose toxic substances without affecting other biochemical molecules, resulting in less side effects; (2) high catalytic efficiency — enzymes usually catalyze the conversion of their substrates with fast kinetics, which is crucial for making effective antidotes for acute intoxication; and (3) the most direct method to treat intoxications caused by metabolic disorders — enzymes can be delivered to replace the dysfunctional ones to redress metabolic disorders. Because of these advantages, many enzymes have been discovered and developed for decomposing toxic substances [31,32,37–43]. However, native enzymes barely show any detoxification effects, while some of them even caused severe immune responses when administrating systemically. Particularly, exogenous enzymes generally exhibit high immunogenicity and low circulating ability, which result in fast clearance after the administration [44–51]. Although such limitations may be partially mitigated through engineering the enzyme structures, such protein-engineering methods are generally time-consuming and often result in decreased enzymatic activity [51–53].

To circumvent these limitations, nanocarriers were extensively developed, affording a large number of protein therapeutics with improved efficacy and reduced side effects [54–58]. Compared with inorganic nanocarriers (e.g., silica particles), organic nanocarriers were investigated more extensively owning to better biocompatibility and the ease to adjust their chemo-physical properties. To date, various enzyme-nanocarrier architectures have been explored, some of which have been used clinically [54,59]. Such enzyme-nanocarrier architectures mainly include liposome-wrapped enzymes, polymer-conjugated enzymes, and polymer-encapsulated enzymes (Fig. 1). In the following sections, these three architectures will be discussed from the perspective of detoxification achieved through systemic administration. Examples of enzyme-based antidotes and therapeutics will be provided (Table 1) and perspectives in future development of enzyme-based antidotes will be also provided.

2. Nanocarriers for systemic delivery of detoxifying enzymes

2.1. Liposomes

Liposomes have been used as pharmaceutical carriers during the past 30 years [54]. Liposomes are nano-sized artificial vesicles, which can be produced from natural or synthetic phospholipids. Enzymes are typically located in the aqueous core, while other hydrophobic molecules can be dissolved within the bilayers of liposomes [60]. Liposomes provide many advantages for detoxification, including: 1) liposomes are biocompatible; 2) liposomes can stabilize the encapsulated enzymes; 3) hydrophobic toxins can be entrapped into liposomes facilitating their degradation; and 4) the size, charge and surface properties of liposomes can be readily turned by introducing desired lipid moieties such as PEG-conjugated lipids, where PEG stands for poly(ethylene glycol).

A potential problem with liposome-wrapped enzymes, particularly when delivered intravenously, is the rapid removal from the circulation by the reticuloendothelial system [61]. To enhance their circulation half life, "stealth liposomes" have been designed by coating the liposomes with PEG [54,62]. This could be achieved either by constructing liposomes using PEG-conjugated lipids (PEG–lipid) or by post-conjugating PEG on the liposome surface (Fig. 2). Klibanov et al. first reported the preparation of PEGylated liposomes, which increased the circulation half life from less than 30 min to 5 h compared to their non-PEGylated counterparts [63]. The prolonged circulation time is attributed to the large hydrodynamic volume of the PEG chains, which shield around the liposomes and mask the liposomes from immune and metabolic systems [64]. Based on a similar mechanism, other hydrophilic polymers were also used to construct long-circulating liposomes, including poly[N-(2-hydroxypropyl) methacrylamide)] [65], poly-N-vinylpyrrolidones [66], L-amino-acid based polymers [67], and polyvinyl alcohol [68]. However, conjugating with these polymers often decreases the liposome stability, because conjugation of hydrophilic polymers reduces the glass-transition temperature of the liposomes. To maintain necessary stability for this liposome, only a limited amount of polymers could be conjugated, leading to low density of the surface-grafted polymeric layer, which reduce their effects in prolonging the circulation time of liposomes.

To date, various enzymes have been encapsulated into liposomes for detoxification or therapeutic purposes. For example, uricase has been successfully encapsulated within liposomes. Studies showed that liposome-wrapped uricase exhibits more effective management of the uric-acid level than native uricase in hyperuricemia rat model due to the higher uricolytic activity [69,70]. Consistently, L-asparaginase has also been encapsulated within liposomes, resulting in liposome-wrapped L-asparaginase with prolonged circulating time, abrogation of acute toxicity and better retained in vivo antitumor activity [71,72]. For systemic detoxification, Petrikovics et al. co-encapsulated rhodanese and a sulfur donor (thiosulfate) for the detoxification of cyanide. By optimizing their compositions, these liposomes exhibit high encapsulation efficiency, as well as good fluidity for effective cyanide penetration and conversion [73,74]. Promising results have been demonstrated in the detoxification of organophosphates (OPs), in which organophosphorus acid anhydrolase (OPAA) and phosphotriesterase were encapsulated within liposomes and delivered intravenously to eliminate diisopropylfluorophosphate (DFP) and paraoxon in the blood circulation