Injectable nano-antidotes (Nanotidotes) for the treatment of drug overdose and poisoning

Vincent Forster and Jean-Christophe Leroux*

Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zurich, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

*To whom correspondence should be addressed: jleroux@ethz.ch

ABSTRACT

The number of intoxications from xenobiotics has risen tremendously in the last decade, placing poisoning as the leading external cause of death in the United States. This epidemic has fostered an explosion of nanomedicines ("nanotidotes") capable of sequestering or degrading offending compounds *in situ*. Several prototype nanotidotes have shown efficacy in proof-of-concept studies, consistently filling the gap to their clinical translation. As the unmet medical needs in resuscitative care call for safe and effective antidotes, this manuscript critically reviews the recent developments in antidotal nanomedicine.

INTRODUCTION

Drug overdose is the most common form of deliberate self-harm and accidental death in the Western world. Driven by the increase in the number of prescribed drugs with high risk factors (mainly opioid analgesics and benzodiazepines), this intoxication burden has reached epidemic proportions over the past 20 years[1]. Although most of the overdosed patients (*ca.* 2.5 million in the USA) require relatively simple care and have good prognoses, the more severe cases are associated with high morbidity and poor long-term outcomes[2]. In 2010, more than 40,000 people died of drug poisoning (intentional and accidental) in the USA, making poisoning the number one cause of injury-related mortality above motor vehicle crash- and firearm-related fatalities[3]. These poor outcomes are partly due to the lack of adapted, efficient, and versatile detoxification therapies. Indeed, prompt reduction of the body load of a given drug (or toxin) is crucial to guarantee survival. However, apart from a few well-established antidotes (*e.g., N*-acetylcysteine for acetaminophen or naloxone for opioids) and oral decontaminants (*e.g.,* activated charcoal), emergency clinicians mainly rely on moderately effective non-specific life-supporting measures to mitigate the effects of a drug overdose[4]. These measures often include the need for mechanical

ventilation and hemodynamic stabilization and mainly depend on the availability and efficiency of extracorporeal techniques to accelerate drug elimination (*e.g.*, hemodialysis, hemofiltration, and peritoneal dialysis). Although quite efficient at removing the free-fraction of small solutes from the blood, these dialysis modalities are of questionable clinical importance for compounds that have a large volume of distribution and/or a high protein binding affinity.

In view of these limitations, the recent years have witnessed innovative parenteral detoxification approaches involving the use of nanosystems with diverse sizes, shapes, and compositions (Table 1). The motivation in using nanomaterials for biodetoxification purposes – nanotidotes – stems from their modular properties in terms of binding affinity, biodistribution profile and circulation time. Unlike treatments designed to mitigate the effects of a drug or toxin, these nanomedicines are generally engineered to sequester (or sometimes metabolize) the offending compound in the blood. They usually induce a redistribution of the drug from its site of toxicity (*e.g.*, heart or brain) into the blood compartment in a non-bioavailable form. These systems can be divided into three main categories. The largest group – wide spectrum – includes systems relying on fairly non-specific interactions (*i.e.*, partitioning) to sequester the excess drug. They share the advantage of being relatively versatile and, therefore, applicable to a vast range of compounds. While the spearhead of this category is undeniably the intravenous lipid emulsions (ILEs) – a nutritional supplement with proven clinical efficiency towards liposoluble drugs – many other vehicles (such as nanoparticles or liposomes) have shown similar or even higher detoxification efficiencies *in vivo.* The second group of detoxifiers – narrow spectrum – binds toxic agents *via* more specific recognition mechanisms. Antibodies and macromolecular binders are typical examples of sequestering agents in this class of nanotidotes. Finally, the last group of biodetoxifiers – enzymatic – involves nanosystems with catalytic properties. Often referred to as

nanoreactors, these systems contain enzymes that metabolize the drug/toxin of interest into innocuous or less active metabolites. This paper reviews the recent injectable antidotal nanomedicines that have high potential for translation into intensive care units. Because the interactions with blood components and the immune system are potential limitations to the efficacy of these therapies, this article mainly focuses on conceptual strategies that have been validated *in vivo*.

WIDE SPECTRUM ANTIDOTES

In severe overdose cases, biodetoxification is particularly challenging if the specific offending xenobiotic is unknown. Although physical examination, toxidrome recognition, and biochemical tests often provide clues to guide toxicologists towards appropriate specific treatments, the diagnostic process can be time consuming. In an emergency setting, the therapeutic actions taken within the first few hours largely influence the prognosis. In this context, a generic, versatile approach that can rapidly neutralize the toxic compound and/or enhance its elimination, even in case of incomplete or uncertain information, would be of great value to the emergency physician. Strategies based on non-specific interactions (Fig. 1) have demonstrated some potential in sequestering or adsorbing a vast (albeit definite) range of drugs and represent the first attempt to develop a generic treatment.

Intravenous lipid emulsions (ILEs)

The resuscitation effect of ILEs has been well referenced in the scientific literature since Weinberg *et al.*[5] first demonstrated in 1998 an amelioration of the bupivacaine-induced cardiotoxicity in pretreated rats. Only 8 years later, Rosenblatt *et al.*[6] reported the first ILE rescue of a patient under cardiac arrest caused by a combination of bupivacaine and mepivacaine. Thenceforward, there has

been a burgeoning of controlled animal studies and human case reports describing the resuscitative role of ILEs in poisoning scenarios entailing cardiovascular collapse, making ILEs the most investigated antidotal therapies in toxicology[7,8]. A careful analysis of the available data seems to support the benefits of ILEs for the treatment of life-threatening poisoning from cardiotoxic local anesthetics[7,9]. However, despite the large number of enthusiastic publications (mainly case reports), this approach is not devoid of limitations and may be associated with some bias due to the underreporting of unsuccessful treatments[9]. It is important to mention that the lack of controlled, randomized clinical trials along with the reported inefficiency and toxicity relapse of ILEs in some animal studies[10–13] and clinical papers[14,15] have kept this therapy from FDA-approval for any antidotal use.

ILE's resuscitative mechanism of action is still a matter of debate and might be more complex than initially postulated. Initial reports have essentially supported the "lipid sink" theory, which assumes that the emulsion creates an additional plasma lipid phase that extracts the drug from the target tissues, thereby reversing its toxicity. Indeed, a number of studies have shown that ILEs influence the pharmacokinetics of overdosed drugs[10] and even endotoxins[16]. Although this mechanism most likely plays a major role in the case of highly lipophilic compounds with large volumes of distribution, its prominence has been challenged by the successful detoxification of less lipophilic molecules (*e.g.*, atenolol, ethanol, acetaminophen, lamotrigine, mepivacaine, or prilocaine)[7,8,17] and by treatment failures (or even toxicity escalation) in poisonings by some lipophilic drugs[10–13]. There are at least two additional mechanisms that could explain the beneficial effects of ILEs. The first one – enhanced metabolic theory – suggests that ILEs increase the myocytes' provision of energy,-while the second one – positive inotropic theory – stipulates that free fatty acids can improve the calcium flux into the myocardium (*via* voltage-dependent calcium

channels), thus enhancing heart contractility[9]. In contrast, the increase in toxicity occasionally reported after ILE administration could be explained by the "lipid subway" hypothesis recently proposed by Kazemi *et al.*[18]. The authors suggested that, because the entrapment of drugs by the lipid droplets is reversible, ILEs may enhance the drug distribution to well perfused organs such as the brain and heart[10,18]. This pharmacokinetic-related concern could in principle be avoided with systems having better sequestration properties. *In vitro* studies have indeed shown that the modulation of the lipid phase composition[19] and/or droplet size[20] could beneficially influence the partition coefficient of the drug.

Liposomes

Liposomes are hollow spherical vesicles containing an aqueous core surrounded by one or more concentric phospholipid bilayers. Liposomes possessing a transmembrane pH-gradient (Fig. 1, upper right) have been extensively studied for their capacity to efficiently entrap and transport ionizable drugs (mostly weak bases) *in vitro* and deliver them to specific locations *in vivo*[21–23]. In 1999, Mayer *et al.*[24] reported that intravenously injected liposomes with an internal pH of 4 could take up the anticancer drug doxorubicin in the bloodstream and decrease its toxicity. While the objective of this pioneering work was not to use such liposomes in an antidotal fashion, it showed that a transmembrane pH gradient was extremely powerful at extracting drugs from the body. Eight years later, in an *ex vivo* experiment performed on isolated hearts, our group[25] demonstrated that transmembrane pH-gradient multilamellar vesicles counteracted the cardiotoxic effects of high concentrations of amitriptyline. More recently, we provided clear evidence that pretreating rats with long-circulating pH-gradient liposomes reduced the systemic hypotension induced by a high-dose perfusion of diltiazem, a calcium channel blocker[26]. In the treated animals, the reduced volume of distribution and the increased area under the plasma concentration

versus time curve of diltiazem and especially its main active metabolite (deacetyl-diltiazem) revealed that both compounds were sequestered in the blood compartment. The proof-of-concept was then validated in an oral model of intoxication using verapamil, another calcium channel blocker[27]. When injected up to 3 h after the oral intake of verapamil, the liposomal antidote significantly decreased the drug's hypotensive effects. The liposomal formulation was more potent than the commercially available ILEs both *in vitro* (*i.e.,* 20-fold higher drug uptake) and *in vivo* (*i.e.,* recovery time >30% faster). Compared to ILEs, transmembrane pH-gradient liposomes have distinct advantages. These liposomes are less prone to the 'lipid subway' effect because they sequester drugs in an ionized form in their aqueous core, making the back diffusion process across the lipidic membrane much slower. The efficacy of transmembrane pH-gradient liposomes is also less dependent on the partition coefficient, which, in principle, may enlarge the palette of overdosed drugs that could be sequestered. More than 85% of drugs commonly involved in intoxications are low-molecular-weight, amphiphilic, weak bases and are therefore suitable candidates for this antidotal approach.

Nanoparticles

Although the good safety profile, biodegradability, and relative manufacturing simplicity of liposomes and ILEs make these systems particularly attractive, ILEs are often criticized for their lack of stability and limited chemical diversity. In recent years, a considerable amount of work has therefore been invested into the development of other biodetoxifying nanostructures (*e.g.,* nanospheres, nanocapsules, nanotubes, and nanodiamonds)[28–31]. Several studies have reported good extraction efficiencies for drugs or heavy metals under *in vitro* conditions, but most of the systems investigated so far do not possess optimal properties for an antidotal application. The high surface charge[28], non-biodegradability[31] and/or hemolytic effects[29] of several tested systems would

certainly preclude their clinical use, especially considering the high doses that are often needed to counteract the overdosed drugs. However, in two recent publications[32,33], it was shown that nanoparticles could eventually be employed to sequester circulating toxins, which are usually present in much lower concentrations than overdosed drugs. These studies are reminiscent of work initiated 10 years ago with liposomes that were tested as lipoprotein surrogates to trap bacterial endotoxins[16]. Unfortunately, in spite of promising initial data[16], a phase II clinical trial failed to demonstrate the efficacy of liposomes for this application[34]. It is possible that the careful design of nanoparticles with a higher avidity for bacterial toxins may perform better than liposomes *in vivo*.

The first study was conducted by Shea and co-workers[32], who rationally synthesized non-biodegradable, negatively charged, polymeric nanoparticles (50 nm in diameter) based on *N*-t-butylacrylamide and acrylic acid with a high binding affinity to melittin – a cytotoxic peptide isolated from bee venom. The optimized system displayed a binding capacity for melittin more than 10 times greater than that of immunoglobulins. The injected nanoparticles (30 mg/kg) accelerated the clearance of melittin in mice and protected the animals against toxin-induced mortality. In the second publication, Zhang and coworkers[33] engineered a bio-inspired "nanosponge" (85 nm in diameter) that could adsorb *in vitro* at least 3 different pore-forming toxins. This biomimetic system, which consisted of a biodegradable, poly(lactic-*co*-glycolic acid) nanoparticle core surrounded by red blood cell membranes, intercepted the membrane-damaging toxins and diverted them from their cellular target. In mice, the nanoparticles (80 mg/kg) notably increased the survival rate following the injection of the *Staphylococcus aureus* α toxin. However, these promising data need to be balanced by the fact that the nanoparticles only counteracted the α-toxin activity when coated with mouse, but not human, erythrocyte membranes that lack the

toxin-specific receptor. Although further investigations are needed to support the translation of this bio-detoxification approach in human, this interesting platform could open new possibilities in the preparation of antitoxin vaccines[35].

NARROW SPECTRUM ANTIDOTES

Antibodies

Whole antibodies and their fragments represent the most studied class of macromolecular binders. They act as natural detoxifiers by capturing foreign (bio)molecules through a combination of multiple non-covalent interactions between complementary three-dimensional surfaces (Fig. 2a). The concept of a drug-specific antibody, first described more than 45 years ago[36], has fostered the development of two ovine-derived light chain immunoglobulin fragments, Digibind® and DigiFab®, approved by the FDA in 1986 and 2001, respectively, to treat digoxin poisoning. These systems have inspired further endeavors aimed at treating human poisoning induced by miscellaneous xenobiotics including drugs (*e.g.*, colchicine, amitriptyline, nortriptyline, dabigatran), herbicides (*e.g.*, paraquat), venoms, and toxins (*e.g.*, anthrax)[37–41]. Unfortunately, the limitations encountered by these immunotoxicotherapies (*i.e.*, elevated production costs, large doses, and possible immunogenicity) have cast doubts on their commercial potential, causing the discontinuation of Digibind® in 2011 and the recent interruption in the development of TriTab®, an antibody specific to tricyclic antidepressants. However, the latest development of vaccines against substances of abuse (*e.g.*, nicotine, morphine) seems to revive the interest in immunotoxicotherapies[42–44]. Indeed, Treweek and Janda recently showed that high affinity human antibody F(ab')$_2$ fragments (66 mg/kg, i.v.) abolished the mortality induced by the intraperitoneal injection of an LD_{50} cocaine dose (93 mg/kg), and significantly reduced both ataxia and seizure scores in mice[45]. It has to be mentioned that in this study, the antidote was administered only 3 min

after cocaine exposure, and therefore it remains to be established whether the therapy would also be effective at later time points.

Non-immune macromolecular binders

Macromolecular binders (synthetic or semi-synthetic polymers with high binding affinities for exogenous or endogenous substrates) have been investigated for more than a decade in the field of drug biodetoxification[46]. Surprisingly, the development of specific antidotes has been mainly focused on neuromuscular blockers and anticoagulants. Motivated by the steep growth of the blood thinners market, a new generation of potent antithrombotic biopharmaceuticals has recently surfaced. The clinical use of these anticoagulants is, so far, hindered by the lack of compounds to reverse serious bleeding episodes[47]. On this ground, some of the same pharmaceutical companies that propose new coagulation inhibitors are simultaneously developing specific antidotes to these drugs. These antidotes are physically and functionally diverse, ranging from antibody fragments[40] to small synthetic molecules[48], inactivated enzymes (recombinant factor Xa)[49], hemostatic proteins[50], and macromolecular binders[51–54]. The latter class of antidotes was mainly developed to counteract the action of aptameric anticoagulants (nucleic acids that bind to specific coagulation factors). The simplest approach to abolish the activity of aptameric anticoagulants consists of binding them to a polycation[51]. This is the mechanism by which protamine neutralizes heparin in the clinic. In pigs, it was shown that the antidotal activity of some polycations (including protamine) against aptamers was fast (<10 min) at doses ranging from *ca.* 2 to 10 mg/kg which were 4–20-fold higher than the aptamer dose. Although such agents could be considered as generic antidotes for aptamers, their possible interactions with a variety of negatively-charged species and the related unspecific activities of some polycations[55] may hamper their development for this application. Another more specific strategy to counteract the effect of aptamers consists of using

complementary oligonucleotide sequences that inactivate the aptamers upon interaction with the binding region[54] (Fig. 2b). Extensive preclinical studies in mice and pigs have demonstrated that the binding of the oligonucleotide antidotes was quick and relatively long lasting[54]. An anticoagulant aptamer-antidote pair system is currently in clinical trials (phase 2) for the treatment of venous thrombosis and for coronary revascularization procedures[56]. This concept is also now being extended to antiplatelet therapies[57].

Another successful polymeric binder that was developed to reverse the activity of neuromuscular blockers is the marketed cyclodextrin-based drug sugammadex. Cyclodextrins are cyclic oligosaccharides spatially arranged in toroids that can accommodate poorly water-soluble compounds in their inner hydrophobic cavity. The use of cyclodextrins in biodetoxification was introduced by Bom *et al.*[58] in a study where the strong complexation of a γ-cyclodextrin derivative with rocuronium reversed the neuromuscular block of anaesthetized monkeys. In sugammadex, the structure of the cyclodextrin was judiciously modified with eight side chains that enlarged the hydrophobic cavity and with carboxylic acid groups that favored electrostatic interactions with aminosteroid neuromuscular blockers (*e.g.*, rocuronium, vecuronium, and pancuronium) (Fig. 2c). These conformational rearrangements resulted in an injectable nanotidote (2–16 mg/kg) with a remarkably high association constant (10^7 M^{-1}) and low dissociation rate, which proved to be extremely efficient in reversing drug-induced neuromuscular blocks in various clinical scenarios[59]. A similar approach has been recently tested with sulfobutylether-β-cyclodextrin[60] and sugammadex[61] to counteract the toxicity of overdosed verapamil, although with mitigated success despite an ideal scenario where the drug and antidote were infused together[60] or within a very short time interval[61].

Specific nanoparticles

The internal structure and surface of nanoparticles can be modified to increase their specificity and binding affinity towards a particular compound[32]. Stark and co-workers[62] recently proposed the use of magnetic carbon-coated iron nanoparticles decorated with specific antibody fragments in an extracorporeal blood purification circuit to remove toxins and inflammatory cytokines from the body by magnetic separation. The need for an extracorporeal blood device would restrict this biodetoxification strategy to intensive care units that are equipped with hemodialysis facilities. However, compared to the direct injection of the free neutralizing ligand into the bloodstream, the possibility of extracting the particles together with the bound toxic agent from the body is appealing. The validation of this concept will require biodistribution and pharmacokinetic studies to determine the fractions of particles that are cleared by the hepatic system and recovered by magnetic immobilization.

Rather than using ligands such as antibodies, it is possible to increase both the specificity and affinity of nanoparticles using molecular imprinting techniques[63]. It was shown that cross-linked, non-biodegradable nanoparticles molecularly imprinted with melittin exhibited an antibody-like binding affinity for the toxin. When injected intravenously (30 mg/kg) 20 s after melittin, the nanoparticles reduced the mortality by ~50% in mice[64]. Although these data are apparently remarkable, a follow up study from the same group showed an apparent higher *in vivo* efficacy of chemically optimized but not imprinted nanoparticles[32].

ENZYMATIC SCAVENGERS

The design of detoxification systems that mimic biotransformation by oxidizing, hydrolyzing, reducing, or demethylating chemical hazards has been a long-standing objective of antidotal

research. The military field is especially interested in developing prophylactic defenses against cyanide and neurotoxic organophosphates (OPs). In principle, OPs could be detoxified by administering natural enzymatic binders (*e.g.*, butyrylcholinesterase (BChE)), or OP catalytic hydrolyzers (*e.g.*, paraoxonases, OP hydrolase, and OP acid anhydrolase). Unfortunately, the rapid inactivation and clearance of injected enzymes, as well as the potential immunogenicity of non-human proteins are impeding their clinical use. These problems can be circumvented in part by two protection strategies, namely encapsulation and polymer conjugation (Fig. 3), which are briefly described below (see recent review from Szilasi *et al.*[65] for more information on this topic). Although the earliest encouraging approaches took advantage of the extensive circulation time of erythrocytes to entrap and deliver bioscavengers, recent strategies have focused on more controllable, robust, and generally applicable liposomal nanoencapsulation strategies. Petrikovics and colleagues developed sterically stabilized liposome-enzyme complexes (with OP hydrolase or OP acid anhydrolase) that prevented paraoxon and diisopropylfluorophosphate poisoning in mice (*i.e.*, LD_{50} increased by 140-fold for OP hydrolase and OP acid anhydrolase against paraoxon and by 2-fold for OP acid anhydrolase against diisopropylfluorophosphate)[65]. However, this protective effect was reduced by half when the nanocatalytic system was injected after paraoxon contamination[66]. Comparable data were generated when the enzymes were complexed with hydrophobized poly(2-ethyloxazoline) hyperbranched polymers[67]. The modest therapeutic efficacy of these systems can be explained by the very large volume of distribution of paraoxon[68] and emphasizes the importance of the xenobiotic's pharmacokinetic characteristics in the selection of the antidotal strategy.

More recently, linear polymers (*e.g.,* polyethylene glycol (PEG) or polysialic acids, 20-40 kDa) have been conjugated to recombinant human BChE and paraoxonase[69–71]. The modified enzymes

maintained a good activity *in vitro* and provided some protection under a prophylactic regimen (4.2-fold increase in LD_{50} at ~25 mg/kg BChE i.v.). Although BChE may not be optimal for OP decontamination due to intrinsic stoichiometric binding limitations (*i.e.*, 1 mol of enzyme neutralizes 1–2 mol of OP), it is a wide spectrum enzyme with hydrolytic activity for various other molecules, such as cocaine. The investigations surrounding the use of BChE in cocaine detoxification (*i.e.*, in acute toxicity reversal and chronic addiction relief) are noteworthy. Zhan and colleagues have substantially contributed to this field by developing a quadruple BChE mutant that showed a 500-fold improved catalytic efficiency compared to the native enzyme *in vitro*[72]. This mutant was then fused to human serum albumin yielding a highly stable protein therapeutic that demonstrated favorable pharmacokinetic properties and rescued rats from acute cocaine toxicity (enzyme doses: 3–10 mg/kg)[73]. Early clinical trials in 40 recreational cocaine-using volunteers also confirmed the enzyme's safety and efficacy[74]. Moreover, natural cocaine esterases isolated from bacteria living in the soil around coca plants have also been modified by site-specific mutagenesis to confer good catalytic activity at body temperature. The most efficient mutant was sterically stabilized with two 40-kDa PEG chains to increase its circulation time. It exhibited a relatively long-lasting therapeutic efficacy (72 h, 32 mg/kg) in rats overdosed with lethal doses of cocaine[75]. In rhesus monkeys, the PEGylated enzyme (3.3 mg/kg) rapidly diminished the cardiovascular effects of cocaine. These polymer-modified catalytic antidotes are extremely promising, but they still face important challenges in their translation into the clinic, such as the immune response upon the repeated injection of the enzymes[76]. The encapsulation of the enzymes into vesicles such as liposomes might be a way to prevent the *in situ* inactivation of the circulating enzymes by the immune system. However, the entrapment efficiency of enzymes in liposomes is usually quite low[77]. An alternative method to encapsulate multiple enzymes with high efficiency

could be achieved through DNA-directed assembly, as recently shown for alcohol oxidase and catalase in a study reporting the design of a novel nanotidote against ethanol overdose[78]. While very interesting in terms of nano-bioengineering, the complexity such enzyme machineries may also limit their cost-efficient mass production.

PERSPECTIVES

The growing need for efficient countermeasures to treat drug and toxin overdoses has spun substantial progress in the realms of antidotal medicine in the last decade. Although several specific approaches, such as cyclodextrins, antibodies, and oligonucleotides, have been shown to be useful in humans, only a single wide-spectrum system, namely ILEs, is currently available for off-label use in emergency wards. In view of the promising data obtained in preclinical studies, it is likely that more "universal" nanotidotes will enter into clinical trials in the near future. Liposome-based scavengers[26,27] are particularly advantageous due to the recognized safety of liposomes as drug delivery systems. An issue rarely evoked in antidotal studies involving injectable colloids – even those that are claimed biocompatible and/or PEGylated – is the activation of the complement and associated risk of anaphylactoid reactions upon intravenous administration[79]. This could be problematic in weakened, intoxicated patients, especially at the relatively high doses that are often injected in rescue protocols. These reactions will have to be investigated in detail in the future, and protocols to reduce them should be implemented[80]. One step in that direction could be the administration of the larger antidotal particles through alternative routes such as the intraperitoneal route. In this case, systemic exposure can be minimized by limiting the passage of the drug scavengers into the bloodstream and by removing them at the end of the treatment. Upcoming systems will also likely include approaches that combine different antidotal mechanisms to synergistically antagonize the effects of the offending compounds.

Among them, one can cite the co-administration of catalytic enzymes and antibodies[81] and the combination of gene therapy and vaccines[82]. The translation of nanotidotes to the clinic will also require the implementation of better-designed *in vivo* protocols, owing to more stringent ethical regulations, and to the general willingness to reduce the use of animals. The potential clinical value of certain antidotal systems and the validity of many rescue protocols described in the literature can be questioned. In several studies, the nanotidotes were administered either before or few minutes after the drug/toxin, which was often injected intravenously. These administration regimens are not representative of acute intoxications, which generally occur outside medical facilities, are not treated immediately, and involve oral ingestion (*i.e.,* in 85% of the cases). As the treatment timing and pharmacokinetic profile greatly influence the antidotal efficacy, it will be important to better reproduce realistic poisoning scenarios in upcoming antidotal studies.

ACKNOWLEDGEMENTS

This work was supported by the Swiss National Science Foundation [grant number 31003A_124882].

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

REFERENCES

1. Paulozzi, L. J. Prescription drug overdoses: a review. *J. Safety Res.* **43,** 283–9 (2012).

2. Liisanantti, J. H., Ohtonen, P., Kiviniemi, O., Laurila, J. J. & Ala-Kokko, T. I. Risk factors for prolonged intensive care unit stay and hospital mortality in acute drug-poisoned patients: an evaluation of the physiologic and laboratory parameters on admission. *J. Crit. Care* **26,** 160–5 (2011).

3. Murphy, S. L., Xu, J. & Kochanek, K. D. Deaths: final data for 2010. *Natl. vital Stat. reports* **61,** 1–168 (2013).

4. Leroux, J.-C. Injectable nanocarriers for biodetoxification. *Nat. Nanotechnol.* **2,** 679–684 (2007).

5. Weinberg, G. L., VadeBoncouer, T., Ramaraju, G. A., Garcia-Amaro, M. F. & Cwik, M. J. Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. *Anesthesiology* **88,** 1071–1075 (1998).

6. Rosenblatt, M. a, Abel, M., Fischer, G. W., Itzkovich, C. J. & Eisenkraft, J. B. Successful use of a 20% lipid emulsion to resuscitate a patient after a presumed bupivacaine-related cardiac arrest. *Anesthesiology* **105,** 217–8 (2006).

7. Cave, G., Harvey, M. & Graudins, A. Intravenous lipid emulsion as antidote: a summary of published human experience. *Emerg. Med. Australas.* **23,** 123–41 (2011).

8. Jamaty, C. *et al.* Lipid emulsions in the treatment of acute poisoning: a systematic review of human and animal studies. *Clin. Toxicol. (Phila).* **48,** 1–27 (2010).

9. Weinberg, G. L. Lipid emulsion infusion: resuscitation for local anesthetic and other drug overdose. *Anesthesiology* **117,** 180–7 (2012).

10. Litonius, E., Niiya, T., Neuvonen, P. J. & Rosenberg, P. H. No antidotal effect of intravenous lipid emulsion in experimental amitriptyline intoxication despite significant entrapment of amitriptyline. *Basic Clin. Pharmacol. Toxicol.* **110,** 378–83 (2012).

11. Litonius, E. S., Niiya, T., Neuvonen, P. J. & Rosenberg, P. H. Intravenous lipid emulsion only minimally influences bupivacaine and mepivacaine distribution in plasma and does not enhance recovery from intoxication in pigs. *Anesth. Analg.* **114,** 901–6 (2012).

12. Cave, G., Harvey, M., Quinn, P. & Heys, D. Hypertonic sodium bicarbonate versus intravenous lipid emulsion in a rabbit model of intravenous flecainide toxicity: no difference, no sink. *Clin. Toxicol. (Phila).* **51,** 394–7 (2013).

13. Blum, J., Carreiro, S. & Hack, J. B. Intravenous Lipid Emulsion Does Not Reverse Dabigatran-induced Anticoagulation in a Rat Model. *Acad. Emerg. Med.* **20,** 1022–1025 (2013).

14. Levine, M. *et al.* Complications Following Antidotal Use of Intravenous Lipid Emulsion Therapy. *J. Med. Toxicol.* (2013). doi:10.1007/s13181-013-0356-1

15. Bucklin, M. H., Gorodetsky, R. M. & Wiegand, T. J. Prolonged lipemia and pancreatitis due to extended infusion of lipid emulsion in bupropion overdose. *Clin. Toxicol. (Phila).* **51,** 896–8 (2013).

16. Gordon, B. R. *et al.* Neutralization of endotoxin by a phospholipid emulsion in healthy volunteers. *J. Infect. Dis.* **191,** 1515–22 (2005).

17. French, D. *et al.* Partition constant and volume of distribution as predictors of clinical efficacy of lipid rescue for toxicological emergencies. *Clin. Toxicol. (Phila).* **49,** 801–9 (2011).

18. Kazemi, A., Harvey, M., Cave, G. & Lahner, D. The effect of lipid emulsion on depth of anaesthesia following thiopental administration to rabbits. *Anaesthesia* **66,** 373–8 (2011).

19. Dhanikula, A. B. *et al.* Long circulating lipid nanocapsules for drug detoxification. *Biomaterials* **28,** 1248–57 (2007).

20. Morey, T. E. *et al.* Treatment of local anesthetic-induced cardiotoxicity using drug scavenging nanoparticles. *Nano Lett.* **4,** 757–759 (2004).

21. Madden, T. D. *et al.* The accumulation of drugs within large unilamellar vesicles exhibiting a proton gradient – a survey. *Chem. Phys. Lipids* **53,** 37–46 (1990).

22. Wagner, V., Dullaart, A., Bock, A.-K. & Zweck, A. The emerging nanomedicine landscape. *Nat. Biotechnol.* **24,** 1211–7 (2006).

23. Allen, T. M. & Cullis, P. R. Liposomal drug delivery systems: From concept to clinical applications. *Adv. Drug Deliv. Rev.* **65,** 36–48 (2013).

24. Mayer, L. D., Reamer, J. & Bally, M. B. Intravenous pretreatment with empty pH gradient liposomes alters the pharmacokinetics and toxicity of doxorubicin through in vivo active drug encapsulation. *J. Pharm. Sci.* **88,** 96–102 (1999).

25. Dhanikula, A. B., Lamontagne, D. & Leroux, J.-C. Rescue of amitriptyline-intoxicated hearts with nanosized vesicles. *Cardiovasc. Res.* **74,** 480–486 (2007).

26. Bertrand, N., Bouvet, C., Moreau, P. & Leroux, J. C. J.-C. Transmembrane pH-gradient liposomes to treat cardiovascular drug intoxication. *ACS Nano* **4,** 7552–8 (2010).

27. Forster, V., Luciani, P. & Leroux, J.-C. Treatment of calcium channel blocker-induced cardiovascular toxicity with drug scavenging liposomes. *Biomaterials* **33,** 3578–3585 (2012).

28. Chakraborty, S. & Somasundaran, P. Sequestration of drugs using poly(acrylic acid) and alkyl modified poly(acrylic acid) nanoparticles. *Soft Matter* **2,** 850–854 (2006).

29. Jovanovic, A. V *et al.* Surface modification of silica core-shell nanocapsules: biomedical implications. *Biomacromolecules* **7,** 945–9 (2006).

30. Gibson, N. *et al.* Colloidal stability of modified nanodiamond particles. *Diam Relat Mater* **18,** 620–626 (2009).

31. Donkor, A. D., Su, Z., Mandal, H. S., Jin, X. & Tang, X. S. Carbon nanotubes inhibit the hemolytic activity of the pore-forming toxin pyolysin. *Nano Res.* **2,** 517–525 (2010).

32. Hoshino, Y. *et al.* The rational design of a synthetic polymer nanoparticle that neutralizes a toxic peptide in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **109,** 33–8 (2012).

33. Hu, C.-M. J., Fang, R. H., Copp, J., Luk, B. T. & Zhang, L. A biomimetic nanosponge that absorbs pore-forming toxins. *Nat. Nanotechnol.* **8,** 336–340 (2013).

34. Dellinger, R. P. *et al.* Efficacy and safety of a phospholipid emulsion (GR270773) in Gram-negative severe sepsis: results of a phase II multicenter, randomized, placebo-controlled, dose-finding clinical trial. *Crit. Care Med.* **37,** 2929–38 (2009).

35. Hu, C.-M. J., Fang, R. H., Luk, B. T. & Zhang, L. Nanoparticle-detained toxins for safe and effective vaccination. *Nat. Nanotechnol.* **8,** 933–8 (2013).

36. Butler, V. P. & Chen, J. P. Digoxin-specific antibodies. *Proc. Natl. Acad. Sci. U. S. A.* **57,** 71–8 (1967).

37. Flanagan, R. J. & Jones, A. L. Fab antibody fragments: some applications in clinical toxicology. *Drug Saf.* **27,** 1115–33 (2004).

38. Heard, K. *et al.* A Preliminary Study of Tricyclic Antidepressant (TCA) Ovine FAB for TCA Toxicity. *Clin. Toxicol. (Phila).* **44,** 275–281 (2006).

39. Yalindağ-Oztürk, N., Goto, C. S., Shepherd, G., Torres, O. N. & Giroir, B. A pilot pharmacokinetic study of tricyclic antidepressant ovine Fab for TCA poisoning in children. *Clin. Toxicol. (Phila).* **48,** 418–23 (2010).

40. Schiele, F. *et al.* A specific antidote for dabigatran: functional and structural characterization. *Blood* **121,** 3554–62 (2013).

41. Hu, W.-G., Yin, J., Chau, D., Negrych, L. M. & Cherwonogrodzky, J. W. Humanization and characterization of an anti-ricin neutralization monoclonal antibody. *PLoS One* **7,** e45595 (2012).

42. Syed, B. A. & Chaudhari, K. Smoking cessation drugs market. *Nat. Rev. Drug Discov.* **12,** 97–8 (2013).

43. Shen, X. Y., Orson, F. M. & Kosten, T. R. Vaccines against drug abuse. *Clin. Pharmacol. Ther.* **91,** 60–70 (2012).

44. Janda, K. D. & Treweek, J. B. Vaccines targeting drugs of abuse: is the glass half-empty or half-full? *Nat. Rev. Immunol.* **12,** 67–72 (2012).

45. Treweek, J. B. & Janda, K. D. An antidote for acute cocaine toxicity. *Mol. Pharm.* **9,** 969–78 (2012).

46. Bertrand, N. *et al.* New pharmaceutical applications for macromolecular binders. *J. Control. Release* **155,** 200–10 (2011).

47. Ansell, J. Blocking bleeding: reversing anticoagulant therapy. *Nat. Med.* **19,** 402–4 (2013).

48. Dolgin, E. Antidotes edge closer to reversing effects of new blood thinners. *Nat. Med.* **19,** 251 (2013).

49. Lu, G. *et al.* A specific antidote for reversal of anticoagulation by direct and indirect inhibitors of coagulation factor Xa. *Nat. Med.* **19,** 446–451 (2013).

50. Perzborn, E. *et al.* Reversal of rivaroxaban anticoagulation by haemostatic agents in rats and primates. *Thromb. Haemost.* **110,** (2013).

51. Oney, S. *et al.* Development of universal antidotes to control aptamer activity. *Nat. Med.* **15,** 1224–8 (2009).

52. Flight, M. H. Biologics: The quest for a universal antidote. *Nat. Rev. Drug Discov.* **8,** 934 (2009).

53. Rusconi, C. P. *et al.* RNA aptamers as reversible antagonists of coagulation factor IXa. *Nature* **419,** 90–4 (2002).

54. Rusconi, C. P. *et al.* Antidote-mediated control of an anticoagulant aptamer in vivo. *Nat. Biotechnol.* **22,** 1423–1428 (2004).

55. Jain, S. *et al.* Nucleic acid scavengers inhibit thrombosis without increasing bleeding. *Proc. Natl. Acad. Sci. U. S. A.* **109,** 12938–43 (2012).

56. Povsic, T. J. *et al.* A Phase 2, randomized, partially blinded, active-controlled study assessing the efficacy and safety of variable anticoagulation reversal using the REG1 system in patients with acute coronary syndromes: results of the RADAR trial. *Eur. Heart J.* 1–9 (2012).

57. Nimjee, S. M. *et al.* Rapidly regulating platelet activity in vivo with an antidote controlled platelet inhibitor. *Mol. Ther.* **20,** 391–7 (2012).

58. Bom, A. *et al.* A Novel Concept of Reversing Neuromuscular Block: Chemical Encapsulation of Rocuronium Bromide by a Cyclodextrin-Based Synthetic Host. *Angew. Chem. Int. Ed. Engl.* **41,** 265–270 (2002).

59. Aniskevich, S., Leone, B. J. & Brull, S. J. Sugammadex: a novel approach to reversal of neuromuscular blockade. *Expert Rev. Neurother.* **11,** 185–98 (2011).

60. Mottram, A. R., Bryant, S. M. & Aks, S. E. Dose-dependent response to cyclodextrin infusion in a rat model of verapamil toxicity. *West. J. Emerg. Med.* **13,** 63–7 (2012).

61. Ozbilgin, S. *et al.* Evaluation of the effectiveness of sugammadex for verapamil intoxication. *Basic Clin. Pharmacol. Toxicol.* **113,** 280–5 (2013).

62. Herrmann, I. K. *et al.* Device for continuous extracorporeal blood purification using target-specific metal nanomagnets. *Nephrol. Dial. Transplant* 1–7 (2011).

63. Chen, L., Xu, S. & Li, J. Recent advances in molecular imprinting technology: current status, challenges and highlighted applications. *Chem. Soc. Rev.* **40,** 2922–42 (2011).

64. Hoshino, Y. *et al.* Recognition, neutralization, and clearance of target peptides in the bloodstream of living mice by molecularly imprinted polymer nanoparticles: a plastic antibody. *J. Am. Chem. Soc.* **132,** 6644–5 (2010).

65. Szilasi, M., Budai, M., Budai, L. & Petrikovics, I. Nanoencapsulated and microencapsulated enzymes in drug antidotal therapy. *Toxicol. Ind. Health* **28,** 522–31 (2012).

66. Petrikovics, I. *et al.* Comparing therapeutic and prophylactic protection against the lethal effect of paraoxon. *Toxicol. Sci.* **77,** 258–62 (2004).

67. Petrikovics, I. *et al.* Enzyme-based intravascular defense against organophosphorus neurotoxins: Synergism of dendritic-enzyme complexes with 2-PAM and atropine. *Nanotoxicology* **1,** 85–92 (2007).

68. De Schryver, E., De Reu, L., Belpaire, F. & Willems, J. Toxicokinetics of methyl paraoxon in the dog. *Arch. Toxicol.* **59,** 319–22 (1987).

69. Ilyushin, D. G. *et al.* Chemical polysialylation of human recombinant butyrylcholinesterase delivers a long-acting bioscavenger for nerve agents in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 1243–8 (2013).

70. Tiegang, L., Nana, W., Heng, D. & Min, Z. Polyethylene glycosylation prolongs the stability of recombinant human paraoxonase-1. *Toxicol. Lett.* **210,** 366–71 (2012).

71. Geyer, B. C. *et al.* Plant-derived human butyrylcholinesterase, but not an organophosphorous-compound hydrolyzing variant thereof, protects rodents against nerve agents. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 20251–6 (2010).

72. Pan, Y. *et al.* Computational redesign of human butyrylcholinesterase for anticocaine medication. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 16656–61 (2005).

73. Brimijoin, S. *et al.* A cocaine hydrolase engineered from human butyrylcholinesterase selectively blocks cocaine toxicity and reinstatement of drug seeking in rats. *Neuropsychopharmacology* **33,** 2715–25 (2008).

74. Zheng, F. & Zhan, C.-G. Are pharmacokinetic approaches feasible for treatment of cocaine addiction and overdose? *Future Med. Chem.* **4,** 125–8 (2012).

75. Narasimhan, D. *et al.* Subunit stabilization and polyethylene glycolation of cocaine esterase improves in vivo residence time. *Mol. Pharmacol.* **80,** 1056–65 (2011).

76. Sun, W., Luo, C., Tipparaju, P., Doctor, B. P. & Saxena, A. Effect of polyethylene glycol conjugation on the circulatory stability of plasma-derived human butyrylcholinesterase in mice. *Chem. Biol. Interact.* **203,** 172–6 (2013).

77. Chaize, B., Colletier, J.-P., Winterhalter, M. & Fournier, D. Encapsulation of Enzymes in Liposomes: High Encapsulation Efficiency and Control of Substrate Permeability. *Artif Cells Blood Substitutes Biotechnol.* **32,** 67–75 (2004).

78. Liu, Y. *et al.* Biomimetic enzyme nanocomplexes and their use as antidotes and preventive measures for alcohol intoxication. *Nat. Nanotechnol.* **8,** 187–192 (2013).

79. Szebeni, J., Muggia, F., Gabizon, A. & Barenholz, Y. Activation of complement by therapeutic liposomes and other lipid excipient-based therapeutic products: Prediction and prevention. *Adv. Drug Deliv. Rev.* **63,** 1020–1030 (2011).

80. Moghimi, S. M. & Farhangrazi, Z. S. Nanomedicine and the complement paradigm. *Nanomedicine* **9,** 458–60 (2013).

81. Brimijoin, S. *et al.* Anti-cocaine antibody and butyrylcholinesterase-derived cocaine hydrolase exert cooperative effects on cocaine pharmacokinetics and cocaine-induced locomotor activity in mice. *Chem. Biol. Interact.* **203,** 212–6 (2013).

82. Carroll, M. E. *et al.* Combined cocaine hydrolase gene transfer and anti-cocaine vaccine synergistically block cocaine-induced locomotion. *PLoS One* **7,** e43536 (2012).

83. Petrikovics, I. *et al.* Nano-intercalated rhodanese in cyanide antagonism. *Nanotoxicology* **4,** 247–54 (2010).

FIGURES AND LEGENDS

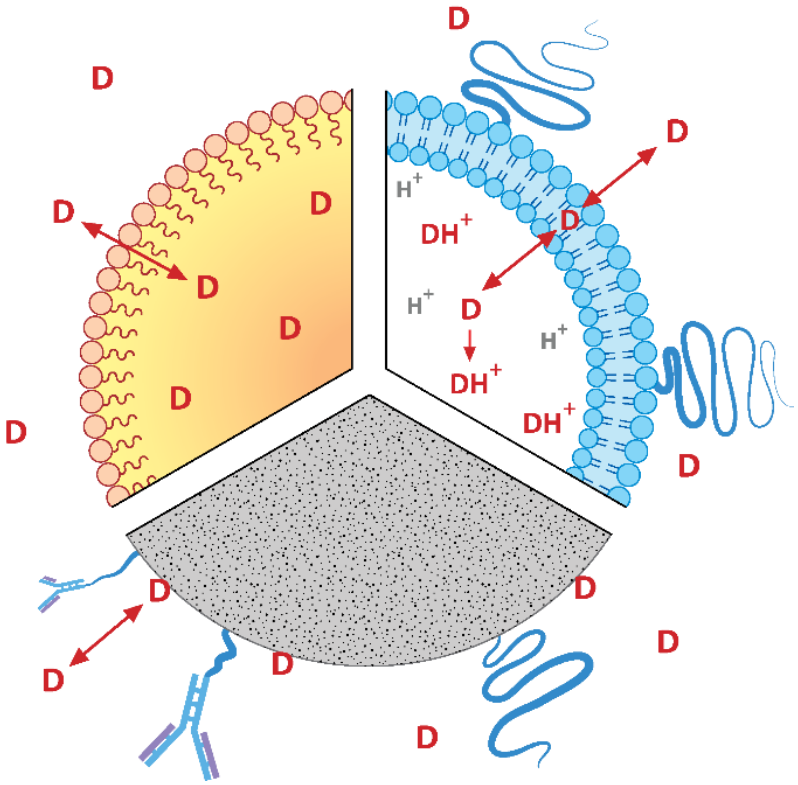

Figure 1. A representation of the three main wide-spectrum nanotidotes used in systemic biodetoxification. While lipid emulsions (upper left) rely on a favorable oil/water partition coefficient to drive the excess of drug (**D**) into their lipid core (or at the interface), pH-gradient liposomes (upper right) capture drugs in their aqueous core through an ionization process. As for polymeric nanoparticles (bottom), they mainly adsorb the offending compounds *via* hydrophobic and/or electrostatic interactions.

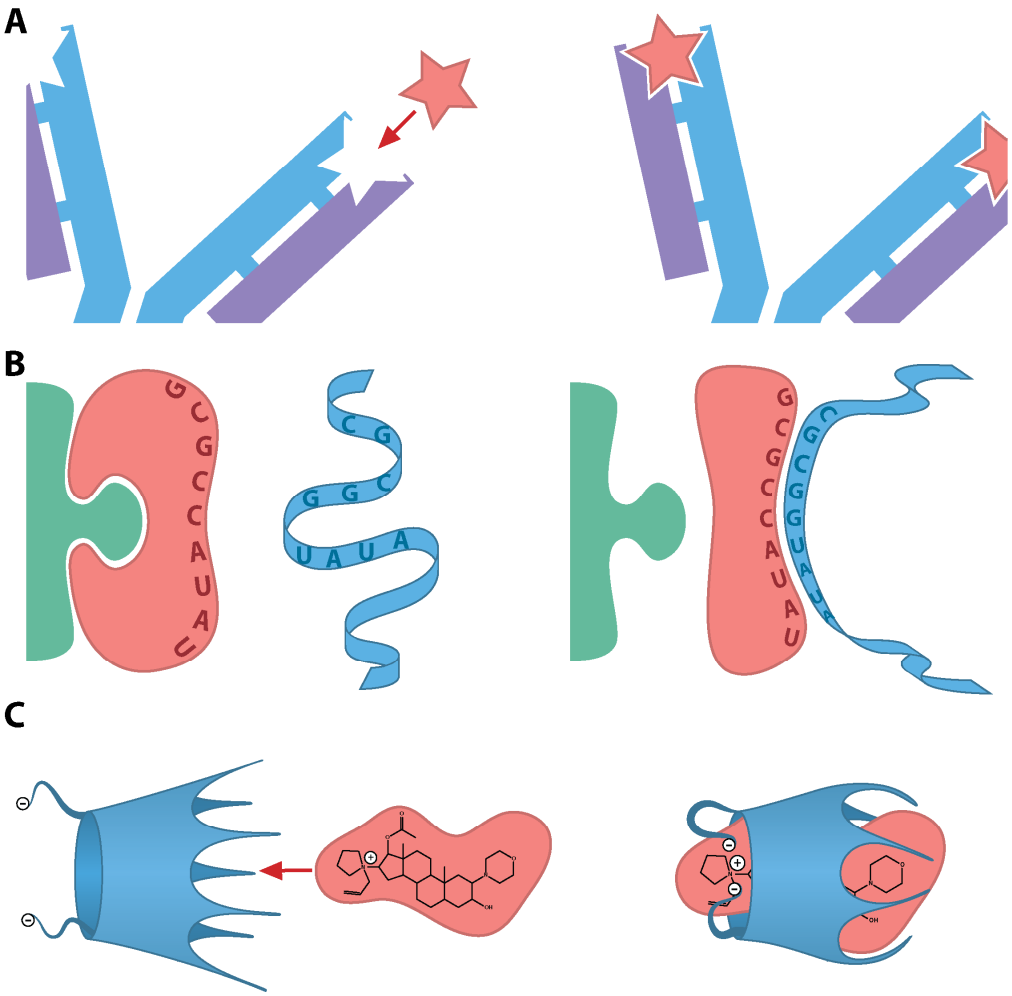

Figure 2. A schematic representation of the sequestering nanotidotes (blue) with enhanced specificity, and their interactions with the drug/toxin (red). **(A)** Monoclonal antibodies or antibody fragments. **(B)** Oligonucleotides with sequences complementary to the active site of aptameric drugs. **(C)** Chemically-modified cyclodextrins capturing rocuronium.

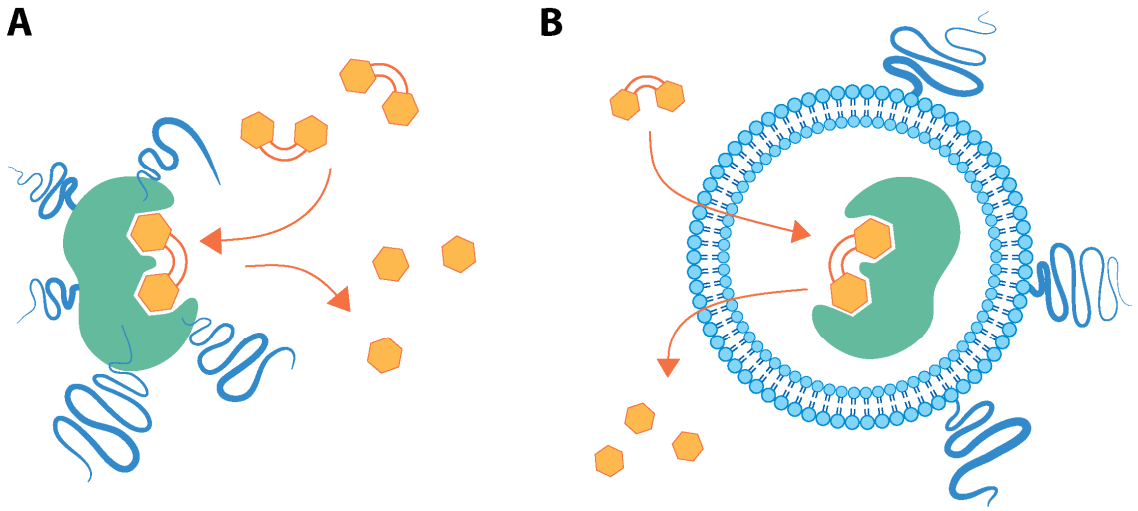

Figure 3. A schematic representation of the catalytic nanotidotes (green) with biotransforming ability for toxic xenobiotics (yellow). These enzymes can either be stabilized by polymers **(A)** or be protected within long-circulating liposomes **(B)**.

TABLES

Table 1. Inventory of nanotidotes with established *in vivo* or clinical efficacies.

System	Specificity	Xenobiotic	Remarks	Status	Ref.
pH-gradient liposomes	+	Calcium channel blockers	◆ Biocompatible/biodegradable ◆ Easy to manufacture ◆ Limited to ionizable compounds ◆ Not adapted to neutralize toxins	Discovery	25–27
Lipid emulsions	+	Calcium channel blockers β-blockers Anesthetics Antidepressants Antiarrhythmics Anticonvulsants Antipsychotics	◆ Evidence of clinical efficiency ◆ Readily available, off-label use ◆ Unclear mechanism ◆ Weak sequestration	Approved (nutrition)	7,8,17
Polycation	+	Aptameric anticoagulants	◆ Easy to manufacture ◆ Potential toxicity	Discovery	51,55
Polymeric nanoparticles	+/+++	Antidepressants Local anesthetics Cardiac glycosides Toxins	◆ High surface area and good stability ◆ Need for more biodegradable systems ◆ Potential toxicity at high doses ◆ High cost for specialized systems (erythrocyte membrane coating) ◆ Possibility to design molecularly imprinted matrices	Discovery	32,33, 62,64
Modified cyclodextrins	++	Neuromuscular blockers	◆ Good safety profile ◆ Proven clinical efficiency	Approved	59–61
Enzymes	++	OPs Cyanide	◆ Degradation of toxic agent ◆ Potential immune response	Discovery	65–67, 69–71, 73,78, 81–83
Oligonucleotides	+++	Aptameric anticoagulants and antiplatelets	◆ High affinity ◆ Narrow spectrum	Phase IIb	49–51, 54,56, 57
Antibodies	+++	Cardiac glycosides Antidepressants Antigout agents Anticoagulants Toxins Recreational drugs	◆ High affinity ◆ Narrow spectrum	Approved	37–41, 45,81, 82