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Will Short Peptides Revolutionize Chelation Therapy?

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Abstract: It will soon be twenty years since the last chelating agent was clinically approved to be used against toxic metals. Even though metal poisoning has been known to humankind for centuries, only about a dozen compounds, all of which are small molecules, compose the pharmaceutical toolbox to expel intrinsically toxic or essential but misregulated metals. These compounds widely suffer from various drawbacks, most critically, poor metal selectivity. Can medicinal inorganic chemistry offer modern solutions to these old challenges? In this perspective, the opportunities and advantages of harnessing short peptides for chelation therapy are described. While broadly aiming to address various toxic metals, achievements in targeting lead (Pb) with peptides reveal the unexplored potential hidden in this chemical space and raise the possibility that peptides may reform chelation therapy.

Keywords: Chelation therapy · Medicinal inorganic chemistry · Peptides · Toxic metals

Michal Shoshan graduated in chemistry and biology at Tel-Aviv University in 2008 and conducted her PhD studies at the Hebrew University of Jerusalem, working on the design of peptidic models for copper metallochaperones, both for mechanistic investigation and as drug candidates for Wilson's disease. After graduating in 2015, she spent three years at ETH Zurich, where she worked as a Marie Curie postdoctoral fellow in the group of Prof. Helma Wennemers on the development of peptide-coated platinum nanoparticles as antitumor agents. Since 2019, Michal has been a group leader at the Department of Chemistry of the University of Zurich, supported by the Swiss National Science Foundation. She works on developing new candidates for chelation therapy and environmental remediation of toxic metals.

1. Toxic Metals

Four metals appear in the list of the top ten chemicals of public health concern by the World Health Organization (WHO); arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg).[1] Apart from arsenic, for which essentiality to humans is debatable,[2,3] these metals are nonessential elements that interfere with numerous biochemical processes of life by replacing native metal ions in metalloenzymes, deactivating proteins by their undesired binding, hampering metal-mediated signal transduction, shifting the oxidative state of cells or organelles, *etc*.[4,5]

In addition to these intrinsically poisonous metals, essential ones can become toxic should their body burden be uncontrolled.[6,7] Within the list of nutritious metals that require tight regulation, the most dominant are copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn). While being crucial structural and enzymatic cofactors, these metals are subjected to sophisticated cascades to control their cellular levels[8] to avoid participating in undesired chemistries, for example, the Fenton reaction, which is catalyzed by redox-active metals such as Fe[9] and Cu[10] to form radicals from hydrogen peroxide.

Genetic mutations in these natural mechanisms responsible for the steady homeostasis of metallic nutrients are the core causes of several chronic disorders such as Wilson's disease (for Cu),[11] primary hemochromatosis,[12] and β thalassemia (for Fe),[13] and hypermanganesemia (for Mn).[14] Furthermore, an unbalanced diet or food and water contaminations can also lead to uptake of essential metals above their healthy amounts.

2. Chelation Therapy

Being intrinsically toxic or essential yet misregulated, metal ions may negatively affect human health due to their undesired accumulation. Chelation therapy is the primary treatment modality against such poisonous metals.[15] It requires administering a chelating agent (CA), mostly a small organic molecule, that should remove the toxic metal of interest from the body through the urinary or the fecal systems (Fig. 1, Table 1).[15]

An ideal CA should fulfill several requirements:[4,16] (i) high aqueous solubility of both the *apo* and *holo* forms, (ii) high metal-binding affinity, (iii) high metal selectivity, (iii) ability to cross tissues and cellular membranes, (iv) low toxicity of both the *apo* and *holo* forms, (v) ability to be eliminated, and (vi) defined complex speciation with the desired metal ion in a preferably 1:1 ratio.

While the current CAs achieve several of these criteria, failing in the other parameters significantly reduces their efficacy and translates into their own toxicity.[17] As a result, in the vast majority of cases, the decision of whether to treat metal poisoning and from which level is often a compromise between the toxicity of the metal and that of the CA itself.

The most critical drawback of these compounds is their inability to distinguish between the toxic metal of interest and essential and physiologically relevant ones, which causes poor metal selectivity (Table 1).[4,5,15,18,19] As a result, essential ions are depleted during treatment, also requiring a high dose to achieve the desired outcome.

Nature approaches the challenges related to metal selectivity with peptides and proteins.[20–24] Furthermore, nature also utilizes peptides and short proteins to solve metal poisoning issues.[25–31] The increase in molecular complexity compared to small molecules enables prioritizing one metal ion over others. This is thanks to a tailored binding pocket that addresses various features affecting metal preferences, such as cavity size and preferred binding moieties, coordination number (CN), and binding geometry.

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Table 1. Common CAs

Name	MW (gr/mol)	Treated metals[a]	Undesired metals[b]	CN[c]
BAL	124.2	**As**, **Hg**, Pb, Cu, Cd	Cu, Zn	2
EDTA	292.2	**Pb**, **Mn**, Cd, Hg, Pu	Ca, Fe, Mn	4 or 6
DMPS	188.3	**As**, **Hg**, Pb, Cd	Cu, Zn	2
Deferoxamine	560.7	**Fe**	Fe, Zn, Ca, Mn	6
Penicillamine	149.2	**Cu**, **Au**, As, Pb	Cu, Zn	1 or 2
TETA	146.2	**Cu**	Cu, Zn, Co	4
DMSA	182.2	**Pb**, **As**, **Hg**, Cd	Cu, Zn	2
Deferiprone	139.2	**Fe**	Fe, Zn, Ca, Mn	2
Deferasirox	373.4	**Fe**	Fe, Zn, Ca, Mn	3
TTM	225.8	**Cu**	Cu, Zn	2

[a]Metals in bold are the most dominant to be treated. [b]Essential metals that can be depleted upon treatment. [c]Coordination number

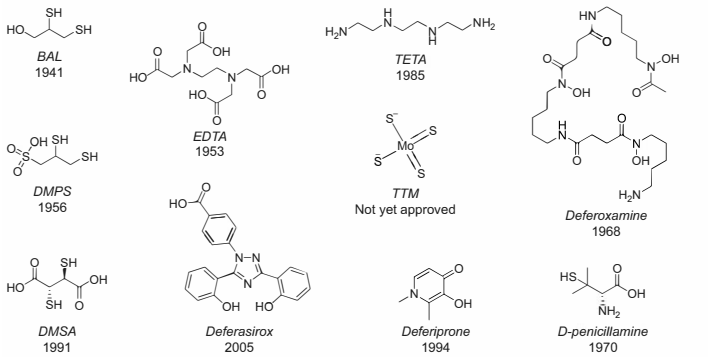

Fig. 1. Most common CAs used in the clinic and their FDA approval year. All compounds except TTM are small organic molecules.

Short peptides are located within the two chemical spaces of small molecules on the one hand and proteins on the other hand. Hence, peptides provide the advantages of each class without their most critical drawbacks.[32,33] For example, peptides are still relatively small compared to proteins, which enable tissue and cellular uptake in addition to their relatively simple synthesis. Furthermore, tuning peptide sequence and structure to allow selective metal binding similar to proteins is easier than in small molecules. We thus anticipate that by overcoming these disadvantages of the two classes by bridging them, peptides can obtain attractive characteristics as next-generation CAs against toxic metals.

Our primary goals are to develop novel peptides as selective metal binders for various purposes, including in medicine, such as modern CAs, radio-based therapy and diagnostics, and in environmental applications. So far, we have mainly focused on Pb, the most harmful metal to humans due to its wide abundance. Herein is a summary of our latest achievements[34–36] in rationally designing short peptides as CAs for Pb.

3. Pb Poisoning

Pb is a nonessential, toxic metal widely spread in the environment and affects vast populations worldwide.[37] No blood Pb level (BLL) is deemed safe,[38] and Pb at even very low concentrations shows destructive effects on various organs. Nevertheless, health authorities determine threshold values at which environmental and medicinal interventions should occur. Most countries adopt the WHO recommendation of 5 μg/dL as this value.[38] In the USA, the CDC recently reduced this value to 3.5 μg/dL, acknowledging the devastating influence of Pb at such levels.[39]

Based on these values, 16% of US children,[40,41] every third child globally,[42] and every second adult in the USA[43] are poisoned with Pb. While at high acute poisoning, Pb kills, lower levels cause various symptoms, mainly kidney impairment, anemia and high blood pressure, fertility issues for both genders, and most critically, cognitive and neurological damages (Fig. 2). The brain and the central nervous system are susceptible to Pb poisoning since Pb(II) ions resemble Ca(II) ions, which are essential in these organs. As a result, Pb as a neurotoxicant easily crosses the blood–brain barrier, resulting in amplified damage to the brain.

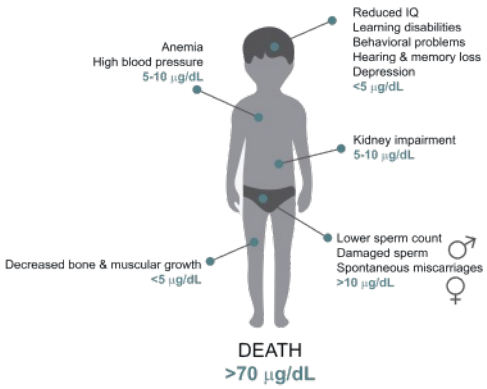

Fig. 2. Primary symptoms of Pb poisoning in children and adults and the typical BLL from which they appear.

The FDA and other authorities approved five small molecules to treat Pb poisoning as CAs (Table 1). Three of the five compounds are no longer used due to severe side effects and poor performance. Only two drugs are still in practice: DMSA and EDTA (calcium disodium salt). These drugs maintain some ability to clear Pb(II) ions but suffer from several disadvantages; most critically, (i) their binding affinity to Pb(II) ions is not as high as to other essential metal ions such as Ca(II), Fe(III), and Zn(II), which results in poor metal selectivity and (ii) they have high intrinsic toxicity due to nonspecific and undesired metal interactions, which cause various side effects, including anemia, headache, vomiting, nausea, muscle and joint pain, fever, diarrhea, and more.

Due to the above, the two drugs are given only in severe cases of Pb poisoning: DMSA is administered at BLL of at least 45 μg/dL and EDTA at values higher than 70 μg/dL. These values are 12 and 20 times higher than the reference value of the CDC, respectively, and 9 and 14 times higher than the WHO one. Notably, people of all ages who suffer from Pb poisoning of up to 45 μg/dL cannot be treated medicinally, leaving them without an effective solution against Pb.

3.1 *Pb-detoxifying Short Peptides*

To design novel peptides that strongly and selectively bind Pb(II) ions, we first analyzed the differences between the toxic ion and other physiologically relevant metal ions. These include mainly Cu(I/II), Ca(II), and Zn(II). While Cu(I/II) ions are relatively rare, the concentrations of Ca(II) and Zn(II) in the blood and cells are relatively high, range 2–4 mM and 150–350 μM, respectively.

Pb(II) and Ca(II) are similar in their ionic radii, of 119 and 114 pm, respectively. However, Cu(I), Cu(II), and Zn(II) are dramatically smaller, ranging between 87–91 pm. Hence, the cavity size of the ligand can play a crucial role in prioritizing Pb(II) ions over the transition metal ions.

Further, Pb(II) is a soft-to-borderline metal, similar to Zn(II) and Cu(I) but different from Ca(II) ions that are considered hard and thus tend not to bind thiols. Lastly, while Pb(II) ion has a broad range of preferred CN of 2–12, at low CN, this ion possesses a unique hemidirected geometry, by which the lone pair electrons in orbital 6s are stereochemically active, hence forcing the binding moieties to be organized in one hemisphere of the ion.

Considering these features, we concluded that our peptide(s) should contain between 2–4 soft-to-borderline binding moieties such as thiols, carboxylic acids, and imidazoles. Such moieties should be facing the same direction to capture the toxic ion in its preferred hemidirected geometry at lower CN. We also aimed to have the groups rigidly preorganized so that selectivity can be achieved by having the ligands in a defined orientation. Noteworthy, since Pb(II) rarely binds to backbone amides, we did not count them as plausible binders.

So far, we have examined three primary scaffolds; two are inspired by the natural tripeptide glutathione (GSH)[35,36] and one by non-peptidic unnatural cyclic ligands based on cyclam.[34] The three families of peptides explored also differ in their length; tri-, tetra-, and octamers, and their backbone rigidity; while the trimer is linear, the other two families are head-to-tail cyclic.

Altogether, we synthesized and investigated over 40 different peptides. We have designed an *in vitro* screening assay that mimics Pb poisoning by using human cells. Once the cells were treated with Pb(II) ions, each peptide was administered at different concentrations, and the viability of the culture was quantified. The results were normalized to poisoned but untreated cells as the negative control. The same measurements were also conducted with the two current drugs, EDTA and DMSA.

Members of all three families succeeded in recovering human cells while outperforming the medications. In particular, peptides SG1, SG2, and SG3 (Fig. 3A), each from a different family, excelled amongst the other investigated peptides.

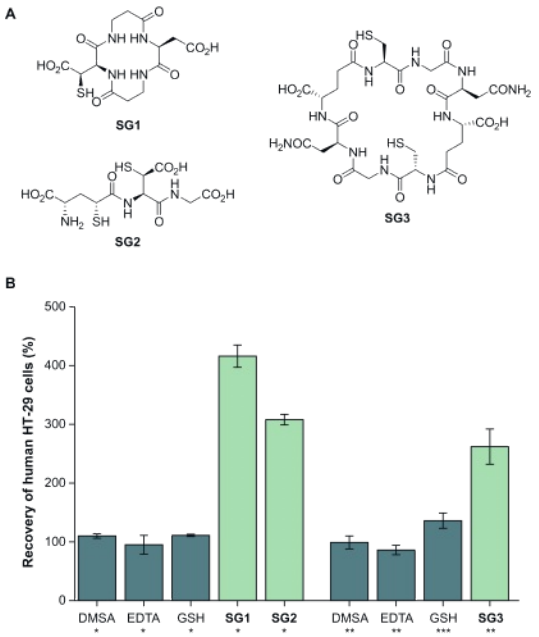

Fig. 3. (A) The three most successful peptides we have developed thus far. (B) Recovery of HT-29 human cells treated with $Pb(NO_3)_2$ (*2 mM; **,***0.2 mM) followed by the addition of the two current drugs, GSH, peptides SG1-3 (1 h after the addition of Pb(II) ions; *10 mM; **1 mM, ***2 mM; values are calculated relative to cells poisoned with Pb(II) ions as the negative control).

These lead peptides were examined for their ability to recover Pb-poisoned human cells (Fig. 3B), Pb-binding affinity, metal selectivity, and toxicity. All indicate the superiority of these peptides in the performed tests compared to the current drugs. Their complexes with Pb(II) ions were also characterized by various techniques, revealing, in most cases, the desired 1:1 binding modes.[34–36]

4. Conclusions

The results acquired so far reveal the great potential of peptides in overcoming the obstacles chelation therapy faces. As a chemical space that bridges small molecules and proteins, peptides benefit from both worlds, if appropriately designed, and enable tailored activities that can then be translated to medicinal applications. Beyond further developing our peptides as next-generation CAs against Pb, we aim to harness our platform towards other toxic metals, showing that, indeed, short peptides can revolutionize chelation therapy.

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