COMMENTARY

Systems approach to metal-based pharmacology

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At least 20 elements of the periodic table are essential to humans, but understanding their biochemistry presents many challenges. The genetic codes for metals such as iron, copper, and zinc are readily identified, but the same cannot be said for vanadium, chromium, nickel, or tin, metals frequently in mineral supplements. The genetic codes for metals are not just for the elements, they are for particular species of the elements. The metal oxidation state, types and numbers of coordinated ligands, as well as the coordination geometry are crucial for recognition by specific uptake, transport, and delivery proteins. Understanding and controlling the biochemistry of metal ions requires speciation on timescales of nanoseconds to years (kinetics of ligand substitution), consideration of metal–ligand affinities (thermodynamics, equilibrium constants), as well as redox potentials (1, 2). Medicines use not only essential, but also nonessential elements. For example, bismuth, the heaviest stable element, has been used for the treatment of infectious diseases and gastrointestinal conditions for over three centuries. Preparations such as bismuth subsalicylate (Pepto-Bismol), colloidal bismuth subcitrate (CBS) (De-Nol), and ranitidine bismuth citrate (Pylorid) are widely used today. However, why is bismuth much more toxic to microbes such as Helicobacter pylori compared with eukaryotic cells? In PNAS, Hong et al. (3) show that a systems pharmacology approach can provide new insights, an approach that, if applied more generally, will greatly advance the rational design of metallo drugs.

The speciation of bismuth is exceptionally challenging: only one natural isotope (209Bi), a quadrupolar nucleus that is of little use for NMR, with no unpaired spins, no useful metal-centered electronic transitions, and a highly variable and fluxional coordination sphere with coordination numbers of 3–9. These features and the strong acidity of Bi3+ tend to (hard) oxygen binding strongly not only to (hard) oxygen ligands, but also to (soft) sulfur, especially thiolates (3-4).

There is an abundance of thiolate sulfur in cells. Most cells contain 2–14 mM glutathione, the tripeptide γ-L-glutamyl-L-cysteinyl-glycine (GSH), not only in the cytoplasm but also in other organelles, e.g., mitochondria (5). Hong et al. have shown that uptake of Bi into HK-2 human proximal tubular cells and bacteria (Escherichia coli) occurs by passive diffusion and is biphasic, the second phase mirroring exponential growth of HK-2, but not E. coli. Moreover, Bi uptake greatly increases GSH levels (approximately four times). Bi uptake is “self-propelled” by a feedback loop. Stimulation of GSH biosynthesis by an external supply of cystine as a cysteine precursor led to a remarkable accumulation of 34 mM Bi in HK-2 cells. In contrast, on depletion of GSH using L-buthionine sulfoximine, an inhibitor of γ-glutamyl-cysteine synthase (glutamate-cysteine ligase), the first step in GSH synthesis, CBS gave rise to HK-2 cell degradation and nucleus malformation. Coordination of Bi3+ to GSH in cells, forming Bi(SG)3, triggers other events. This (conjugation) step may be aided by GSTs, which constitute up to 10% of cytosolic proteins.

The wide family of multidrug resistance-associated proteins (MRPs) mediate ATP-dependent transport of GSH conjugates, so decreasing cellular drug accumulation (confering resistance). MRPs also pump conjugates into perinuclear vesicles, where Bi is seen as dense black deposits, probably Bi sulfide (inclusion bodies). The nature of Bi (as well as Ag and Pb) “intranuclear inclusion bodies” has long been a puzzle (6, 7). Importantly, detoxification of Bi in human cells is not so effective in bacteria where GSH is less prevalent. Where does the sulfide come from? —via the action of cysteine desulfurase on cysteine as it does for ferredoxins? Perhaps it arises from cleavage of the γ-glutamyl residue of Bi(SG)3 by γ-glutamyltransferase (γ-GT), which initiates mercapturic acid synthesis. In many anaerobic bacteria, GSH is absent and cysteine is the major thiol. Hence Bi drugs can be effective and selective against

Fig. 1. Involvement of GSH, some GSH-dependent enzymes, and membrane pumps in cellular reactions of As(III), Pt(II), Bi(III), and Au(I) drugs and essential Cu(I) Lysosomal deposits (gold aurosomes) or intranuclear inclusion bodies (Bi) are curious features also seen for some other metals. Pt(II) exchanges ligands more slowly and its reactions have some different features. These pathways can detoxify metals; their inhibition might be built into, e.g., an anticancer strategy. Speciation of metal ions in intact cells is a major challenge. GSH also has redox functions in cells.
Many other soft metal ions have a high affinity for thiolate sulfur and GSH sequestration pathways might be expected to be involved in their metabolism too. These include anticancer drugs based on As(III) and Pt(II), antiarthritic Au(I) drugs, as well as the essential metal ion Cu(I) (Fig. 1). The As(III) drug Trisenox [AsO$_3$, As(OH)$_3$, in aqueous solution], the drug of choice for promyelocytic leukemia, forms GSH conjugates that have been related to poor patient outcome. These GSH-adducts are GST-substrates easily detoxified by cancer cells (8). In contrast, the drug candidate Zinapar (darinaparsin, ZIO-101), dimethylarsenic conjugated to glutathione, takes advantage of the excessive cystine/cysteine requirement of cancer cells. This As(III) complex appears to be transformed by γ-GT at the cell surface with formation of the dimethylarsino-cysteine adduct (DMAC), which is readily taken up via cystine/cysteine importers. Hence increased cellular accumulation of As from ZIO-101 occurs. This complex might be favored for multidrug-resistant tumors where chemoresistance is mediated by high GSH levels (9).

In the case of Pt(II)-based anticancer drugs, GSH may be responsible for the inactivation of compounds such as cisplatin, cis-diamminedichloroplatinum(II), or its derivatives carboplatin and oxaliplatin. Indeed, at least 60% of cellular Pt is present as GSH-adducts and increased levels of intracellular GSH are directly linked to the development of resistance to Pt(II) drugs in lung and ovarian cancers (10). For ovarian cancer, proteomic studies have related differentially expressed GSTs to Pt chemoresistance. Both cisplatin and carboplatin generate adducts in which Pt is bound to the CXXC copper-binding motif of methyl-CpG binding domain 2 (MBD2), and then excreted by ATP7B, a Cu exporter (11).

Injectable Au(I) thiolate drugs such as Myochrysine (aurothiomalate) and the oral drug Ridaura [auranofin; (tetraacetylthioglucose)Au(PET)$_3$] are used for treating rheumatoid arthritis. It has long been known that gold accumulates in lysosomes in cells as dense deposits (aurosomes), usually believed to be sulfur-rich (12), but they are not well characterized. In view of the observations on Bi by Hong et al. (3), it now seems to be the time to reexamine aurosomes. Myochrysine inhibits GSH-peroxidase in vivo, which contributes to increased oxidative stress (13). Auranofin exerts its antiinflammatory activity by inhibiting mitochondrial thioredoxin reductase (TrxR) (14); nonetheless, its anticancer activity can be enhanced by direct binding and subsequent inhibition of GST (15). Interestingly, a member of the GST family, TDR1, activates antiparasitic antimonial prodrugs by deglutathionylation of Sb(V)-glutathione adducts with reduction and release of Sb(III) (16).

There is a link to the metabolism of essential metal ions. GSH stimulates the uptake of copper via the transporter Ctrl. Initial formation of [Cu(SG)$_3$]$^{2-}$ may be followed by transport to F$_{1}$β$_{1}$-type ATPases and superoxide dimutase mediated by the dynamic transfer of Cu(I) between cysteine thiolates of chaperones Atx1 and CCS in the cytoplasm (11).

The field of bioorganic chemistry and its applications in medicine are fast-growing areas of research that use the rich structural diversity of metallic scaffolds for drug design. Metal-based drugs can provide in-cell chemical reactions that are not accessible to purely organic drugs, accompanied by multitargeting that can combat resistance (17). However, there is an urgent need for further development of methods to elucidate the spatial and temporal speciation of metallo-drugs. This requires a multidisciplinary effort with contributions like those of Hong et al. (3) to understand fully the implications of the metal center in the metabolism and pharmacokinetic/dynamic properties of candidate drugs.

It is evident from this brief commentary that progress in the design of metallo-drugs requires a systems pharmacology approach. Not only do we need a good understanding of the kinetics and thermodynamics of metal–ligand exchange and redox reactions, but also of the responses of cells in terms of attack on multiple targets, self-propelled transport, and delivery processes related to feedback loops (Fig. 1). For metallo-drugs, it will often not be appropriate to describe their mechanisms of action in terms of a single target site. This “polypharmacology” offers potential advantages because it is now becoming apparent that resistance to single-target drugs can readily develop. The introduction of systems pharmacology approaches to unraveling the mechanism of action of metallo-drugs should lead to advances in their design and in their use so as to avoid unwanted side effects.