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Viewpoint

Drug Discovery and the Supramolecular Factor

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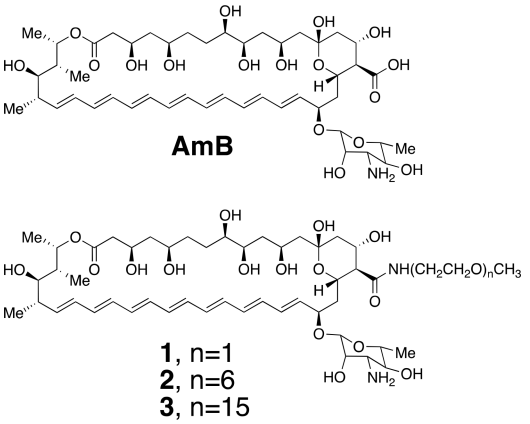
Creating therapeutic agents that are effective, minimally toxic, and low in cost is the major goal in all drug discovery efforts. Classically, drug discovery has been largely based on the use of structure−activity relationships (SAR) where the biological activity of a potential drug is predicted based on the known biological activities of closely related compounds.[1,2] A modern approach toward drug discovery that is now rapidly evolving employs artificial intelligence (AI).[3] Here, machines that mimic human intelligence are "being asked" to assess databases so that potential drugs can be identified more quickly.[4] In this Viewpoint, I wish to highlight a factor that I believe has been overlooked in virtually all SAR and AI approaches. It is *the difference in activity between monomeric and aggregated forms of biologically active agents*. I will refer to this difference as the "supramolecular factor". Let me explain why I think this factor is important.

Previously, we showed that the attack of micelles of Triton X-100 on cholesterol-rich liposomes resulted in the catastrophic rupture of their membranes and the complete release of their contents.[5] In sharp contrast, attack by corresponding monomers left these same liposomes intact while making them somewhat leaky. Our discovery of this aggregate/monomer dichotomy led us to hypothesize that supramolecular factors could play an important role in drug discovery, especially for those agents that operate at the membrane level. Specifically, we posited that by eliminating the presence of aggregates and, consequently, membrane rupture pathways, greater cell selectivity should be possible.

To test our hypothesis, we synthesized a series of derivatives of amphotericin B (AmB) in which poly(ethylene glycol) chains of varying length were conjugated to the macrolide's carboxylic acid group via amide formation (Chart 1).[6] These modifications were expected to alter the drug's hydrophilic−lipophilic balance (HLB) and its critical aggregation concentration (CAC). Amphotericin B was chosen for this study because it was the *gold standard* for treating systemic fungal infections despite its high toxicity.

We then examined the antifungal and hemolytic activities of each conjugate. As shown in Figure 1, our results were dramatic. In contrast to the native drug, which exhibited a CAC value that was virtually identical to its minimum inhibitory concentration against *Candida albicans*, as well as the concentration needed for 50% hemolysis of red blood cells, a clear separation of antifungal and hemolytic activity was observed for each of the poly(ethylene glycol) conjugates. Thus, we found that (i) extending the length of the poly(ethylene glycol) chain significantly increased the drug's CAC value, (ii) hemolysis was observed only when the concentration of the conjugate exceeded its CAC value, (iii) the antifungal activity for each conjugate was only modestly reduced relative to the native drug, and (iv) the separation of antifungal from hemolytic activity increased as the CAC value increased. We were most gratified to learn of an independent study, which provided alternative evidence confirming that aggregates of AmB are responsible for its poor cell selectivity.[7]

Chart 1



Based on the results shown in Figure 1, we decided to carry out animal studies to assess antifungal activity and toxicity *in vivo* for one such conjugate, i.e., **2**.[8] In brief, we found that the potency of **2** for treating mice, which were infected with *Candida albicans*, was similar to that of AmB and that dosages as high as 30 mg/kg of body weight were well-tolerated. In sharp contrast,

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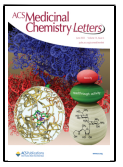

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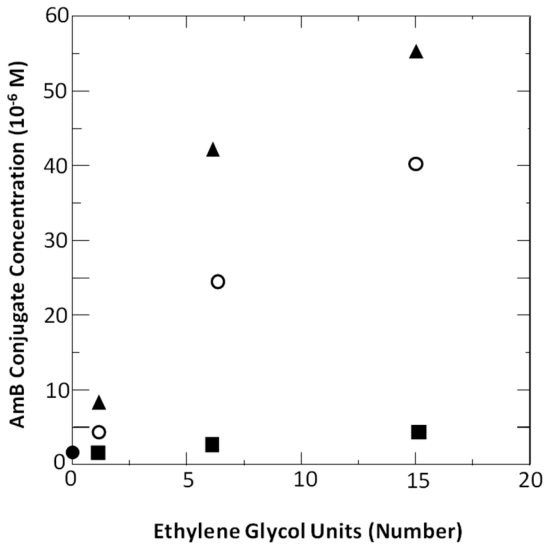

Figure 1. Plot of the number of ethylene glycol units present in **1**, **2**, and **3** as a function of their (■) minimum concentration needed to inhibit the growth of *Candida albicans*, (○) critical aggregation concentration (CAC), and (▲) concentration needed for 50% hemolysis. An overlap region (●) that includes the MIC, CAC, and K_{50} values for underivatized amphotericin B is also shown.

similar dosages of the native drug proved lethal.[8] Today, liposomal formulations of AmB are commonly used to treat systemic fungal infections due to lower toxicity. While the basis for this reduction in toxicity has not been established, I believe it is likely that these liposomes are simply acting as a reservoir for releasing AmB monomers that circumvent nonselective, membrane rupture pathways.[6]

An obvious question that can be raised is whether the aggregate/monomer dichotomy that we discovered for AmB is unique to this drug or whether it represents a general phenomenon. Although more studies are clearly needed to answer this question, definitively, I will cite a recent study that points toward generality. Here, the antibacterial properties of a simple quaternary ammonium compound derived from L-phenylalanine was examined above and below its CAC.[9] When tested below its CAC, significant antibacterial activity was observed with negligible hemolytic activity. In contrast, at concentrations greater than its CAC, significant hemolytic as well as antibacterial activity was found.[9] The fact that an aggregate/monomer dichotomy has been documented for an *antibacterial* agent that is vastly different in structure from AmB implies that this phenomenon is likely to be general.

As noted by other researchers, the attachment of poly(ethylene glycols) (PEGs) to peptide and protein drugs has often been used to reduce their toxicity.[10] For example, attachment of a 2.0 kDa poly(ethylene glycol) to LyeTx I-b (a 24 residue-long peptide derived spider venom of *Lycosa erythrognata*), allows the peptide to maintain antibacterial activity while reducing its toxicity toward VERO cells.[10] Unfortunately, no CAC data was reported for LyeTx I-b and its poly(ethylene glycol) conjugate. A simple explanation for this reduction in toxicity toward VERO cells is that by increasing the CAC of the peptide, the antibacterial activity of monomers is increasing in importance.

Drug discovery that is based on SAR and AI approaches will, undoubtedly, lead to further advances in the future. If supramolecular factors were also considered, especially for those drugs that possess limited water solubility, I believe that many of these advances would come a lot sooner.

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Notes

The author declares no competing financial interest.

ABBREVIATIONS

AmB, amphotericin B; AI, artificial intelligence; CAC, critical aggregation concentration; HLB, hydrophilic−lipophilic balance; K_{50}, concentration needed for 50% hemolysis; LyeTx I-b, 24 residue long peptide derived spider venom of *Lycosa erythrognata*; MIC, minimum inhibitory concentration; PEGs, poly(ethylene glycols); SAR, structure−activity relationships

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