Copper ion as a delivery platform for taxanes and taxane complexes

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ABSTRACT

A number of delivery agents, such as proteins, liposomes, micelles, and nanoparticles, are utilized for transporting pharmaceutical agents in a physiological environment. This Letter focuses on the use of the copper(II) ion and its potential role as a delivery agent for the taxanes and taxol couple to a malaria drug. Nuclear magnetic resonance (NMR, ^1H, ^{13}C, ^{15}N), Mass Spectrometry (LC-MS, MALDI-TOF, FT-ICR) and computational methods are used to examine the structure of the complex. The National Cancer Institute's benchmark 60 cell line panel is used to compare the efficacy of the copper–taxol and copper–taxol–hydroxychloroquin complexes to that of iron–taxol and pure taxol.

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Taxol (paclitaxel), which is derived from the Pacific Yew Tree, has been used to treat cancers such as oral, lung, breast, ovarian, and central nervous system (CNS) cancers since the 1960s.[1] Taxol interferes with the growth of cancer cells by stabilizing spindle microtubules. In addition, this disruption of the mitotic process helps reduce migration.[2,3] However, the poor water solubility of taxol reduces its ability to effectively reach target cells. Like many organic molecules with low water solubility, the scientific community has been working to increase their value by attaching or binding the medicinal agent to a secondary structure.

Several methods exist to improve drug delivery. Many, such as nanoparticles, liposomes, micelles, and aptamers, help increase efficacy by binding or surrounding drugs thereby allowing the drug to reach its target efficiently. Nanoparticles composed of various organic and inorganic compositions have been established and are in various stages of development for the delivery of medicinal agents.[4] Nanoparticles enter the cell through endocytosis, in which the material is engulfed by the cell wall. One of the first nanometer-sized delivery systems tested were liposomes. These systems are biological micelles with an external component that is polar and an internal component that is nonpolar. For liposomes, the drug is encased within the phospholipid bilayer in order to change the water solubility of the compound and allow the drug to reach the target.[5] Aptamers are nucleic acid sequences which can bind specific targets.[6] Drugs bound to aptamers utilize these sequences to reach highly specific sites in the target region.[7,8] For example, the formulation of aptamer-coated particles containing paclitaxel–polylactide nanoconjugates were developed to target cancer cells.[9]

Methods have been developed that focus on specifically delivering amine containing pharmaceutical agents that are currently on the market. Albumin, which is abundant throughout the human body, has been demonstrated as a delivery vehicle for taxol.[10] The use of the albumin is referred to as a nanoparticulate formulation despite being a naturally occurring biomolecule.[11] Albumin has higher water solubility than taxol. The combination of albumin and taxol, known as Abraxane, was approved in 2005 for applications in patients with metastatic breast cancer who have been through other treatments but failed. Current studies with Abraxane have also demonstrated improvements in over thirty-three percent of pancreatic patients.[12] Another product exists that outlines a method of using bases to increase the permeation of amine drugs across the skin. The patent covers a wide range of amine drugs which include a variety of compounds used to treat Alzheimer's disease, enlarged prostates, and acid reflux disease.[13]

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While there is an extensive body of work related to the pharmaceutical delivery of taxol and other taxanes, little exists on its binding to cations, particularly any of the transition metals. The iron–taxol complex was synthesized and tested against the National Cancer Institute's sixty cell line cancer panel. The iron–taxol complex had lower *anti*-cancer activity compared to pure taxol.[14] Rather than enhance the taxol efficacy, iron(III) binding to the pharmaceutical agent lowered its pharmaceutical activity. Subsequently, the binding of copper(II) ion to hydroxychloroquin was structurally evaluated and tested against the single dose NCI 60 cell line panel.[15] Malaria drugs have garnered recent interest to the cancer research community because their activity against pancreatic cancer cell lines.[16] In our work with cations and natural products with multiple oxygen and/or amine groups, we developed the concept of a polarity adaptive molecules.[17,18] In this metal–molecule complex in the aqueous phase, the cation moves from binding site to binding site on the molecule. While it may prefer one site over another, NMR studies demonstrate a cation can migrate around a molecular structure.

Copper sulfate ($CuSO_4$) has a lethality dose (LD_{50}) of approximately 30 mg of the copper salt per kilogram of rat (30 mg/kg). In adult humans, it requires gram quantities of copper sulfate to be lethal. In drinking water, the suggested safe level of copper is approximately 2 ppm or 2.0 mg/liter.[19] In all applications proposed here, substantially lower levels of copper are proposed and the levels that would result from a typical copper(II) cation-pharmaceutical agent complex would be on par with the copper intake in a healthy diet.

Binding the copper(II) ion to taxol in a 1:1 complex, means that for every one mole of taxol (853 g/mol) there would be 1 mol of copper ion (63.5 g/mol) or the mass of copper would be less than ten percent the mass of the complex. Currently, taxol is sold in different formulations such as 30 mg (in 5 mL); 100 mg (in 16.7 mL), and 300 mg (in 50 mL) in multidose vials. In this commercially available formulation, each milliliter of the sterile solution contains 6 mg of taxol (paclitaxel), 527 mg of Cremophor® EL (polyoxyethylated castor oil) and 50% (volume/volume) of a dehydrated alcohol. In these formulations, if copper was included, the dose would contain less than 1 mg of the copper cation.

The copper(II) cation has been shown to promote angiogenic responses.[20] These observations have led to the development of *anti*-copper-based, *anti*-angiogenic strategies for the treatment of different types of cancer. Many researchers believe that the copper ion is a switch that turns on the angiogenesis process in tumor cells. It has been observed that patients with many types of progressive tumors typically have very high copper levels in the tumor region. Binding an amine containing drug to a copper ion will serve to block that amine site from being sidetracked by existing copper ions, in their different physiological environments. This allows the free copper–amine complex (i.e., Cu–taxol) to by-pass the existing copper complexes, existing in the cancerous regions, and attack its medicinal target.

Computational studies on chemical complexes can provide unique insights into some of the physical and chemical properties and geometries. The concept of a polarity adaptive molecule can be powerful parameter for medical agents. Taxol has low water solubility which impacts its efficacy in medicinal applications. A detailed computational study of the copper(II) ion binding to taxol was performed using semiempirical methods.[21] The ratio of the dipole moment (D) to molecular volume (V) or the D/V ratio of the complex ranged from 0.0065 to 0.024 Debye/Å³, depending on which electronegative elements (N, O) were bound. Comparing this range to the D/V ratios of some common solvents (see Table 1), it is evident the copper ion can dramatically shift the polarity of the taxol complex.

Table 1
A list of common solvents with their calculated dipole moment (D), molecular volume (Å³), and D/V ratio (Debye/Å³)

Name	Dipole moment (D)	Molecular volume (Å³)	D/V (Debye/Å³)
Water	1.74	19.24	0.09
Methanol	1.54	40.66	0.038
Ethanol	0.148	59.08	0.025
1-Propanol	0.159	77.37	0.02
1-Butanol	1.6	95.69	0.017
1–3 Butanediol	3.23	102.19	0.031
1-Pentanol	1.41	114.06	0.012
1-Octanol	1.62	168.95	0.0096
Hexane	0	124.8	0

Table 2
Total possible number of copper(II)–taxol combinations based on the permutations equation $n!/(r!(n − r)!)$

	Possible combinations $n!/(r!(n − r)!)$		
Group	n	r	Total
Cu–taxol	14	5	2002
Cu–taxol–H_2O	14	4	1001
Copper(II)–taxol refined	9	5	126
Cu–taxol–H_2O refined	9	4	126

Computational work also suggests how mobile the cation can be on the taxane structure. Permutations calculation is used to determine the total number of possible combinations for both the hydrated and non-hydrated Cu–taxol complexes assuming at least one Cu—N bond. Using the equation $n!(r!(n − r)!)$, where n is the number of possible oxygen binding sites, and r is number of oxygen atoms bound to central copper atom. It was determined that there were over two hundred and fifty possible non-hydrated Cu–taxol and monohydrated Cu–taxol geometries that were possible that meet the stability requirements (see Table 2).

There are several pieces of complimentary analytical data that supports this polarity adaptive molecule assertion for the copper–taxol complex. First there were three sets of NMR data obtained (^1H, ^{13}C, ^{15}N). Using the atom numbering scheme outlined in Figure 1, the spectral features for ^1H and ^{13}C for taxol, Cu–taxol and literature values for taxol are compared in Table 3 (see Fig. 2, Table 3). This data indicates the copper ion interacts with many of the oxygen atoms, the amine group as well as forms some Cu—π bonds with the aromatic structures. A Nitrogen-15 NMR analysis of taxol and Cu–taxol was also undertaken. Taxol analyzed by N^{15} NMR gave two spectral features at 73 and −138 ppm, suggesting two configurations for the medicinal agent. The copper–taxol complex N^{15} NMR resulted in a single spectral feature at 73 ppm, suggesting the copper ion was binding the amine and that the copper ion increased the rigidity of the structure into a single geometry (Fig. 3). This single geometry effect will be discussed below in an analysis of the cell line datum.

We have utilized several mass spectrometric techniques to study the Cu–hydroxychloroquin, Cu–taxol and C–hydroxychloroquin–taxol complexes including LC-MS, FT-ICR and MALDI-TOF-MS. All have provided valuable data concerning the various taxane complexes. For example, Figure 4 provides sample FT-ICR spectra for the Cu–docetaxil complex ($Cu_1C_{43}H_{52}NO_{14}$; 869.271 m/z). The loss of a proton from docetaxil ($C_{43}H_{53}NO_{14}$) in the copper complex likely occurs at the amine. MALDI-TOF-MS spectra (see Fig. 5) shows the feature corresponding to Cu–taxol (Cu-$C_{47}H_{51}N_1O_{14}$, 916 g/mol).