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Nanogel–drug conjugates: a step towards increasing the chemotherapeutic efficacy

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Networking drugs

“The universal nanogel–drug conjugate platform can benefit chemotherapies with existing drug solubility and cellular internalization problems, and further advance drug targeting and its ability to cross biological barriers.”

Nanogels are defined as tiny, spherical, semipermeable polymer networks with interior volumes that can encapsulate molecules of various sizes and are highly soluble drug delivery systems (DDSs) [1]. With these unique properties, the drug release from nanogels can be controlled by either the molecular weight of entrapped molecules and network density, or the charges of the drug and the network. Entrapped drugs can diffuse out of nanogels as a result of network degradation, changes in pH, temperature and ionic strength, or substitution by other molecules. For charged hydrogels, however, drug release kinetics become more complicated. We have extensively studied cationic nanogels as DDSs for negatively charged oligonucleotides, and 5'-triphosphates of cytotoxic or antiviral nucleoside analogs (NAs) [2,3]. They formed neutral 'core-shell' complexes that could deliver labile drugs with high efficacy, and demonstrated enhanced activity in preclinical models. The surface of nanogel DDSs can be decorated by targeting molecules (e.g., folate, peptides or antibodies) to enhance drug transport through biological barriers or accumulation in tumors [2-4]. Despite the many advantages of nanogel DDSs, they also have certain limitations, including fast release of small molecules, potential toxicity of cationic carriers and oral administration.

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The step to drug conjugates

Macromolecular pharmacology (when drug molecules are conjugated to a polymer molecule) is currently one of the preferred approaches to enhance biostability of protein drugs (e.g., PEGylated interferon, Pegasys®; Hoffman–La Roche Inc., Basel, Switzerland). Discovery of the enhanced permeability and retention effect, a preferred accumulation of macromolecular drugs in tumors caused by leaky neovasculature, additionally increased the attractiveness of this approach [5]. Numerous macromolecular drugs have been tested, but consequently failed due to the high viscosity and significantly reduced activity of conjugated drugs. Recently, we proposed an essential improvement through the application of nanogel–drug conjugates (NDCs) instead of simple polymer–drug conjugates. Compared with ionic nanogels, the drug release from the NDC is significantly extended and can be controlled by linker stability. In addition, these drugs are efficiently internalized and transferred across the cellular membrane into the cytoplasm, where the drug targets are located. Compact nanogels have a much lower viscosity compared with linear polymer-conjugated drugs. Finally, certain NDCs may be administered orally. The simplest NDC can be obtained by drug conjugation to multivalent cholesterol-modified polymers or polysaccharides, which easily self-assemble in compact nanogels. Shimoda *et al.* extensively studied the encapsulation of proteins in cholesteryl pullulan nanogels [6]. At low cholesterol concentrations, the cholesteryl pullulan conjugates formed a nanogel network engulfing and protecting dissolved protein molecules. Usually, the attached cholesterol moieties become organized in stacking packs after intensive sonication on nanogel dispersions. Similar self-assembling nanocarriers are known as polymeric micelles. Nishiyama *et al.* introduced drug-conjugated polymeric micelles formed of a cisplatin conjugate with PEGylated poly(benzyl-aspartate) and a corresponding folate-modified DDS with enhanced anti-tumor activity [7]. The selection of biodegradable linkers may be crucial for macro-molecular drug design. Ideally, the drug must be released only at the site of action; for example, after DDS accumulation in the tumor. Drugs binding via low pH-cleavable hydrazone bonds can allow drug release predominantly in tumors characterized by an acidic microenvironment [8]. Enzyme-sensitive peptide linkers, which are sensitive to enzymes produced at elevated levels in tumors, can be used in polymeric drugs, such as the angiogenesis inhibitor *N*-(2-hydroxypropyl) methacrylamide conjugate [9].

For nanogels, we have designed a tetra-phosphate linker for binding NA drugs [10]. Anticancer NAs are prodrugs requiring phosphorylation to become active. The linker was stable in the gastrointestinal (GI) tract, but could be degraded by cellular phosphatases or phosphodiesterases allowing the release of active NA. The NDC of floxuridine (5-fluorouracil nucleoside) demonstrated stronger inhibition of tumor growth than the free drug. The NDC of gemcitabine showed high efficacy against various gemcitabine-resistant tumors [11].

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In a second approach, we introduced drug conjugates with cholesteryl-hyaluronic acid (CHA) nanogels for targeting drug-resistant tumors and cancer stem cells (CSCs) [12].

Several potentially active compounds against CSCs, such as etoposide, salinomycin and curcumin, have been attached via biodegradable ester bonds. All of them were found to be more efficient than free drugs against drug-resistant cancer cells. In comparison, hyaluronic acid drug conjugates demonstrated lower activity than free drugs. Evidently, the structure of the NDC is a critical factor for this observed activity.

Measure of drug efficacy: cellular penetration

Drug fate after administration is not always well understood. Many drugs bind serum components, while other drugs are degraded by enzymes or acid in the GI tract. A significant barrier to drug activity against intracellular targets is the cellular membrane, because hydrophilic and charged drug molecules can only penetrate it if the cell has specific drug-binding or scavenger receptors. Lipophilic drug molecules may effectively bind to the cellular membrane; however, their release inside the cell is limited. Thus, membrane binding and release are key steps that determine drug activity. Nanocarriers can be subject to the same restrictions. Binding to certain membrane components results in absorption or receptor-mediated endocytosis of nanocarriers. The nanoencapsulated drugs, which cannot escape endosomes, are degraded in lysosomes or recycled out of the cells. There are only two ways for a nanocarrier-loaded drug to escape endosomes: by disrupting endosomes and becoming a part of the membrane by forming a semipermeable patch. Preferably, nanocarriers are anchored into the cellular membrane via membranotropic molecules, such as lipids or cholesterol (e.g., in liposomes), or amphipathic peptides. We recently suggested that intracellular drug release can be boosted by the formation of nanogel patches in the cellular membrane. These interactions could clearly be observed for cationic nanogels and nanogels built from amphiphilic polymers [13]. Thereafter, we found that cholesterol-modified nanogels have better cellular accumulation than cationic nanogels at an optimal cholesterol content of 3–5%, which had been utilized in the design of NDCs. For these, the putative mechanism of cellular drug accumulation included steps involving nanogels binding and fusing with the membrane, resulting in sustained drug release in the cytoplasm. Unmodified polymer–drug conjugates are usually incapable of fusing with the cellular membrane and, therefore, release the drug inside the cell. Furthermore, compact NDCs have a higher rate of endocytosis compared with larger polymer–drug conjugates. This synergy works seamlessly for NDCs and results in significant enhancement of drug delivery inside targeted cells.

Successful beginnings

Chemotherapy eventually induces drug resistance in cancer cells and cannot prevent tumor relapse. The phenomenon of drug resistance is complex and involves intensive drug efflux, restrictions in drug transport and activation, drug metabolism and activation of antiapoptotic mechanisms. In addition, accumulation of CSCs in treated tumors may play a significant role in post-therapeutic cancerogenesis and tumor relapse [14]. NDCs of cytotoxic NAs demonstrated up to 120-times higher efficiency against drug-resistant cancer cells compared with free drugs [10,11]. These conjugates have a sufficient stability in GI conditions, allowing them to be safely administered orally. The efficacy of oral administration was confirmed in several tumor xenograft models of both wild-type and drug-resistant cancers, where a three- to four-fold tumor growth inhibition was observed [11]. Gemcitabine, which

had never previously been orally administered due to its GI instability, was conjugated in a protected form with nanogels based on biocompatible and mucoadhesive poly(vinyl alcohol) modified with cholesterol. This NDC was stable in GI conditions and demonstrated pronounced tumor growth inhibition following intraperitoneal or oral administration [11]. In preclinical studies, an oral NDC of gemcitabine extended the survival time for animals with aggressive drug-resistant xenografted tumors fourfold. The anticancer activity of these NDCs was evidently increased by the enhanced permeability and retention effects; however, it is possible to further increase these effects by targeting tumors with combinatorially selected peptoid vectors that are stable in the GI tract.

“All components of the cholesteryl-based nanogels are biocompatible, nontoxic and, in many cases, biodegradable; providing simple regulatory approval.”

The NDCs of antineoplastic compounds with enhanced activity against CSCs have recently been evaluated against cancer cells and spheroids [14]. Many drug-resistant cancers and CSCs express high levels of the CD44 receptor, which binds hyaluronic acid. Hyaluronic acid in CHA nanogels can directly target CD44 receptors. We demonstrated penetration and strong activity of CHA–etoposide in cancer spheroids modeling quiescent CSCs. Tumor growth in auto-chthonous and xenograft animal models, including drug-resistant tumors, was reduced with both intraperitoneal and oral administration [11]. These NDCs have significant clinical potential owing to their specificity to drug-resistant cancers and extraordinary activity.

Analogous NDCs of nucleoside reverse transcriptase inhibitors (nano-NRTIs) can be applied to treat HIV infection. Macrophages effectively capture nanogels and, being the virus hosts, the macrophages can be treated with nano-NRTI [15]. We have demonstrated high antiviral activity of several nano-NRTIs obtained from cholesteryl–dextran and cholesteryl ϵ -polylysine in HIV-infected human macrophages. This effect was maintained for at least 10 days with doses two- to three-times lower than when using free NRTIs. This extended-release approach provides a much safer and more comfortable alternative to many existing antiviral therapies. Ultimately, our ambition is to make oral nano-NRTIs, alone or in combinations, similar to those used in highly active antiretroviral therapy.

Future advances & applications

In conclusion, the nanogel–drug coupling makes the initial drug more active and beneficial. All components of the cholesteryl-based nanogels are biocompatible, nontoxic and, in many cases, biodegradable; providing simple regulatory approval. Modification of linkers in NDCs can adapt the DDS to selective enzymatic or cellular activities, making the drug release organ- or tumor-specific, as well as extending the drug release. Conjugation of existing drugs to nanogels can increase the area of their application and reduce harmful side effects. The universal NDC platform can benefit chemotherapies with existing drug solubility and cellular internalization problems, and further advance drug targeting and its ability to cross biological barriers.

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