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Opinion

Opinion on Cyanine Dye Conjugates for Biomedical Applications

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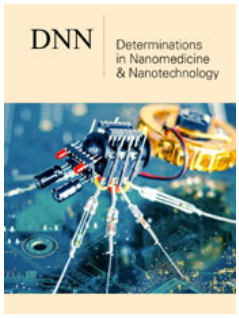
ISSN : 2832-4439



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Submission: January 2, 2024
Published: January 19 , 2024

Volume 3 - Issue 2

How to cite this article: Guliz Ersoy, Zaryab Gul and Maged Henary*. Opinion on Cyanine Dye Conjugates for Biomedical Applications. Determ in Nanomed & Nanotech. 3(2). DNN. 000559. 2024.
DOI: 10.31031/DNN.2024.03.000559

Abstract

Cyanine dyes are excellent candidates as bioimaging and therapeutic agents. Their brightness, high quantum yields and highly conjugated and rigid structure with combination of several modification sites give them unique properties. They have absorbance in NIR I region (650-900nm) and NIR II (900-1500nm), which are known as therapeutic window, promoting deeper penetration, less light scattering, and lower fluorescence signal from biomolecules. By increasing the conjugation between heterocycles their absorbance can be shifted to NIR II region to improve their imaging properties. Their structural malleability allows for a variety of different modifications. For example, by incorporating different moieties on the heterocycles to increase solubility and altering at the meso position to introduce pH sensitivity for activable targeting.

Presence of different functional groups provide versatile conjugation sites on the cyanine scaffold for various active groups and small molecules e.g., targeting ligands, antibodies, and drugs etc. While cyanine dyes can be used as small molecules for bioimaging, they can be linked to targeting moieties to be used as active targeting agents or they can be encapsulated with nanoparticles to enhance their solubility and circulation time in body. In addition to nanoparticles, they can be linked to nanorods, proteins, polymers, and micelles. Herein, the structural modifiability of cyanine scaffold will be discussed for different conjugation modalities including drug delivery, targeted therapy, and targeted imaging.

Introduction

Cyanine dyes containing two heterocyclic units at each end connected by a conjugated polymethine chain possess unique properties. They are named according to the length of the polymethine chain as; monomethine cyanine, trimethine cyanine, pentamethine cyanine and heptamethine cyanine [1]. Cyanine dyes can contain a polymethine chain or a cyclohexene ring in the middle. The cyclohexene ring is preferred to introduce rigidity to the structure [2]. One of the heterocyclic units is positively charged and the other unit is neutral, which creates a 'push pull' type of system. Cyanine dyes with shorter polymethine bridge such as monomethine and trimethine cyanines generally have absorbance in UV-vis region. However, it can be extended to NIR region by changing the heterocycles [3]. On the other hand, ones with longer polymethine chain like heptamethine cyanine dyes have absorbance in NIR region. When an additional CH=CH bond is added to the polymethine chain, the absorbance shifts approximately 100nm [1]. In NIR region there is less autofluorescence from biomolecules and NIR light penetrates deeper into tissue resulting in higher signal to noise ratio [4].

Due to these reasons, cyanine dyes absorbing in NIR region have been used in various biomedical applications such as bright bioimaging probes [5-7], pH sensitive activatable probes [8] and structure-inherent tumor targeting using tumor associated immune cells [9]. One of the important cyanine dyes is Indocyanine Green (ICG), which is an FDA approved probe that is used for bioimaging. However, ICG has low quantum yield, it is taken by liver, causes

Gastrointestinal (GI) tract to get contaminated and it is not stable in aqueous solutions. A common issue of low solubility of cyanine dyes was overcome by encapsulating with nanoparticles, which also improved circulation in the body. Pentamethine cyanine dyes encapsulated with tumor targeting nanoparticles were reported to have enhanced optoacoustic intensity to detect pancreatic tumors [10].

Structural malleability and reactivity: Antibody-dye conjugate

One of the most sought-after strategies to modify cyanine structures for conjugation with biologically functional groups has been the C4' meso Cl position on the polymethine chain. This strategy has been widely employed to replace meso Cl with amine, thiol and phenol substituents via SRN1 mechanism [11,12]. Moreover, Smiles rearrangement have also been used to access O-alkyl substituted cyanines with a prominent contribution being the development of FNIR-774 by Schnermann group [12,13]. Furthermore, FNIR-774 that bears a carboxylate group, has been conjugated with EGFR-targeting monoclonal antibodies, cetuximab and panitumumab, and compared with another commercially available IR-800CW conjugated with the same EGFR-targeting antibodies [14].

The analysis of pharmacokinetic parameters and targeting ability of the mAb-FNIR-774 showed better results compared to those of mAb-IR-800CW [14]. This also signifies the impact of conjugation site of the optical label as in the case of IR-800CW, the conjugation occurs at the carboxylate end on the alkyl chain connected to indole N. While in FNIR-774, the conjugation site is the O-alkyl carboxylate linked to C4' position. Given the overall similar structural functionalities and conjugations of FNIR-774 an IR-800CW, this study gives important insights form both synthetic point of view as well as for practical applications of cyanine mAb-conjugates for tumor targeted imaging and diagnostic purposes. Antibody drug conjugates (ADCs) have attracted significant research in targeted drug delivery with the ultimate goal being the targeted specific release of the drug to avoid off drug related side effects.

Dye-Ligand conjugates: Fluorescence image guided surgery

Fluorescence image guided surgery has been long anticipated as a real time, intraoperative fluorescence guide for improved surgical outcomes especially in cancer and tumor surgeries. Usually, surgeons rely on preoperative diagnostic tools and have only visible light and palpation at their disposal during the surgery to conduct tumor resection, which is a far from ideal strategy despite the technological advancements. Cyanine fluorophores has been at the frontiers to overcome this challenge through fluorescence guided surgery by using ICG [15,16]. Moreover, a recently approved intraoperative imaging agent for ovarian and lung cancer surgery, Pafolacianine, is a cyanine-based imaging agent. Pafolacianine consists of S0456 cyanine dye conjugated with a folate analog that binds with Folate Receptor Alpha (FRα) overexpressed in certain tumors including ovarian cancer [17-19].

Pafolacianine also employs the C4' meso Cl site of SO456 cyanine dye for conjugating the folate analog through phenolic linkage [17]. Similarly, ZW800-1 has been conjugated with cRGD (Cyclic Pentapeptide), for tumor targeted intraoperative imaging in colorectal cancer with encouraging clinical results [20,21]. NIR-II imaging has greater signal to noise ratio in fluorescence imaging [22]. Hu and coworkers reported the synthesis of a heptemethine cyanine dye, IR808, and crizotinib, which is an FDA approved ATP competitive inhibitor against c-Met, conjugate to study NIR-II imaging-guided synergistic chemophototherapy in colorectal cancer. Crizotinib-IR808 conjugate showed exceptional $^1O_2$ generation and in vivo therapeutic abilities, especially higher water solubility was achieved after encapsulating with Bovine Serum Albumin (BSA) [23].

Photodynamic therapy and photoacoustic imaging

Besides fluorescence imaging, Photodynamic Therapy (PT) is an emerging technique. Photodynamic Therapy (PDT) was first discovered during 1900s and developed further since. PDT uses Photosensitizers (PS), which are activated via light energy to generate Reactive Oxygen Species (ROS) [24]. Iodinated derivative of ICG reported to have enhanced ROS generation [25], was used together with triapazamine and co-loaded on liposomes to generate synergistic phototherapy and PDT induced chemotherapy [26]. Photoacoustic imaging (PA) is another developing imaging technique in which light energy is used to excite a molecule resulting in energy to be released in another form to image the tissue [27].

Lorenz and coworkers studied the effect of alkyl chains and halogens in a library of PEG-PCL encapsulated heptamethine cyanine dye nanoparticles on their Photoacoustic (PA) and heat producing abilities. The dye-nanoparticle conjugate containing tertiary butyl incorporated cyclohexene unit with benzoeindolium and butyl chains showed highest PA signal while the one with chlorine containing indole units reported to have the highest temperature change [28]. In vitro studies of selected analog were evaluated in ovarian cancer cells and showed sharp and narrower PA signals [28].

Drug-Dye conjugates: anti-cancer therapy and NIR imaging

Cyanine drug-dye conjugates have attracted tremendous preclinical research interest for cancer theragnostic as it combines the therapeutic activity of the drug with the targeted optical ability of the cyanine to serve as NIR fluorescence imaging agent [29]. In this regard different conjugation sites of cyanine scaffolds have been employed for conjunction with cytotoxic, anti-cancer agents. A kinase inhibitor, dasatinib, based theragnostic agent has been reported, which conjugated dasatinib with heptamethine cyanine dye via covalent bonding between carboxylate group linked to N of the indolium unit and hydroxyl group on dasatinib [30]. A similar approach was reported by Kang and coworkers that combined the targeted cancer therapy with NIR optical imaging [29]. The design of targeted drug like conjugate consisted of Ne3TA iron chelating anti-cancer agent that was linked to tumor targeting ligand, transferrin.

This unit was conjugated with Cy5.5 dye through an ester linkage on the alkyl chain of indolium [29].

Cyanine NIR activated ADCs: Photocages for drug delivery

Moreover, cyanine dyes have been reported for their use as photocages for targeted drug delivery [31]. Photocages are light sensitive compounds that hide the potential activity of the molecule until it is irradiated with light at the desired location [31,32]. An added advantage of using cyanine dyes for photocaging is that the external light stimulus required for activation and subsequent release of small molecule is NIR light, which has deeper tissue penetration and higher biocompatibility as it falls within clinically employed safer spectral window for theragnostic applications [4]. An example of rather simpler application of cyanine based photocage for variety of carboxylic acid groups conjugated to heptamenthine cyanine dyes has been reported with NIR light irradiation (up to 820nm) by Stacko and co-workers [33].

The proposed mechanism of ultimate photo-uncaging of carboxylic acid group involved photooxygenation leading to the oxidative cleavage of polymethine chain [33]. Another interesting instance involving both the Antibody-Drug Conjugates (ADCs) in the context of NIR cyanine based photocage was reported by Nani and colleagues [34]. The clever design of this reported NIR-activated ADC combined targeted drug delivery of ADC with optical labeling provided by cyanine dye. The cyanine dye acted as photocage for ADC (panitumumab- combretastatin A4) via a photolabile linker at C4' that when irradiated with NIR light (690nm) would release the drug at the targeted location through photooxidative cleavage [34].

Conclusion

In summary, cyanine dyes are exceptional candidates for therapeutic applications. The underlying factor that makes cyanine scaffold such an interesting candidate for biomedical applications, in addition to its optical characteristics, is the structural malleability and the availability of numerous modifiable sites on the structure. This allows for several applications of cyanine conjugates such as fluorescence imaging in NIR-I and NIR-II window, photoacoustic imaging and targeted drug delivery. Cyanines conjugated with nanoparticles, drugs and other targeting biomolecules are developing further to be ideal agents for biomedical applications.

Acknowledgement

Authors would like to thank Department of Chemistry at Georgia State University. GE and MH would like to thank Molecular Basis of Disease at Georgia State University.

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