Nanomedicine & Biotherapeutic Discovery

Extended Abstract

Protamine conjugated fluorochromes: A new photosensitizer for photodynamic tumor therapy

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Abstract

The Photodynamic Therapy (PDT) is a promising alternative therapy that could be used adjunct to chemotherapy and surgery for curing cancer causing tissue destruction by visible light in the presence of a Photosensitizer (PS) and oxygen. Protamine is a high arginine peptide with membrane trans-locating and nuclear localizing properties. The reaction of an NHSester of Methylene Blue (MB) and clinical Protamine (Pro), to yield MB-Pro, was described in this context and demonstration of photo-toxicity which clinical protamine improved PDT effect was performed. The reaction between clinical Protamine (Pro) an NHS ester of MB is a solution phase reaction with the complete modification of the protamine peptides which feature a single reactive amine at the N-terminal proline and single carboxyl group at the C-terminal arginine. The aim of this study was to find a new type of Photosensitizer (PS) for PDT on in vitro and in vivo experiments and to assess the anti-tumor effect of PDT using the protamine conjugated-PS on the cancer cell line. Photodynamic cell death studies show that the MB-Pro produced has more efficient photodynamic activities than MB alone, causing rapid light induced cell death. The attachment of MB to clinical Pro, yielding MB-Pro, confers the membrane internalizing activity of its high arginine content on MB and can induce a rapid photodynamic cell death, presumably due to cell membrane rupture induced by light. The PDT using MBPro for HT-29 cells was very effective and those findings suggest that MB-Pro is one of candidate for photosensitizer in solid tumors.

Photodynamic therapy (PDT) is a clinically approved therapeutic method for the treatment of many malignant carcinomas. It involves the selective accumulation of a photosensitizer (PS) in tumor cells activated by irradiation of a specific wavelength, causing selective antitumor effects: direct cytotoxicity in tumor cells, involving the mitochondria-associated pathway and endoplasmic reticulum stress, due to the production of reactive singlet oxygen; devascularization of tumors; direct immune response by cytotoxic T cells against tumors; and elicitation of an immune response in the cells triggered by shutdown of the tumor vasculature, leading to local depletion of nutrients and oxygen in the tumor and causing secondary necrosis. These effects of PDT lead to several forms of cell death, including apoptosis, necrosis, and autophagy; different mechanisms account for these different forms of cell death. Apoptosis is the major process of cell death activated by PDT; however, cell death due to PDT generally occurs as a combination of these three mechanisms, and no single pathway of PDT leads to cell death. The factors determining mechanisms of cell death depend on various parameters: type and dose of PS, localization of PS, light dose, and oxygen concentration in the cell. Determination of major factors of cell death requires further study, but one factor postulated is the dosage of PS: high dose of PS leads to necrosis, while low dose of PS tends to activate apoptosis.

Since PDT was first used clinically in 1898, many of its advantages have been exploited. A combination of PDT and surgery, radiotherapy, or chemotherapy features low- or noninvasiveness, a low incidence of side effects, good compatibility with other treatments, fewer risks over repeated treatments, short treatment time, cost-effectiveness, and non-immunosuppression. However, there were also several drawbacks to PDT, preventing it from being used as a major treatment modality: dependence on certain types of PSs and light sources (wavelength, time exposure, pulse duration, and pulse frequency), low delivery accuracy of the PSs, and limited treatment depth. Therefore, to improve the efficiency of PDT and expand its applicability in the treatment of various cancers, the limitations regarding its clinical efficacy should be reduced. PDT requires light irradiation at a specific point in a

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tumor; therefore, monitoring of the tumor location is crucial for effective PDT with low side effects. Another limitation is that singlet oxygen has a very short half-life of less than 40 ns and a proliferation range of 10–20 nm; thus, effective delivery of the PS is required to enhance the availability of singlet oxygen in the cytoplasm and to direct it towards the organelles of tumor cells. To overcome the limitations of PDT, it is necessary to generate efficient methods for monitoring the accumulation of PSs and for delivering them to a selected site at a low dosage. We developed a new approach to the delivery of PSs by conjugation of a cell-penetrating peptide (CPP) with a typical fluorescent dye, rhodamine (Rho). CPPs are interesting, as they can easily translocate through cellular membranes. CPP typically contains 20–30 amino acid residues and is classified as amphipathic in nature, although cationic CPP has fewer amino acids with positively charged residues (arginine and lysine). CPPs offer an efficient way for drugs to pass easily through cellular membranes. Notably, CPPs rich in arginine penetrate cell membranes very readily. Arginine-rich peptides have membrane-translocating and nuclear-localizing sequences, which have led to their use in various drug delivery methods. Protamine (Pro), used to neutralize the anticoagulant effects of heparin, is a mixture of four similar arginine-rich peptides with membrane-translocating and nuclear-localizing properties. The advantage to using clinical Pro as a CPP is that the sequence of clinical Pro features a single reactive amine at the N-terminal proline and a single reactive carboxyl group at the C-terminal arginine providing simple steps for manipulation or modification of Pro by the addition of Rho.

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