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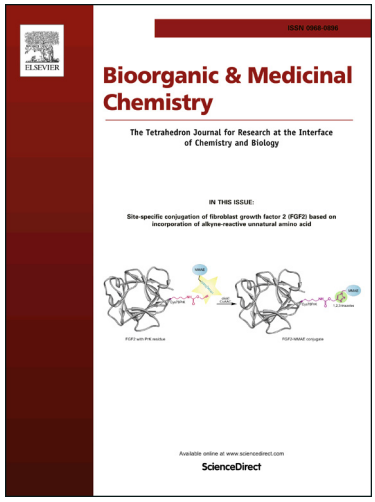

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Ultrasound-dependent cytoplasmic internalization of a peptide-sonosensitizer conjugate

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ABSTRACT

A method to induce cytoplasmic peptide delivery, using ultrasound, was demonstrated using a molecular conjugate of a cell-penetrating peptide (CPP), a functional peptide, and a sonosensitizer. As a model of such molecular conjugates, TatBim-RB, consisting of the Tat CPP, the Bim apoptosis inducing peptide, and the sonosensitizer rose bengal was synthesized. CPPs have been widely used for intracellular delivery of various cargos; however, CPP-fused molecules tend to become entrapped in endosomes, as was observed for TatBim-RB molecules applied to cells. To promote escape of the entrapped TatBim-RB molecules, cells were irradiated with ultrasound, which successfully induced endosomal escape and cytoplasmic dispersion of TatBim-RB, and subsequently apoptosis. Our results suggest that this peptide-sonosensitizer conjugate strategy may facilitate numerous kinds of medicinal chemistry studies, and furthermore, this specific conjugate may exhibit potential as a novel therapeutic agent for the promotion of apoptosis.

1. Introduction

Recently, the fusion of peptides and proteins to cell-penetrating peptides (CPPs) to facilitate their transport into cells has been widely used for therapeutic and biological purposes.[1,2] However, this strategy is often limited by the inefficient transfer of the CPP-fused peptides and proteins to the cytosol consequent to their endosomal entrapment.[3] One of the methods to overcome this problem is to use photosensitizers and light to mediate endosomal escape.[4,5] In this method, the light causes the entrapped photosensitizer to generate reactive oxygen species (ROS), which disrupts the surrounding endosomal membrane. For example, we previously designed a TatBim-Alexa molecule,[6] comprising a conjugate of Tat CPP from the HIV-1 transactivator of transcription (TAT) protein,[7] the BH3 domain derived from Bim apoptosis-inducing protein,[8,9] and the Alexa Fluor 546 dye as a photosensitizer. TatBim-Alexa molecules enter cells by the endocytic pathway, are entrapped in endosomes, and then escape from the endosomes and induce apoptosis by photoirradiation. Similar fusion molecules, such as TatU1A-Alexa and TatU1A-DY750 for photo-dependent cytoplasmic RNA delivery, were also reported.[10–13] However, the poor tissue penetration of light limits the application of these photosensitizing molecules. Alternatively, ultrasound (US) represents a promising substitute for light as an external stimulus because of its deeper penetrating property. The high tissue-penetrating ability has prompted extensive evaluations of US for medical purposes.[14,15] Recently, high-intensity, focused ultrasound has attracted attention because it can specifically irradiate a target tissue and provides a potential noninvasive therapeutic strategy.[16]

Sonosensitizers are known as molecules that generate ROS in a US-dependent manner. Sonosensitizers include inorganic materials (*e.g.* titanium dioxide)[17] and organic dyes (*e.g.* porphyrin derivatives and rose bengal).[18–20] In the current study, a sonosensitizer- and CPP-fused functional peptide was developed as a molecule exhibiting US-dependent intracellular function. As an example of this design, TatBim-RB, containing the Tat CPP, the Bim apoptosis-inducing peptide, and the sonosensitizer rose bengal was synthesized. The use of a sonosensitizer and US instead of a photosensitizer and light was attempted to facilitate endosomal escape of the CPP-fused functional peptide (Fig. 1).