Photodiagnosis and Photodynamic Therapy (2014) 11, 48–54

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/pdpdt

A novel photodynamic therapy for drug-resistant prostate cancer cells using porphyrus envelope as a novel photosensitizer

Masaya Yamauchi[a,*], Norihiro Honda[a,b], Hisanao Hazama[a], Shoji Tachikawa[c], Hiroyuki Nakamura[c], Yasufumi Kaneda[d], Kunio Awazu[a,e,f]

[a] *Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *Institute for Academic Initiatives, Osaka University, 1-1 Yamadaoka, Suita, Osaka 565-0871, Japan*
[c] *Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588, Japan*
[d] *Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[e] *Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871, Japan*
[f] *The Center for Advanced Medical Engineering and Informatics, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*

Available online 19 October 2013

KEYWORDS
Photodynamic therapy;
Prostate cancer;
Drug resistance;
Hemagglutinating virus of Japan envelope;
Drug delivery system

Summary
Background: In the clinic, it is often very difficult to treat drug-resistant advanced prostate cancer by conventional treatments. Photodynamic therapy (PDT) is a minimally invasive treatment that takes advantage of photochemical reactions between a photosensitizer and light. On the basis of several of its key characteristics, PDT is considered to be a promising novel method for treating drug-resistant prostate cancer.
Objectives: For effective treatment of drug-resistant prostate cancer, we developed a novel agent termed porphyrus envelope, which was produced from PpIX lipid and hemagglutinating virus of Japan envelope (HVJ-E).
Materials and methods: We inserted PpIX lipid into HVJ-E by centrifugation, and used the resultant porphyrus envelope in PDT of two drug-resistant prostate cancer cell lines, PC-3 and PC-3-DR.
Results: Porphyrus envelope enhanced uptake of PpIX, and cytotoxicity of PDT, relative to free PpIX lipid or PpIX induced by 5-ALA.
Conclusion: PDT using porphyrus envelope has potential as a method for treating drug-resistant prostate cancer.

* Corresponding author. Tel.: +81 66879 4735; fax: +81 66879 7363.
E-mail address: yamauchi-m@see.eng.osaka-u.ac.jp (M. Yamauchi).

1572-1000/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.pdpdt.2013.10.001

Introduction

Prostate cancer usually occurs in older men. Whether or not a prostate cancer is metastatic, the tumor needs androgen in order to grow. Therefore, cutting off the supply of androgen is an effective way to inhibit the growth of advanced prostate cancer. However, prostate cancer often gradually acquires resistance to such treatment and recurs [1,2]. Improved methods of medication, aimed at increasing the time before prostate cancers acquire resistance, are currently being investigated; these methods include administration after some interval and the simultaneous use of multiple drugs [3]. However, the problem remains fundamentally unsolved. Docetaxel is often used to treat such hormone drug-resistant prostate cancers. However, the response rate of docetaxel is not good (44.2% when used with prednisolone [4]). Because of the current difficulty of treating drug-resistant prostate cancer, new methods of treating such tumors are urgently needed.

Photodynamic therapy (PDT) is a photochemical modality approved for the treatment of various cancers, skin diseases, and diseases with neovascularization [5,6]. This method is based on photochemical reactions between light and tumor tissue treated with exogenous photosensitizer. Because of the selectivity of the photosensitizer, PDT is a less invasive treatment method, and is therefore considered to be a promising novel treatment for drug-resistant prostate cancer. When PDT is applied to such prostate cancers, greater tumor selectivity is needed in order to effectively treat advanced metastatic tumors and to decrease side effects such as photosensitivity. In order to establish the ideal PDT, it is important to develop novel photosensitizers. To improve the selectivity of PDT, photosensitizers should be delivered by a drug-delivery system (DDS). A DDS is a technology designed to optimize drug therapy by controlling drug disposition and selectively transporting the drug to a target site at a preferred density or time. An ideal DDS also effectively reduces the side effects and improves the safety of a given drug.

Hemagglutinating virus of Japan envelope (HVJ-E) is a non-viral DDS carrier with a bilayer structure. HVJ-E can serve as an active targeting system with antibodies, as well as with liposomes or micelles [7—11]. HVJ-E has two interesting characteristics that distinguish it from other drug carriers. First, it has the ability to undergo membrane fusion. As shown in Fig. 1, HVJ-E contains the F and HN proteins. HN protein conjugates with the sialic acid receptor, and F protein mediates membrane fusion [9,12]; consequently, the contents within HVJ-E are delivered directly into the cell, improving the rate of photosensitizer accumulation. Second, HVJ-E alone has an antitumor effect [12—15]. This effect has been investigated in pre-clinical trials, and is currently being investigated in a clinical trial [16].

In this study, we inserted protoporphyrin IX lipid (PpIX lipid) [17] into HVJ-E. PpIX is a photosensitizer widely used in topical PDT. PDT with PpIX is based on the endogenous accumulation of PpIX after topical or systemic administration of 5-aminolaevulinic acid (5-ALA) [18]. PpIX has two advantages as a photosensitizer: low cost of production and low cytotoxicity without light. HVJ-E can deliver PpIX directly and achieve higher concentrations of PpIX than 5-ALA. We used not PpIX itself, but PpIX lipid, which contains two lipid chains and two Li groups in order to enhance the loading rate. The structure of PpIX lipid is shown in Fig. 2. The two lipid chains of PpIX lipid are reminiscent of the molecular structure of the phospholipid bilayers such as those surrounding cells and HVJ-E. Therefore, PpIX lipid can be inserted into not only in the inner space of HVJ-E, but also into the lipid layer. Because of its high molecular weight, PpIX lipid does not leak from HVJ-E at a significant rate. Furthermore, PpIX lipid forms micelles and improves the water solubility of PpIX.

In order to establish a novel method for PDT to treat advanced drug-resistant prostate cancer, we examined the effectiveness of PDT of two drug-resistant prostate cancer cell lines (PC-3 and PC-3-DR) using PpIX lipid inserted into HVJ-E, which we call ''porphyrus envelope''.

Materials and methods

Photosensitizer

5-ALA (A7793, Sigma—Aldrich Co., LLC, USA), PpIX lipid, and porphyrus envelope were used as photosensitizers. ''Porphyrus envelope'' is a novel photosensitizer generated from PpIX lipid and HVJ-E. PpIX lipid is a chemically modified protoporphyrin IX; the structure of PpIX lipid is shown in Fig. 2. PpIX lipid contains two lipid chains (lipophilic) and two Li groups (hydrophilic). PpIX lipid was produced as previously described [19]. PpIX lipid forms micelles and is more soluble in water than PpIX. In addition, PpIX lipid can be inserted to the lipid layer of HVJ-E.

Synthesis of porphyrus envelope

A suspension of hemagglutinating virus of Japan (HVJ) was inactivated by irradiation of ultraviolet light with an energy density of 99 mJ/cm^2. To 2.5×10^{10} particles of HVJ-E (2500

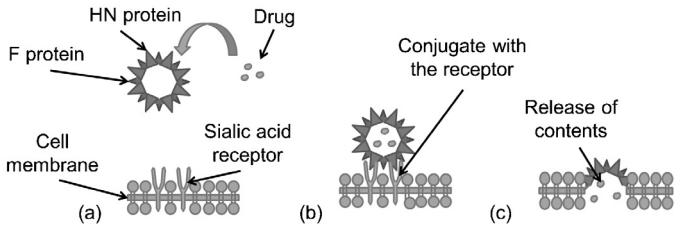

Figure 1 Membrane fusion of HVJ-E. Introduction of drug into HVJ-E (a). Conjugation of HN protein with receptors on the cell surface (b). Membrane fusion and drug release, mediated by F protein (c).