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Photodynamic activity of thiophene-derived lysosome-specific dyes

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ABSTRACT

The photodynamic activity occurring through the lysosome photo-damage is effective in terms of triggered synergic effects which can avoid chemo-resistance pathways. The potential photodynamic activity of two fluorescent lysosome-specific probes was studied providing their interaction with human serum albumin, demonstrating their *in vitro* generation of singlet oxygen and investigating the resulted photo-toxic effect in human cancer cells.

1. Introduction

Photodynamic activity is a photochemistry-based mechanism in which a light-activatable dye, the photosensitizer (PS), is capable of generating reactive oxygen species (ROS) upon light irradiation [1,2].

This combination of light and non-toxic PS is the basis of the emerging photodynamic therapy (PDT), a minimally invasive treatment of various pathologies including cancer [3–5]. PDT has several advantages compared to conventional protocols (such as chemotherapy and radiotherapy) due to its selectivity, non-invasiveness, loss of systemic side effects and activation of pathways which overcome cancer drug resistance [6]. The produced ROS can trigger cascading biochemical and molecular events which finally lead to cell death and cancer disruption due to the oxidation of biological molecules such as proteins, DNA and phospholipids [7–10]. So the photo-damage acts to various levels inducing apoptosis or necrosis and bypassing the cancer cell chemo and radioresistance pathways. PDT can directly induce damage to proteins involved in cancer resistance mechanisms such as the antiapoptotic BCL-2 family proteins which are one of the principal drug resistance pathways [6,11]. A number of PSs are already in clinical use or in clinical trials for the treatment of different solid tumors in skin, brain, lung, bone, cervix, prostate and ovary [12]. PSs are commonly divided into three main classes: the first generation of PSs includes porphyrin-based dyes with a good photodynamic activity, widely used for the treatment of lung and esophageal cancers [13]; the second generation includes PSs with improved pharmacokinetic and reduced tissue photosensitivity because of their near-infrared (NIR) wavelength absorption such as benzoporphyrin derivatives, Bodipy dyes, phthalocyanines and many others which were projected with selected properties [14]. Currently photochemistry research goes toward two-photon bio-imaging [15] that offers new PSs, the third generation, which can be excited with a double wavelength providing a better tissue penetration and a more sensitive therapeutic effect [16,17]. Many factors can influence the PS activity: absorption band, fluorescence quantum yield, photostability, dark toxicity and hydrophobicity. A PS should have relatively high absorption bands (>20,000–30,000 M^{-1} cm^{-1}) in order to minimize the dose of PS to achieve the desired effect [12]. The fluorescence of the PS led to the development of protocols for dye quantification in cells and tissues and allows bio-imaging techniques to locate and monitor the disease development [2,18]. For clinical applications an important issue is the solubility of PS in water or in a harmless aqueous

Abbreviations: DPBF, 1,3-diphenylisobenzofuran; HSA, human serum albumin; lyso-PDT, photo-damage of lysosomes; mito-PDT, photo-damage of mitochondria; PDT, photodynamic therapy; PS, photosensitizer; ROS, reactive oxygen species.

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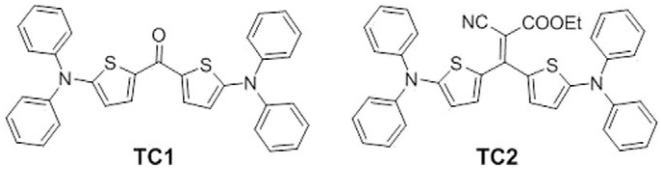

Fig. 1. Structures of TC1 and TC2 dyes.

solvent mixture without phenomena of aggregation which could determine the loss of photodynamic activity [19]. Another important characteristic of the PS is its hydrophobic nature that may interfere with the solubility but may facilitate the crossing of biological membranes optimizing PS cellular uptake. The PDT treatment implies the PS accumulation in cancer tissue through two mechanisms: using a selective drug delivery system (active targeting) or exploiting the enhanced vascular permeability and retention (EPR) effect (passive targeting) [20,21]. Therefore it is possible to perceive that the PS biodistribution and the achievement of the target site are linked to various factors such as their hydrophobic nature, the undesirable uptake by phagocytic cells and the binding of plasma molecules [22]. In this context the human serum albumin (HSA) plays a crucial role. HSA is the main plasma protein and it is responsible for the binding and transport of many endogenous and exogenous molecules including drugs [23,24]. The nature and the efficiency of the binding between the PS and serum albumin play an important role for PDT efficiency. Recently, it has been shown that if a molecule possesses affinity for serum albumin, it would probably exhibit efficient PDT applications [25,26]. The effective cancer cellular uptake is essential but the triggered cell mechanisms and the degree of photo-damage is closely linked to the subcellular localization of the PS [27–29]. ROS have high reactivity, short half-life and their radius of action is of the order of few nm, only the biomolecules proximal to the area of its production are directly affected by PDT [30]. Therefore the subcellular localization of PS is a crucial factor which depends on the target of the photo-damage and on the induced cell death mechanism [31]. Most PSs preferentially localize in plasma membrane, Golgi apparatus, endoplasmic reticulum, nucleus, mitochondria and lysosomes [2]. The two most projected and exploited PDT methods are the photo-damage of mitochondria (mito-PDT) and the photo-damage of lysosomes (lyso-PDT). The mito- and lyso-PDT promote the pro-apoptosis pathways bypassing cancer drug resistance [6]. In particular lysosomes had shown a relevant role in cell death, representing a promising target for effective PDT [32]. In fact the lyso-photodamage in cancer cell models causes the lysosome permeabilization with release of proteolytic enzymes that trigger the apoptosis through different pathways such as the activation of pro-apoptotic factors [33–37]. Thus the advantage of lyso-PDT is the multiple response bypassing the autophagic mechanism [38]. The lyso-PDT response implies not only the destruction of these organelles, but also the permeabilization of mitochondria membranes with the loss of membrane potential, release of cytochrome c and activation of caspases [39–41]. The PDT mechanism is influenced also by the photodynamic dose [42]. Therefore recent works have demonstrated a synergic enhancement of photo-damage by using PS with different compartments targeting [43–45]. In particular an enhanced photo-toxic effect is obtained with a sequential low dose lyso- and mito-PDT [46]. The lysosomal PSs are also good candidates for the photochemical internalization process (PDI) that is a drug delivery mechanism allowing the internalization of therapeutic macromolecules which do not reach their target, remaining entrapped in lyso- and endocytic vesicles. In this case the photodynamic activity of PS is exploited to induce the destabilization of lysosome membranes allowing the drug release in the cytosol [47]. In our recent paper, we have synthesized and characterized two thiophene-derived lysosome-specific dyes (TC1 and TC2), whose structures are shown in Fig. 1 [48]. These dyes showed high selectivity for fluorescent staining of lysosomes (co-localization coefficient of >95%), high photostability, low toxicity, moderate fluorescence quantum yield and extinction coefficient. These features make TC1 and TC2 perfect candidates as PSs.

In this present study we investigate the photodynamic activity of TC1 and TC2 studying the interaction of these dyes with HSA, ensuring the *in vitro* singlet oxygen production and their phototoxic effect in human fibrosarcoma cells.

2. Materials and Methods

2.1. Materials

All reagents were of analytical reagent grade and double distilled water was used. The singlet oxygen probe 1,3-diphenylisobenzofuran (DPBF), human serum albumin (HSA), intracellular probe for oxidative stress 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. The WST-1 cell proliferation assay was purchased from Roche Applied Science (Penzberg, Germany). The thiophene-based fluorescent probes, TC1 and TC2, were synthesized as already described in our previous work [48].

2.2. Spectroscopic Measurements

UV–Vis measurements were recorded using a Cary 300 spectrophotometer. Fluorescence measurements were recorded using a

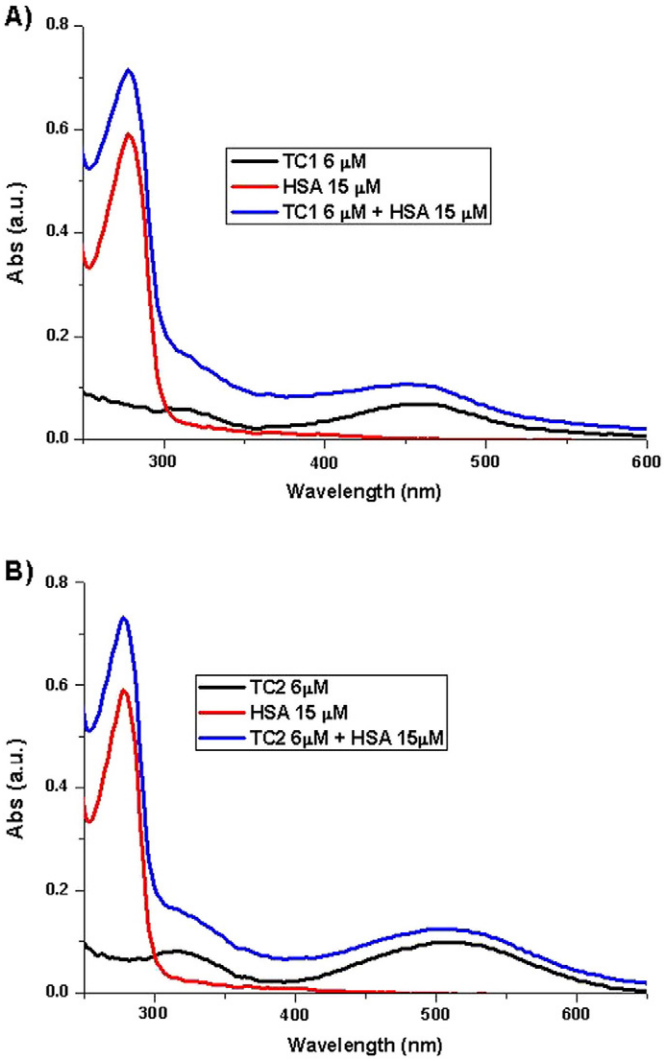

Fig. 2. UV–Vis spectra of A) TC1-HSA interaction and B) TC2-HSA interaction.