Photoswitchable Drugs

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Direct Photocontrol of Peptidomimetics: An Alternative to Oxygen-Dependent Photodynamic Cancer Therapy

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Abstract: *Conventional photodynamic treatment strategies are based on the principle of activating molecular oxygen in situ by light, mediated by a photosensitizer, which leads to the generation of reactive oxygen species and thereby causes cell death. A diarylethene-derived peptidomimetic is presented that is suitable for photodynamic cancer therapy without any involvement of oxygen. This light-sensitive molecule is not a mediator but is itself the cytotoxic agent. As a derivative of the cyclic amphiphilic peptide gramicidin S, the peptidomimetic exists in two thermally stable photoforms that are interconvertible by light of different wavelengths. The isomer generated by visible light shows much stronger toxicity against tumor cells than the UV-generated isomer. First in vivo applications are demonstrated on a tumor animal model to illustrate how the peptidomimetic can be administered in the less toxic form and then activated locally in a solid tumor by visible light.*

Drug candidates for the treatment of cancer tend to have a low success rate. The chances for approval of a new anticancer chemotherapeutic after entering phase I clinical trials were amongst the lowest of all drug candidates tested during the period from 2003 to 2011.[1] This situation reflects the fundamental challenge in cancer chemotherapy of having to eliminate the transformed cells selectively without affecting the healthy tissue. Targeted anticancer drug delivery has emerged as a strategy to overcome this problem and has already yielded successful candidates that are being tested clinically.[2] A different approach to tumor-selective and minimally invasive cancer treatment relies on photodynamic therapy (PDT), which is also on the rise.[3]

PDT can be generally defined as treatment of localized lesions based on the use of drugs that are directly or indirectly activated by light. Initial observations that laid the groundwork for all subsequent developments date back to the year 1900.[4] The first PDT agents that induce cancer cell death upon irradiation of tumors in vivo were introduced into the clinic in the second half of the last century.[5] Notably, currently used PDT agents are not the actual cytotoxic compounds. Instead, they act as photosensitizers to mediate energy transfer from photons to molecular oxygen, which is thereby activated to its reactive singlet state. The oxidation reaction cascades initiated by singlet oxygen eventually destroy the surrounding cells, thereby leading to the therapeutic effect.[6] Current anticancer PDT approaches are thus oxygen-dependent; they require molecular oxygen to be present in tumors at a sufficient level. However, cells within solid malignancies grow and proliferate so rapidly that their consumption of oxygen tends to elicit an insufficient oxygen supply. The development of such hypoxic microenvironments in tumors can then induce cell phenotypes with elevated resistance and invasivity, which bear a high risk for generating metastases and relapse.[7] Obviously, a conventional oxygen-dependent PDT treatment is less effective in such cases.[8] Different ways to circumvent this problem have been proposed, however, there have been limited applications in vivo so far.[9]

Herein, we report the design, synthesis, and in vitro and in vivo evaluation of a promising candidate for an oxygen-independent PDT for cancer: a photocontrollable cytotoxic peptide analogue (peptidomimetic). The idea behind controlling the biological activity of peptides and proteins with light relies on the incorporation of "molecular photoswitches" (reversibly photoisomerizable fragments) into the backbone or side chains. The structure and properties of these products can thus be switched by photoisomerization, as has been

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demonstrated for many peptidomimetics[10] and modified proteins.[11]

Early progress in the design of peptidomimetics with clear-cut therapeutic effects in vivo was slow, even though compounds of this type had been recognized as highly promising for clinical applications.[12] Recently, we prepared a new class of such compounds containing a photoswitchable diarylethene core,[13] using the natural peptide antibiotic gramicidin S (GS) as a template (Figure 1).[14] With its amphiphilic cyclic structure, this cationic peptide is a well-known membranolytic agent.[15] Our first-generation GS derivatives were constructed by using the hydrazide-acid photoswitch (Sw) as a building block. With these analogues, we achieved good photocontrol of antibacterial activity. As a further development, we explored second-generation diarylethene-based peptidomimetics as compounds with photocontrolled cytotoxic activity against cancer cell lines, and we demonstrate herein for the first time the utility of the oxygen-independent PDT approach in vivo.

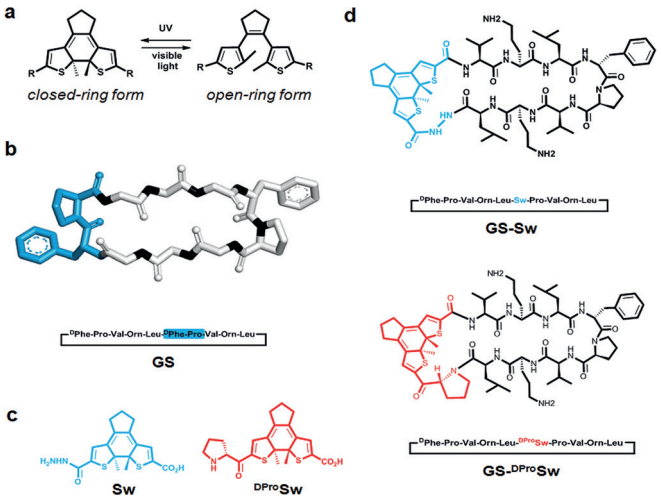

Figure 1. a) Reversible photoisomerization of the diarylethene core with UV and visible light. b) Sequence and backbone structure of GS, used here as a template (Orn = ornithine, DPhe = D-phenylalanine, amino acid side chains outside the β-turns are omitted). The fragment changed by the photoswitch is highlighted in blue. c) Structures of Sw and ^{DPro}Sw (shown in the closed-ring forms). d) Sequences and structures of the first-generation (GS-Sw) and second-generation (GS-^{DPro}Sw) photocontrollable peptidomimetics.

The natural peptide GS is known to be highly cytotoxic not only to prokaryotes but also to eukaryotic cells, including various transformed cell lines, and it can also inhibit tumor growth in vivo.[16] However, it also demonstrates high systemic toxicity, which is caused by its low in vivo selectivity towards the tumor cells.[16] We therefore envisaged that photocontrolled GS analogues could alleviate this problem when properly applied: they should be administered in their inactive form followed by local light-triggered activation in tumors only.

To implement this idea, we initially considered the first-generation analogues described above to evaluate their anticancer activity, but we identified several issues that could hamper their application in PDT. Specifically, these compounds contain the chemically unstable and potentially toxic hydrazide fragment. Furthermore, the overall yields of their synthesis were low in our hands. Most importantly, the long-wavelength maximum in the absorbance spectra of their closed-ring forms is around 530 nm, far from the near-infrared (NIR) window in which light best penetrates biological tissues. Activation of PDT agents by NIR light is an important prerequisite for successful clinical usage.[3,17] It should be noted that significant advances in the design of red- and NIR-light-controlled photoswitches have been made recently with azobenzenes,[18] diarylethenes,[13a,c] spiropyranes[19] and other types[20] of photoswitching systems. Taking into account these achievements, we designed a new photoswitchable building block for peptidomimetics of a second generation, in which the diarylethene fragment is connected to the peptide backbone via a keto group (^{DPro}Sw, Figure 1c). The resultant peptidomimetic (GS-^{DPro}Sw; Figure 1d) was obtained in approximately 80% yield at the cyclisation step, and the preparation of gram quantities was practically feasible. The absorption maximum of the closed-ring GS-^{DPro}Sw had shifted by around 46 nm to the NIR window compared to GS-Sw (Figure 2).

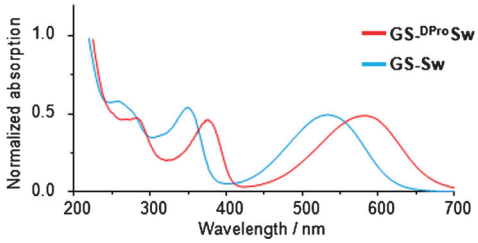

Figure 2. Absorption spectra of the closed-ring forms of the peptidomimetics.

Next, we proceeded to characterize the two GS-^{DPro}Sw photoforms in terms of their activity against human cancer cells in vitro. The cytotoxicity of the open- and closed-ring forms was assessed in a standard MTT assay against HeLa (cervical cancer) and COLO-205 (colorectal cancer) cell lines. A BALB/c mouse aortic endothelial cell line (MAEC) was used to estimate the possible damage that may be caused by the peptidomimetic against healthy eukaryotic cells. The open-ring form exhibited significant cytotoxicity against all tested cell lines (Figure 3). Moreover, its half maximal inhibitory concentration (IC_{50}) values were very close to those of the wild-type GS (IC_{50} of ca. 6 μM in all cases). At the same time, the closed-ring photoisomer showed about 5.5- to 8.0-fold lower cytotoxicity. Notably, the cytotoxicity of the closed-ring form of the peptidomimetic was the lowest against MAEC, which is in line with the reported moderate intrinsic selectivity of GS for cancer cells. These findings encouraged us to address the possibility of using light to activate the cytotoxicity in vivo as a next logical step towards assessing the potential of such peptidomimetics for PDT of cancer.

Before carrying out experiments using an animal cancer model, we evaluated crucial parameters for the treatment regime: the irradiance, wavelength, and treatment schedule.

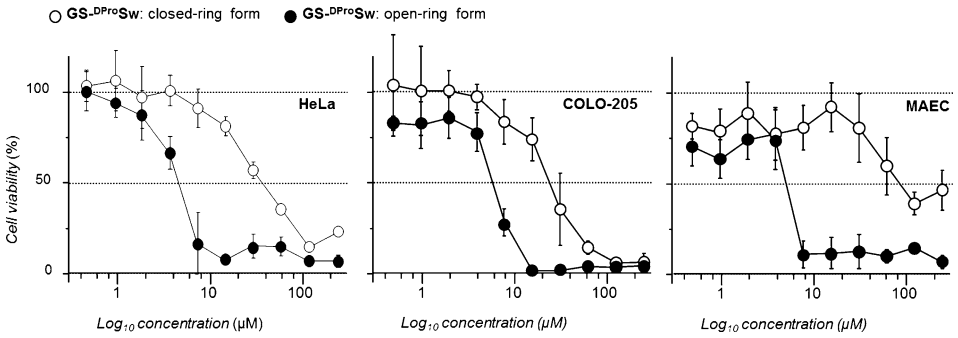

Figure 3. In vitro cytotoxic activities of the two GS-^{DPro}Sw photoforms as measured in the MTT test.

As we learned from the in vitro tests, GS-^{DPro}Sw is more active in its open-ring form. Therefore, the UV-generated isomer has to be administered first, and then the active compound has to be formed locally in the tumor tissue upon illumination with visible light. The optimal and practical light parameters to achieve this were determined in model tissues. Samples of surrogate tissue were mixed with the closed-ring GS-^{DPro}Sw, exposed to light of variable wavelength and intensity, and then analyzed by HPLC. It is reasonable to apply light of high intensity in order to reduce the irradiation time and activate molecules deeper within the tissues, but at the same time, overly strong irradiation could cause heat-shock damage to the tissues. We thus compromised with irradiance between 100–600 mW cm^{-2}. Irradiation with wavelengths around 620–680 nm was found to be best in terms of photoisomerisation efficiency and light penetration into the tissue (Figure 4a). In one of the experimental settings, irradiation by 664 nm light at an irradiance of 400 mW cm^{-2} for 30 min caused around 50% transformation of the peptidomimetic from the closed-ring to the open-ring form, up to 12 mm deep into the model tissue.

Another important issue is the pharmacokinetic parameters of the peptidomimetic. First of all, we wanted to know 1) whether the compound was sufficiently stable in blood and tissue, and 2) whether it would accumulate in tumors in therapeutically significant concentrations upon administration. We obtained a positive answer to the first question: incubation of GS-^{DPro}Sw in human blood serum at 37 °C for 16 h resulted in negligible degradation. This was expected because cyclic peptidomimetics are known to be extremely resistant to proteolytic degradation in vivo.[21] Regarding the second question, we performed initial pharmacokinetic studies in mice, using the Lewis lung carcinoma (LLC) allograft as a well-established rodent model. The closed-ring form was administered intraperitoneally (5 mg kg^{-1} as single dose), and its distribution profile and spontaneous conversion was monitored for up to 2 hours by HPLC analysis of the blood and tumor tissue from a series of sacrificed animals. In blood, the administered closed-ring form reached the highest concentration (ca. 10–14 μg mL^{-1}) within less than 15 min, and this concentration did not change significantly over 2 hours. As expected, the background concentration of the open-ring GS-^{DPro}Sw in blood did not exceed 5–10% of that of the closed-ring form when the mice were kept in the dark. Importantly, the closed-ring photoform was found to accumulate in the tumor tissue after injection. Subsequent irradiation of the peptidomimetic-treated animals in the areas of their tumors (Figure 4b) with visible light (570 nm, 550 mW cm^{-2}, 10–20 min) caused photoconversion (> 75%) of the closed-ring into the open-ring form in the tumor tissues. At the same time, the concentration of the open-ring form in the blood did not change significantly (< 12% photoconversion).

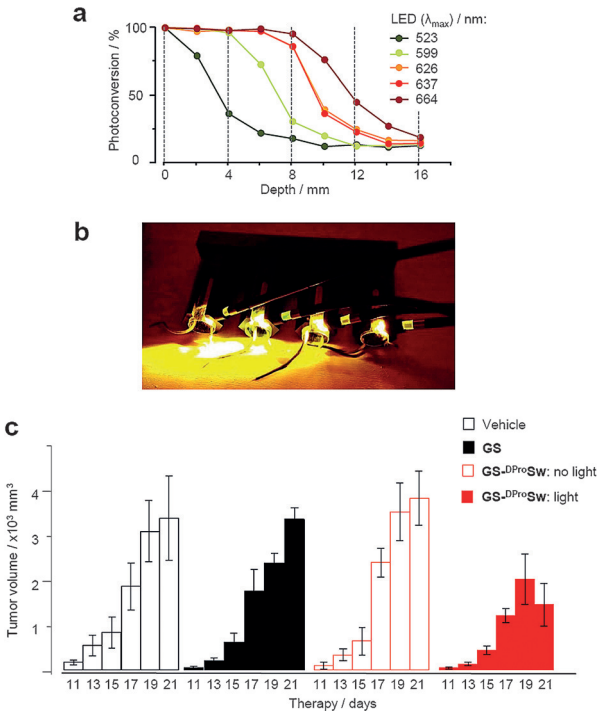

Figure 4. a) Conversion of the closed-ring form into the open-ring form, monitored in a model tissue exposed to illumination by LEDs with different emission wavelengths λ_{max} (illumination time 30 min, irradiance 100–400 mW cm^{-2} depending on the LED characteristics). The percentage conversion was plotted against the light penetration depth into the tissue, as measured from the illuminated surface (see the Supporting Information for details). b) A photograph of the experimental setup. c) Antitumor efficiency of PDT therapy with GS-^{DPro}Sw, as observed in the LLC model.

Finally, an in vivo experiment was set up, again using the LLC model in C57Bl/6 mice. Seven groups, each consisting of eight animals with palpable tumors, received a daily intraperitoneal injection of either the vehicle (ethanol/saline 1:10 vol.; group 1), GS (ca. 1.0 mg kg^{-1} and 9.1 mg kg^{-1}; groups 2,3), or the closed-ring form of GS-^{DPro}Sw (ca. 1.0 mg kg^{-1}, groups 4,5 and 9.1 mg kg^{-1}, groups 6,7). The tumors in groups 4,6 (receiving the peptidomimetic) were locally irradiated with visible light (ca. 100 mW cm^{-2}, 15 min after the injection) for 20 min to model PDT. The results showed that animal survival in the groups that received the

PDT was improved by 60% compared to the control, while it was reduced in the animals treated with wild-type GS as expected. Post-mortem examination of the tumors in the treated animals showed that the tissues were necrotic in the case of the PDT, and they had shrunk dramatically (Figure 4c). The PDT scheme (groups 4, 6) thus led to a significant antitumor efficiency after approximately 20 days compared to both the control groups (1–3) and to the other groups that had received the peptidomimetic but were not exposed to irradiation. In one animal treated with GS-^{DPro}Sw in combination with light, the tumor had vanished completely by day 21.

In summary, we have created a diarylethene-based photoswitchable peptidomimetic, shown the practical possibility of cytotoxicity photoregulation in vitro, and performed a proof-of-the-principle demonstration of this oxygen-independent PDT for cancer.

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