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A PHOTOACTIVATED PRODRUG

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Abstract: A photolabile derivative (**1**) of the anticancer drug, 5-fluorodeoxyuridine (**2**), was designed and synthesized as a model prodrug. Photolysis of **1** with long-wavelength UV light rapidly released **2** in solution. While compound **1** alone is nontoxic to cells, the presence of both **1** and UV irradiation (λ = 350 nm) resulted in potent inhibition of cell growth.

Introduction

A major limitation in cancer chemotherapy is the inadequate selectivity of most anticancer drugs,[1,2] which usually have nonspecific cytotoxic properties and cause serious damage to normal proliferating tissues such as the bone marrow. These side effects often limit the dosage below the optimal level required to completely eradicate the tumors. Numerous strategies have been explored to improve the effectiveness and specificity of cancer chemotherapy. For example, anticancer drugs conjugated to tumor antigen-specific monoclonal antibodies have been delivered to tumor sites.[3–6] This strategy improves drug selectivity by achieving a higher drug concentration at the tumor site relative to normal tissues. However, there are a number of problems inherent with this strategy, especially in the case of treating solid tumors.[7–11] Effort has also been focused on the development of relatively nontoxic prodrugs that can be activated by tumor-specific enzymes.[12] This approach is very attractive since the prodrugs are, in principle, only activated at the tumor sites provided that highly specific enzymes can be found. The later part of the above approach has proved quite challenging due to the difficulties in finding enzymes that are specifically tumor-associated. Yet another strategy for delivering anticancer drugs to tumor sites involves the use of tumor-specific monoclonal antibodies to deliver catalysts such as enzymes to tumor cell surface.[13] The enzymes can generate low molecular weight cytotoxic drugs from the corresponding nontoxic prodrugs within tumor mass. A large number of drugs can be generated per conjugate molecule. The released drugs, as small molecules, can easily penetrate into nearby tumor cells, many of which would otherwise be inaccessible to the enzyme-antibody conjugate. A number of enzymes have been used to activate prodrugs.[3,13] The major problems associated with natural enzyme activation of a prodrug are: (1) the widespread distribution of nonselective enzymes that may prematurely activate a prodrug,[13] thus causing damage to normal tissues; (2) if the enzyme used for prodrug activation is of alien origin, immune response from humans/animals will eliminate the enzymes relatively rapidly. Therefore, a major challenge for prodrug design is to design and discover non-toxic prodrugs that can only be activated by highly specific and efficient methods but remain inert toward natural enzymes. Catalytic antibodies have been designed for activating prodrugs in model systems.[14–16] The use of catalytic antibody should allow the activation of prodrug

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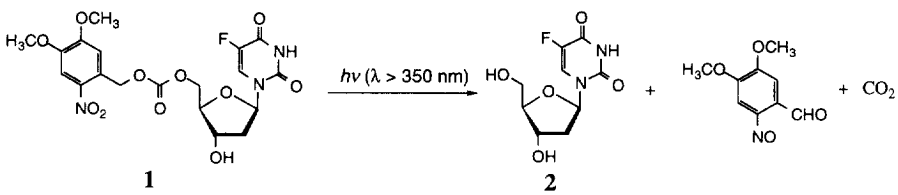
via a reaction not catalyzed by endogenous enzymes and minimize immunogenicity. This approach, however, has to wait for the development of highly efficient catalytic antibodies that can catalyze reactions with no parallels in nature.

We now report a new model system of prodrug activation via photolysis. 5-Fluorodeoxyuridine **2** (5-FdU) and its photolabile carbonate derivative **1** were chosen as the parent drug and prodrug respectively, in this model system (Scheme 1). 5-FdU **2** is chosen because it has been widely used for designing anticancer prodrugs.[15] It is converted in vivo into 5-fluorodeoxyuridine 5'-monophosphate, which acts as a mechanism-based inhibitor of thymidylate synthase.[17] Thus, the carbonate ester **1** should be significantly less toxic than 5-FdU since **1** cannot undergo 5′-phosphorylation. Among the numerous photolabile protecting groups available, we chose the ones derived from 4,5-dimethoxy-2-nitrobenzyl (DMNB) group for three reasons: (1) the DMNB group shows favorable absorption characteristics (λ_{max} = 365, ε = 5,000 M^{-1} cm^{-1}),[18] (2) irradiation by long-wavelength UV (UV-A, $\lambda > 350$ nm) light sources alone was reported to have little effect on normal cells[19] and, (3) the chemistry of the DMNB group has been well studied. It has been used to synthesize a variety of "cage" compounds with carbonate, carbamate, ether, and ester linkages.[20]

Results and Discussion

Scheme 1 shows the structure of prodrug **1** which, upon photolysis, should release the free 5-FdU **2** and 4,5-dimethoxy-2-nitrosobenzaldehyde.

Scheme 1



1 **2**

Photolytic activation of the prodrug **1** was first carried out in vitro by irradiating with long-wavelength UV light (λ > 350 nm) and analyzing the cleavage products by reverse-phased HPLC (Figure 1a). Prior to

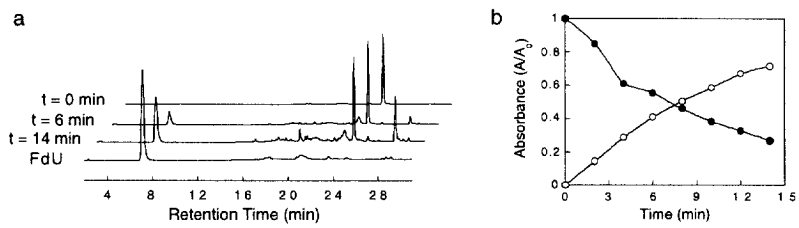


Figure 1. In vitro activation of prodrug **1** by photolysis. (a) HPLC analysis of release of FdU; (b) the kinetics of prodrug disappearance (closed circles) and FdU formation (open circles).

photolysis, prodrug **1** eluted as a single sharp peak with a retention time (R_T) of 24.7 min. Upon UV irradiation, two new peaks appeared at R_T values of 7.1 and 28.4 min, respectively. The species at R_T = 7.1 min is apparently the released 5-FdU, as it co-elutes with an authentic 5-FdU sample. The species at 28.4 min was shown to have a molecular weight of 195 amu by mass spectrometric analysis. Thus, this species is the other

product of the photolytic cleavage reaction, 4,5-dimethoxy-2-nitrosobenzaldehyde. The photolysis reaction displayed first-order kinetics, with $t_{1/2} \sim 6$ min (0.1 mM of prodrug **1** in 20 mM sodium phosphate buffer, pH 7.4) (Figure 1b). No significant cleavage of **1** was detected when incubated in the dark. This result demonstrates the viability of the prodrug approach.

We then used bacterial cells as a model system to test the ability of prodrug **1** to inhibit cell growth. While addition of 100 μM free 5-FdU (**2**) to freshly diluted overnight culture of *Escherichia coli* DH5α cells completely arrested the cell growth, 100 μM **1** resulted in only slight growth inhibition (Figure 2).[21] However, when the cell culture containing 100 μM prodrug **1** was irradiated with a 350 nm lamp for 10 min at 4 °C prior to incubation at 37 °C, the cell growth was almost completely inhibited. Irradiation with 350 nm light alone caused a only a slight delay in growth rate. These data demonstrate that the prodrug (**1**) can indeed be specifically activated by UV light, resulting in specific inhibition of bacterial cell growth.

Compared to previously reported methods of prodrug activation, this approach has the potential of designing prodrugs that are resistant to most of natural enzymes. For example, instead of using the relatively labile carbonate linkage to block the parent drug, the DMNB group can also be coupled to cytotoxic drugs via an ether linkage that is known not to be cleavable by any natural enzymes. The corresponding work is in progress. Combined with the numerous photo-labile protecting groups currently being used in chemistry and biology, the approach described here can convert a wide variety of extremely potent agents, such as nitrogen mustards and palytoxin, into virtually non-toxic prodrugs that can be specifically activated at tumor sites. The photolabile prodrugs, in conjunction with up-to-date technology such as fiber optics that is extensively used for delivering light sources in medicine,[22] may provide a powerful new approach for cancer chemotherapy.

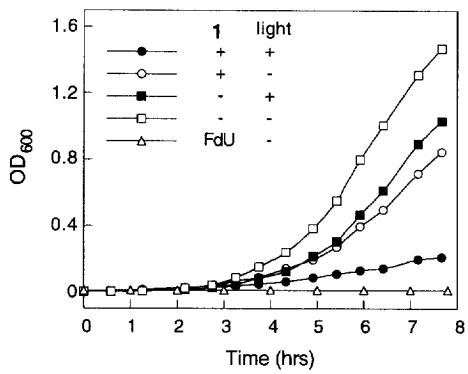


Figure 2. Inhibition of *E. coli* cell growth by prodrug **1**.

Experimental Section

Synthesis of Prodrug 1

Compound **1** was prepared by reacting 5-FdU (**2**) with 4,5-dimethoxy-2-nitrobenzyl chloroformate in pyridine based on a procedure similar to that reported before.[23] The product was purified by column chromatography (silica gel, eluted with ethyl acetate/methanol).

Photolytic Cleavage of 1

An 100 μM solution of 1 in 20 mM sodium phosphate buffer (pH 7.4) in a transparent glass vial was incubated in a photochemical reactor equipped with 16 350 nm lamps (Southern New England Ultraviolet Co.)

at 4 °C. Fifty-μL aliquots were withdrawn at various time points and subjected reverse-phased HPLC (C-18; Solvent A: 0.05% trifluoroacetic acid in ddH_2O and solvent B: 0.05% trifluoroacetic acid in CH_3CN; Gradident: 100% A for 5 min, then 0 to 50% B in 25 min at 1 mL/min). The areas (A) underneath the peaks for **1** and FdU were integrated and the A/A_0 (for **1**) and A/A_∞ (for FdU) values were ploted against time (min). A, area at time t; A0, area of **1** at time 0; A_∞, area of FdU at time when **1** is completely cleaved.

Bacterial Growth Inhibition Assay

An overnight culture of DH5α cells was diluted 1000 fold into fresh LB medium. After addition of 100 μM prodrug **1** (final concentration), the cell culture was irradiated with the 350 nm lamps for 10 min at 4 °C. The cells were then incubated in a 37 °C shaker and cell densities were monitored by following OD at 600 nm.

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