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Modulation of the UVB-induced lethality by furocoumarins in *Staphylococcus aureus*

Emanuelle B.F. Silva [a,*], Ideltônio J.F. Barbosa [a], Humberto M. Barreto [b], José P. Siqueira-Júnior [a]

[a] Departamento de Biologia Molecular, Universidade Federal da Paraíba, João Pessoa, Brazil
[b] Departamento de Ciências da Natureza, Universidade Federal do Piauí, Floriano, Brazil

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ABSTRACT

Furocumarins (FCs) are photoactive compounds capable of binding to DNA, and once excited by UVA light (~365 nm), they form photoadducts which can lead to mutagenicity and lethality. However, the biological effects of FCs combined with UVB light (312 nm) is still little investigated. In the present study, the lethal effect of UVB light alone and combined with different concentrations of 8-methoxypsoralen (8-MOP), 4,5′,8-trimethylpsoralen (TMP) and 3-carbethoxypsoralen (3-CPs) was evaluated in a strain of *Staphylococcus aureus*. 8-MOP-UVB and TMP-UVB were more effective in inducing lethality compared to UVB alone, indicating that these FCs act as photosensitizing agents for UVB. The increase in concentration of 8-MOP resulted in a greater mortality. On the contrary, a decrease in mortality was found with an increase in TMP concentration. 3-CPs protected bacteria against damage induced by UVB, which can be attributed to the inhibition of cyclobutyl pyrimidine dimer formation. The different modulatory effects on lethality induced by UVB shown by the FCs tested could be related to differences in the specificity of each compound for particular nucleotide sequences, as well as other chemical characteristics of each molecule could influence the number and types of adducts formed, contributing to the photosensitizing or photoprotective effects observed.

1. Introduction

Furocoumarins (FCs), an important class of photoactive compounds, are natural or synthetic tricyclic compounds formed by the fusion of a furan ring with a coumarin (1,2-benzopyrone). FCs in the presence of UVA (320–400 nm) cause lethal and other biological effects in a variety of cell systems because of their photoreactivity with DNA and other macromolecules and cell components [1].

The photochemical reactions of FCs with DNA are the most well characterized [2,3]. In the dark, DNA and FCs form an intermolecular complex by the intercalation of FC between DNA base pairs; upon exposure to UVA (~356 nm), they bind covalently to pyrimidine bases at the 5,6 double bond, forming 4′,5′ furan- or 3,4 pyrone-side monoadducts (MA). Regarding bifunctional FC, such as 8-methoxypsoralen (8-MOP) and 4,5′,8-trimethylpsoralen (TMP), a second photochemical reaction, involving a pyrimidine located on the opposite strand in an adjacent base pair, converts furan-side MA to interstrand cross-links (CL). Pyrone-side MA are only converted to biadducts (BA) at a wavelength <330 nm. The monofunctional FC 3-carbethoxypsoralen (3-CP) forms only furan-side MA in view of the blocked 3,4-double bond [4].

The effect of FCs in combination with UVC (~254 nm) has been studied far less. Igali et al. [5] found that treatment with 8-MOP before UVC irradiation decreased the yield of prototrophic mutants in *Escherichia coli*. Bridges [6] and Hass and Webb [7] showed that such pretreatment with 8-MOP protects *E. coli* against the lethal damage of UVC. The same protective effect was demonstrated in *Staphylococcus aureus* not only for 8-MOP but also for TMP, 3-CPs and angelicin [8]. In addition, UVC lethality is increased if 8-MOP is present in the post-irradiation plating medium, due to inhibition of DNA-repair [6,9,10].

Studies of the effect of FCs in combination with UVB (~313 nm) is most limited to 8-MOP and phototherapy issues [11 and references there in], or tacitly treated in the course of an "action spectra" investigation [7,12–15]. To gain more insight into this matter, we were prompted to determine the effect of several FCs in combination with UVB in *S. aureus*.

2. Materials and methods

2.1. Bacterial strain and culture media

Strain ISP 255 (= 112) of *S. aureus*, phenotypically characterized as proficient in DNA repair [16], was used throughout this work. The cells were normally grown in brain heart infusion broth

* Corresponding author. Tel.: +55 83 3216 7436; fax: +55 83 3216 7787.
E-mail address: manubatistapb@gmail.com (E.B.F. Silva).

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(BHI; Difco) without shaking; blood agar base (BAB; Difco) was used for plating.

2.2. Furocoumarins

8-MOP and TMP were purchased from Sigma–Aldrich Co.; 3-CPs was synthesized by Dr. E. Bisagni (Institut Curie, Orsay, France) and kindly supplied by Dr. J.A.P. Henriques (Universidade Federal do Rio Grande do Sul, Brazil). Stock solutions (2.3 mM) of the FCs were prepared in 40% ethanol (8-MOP), 50% ethanol (3-CPs), or absolute ethanol (TMP) and stored in the dark at 4 °C. They were used at the following concentrations: 0.02875, 0.0575, 0.115 and 0.23 mM.

2.3. Treatment with FCs plus UVB light

Bacteria were grown in BHI until they reached stationary phase (18–24 h at 37 °C), and were resuspended in saline at a density of *ca.*10^8 cfu mL^{-1}. FC was added at the proper concentrations, and the suspension incubated in the dark for 20 min at room temperature. The cells were irradiated at room temperature with UVB in an open Petri dish with gentle shaking in a yellow-light environment using two Philips TL20W/12RS lamps emitting chiefly the 312 nm line. The fluence rate was 13.3 W/m^2, monitored with a Vilber Lourmat VLX-3W radiometer (UVB photocell). A bacterial suspension containing no FC was also irradiated. The doses of irradiation were given cumulatively using the same bacterial suspension. After each irradiation, 0.1-mL aliquots were taken, serially diluted in saline solution and spread-plated onto BAB. After 24 h incubation at 37 °C for 24 h, colonies were counted and survival rates calculated (bacterial titer at a irradiation time/bacterial titer at time 0). None of the FCs tested in the dark (at any concentration used for up to 30 min) produced significant cell kill.

3. Results

The survival fraction was plotted against dose (Figs. 1–3). All experiments were carried out in triplicate. The lethal effect caused by the 8-MOP-UVB and TMP-UVB combinations appeared to be dependent on concentration of FC as well as dose of irradiation. Increasing concentrations of 8-MOP caused an increase in bacterial sensitivity to UVB. In contrast, the bacteria showed a greater sensitivity to UVB at a lower concentration of TMP, with a gradual reduction in sensitivity as the concentration of this FC was raised. The results rather indicate that 3-CPs provides protection at all the concentrations tested.

4. Discussion

The results obtained indicated that 8-MOP and TMP act as photosensitizing agents for UVB. Comparing the results for 8-MOP-UVB presented here with those obtained earlier with 8-MOP-UVA [10], a higher lethal effect was observed for the 8-MOP-UVB treatment. In the absence of FC (or some other xenobiotic excitable by UV), UVA and UVB show different effects in cell systems. The primary target of UVB is DNA, whilst most UVA-mediated biological events are oxygen-dependent [17]. Therefore, FCs-UVA and FCs-UVB probably could affect the cell in different ways, but without discarding some mechanism in common for cellular inactivation [18]. FCs can bind reversibly in the dark and form MA and BA on DNA after exposure to radiation with a wavelength greater than 300 nm [7,12,14]. Thus, the photosensitizing effect demonstrated by the combinations 8-MOP-UVB and TMP-UVB can be attributed to the accumulation of MA and BA formed on DNA.

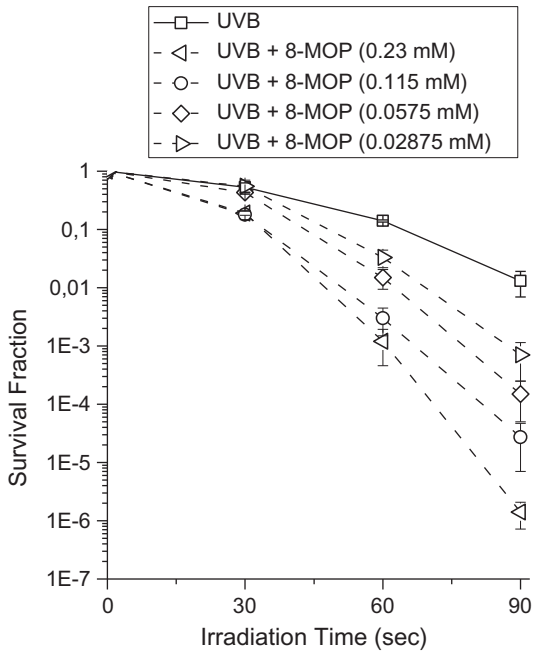

Fig. 1. Survival of *Staphylococcus aureus* ISP 255 after UVB irradiation in the absence and presence of different concentrations of 8-MOP (mM). SEM are indicated.

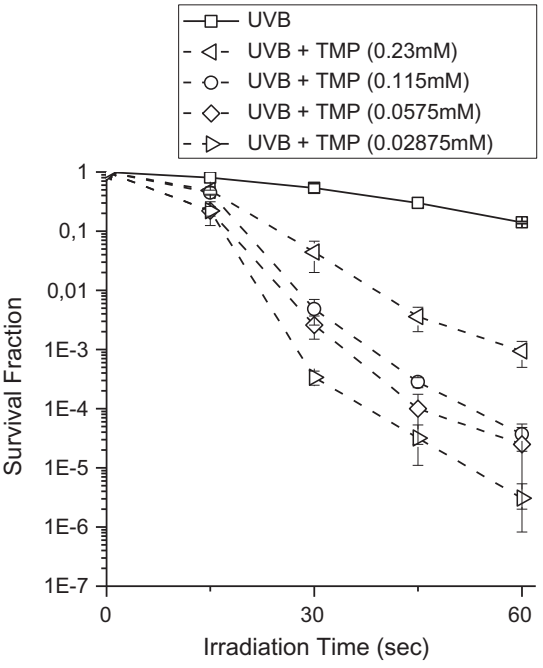

Fig. 2. Survival of *Staphylococcus aureus* ISP 255 after UVB irradiation in the absence and presence of different concentrations of TMP (mM). SEM are indicated.

UVB, in the same way as UVC, causes the formation of cyclobutyl pyrimidine dimers (CPDs) [19,20], whose formation, can be inhibited by intercalated FCs, which can be explained by the concept of energy transfer, resulting in a photoprotective effect [8]. Therefore, it is possible that a survival curve obtained after treatment with 8-MOP-UVB or TMP-UVB is the reflection of two effects elicited by FC: photosensitization, due to the formation of MA and BA and photoprotection, as a consequence of the inhibition of CPDs formation. However, the contrasting results obtained between 8-MOP-UVB and TMP-UVB when the bacterial strain was treated

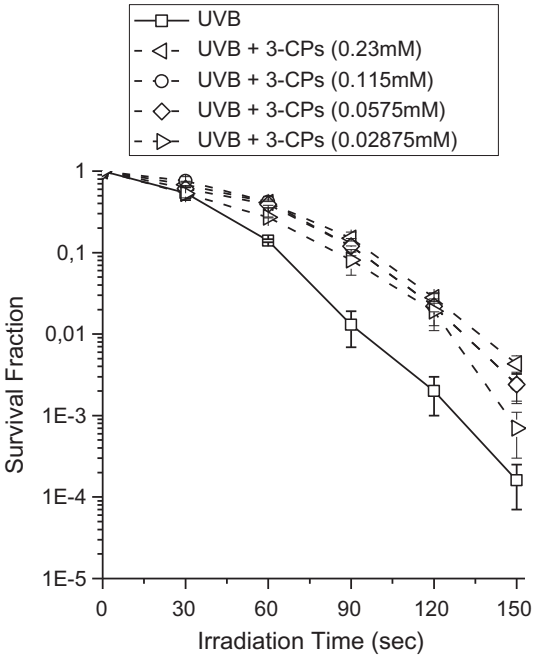

Fig. 3. Survival of *Staphylococcus aureus* ISP 255 after UVB irradiation in the absence and presence of different concentrations of 3-CPs (mM). SEM are indicated.

with different concentrations of each FC could be explained by the differential specificity of intercalation of the two FCs for certain nucleotide sequences.

8-MOP shows a high specificity for $(AT)_n$ sites [21–23], which are prone to undergo a BA formation due to the proximity of thymines on opposite DNA strands [24]. Such sequences are important for cell viability, since they are often located at origin of replication sites, integration sites, recombination sites and gene promoters [21,25]. Therefore, preferential intercalation of 8-MOP in these biologically important sequences may result mainly in BA formation, which together with MA and CPDs contribute to the photosensitizing effect observed. A possible explanation for the greater sensitization of the strain with increase in concentration of 8-MOP could be that the repair of these lesions would be slower and more complex, resulting in higher mortality [26,27].

The increase in TMP concentration resulted in a reduction in sensitivity of the bacteria to UVB. TMP, like other methylated FCs, shows a high reactivity with DNA and a low specificity for strong sites $(AT)_n$, but it is capable of binding to sites containing adjacent or isolated thymines, which are considered weak sites or non-preferential sites for photoreaction of different FCs with DNA [21,28,29]. In this case, it is possible that the reduction in sensitivity with increase in concentration occurred due to a greater availability of molecules for intercalation in DNA, leading to a greater inhibition of CPDs formation. Despite the greater availability of molecules for intercalation, a smaller number of BA will be formed because of the low specificity of TMP for $(AT)_n$ sites [24].

On the other hand, we cannot rule out the possibility of protein binding in the dark affects the bioavailability of a given FC for intercalation into DNA. The association constant of dark complexation with protein is very high for TMP – 15 times higher than for 8-MOP [30]. Indeed, it is noteworthy that TMP protects against UVC only at concentrations $\geqslant$0.23 mM (previous unpublished results).

3-CPs have a low affinity for DNA, binding, almost exclusively to strong sites [21,25]. Given its monofunctional nature, due to the presence of the carbethoxy group on carbon 3, this compound forms only 4′,5′-MA. Dall'acqua et al. [31] suggested that 3,4-MA corresponds to 74–93% of all DNA lesions following psoralen-UVA (365 nm) treatment, whereas 4′,5′-MA and BA lesions are less frequent. The photoprotective effect of this compound may be related to inhibition of CPDs formation at sites important for cell viability, to its incapacity to form BA, to the low number of MA induced and to the efficient repair of remaining MA and CPDs.

These results provide evidence that 8-MOP and TMP act as strong photosensitizing agents for UVB, which is probably related to their capacity to form BA. This indicates that combinations of bifunctional FCs and UVB could be used in photodynamic therapy of skin diseases, such as psoriasis [32]. On the other hand, in virtue of the photoprotective effect exerted by 3-CPs against damage induced by UVB in *S. aureus*, it would be interesting to perform studies utilizing other biological systems, including studies *in vivo* to better evaluate the therapeutic potential of the 3-CPs-UVB combination.

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