Phototoxicity of the tetracyclines: Photosensitized emission of singlet delta dioxygen

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ABSTRACT The spectroscopic observation of 1268-nm emission of singlet oxygen photosensitized by tetracyclines in oxygenated solutions at room temperature is reported. In the series demeclocycline, tetracycline, and minocycline, the efficiency of singlet oxygen generation is found to parallel the clinical observation of relative frequency of phototoxicity of these antibiotics, suggesting singlet oxygen generation as the origin of their phototoxicity.

The tetracyclines are one of the most frequently prescribed groups of antibiotics, deriving their bacteriostatic effect by preventing the binding of the aminoacyl-tRNA to the aminoacyl (A) site of the ribosome thus inhibiting protein synthesis (1). A side effect associated with tetracycline therapy is cutaneous phototoxicity. The relative phototoxicities of the tetracyclines, estimated from clinical reports in the literature, clearly indicate that the chloro derivatives chlortetracycline and demeclocycline are the most potent photosensitizers within the tetracycline family (2-7). The nature of the active species causing this phototoxicity is not established, although activated oxygen species have been postulated (8, 9). In this report we present the first direct evidence of tetracycline-photosensitized generation of singlet delta oxygen in solution, identified by the 1268-nm emission spectrum of O2 (1Δg). We find that the yield of photosensitized singlet oxygen follows the same order as the reported phototoxicity within the tetracycline family, suggesting that the basis for variation of the phototoxic potential within the tetracycline series may lie in their relative efficiency of generating singlet oxygen upon photoexcitation.

Consistent with clinical reports, we have previously shown (9) that chlortetracycline and demeclocycline are also the most effective photosensitizers in vitro, although the detailed mechanism of tetracycline-induced phototoxicity is still not clear. Based mainly on the ability of the tetracyclines to photoxidize the singlet oxygen scavengers dimethyl furan and limonene, Weibe and Moore (10) suggested that tetracycline photosensitization in solution involved photooxygenation rather than a free radical process. However, the specificity of the chemical scavengers used is not high enough to constitute definitive proof of the involvement of singlet oxygen. In cellular systems, based on the observation that in vitro photosensitization was enhanced in ΩH2O as compared to H2O, we (9) and others (8) have evoked singlet oxygen as a possible reactive intermediate in tetracycline-induced phototoxicity. This observation of ΩH2O enhancement of photosensitization, although supportive, is nevertheless indirect evidence of singlet oxygen involvement (11). In fact to our knowledge, prior to this study no direct demonstration of the tetracycline-photoinduced generation of singlet oxygen in solution or in cellular systems has been reported.

Table 1. Structure of tetracyclines

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
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<tbody>
<tr>
<td>Demeclocycline</td>
<td>Cl</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>H</td>
<td>CH3</td>
<td>OH</td>
</tr>
<tr>
<td>Minocycline</td>
<td>N(CH3)2</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Table 1 shows the structure of the three tetracyclines used in this investigation: tetracycline, demeclocycline, and minocycline. Tetracycline hydrochloride, demeclocycline hydrochloride, and minocycline hydrochloride (Sigma) were prepared in oxygen-saturated CCl4 (Mallinckrodt; spectrophotometric grade) by first solubilizing the antibiotics in dimethyl sulfoxide (Me2SO) (Mallinckrodt; analytical reagent), then adding a fixed volume of the Me2SO solution to oxygenated CCl4. The final solution contained 0.6% (vol/vol) Me2SO in CCl4. The solutions containing the tetracyclines were of comparable optical density, 0.070 ± 0.006 (1-cm cells) at 392 nm. The solutions were photoexcited on the long wavelength edge of the absorption bands of the tetracyclines. An excitation band at 392 nm (389-404 nm) was isolated from a 75-W Oriel xenon lamp by a combination of glass and liquid filters (Corning filters CS 7-39, CS 3-75, and 5 cm of 4% (wt/vol) CuSO4 and 4% (wt/wt) CoSO4 in H2O, cf. Fig. 1). The emission spectra were isolated (Corning filter CS7-56) and recorded with an ultrasensitive near-IR spectrometer based on an ADC 403L (Applied Detector, Fresno, CA) liquid nitrogen-cooled germanium detector, Spex Minimate II, f/4.0 monochromator (Spex Industries, Metuchen, NJ) fitted with a 1.25-μm blazed grating, followed by a low noise amplifier PAR model CR4, (E.G.&G. Princeton Applied Research, Princeton, NJ) lock-in amplifier (PAR model HRB), followed by a Spex Datamate with digital storage and printout. The spectrometer is a modified version of the one reported (12).

Quantum yields of photooxygenated singlet oxygen by tetracyclines were estimated relative to the photoquantum yield of singlet oxygen generated by aerated alkaline (10 mM KOH) ethanolic rose bengal at room temperature reported to be 0.75 (13). The areas under the peak of the 1268-nm singlet oxygen emission band photosensitized by demeclocycline in CCl4/Me2SO solvent were compared to the same peak area in the ethanolic rose bengal photosensitization, corrected for singlet oxygen lifetime in the solvent. The estimate of quantum yield
for tetracycline and minocycline was made with respect to the demeclocycline value. The results are presented in Table 2.

**RESULTS**

Electronic absorption spectra of the tetracyclines are characterized by major bands around 365 and 268 nm in solution at room temperature and are presented in Fig. 1 for the three tetracyclines used in this investigation. Fig. 2 is the emission spectral scan between 1200 nm and 1340 nm taken at a rate of 1 nm/sec with wide slits (8 mm x 8 mm) using the near IR spectrometer. Two scans were accumulated, and the background containing solvent emission subtracted, showing the 1268-nm singlet oxygen emission band of (0,0)Δ→3Σg⁻. The three traces shown are for demeclocycline, tetracycline, and minocycline as labeled. The trace for minocycline shows no emission in this spectral region. The estimated relative yields of singlet oxygen are presented in Table 2 and follow the trend demeclocycline > tetracycline > minocycline.

The self-sensitized photodestruction of the tetracyclines also exhibits a molecular and structural dependence—demeclocycline > tetracycline > minocycline—but the absolute rate for this photoreaction is slow. The 6-min exposure to excitation radiation necessary to accumulate each spectrum does not significantly alter the results.

**DISCUSSION**

Electronically excited singlet molecular oxygen is now recognized as a distinct chemical reagent (14-17). The photogeneration of singlet oxygen involves transfer of excitation energy from an excited sensitizer molecule to ground state oxygen (3Σg⁻). The dominant route of this electronic energy transfer is via the triplet state of the photoexcited sensitizer molecule (18-20). In an halogen substituted series of aromatic molecules the photopopulation of the triplet state follows the order H < Cl < Br < I, resulting from mixing of the electronic singlet and triplet states (21, 22). This expectation is consistent with the observed higher efficiency of singlet oxygen generation for demeclocycline compared to tetracycline. Minocycline, which is not phototoxic, did not generate singlet oxygen in our experiments. Minocycline is the only member of the series that has a dimethyl amine substitution on the aromatic ring. Aromatic amines are well known singlet oxygen quenchers (23).

The site of tetracycline localization within the cell is not clearly established. There are reports where tetracyclines have been shown to combine specifically with the mitochondria of living cells both in tissue culture and in fresh preparations from various organs (24). Some evidence exists that this is so in vivo (25) and that most of the intracellular tetracycline in fact resides in the mitochondria (26) of eukaryotic cells. A large proportion of the cellular oxidative processes occur in the mitochondria, and, if the mitochondria are considered potential subcellular targets for tetracycline-

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**Table 2. Singlet oxygen photogeneration by the tetracyclines**

<table>
<thead>
<tr>
<th>Tetracycline</th>
<th>Demeclocycline</th>
<th>Minocycline</th>
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<tbody>
<tr>
<td>%</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Estimated quantum yield of O₂ scavenging photogeneration</td>
<td>0.08</td>
<td>0.05 (6)</td>
</tr>
<tr>
<td>Clinical observation</td>
<td>Most phototoxic</td>
<td>Intermediate phototoxic</td>
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</table>

*Singlet oxygen generation efficiency relative to demeclocycline.
†Singlet oxygen quantum yield relative to alkaline rose bengal (ref. 13).
‡From refs. 2-7.*

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![Fig. 1.](image1.png) **Fig. 1.** Electronic absorption spectra of tetracyclines in 50% (vol/vol) Me₂SO in MeOH between 234 nm and 550 nm at room temperature. For demeclocycline (DMCT), the molar extinction coefficient at 372 nm is 13.88 x 10⁵ liter per mol per cm; for tetracycline (TC), the molar extinction coefficient at 366 nm is 14.15 x 10⁵ liter per mol per cm; for minocycline (MC), the molar extinction coefficient at 350 nm is 11.39 x 10⁵ liter per mol per cm.

![Fig. 2.](image2.png) **Fig. 2.** Near IR emission of singlet oxygen in oxygenated [99.4% CCl₄/0.6% Me₂SO (vol/vol)] solvent at room temperature, photo-sensitized by demeclocycline (DMCT), tetracycline (TC), and minocycline (MC). The intensity is in arbitrary units.
induced phototoxicity, the strong oxygen dependence and possible singlet oxygen involvement are consistent.

In summary we report the first direct demonstration of tetracycline-photosensitized singlet oxygen generation in solution and find a qualitative correlation between the $^{1}O_2$ yield and the phototoxic potential of three tetracyclines. More conclusive information is expected from in vitro and from in vivo experiments.

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