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PHOTOTOXICITY OF TETRACYCLINES AS RELATED TO SINGLET OXYGEN PRODUCTION AND UPTAKE BY POLYMORPHONUCLEAR LEUKOCYTES

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Abstract—The photo-induced singlet oxygen production of six tetracyclines was measured as tryptophan degradation. Demethylchlortetracycline was the most efficient singlet oxygen producer followed by doxycycline. The least efficient producer was minocycline. Doxycycline, however, was the most potent inducer of photodamage to polymorphonuclear leukocytes (PMNLs) followed by demethylchlortetracycline. Accordingly, the singlet oxygen production during irradiation did not correlate with the induction of photodamage to the PMNLs. However, the uptake of doxycycline by the cells was 3 times higher than that of demethylchlortetracycline, and the tetracycline-induced photodamage to the PMNLs correlated with the product of singlet oxygen production during irradiation and the drug uptake by the cells.

Photosensitivity reactions sometimes occur in patients treated with tetracyclines [1, 2]. At the most commonly used doses (serum concentrations 1–10 μg/ml), demethylchlortetracycline seems to be the most phototoxic followed by doxycycline [3, 4]. The reason for the photosensitivity reactions is not known, but production of toxic oxygen radicals may be an important factor [5]. In addition, it has been indicated that polymorphonuclear leukocytes (PMNLs) are involved in the damage [6]. Using PMNLs as indicator cells, we have shown that doxycycline induced photodamage is mediated by singlet oxygen production during irradiation [7, 8]. In contrast to skin damage, however, doxycycline was a more potent inducer of cell damage than demethylchlortetracycline [7]. The reason for this discrepancy is not known but may in part be due to differences in drug uptake by the cells [9].

In the present study, we have examined the singlet oxygen production during irradiation of six tetracyclines and the uptake of the drugs by PMNLs. The results have been related to the impairment of PMNL functions.

MATERIALS AND METHODS

PMNL preparation. Heparinized (18 U/ml) venous blood from healthy volunteers were diluted 1:2 with phosphate buffered saline (PBS). Four ml were layered on the top of a discontinuous density gradient in plastic tubes [10]. After centrifugation at 600 *g* for 30 min at room temperature, the PMNLs were obtained by aspiration of the cell band at the gradient interface. The cells (of which 96–99% were PMNLs) were then washed twice and resuspended in PBS for the measurement of tetracycline uptake.

Leukocyte preparation. Ten parts heparinized (18 U/ml) venous blood mixed with three parts Dextran 70 solution (60 mg/ml, Pharmacia Fine Chemicals, Uppsala, Sweden) were sedimented at room temperature. After centrifugation of the supernatant (500 *g*, 5 min) the remaining red cells were lysed with ammonium chloride solution (0.15 M, pH 7.4) and the leukocytes (of which 60–80% were PMNLs) were washed twice in PBS. Cells to be irradiated were suspended in PBS and preincubated with tetracyclines (10 μg/ml) for 15 min at 37°.

Irradiation. Irradiation was carried out in test tubes (10 mm diameter) with a photochemotherapy unit (PUVA, H. Waldmann, D-722; Schwenningen, Federal Republic of Germany) containing 14 fluorescent tubes (F8T5/BL PUVA; GTE Sylvania Lightening Products Group, Danvers, MA) in a bank. About 70% of the emission energy of this unit is between 340 and 380 nm, and the light fluence was 66 W/m^2 at sample level as measured with an UDT model 80X optometer equipped with a radiometric filter (United Detector Technology Inc. Santa Monica, CA). Irradiated cells were washed once in PBS prior to resuspension for the tube migration test.

Tube migration. Migration of leukocytes in capillary tubes was studied by a method described by Ketchel and Favour [10] and modified by Schreiner and Hopen [12]. Briefly, leukocyte suspensions (5×10^6 cells per ml) in autologous plasma were aspirated halfway into hematocrit capillary tubes which were sealed at the bottom end. The tubes were centrifuged at 2500 *g* for 3 min in a hematocrit centrifuge and mounted in five parallels on microscope slides. After incubation for 2 hr at 37°, the zone of migrating leukocytes on the tube wall was measured with an ocular micrometer. Tube migration was expressed in millimeters (mean value of five tubes for each test condition). No discrepancy in test results was observed using pure PMNL suspensions instead of mixed leukocyte suspensions.

Tryptophan degradation. Singlet oxygen production was measured as photo-induced degradation of tryptophan. Tryptophan (100 μM) suspended in

PBS containing tetracycline was irradiated at 22° for 30 min. The quantum yield was determined at a concentration of 50 μg/ml of the different tetracyclines. During the irradiation, oxygen was bubbled through the solution. The degradation of tryptophan was determined after 60 fold dilution in PBS as the fluorescence decrease at excitation wavelength 280 nm and emission wavelength 355 nm.

Uptake of tetracyclines. PMNLs suspended in PBS (10^7 cells per ml) were incubated with 10 μg tetracycline per ml at 37° for 15 min. The samples were rapidly cooled and centrifuged at 4°. The cells were then washed twice and resuspended in ice cold PBS. Cell associated tetracycline was determined by a chloroform extraction method essentially as described by Wilson *et al.* [13]. Briefly, the tetracycline containing cells and tetracycline standards were suspended in 4 ml barbital- Ca-acetate buffer, pH 9.0. A solution (1.6 ml) consisting of one part Triton X-100 and four parts chloroform was added. After shaking for 15 min, the samples were centrifuged for 10 min at 2500 *g*. The fluorescence intensity at 525 nm was measured with a spectrofluorimeter (Perkin-Elmer LS-5 Luminescence Spectrometer, Buckinghamshire, U.K.).

Chemicals. Doxycycline and oxytetracycline were kindly supplied by the Pfizer Corp., Brüssels, Belgium; minocycline was supplied by Lederle Laboratories, Pearl River, NY; and lymecycline was supplied by Pharmitalia Carlo Erba, Milan, Italy. Demethylchlortetracycline and chlortetracycline were obtained from Sigma Chemical Company, St. Louis, MO. Other chemicals were of the highest commercially available purity.

RESULTS

Demethylchlortetracycline was the most efficient singlet oxygen producer followed by doxycycline and lymecycline (Table 1). The amount of tetracycline accumulated in PMNLs varied from 0.33 μg/10^7 cells for doxycycline to 0.09 μg/10^7 cells for lymecycline. In the presence of 4 mM Mg^{2+} the uptake of doxycycline was markedly reduced (Table 1). Due to the low fluorescence intensity of minocycline the uptake of this drug could not be determined. The product of singlet oxygen production and uptake of the tetracyclines by PMNLs was highest for doxycycline followed by demethylchlortetracycline (Table 1).

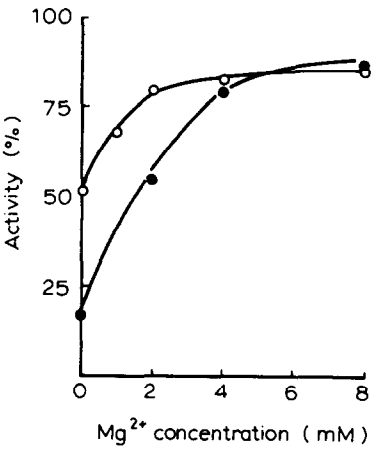

Fig. 1. Tube migration of PMNLs irradiated for 20 min at 4° in the presence of doxycycline (●), and demethylchlortetracycline (○). Effect of increasing concentrations of Mg^{2+} in the cell suspension during irradiation. Results are given as percent activity of non-irradiated cells.

Doxycycline was the most phototoxic drug as measured by the tube migration test (Table 1). Oxytetracycline, lymecycline and minocycline were poor photosensitizers. Doxycycline- and demethylchlortetracycline-induced photodamage to PMNLs was gradually diminished in the presence of increasing concentrations of Mg^{2+} during preincubation and irradiation (Fig. 1). In contrast, the singlet oxygen production remained unchanged when the irradiation was carried out in the presence of Mg^{2+} (Table 1).

DISCUSSION

Patients taking tetracyclines sometimes develop skin photosensitivity [1–4]. These reactions are similar to those experienced in porphyria cutana tarda [14], and the term pseudoporphyria has been adopted [15]. The reason for the tetracycline induced phototoxicity is not known but, as in porphyria cutanea tarda, singlet oxygen has been indicated as a contributing factor [5, 6]. Previously we have studied the mechanism of tetracycline-induced photodamage to tryptophan [7]. After irradiation in the presence of different quenchers, NaN_3 and D_2O, we concluded that singlet oxygen was responsible for

Table 1. Tetracycline-induced singlet oxygen production (ϕ) during irradiation relative to doxycycline, uptake of the drugs by polymorphonuclear leukocytes and polymorphonuclear leukocyte migration after irradiation of the cells for 10 min at 4°

	ϕ	Uptake (μg/10^7 cells)	ϕ × uptake	migration (% of controls ± SD)
Chlortetracycline	0.6	0.22	0.13	79
Demethylchlortetracycline	1.5	0.11	0.17	76
Doxycycline	1.0	0.33	0.33	56
Doxycycline + 4 mM Mg^{2+}	1.0	0.06	0.06	93
Lymecycline	0.9	0.09	0.08	100
Minocycline	0.2	—	—	91
Oxytetracycline	0.8	0.14	0.11	97

The concentration of tetracyclines during the experiments was 50 μg/ml. The results are mean of four experiments.

the damage. In this study, demethylchlortetracycline and doxycycline were the most potent singlet oxygen inducers. In patients with phototoxic skin lesions, these two agents also seem to be the most toxic tetracyclines [3, 4]. The low singlet oxygen production during irradiation of minocycline suspensions is also compatible with the low incidence of photoinduced skin lesions in patients receiving this drug [15]. However, doxycycline was more phototoxic to the PMNLs than demethylchlortetracycline. This is not in agreement with the findings in patients where demethychlortetracycline has been incrimented as the most phototoxic agent followed by doxycycline. According to our findings, therefore, other factors than singlet oxygen production also contribute to the tetracycline induced PMNL photodamage.

In our study, the product of singlet oxygen production during irradiation and the drug accumulation by the PMNLs correlated better with the impaired cell function than the singlet oxygen production alone. The results obtained when Mg^{2+} was present in the suspensions during preincubation and irradiation support the hypothesis that tetracycline induced photodamage to PMNLs also depends upon the drug uptake by the cells. The singlet oxygen production *per se* was not influenced by adding Mg^{2+} to doxycycline suspensions during irradiation but the photodamage to the PMNLs was gradually abolished in the presence of increasing concentrations of this ion which prevents drug uptake by the cells. Since the cell damage is caused by singlet oxygen production by the intracellular drug, changes in environment of the drug may influence the singlet oxygen yield. However, since the product of the tetracycline uptake and the measured singlet oxygen production correlates well with the photodamage, it is likely that the singlet oxygen production of the different dyes relative to doxycycline remains unchanged in the cells. Most likely, the impaired PMNL function due to tetracycline induced photodamage depend on the singlet oxygen production potential of the drug as well as the drug uptake by the cells. The discrepancy between skin and PMNL photodamage induced by tetracyclines may indicate that a cell damage is not responsible for the skin lesions.

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