DNA Strand-Breaking Activities of Quinolone Antimicrobial Agents under Visible Light Irradiation

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The DNA strand-breaking activities of 9 quinolones, widely used as antimicrobial agents and suspected to induce photo-dermatological disorders in humans, were examined under irradiation using standard domestic fluorescent lamps. Two quinolones, tosufloxacin and enoxacin, converted the supercoiled covalently closed circular plasmid DNA to the open circular form under visible light irradiation. The maximum of photodynamic single strand-breaking activity for tosufloxacin was found to be 12% at 50 μ M and that of enoxacin was 33% at a concentration of 2 mM. The DNA strand-breaking activities of photoirradiated tosufloxacin and enoxacin were markedly inhibited by NaN_3 , but only partially by D-mannitol, superoxide dismutase (SOD) and catalase did not inhibit these activities.

These results suggest that some quinolone antimicrobial agents can be activated by visible light and induce phototoxic DNA damage in various organisms. We should carefully investigate such phototoxic activities during the development of new quinolones to avoid producing phototoxic disorders in man.

Keywords phototoxic; new quinolone; DNA strand-breaking; tosufloxacin; enoxacin; Type II

Quinolones are inhibitors of bacterial DNA gyrase and are used clinically as first choice antimicrobial agents for beating general bacterial infectious diseases. However, undesirable side effects such as digestive and central nervous system disorders or phototoxicity have been reported.^{1,2} Nalidixic acid,^{3,4} a prototype fluoroquinolone, enoxacin⁵⁻⁸ and others already on the market have been reported to be phototoxic.⁹⁻¹² The *in vivo* phototoxic activity of quinolones have been investigated by measuring incremental changes in the ear thickness of BALB/c mice.¹³⁻¹⁵ Recently, assay systems to determine the phototoxic activity of chemicals in terms of an induction of DNA strand-breaking have been developed. In previous work¹⁶ we examined the photodynamic DNA strand-breaking activities of 9 drugs which are used clinically and showed that ofloxacin and enoxacin, a quinolone antimicrobial agent, are active photosensitizers. We also compared quantitatively the activities of acridine compounds,¹⁷ which are well known photosensitizers of yeast.

In this communication we have investigated the photodynamic DNA strand-breaking activities of 9 quinolone antimicrobial agents under visible light irradiation.

MATERIALS AND METHODS

Plasmid DNA Plasmid YRp7 DNA was purified from *Escherichia coli* JA221/YRp7 by the method described in a previous report.¹⁶

Determination of the Photodynamic Single-Strand Breaking (SSB) Activity of New Quinolones Ten μ l of plasmid DNA stock solution (415 ng) was mixed with 10 μ l dimethyl sulfoxide (DMSO) containing one of the drugs

(final concentration, 0.1–1 mM) in 1.5 ml Eppendorf tubes. Sample tubes were hung between two 15 W standard domestic fluorescent lamps (Toshiba, mellow white FL 15N), maintained at a distance of 10 cm. These lamps have broad emission spectra from 350–750 nm and the tubes were irradiated for 1 h at 9000 lx (measured using a Topcon IM-3 illuminometer (Tokyo Optical Co.)). The total dose was 425 mJ/cm² (measured with Topcon UVR-1 UV meter). This type of lamp was used to examine the phototoxicity of quinolones under illumination condition produced by standard fluorescent light.

Agarose Gel Electrophoresis Electrophoresis was performed by the method described in a previous report.¹⁶ After migration, the gels were photographed using Polaroid type 667 positive film, using a red filter on a transilluminator (Funakoshi TM-20) or scanned with a Shimadzu chromatoscanner (CS-910) at 600 nm (excitation wavelength at 330 nm). The relative fractions due to the open circular (OC) and closed circular (CC) DNA forms were calculated from the resulting peak areas, as described previously.¹⁶

Chemicals Quinolones were supplied by the pharmaceutical companies, responsible for their manufacture. Fleroxacin and norfloxacin were gifts from Kyorin Pharmaceutical Co., Ltd., enoxacin, pipemidic acid and piromidic acid were from Dainippon, ofloxacin was from Daiichi, lomefloxacin was from Hokuriku Seiyaku, tosufloxacin was from Dainabot Co., Ltd. and ciprofloxacin was from Bayer Yakuhin. Superoxide dismutase (SOD) and catalase (C-100) were purchased from Sigma, D-mannitol and sodium azide was obtained from Nakarai Chemical Co.

All other chemicals were of reagent grade. The chemical structures of quinolones used in this work are shown in

Fig. 1.

RESULTS

Photodynamic DNA SSB Activity of Quinolones

Plasmid YRp7 DNA was irradiated using fluorescent lamps in the presence of 100 μM (final concentration) of different quinolones whose chemical structures are shown in Fig. 1. Samples were subjected to electrophoresis on 0.7% agarose slab gels. Two quinolones, tosylloxacin and enoxacin, induced single strand-breaks in plasmid YRp7 DNA and converted the CC DNA form to the OC form, as shown in Fig. 2. The SSB activities of tosylloxacin and enoxacin were dose-dependent under these experimental conditions. As shown in Fig. 2a, most plasmid DNA in

the sample photoirradiated without drug was in the CC form. Addition of 10 μM tosylloxacin did not induce appreciable OC formation even after photoirradiation. However, 50 μM tosylloxacin produced clear conversion to the OC form while the OC formation (%) decreased somewhat with increasing concentration of tosylloxacin under photoirradiation. A dose-dependent increase in OC (%) and a decrease in CC (%) were observed in the case of photosensitization with enoxacin (Fig. 2b). No induction of OC formation was observed in the sample incubated with 1000 μM enoxacin without photoirradiation.

Dose-Dependent Increase in the OC Form (%) Produced by Photoactivated Tosylloxacin and Enoxacin Following electrophoresis, the gels were scanned with a Shimadzu

Fig. 1. Chemical Structures of Quinolones

Fig. 2. Induction of OC Form by the Photodynamic Action of Tosylloxacin (a) and Enoxacin (b)

Supercoiled plasmid YRp7 DNA photoirradiated with different concentrations of quinolone was subjected to electrophoresis on 0.7% agarose including 0.5 $\mu\text{g}/\text{ml}$ ethidium bromide. After migration, gels were photographed using Polaroid type 667 positive film, using a red filter on a transilluminator. a: DNA was irradiated for 1 h with 0, 10, 20, 50, 100, 200, 500 or 1000 μM tosylloxacin (lanes 1–8) or 500 (lanes 9, 10) or 1000 μM tosylloxacin (lanes 11, 12). Samples of lanes 9–12 were subjected to electrophoresis after butanol extraction. b: DNA was irradiated for 1 h with 0, 10, 20, 50, 100, 200, 500, 1000 or 2000 μM enoxacin (lane 1–9) or left for 1 h in the dark with 200, 500, 1000 or 2000 μM enoxacin (lane 10–13).

Fig. 3. Dose-Dependent Increase in the OC Form (%) Produced by the Photoactivated Tosufloxacin and Enoxacin

Following electrophoresis agarose gels were scanned using a Shimadzu chromatoscanner (CS-910) and the fluorescence of ethidium bromide bound to DNA was excited at 330 nm and measured at 600 nm. OC form (%) was calculated as described in the Materials and Methods. ●, tosufofloxacin; ○, enoxacin.

Fig. 4. Irradiation Time-Dependent Increase in the OC Form (%) Produced by Tosufloxacin and Enoxacin

Plasmid DNA was photoirradiated with tosufofloxacin (●, 0.05 mM; ○, 1.0 mM) or enoxacin (○, 0.1 mM; ●, 1.0 mM) for 30–180 min. a, tosufofloxacin; b, enoxacin.

chromatoscanner (CS-910) and the fluorescent intensity of ethidium bromide bound to DNA was measured at 600 nm using an excitation wavelength of 330 nm. The relative fractions of the OC and CC forms were calculated from the peak areas. As shown in Fig. 3, the maximum induction rate of OC DNA by photoactivated tosufofloxacin was achieved at 50 μM and then decreased somewhat at higher drug concentrations, while that of enoxacin increased with increasing concentration of enoxacin. Mixing 10 μl of the plasmid DNA solution and 10 μl of concentrated tosufofloxacin solution in DMSO give rise to an opaque solution. Therefore, light transmission might be inhibited at high drug concentrations. The ED₅₀ val-

TABLE I. Effects of Active Oxygen Scavengers on the Photodynamic SSB Activities of Quinolones

Scavenger		Inhibition (%) ^{a)}	
		Tosufloxacin	Enoxacin
NaN ₃	50 mM	78.3	97.9
SOD	1000 U/ml	0	2.3
D-Mannitol	100 mM	15.8	25.6
Catalase	1000 U/ml	6.4	5.2

Plasmid DNA was irradiated for 2.5 h with 50 μM tosufofloxacin or for 1 h with 1 mM enoxacin in the presence of active oxygen scavenger. a) Inhibition rates were calculated as described in the Materials and Methods.

ues, doses able to induce 50% of the OC form after 1 h photoirradiation, of enoxacin or tosufofloxacin were not obtained because of the low activity shown by both drugs.

Irradiation Time-Dependent Increase in the OC Form (%) Produced by Photoactivated Tosufloxacin and Enoxacin Plasmid DNA in the CC form was irradiated for various times with 0.05 or 1.0 mM tosufofloxacin (Fig. 4a) and 0.1 or 1.0 mM enoxacin (Fig. 4b). The percentage of the OC form increased in a time-dependent fashion under every set of experimental conditions employed, although the activities at a concentration of 0.05 mM tosufofloxacin were higher than those at 1.0 mM, at all irradiation times.

Effects of Active Oxygen Scavengers on the Photodynamic SSB Activities of Tosufloxacin and Enoxacin The inhibitory effects of different active oxygen scavengers on the photodynamic SSB activities of tosufofloxacin and enoxacin were examined. As shown in Table I, the photodynamic SSB activities of both drugs were markedly inhibited by NaN₃ partially inhibited by D-mannitol and only very weakly inhibited by SOD or catalase.

DISCUSSION

Many different kinds of drugs in clinical use induce photodermatological disorders, such as erythema, edema, eczema and pigmentation, in patients. However, it is often difficult to pinpoint which drug causes these effects particularly in patients simultaneously receiving several agents. For this reason it is necessary to establish a simple screening system to detect drug phototoxicity.

We have reported that some food additive dyes induce cell inactivation of yeast *Saccharomyces cerevisiae*¹⁸⁾ by their photodynamic action while photoactivated acridine compounds induce not only nuclear mutation such as trp⁻ reversion but also mitochondrial mutation ("petite" mutation) in addition to cell inactivation.^{19–22)} Several groups have demonstrated that DNA strand-breaks can be induced *in vitro* by the photodynamic action of certain chemicals, such as thiopyronine,²³⁾ proflavine,^{24,25)} riboflavin,²⁶⁾ bilirubin,²⁷⁾ promazines,^{28–30)} tetracyclines³¹⁾ and non-steroidal antiinflammatory drugs.³²⁾ We have also demonstrated that afloqualone and enoxacin, a quinolone antimicrobial agent, can induce single strand-breaks in plasmid DNA *in vitro* following illumination produced by standard domestic fluorescent lamps.¹⁶⁾ Using this system we compared the phototoxic activity

of 9 quinolones. Only two of these, tosufloxacin and enoxacin, induced photodynamic DNA strand-breaking under the experimental conditions used. Patients given these drugs should not be exposed to sunlight or brought into close proximity to artificial light sources, including standard domestic fluorescent lamps, for long periods.

The photodynamic SSB activities of tosufloxacin and enoxacin were strongly inhibited by NaN_3 , a singlet oxygen scavenger, and partially inhibited by D-mannitol, a hydroxyl radical scavenger. There was hardly any inhibition by SOD or catalase. These results suggest that the use of singlet oxygen scavengers may protect against the phototoxic side-effects of these agents. Hydroxyl radical ($\text{HO}^\bullet$) involvement in SSB induction has been already demonstrated in photosensitization produced by non-steroidal antiinflammatory drugs.¹⁰⁾ Therefore, it is important to determine what kinds of active oxygen species are involved in phototosensitization produced by each agent to prevent any photodermatological disorders caused as a consequence of their use.

This experimental system may be useful for screening phototoxic substances in our environment and for investigating the photosensitization process produced by various agents.

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