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Sparfloxacin phototoxicity: potential photoaugmentation by ultraviolet A and B sources

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Abstract **Sparfloxacin, a quinolone antibacterial agent, frequently elicits photosensitive skin reactions. Our clinical studies of patients treated with sparfloxacin have demonstrated that this photosensitivity is primarily phototoxic and that a marked erythematous response is induced by sequential irradiation with ultraviolet A (UVA) and B (UVB) but not UVA or UVB alone, suggesting potential synergism between UVA and UVB. We evaluated the phototoxicity of this agent using in vitro DNA breaking activity and in vivo murine cutaneous responses. Sparfloxacin induced DNA strand breaks in vitro and converted the supercoiled closed circular form of plasmid DNA to the open circular form by its photodynamic action. In mice, the topical application of sparfloxacin and subsequent irradiation with UVB, but not UVA, induced ear swelling responses. However, the UVB-induced ear swelling response was augmented by irradiation with UVA before or after UVB exposure. Such interaction between UVA and UVB in the production of ear swelling was further confirmed by systemic administration of sparfloxacin. Our study suggests that sparfloxacin is a unique phototoxic agent in that photosensitivity dermatitis is evoked by photoaugmentation between UVA and UVB.**

Key words DNA strand-breaking activity · Photoaugmentation · Phototoxicity · Sparfloxacin

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Introduction

Quinolone antibacterial agents, including nalidixic acid, enoxacin, ofloxacin, ciproxacin, lomefloxacin and norfloxacin, have been reported to induce both phototoxic and photoallergic reactions [1–8]. Sparfloxacin, a new quinolone derivative, has been marketed in Japan since 1993. Since then there has been an extremely high incidence of photosensitivity in patients treated with this drug. In our clinical and phototesting studies [9], sparfloxacin photosensitivity has been characterized as summarised in Table 1. These features suggest that photosensitivity dermatitis evoked by sparfloxacin is a photo-

Table 1 Summary of sparfloxacin photosensitivity in five patients

Typical cutaneous manifestations	Sunburn-like eruption; one patient showed a lichenoid eruption following sunburn-like lesions
Incidence of photosensitivity	High; 5 out of 60 patients treated
Time from oral administration to occurrence of photosensitivity dermatitis	4 to 40 days
Dose dependency of intensity of photosensitivity	Present; a patient treated with 600 mg daily showed more severe photosensitivity dermatitis than those treated with 200 mg daily
Action spectrum	UVA: unsuccessful induction of erythema in 4 out of 5 patients with 4.8–6.0 J/cm^2 at 365 nm UVB: modest reduction of minimal erythemal doses in two patients UVA + UVB: photosynergistic induction of erythema by UVA (1.2–4.8 J/cm^2 at 365 nm) and UVB (45 mJ/cm^2 at 305 nm) in a normal subject treated with sparfloxacin

toxic, but not photoallergic, reaction, although some patients show a prolonged eruption with a lichenoid tissue reaction following sunburn-like lesions.

The action spectrum of sparfloxacin photosensitivity in patients and healthy volunteers is enigmatic. In spite of successful provocation by sunlight, exposure to substantial doses of ultraviolet A (UVA) does not induce erythema and minimal erythemal doses (MED) of ultraviolet B (UVB) are only modestly decreased. In a healthy volunteer (MED of UVB, 90 mJ/cm^2) given the usual dose of sparfloxacin (200 mg daily for 7 days), no erythema was evoked by irradiation with UVA at doses of 1.2–3.6 J/cm^2 and minimally perceptible erythema was observed at 4.8 J/cm^2. Preirradiation with UVA at suberythematogenic doses of 2.4 and 3.6 J/cm^2 markedly augments the response to UVB at a dose of 45 mJ/cm^2, a dose that usually produces barely perceptible erythema [9]. These findings suggest that synergism between UVA and UVB is critical in sparfloxacin photosensitivity.

The present study was conducted to demonstrate the phototoxicity of sparfloxacin using an in vitro method [10] and to clarify its UVA/UVB photointeraction employing an in vivo quantitative system. For this purpose, we tested the ability of sparfloxacin to break plasmid DNA, a known highly sensitive method for detecting phototoxicity, and measured murine ear swelling responses to determine quantitatively its cutaneous phototoxicity in vivo.

Materials and methods

Sparfloxacin

Sparfloxacin (Spara) was a kind gift from Dainippon Pharmaceutical Co., Osaka, Japan. The chemical structure and absorption spectrum are shown in Fig. 1. As monitored with a spectrophotometer (EMC-418; Japan Spectroscopic Co., Tokyo, Japan), sparfloxacin had two absorption peaks at 290 and 365 nm, which corresponds well to the UVB and UVA wavelength ranges, respectively. The former absorbance was greater than the latter.

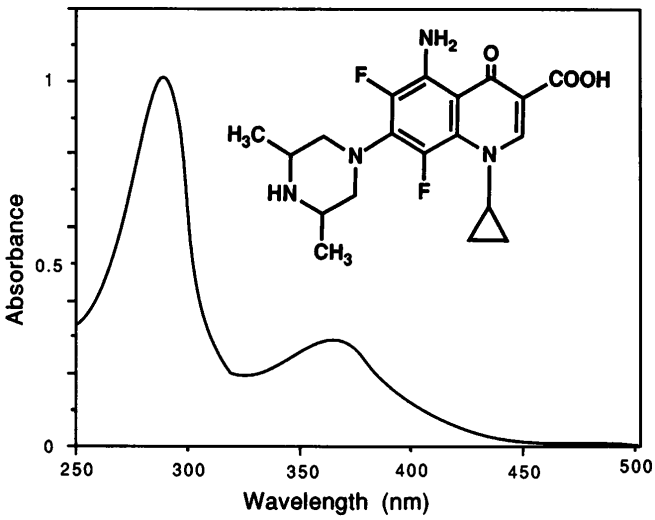

Fig. 1 Chemical structure and absorption spectrum of sparfloxacin. Sparfloxacin was dissolved in phosphate-buffered saline (PBS; pH 7.4) at 50 μ*M*. The spectrum was derived by subtracting the absorbance of PBS

Irradiation of plasmid DNA in the presence of sparfloxacin

Plasmid YRp7 DNA was purified from *Esherichia coli* JA221/YRp7 as reported previously [11]. Crude plasmid DNA was purified by caesium chloride-ethidium bromide equilibrium density gradient ultracentrifugation. The covalently closed circular (CC) form was collected and ethidium bromide was removed by shaking in *n*-butanol. After dialysis against Tris-HCl (10 m*M*)/EDTA (1 m*M*) buffer (TE buffer, pH 8.0), the purified plasmid DNA was stored at 4° C. The method for irradiation of DNA in the presence of a phototoxic agent has been described previously [6, 7, 10]. Briefly, 10 μl of a stock solution of plasmid DNA (415 ng in 80 m*M* TE buffer, pH 8.5) was mixed with 10 μl of a solution of sparfloxacin in DMSO (final concentration, 0.001–1 m*M*) in 1.5 ml Eppendorf tubes. In this 50% DMSO solution, the absorption spectrum was altered only at wavelengths less than 265 nm. The sample tubes were hung between two 15 W common fluorescent lamps (mellow white FL 15N; Toshiba Electric Co., Tokyo, Japan) at a distance of 10 cm: These lamps have a broad emission spectrum from 350 to 750 nm and provided an illuminance of 9400 lux (measured with a Topcon IM-3 illuminometer; Tokyo Optical Co., Tokyo, Japan) [10]. The total dose from a 60-min irradiation, measured with a Topcon UVR-1 UV meter (Tokyo Optical Co.), was 425 mJ/cm^2.

Agarose gel electrophoresis

Electrophoresis was performed with horizontal 0.7% agarose slab gels containing 0.5 μg/ml ethidium bromide in Tris-borate (0.089 *M*)/boric acid (0.089 *M*)/EDTA (0.002 *M*) buffer (TBE buffer, pH 8.3). Photoirradiated DNA solution (20 ml) was mixed with 2 ml gel loading buffer (0.25% bromophenol blue, 40% w/v sucrose in distilled water) and electrophoresed for 3 h at 115 mA (100 V). After migration, the migration patterns were photographed on Polaroid type 667 positive film through a red filter on a transilluminator.

Mice

Male BALB/c mice, 8 to 10 weeks old, obtained from Japan SLC, Hamamatsu, Japan, were maintained in our conventional animal facility. Each group consisted of four or more mice.

Murine cutaneous responses to topical application of sparfloxacin and ultraviolet radiation

Black light lamps (FL20BLB, Toshiba Electric Co.) emitting UVA ranging from 320 to 400 nm with a peak emission at 365 nm and sunlamps (FL20SE, Toshiba Electric Co.) emitting UVB ranging from 280 to 320 nm with a peak emission at 305 nm were used as UVA and UVB sources, respectively. The energy output was measured with a UV radiometer (Eisai Co., Tokyo, Japan).

To assess the in vivo phototoxic effect of sparfloxacin, we used the elicitation procedure of the murine contact photosensitivity system [12], except that the mice were challenged without prior sensitization. Both sides of the earlobes of the mice were painted with 25 μl 0.01–0.3% sparfloxacin (w/v) in absolute ethanol. The mice were irradiated 30 min after application with UVA or UVB by placing the cages containing the mice over the lamps, as previously described [13]. Three black light lamps and sunlamps were used as UVA and UVB sources, respectively. The black light (UVA) was passed through a pane of 3-mm thick glass to ensure that no radiation below 320 nm reached the skin. In some experiments, mice received sequential irradiation with UVA and UVB or vice versa. The daily sparfloxacin painting plus UV irradiation was performed on two consecutive days. Ear thickness was measured

before the application of sparfloxacin and 24 h after each daily irradiation, and was expressed as the mean increment in thickness above the baseline control value.

For histopathology, earlobes taken 24 h after topical treatment were fixed in 10% formaldehyde solution and 4-μm sections were stained with haematoxylin-eosin.

Murine cutaneous responses to systemic administration of sparfloxacin and irradiation with UV

The mice were treated orally with sparfloxacin (100 mg/kg body weight). The mice were placed in the cage 1 h after treatment and irradiated with UVA and/or UVB as mentioned above. Ear thickness was measured before and 24 h after irradiation.

Statistical analysis

Students *t*-test was employed to determine the significance of differences between the means.

Results

Photosensitized DNA single strand-breaking activity of sparfloxacin

Initially, plasmid DNA was irradiated with the 15-W fluorescent lamps in the presence of 1–1000 μ*M* sparfloxacin for 60 min, a time sufficient for detecting DNA strand-breaking activity of phototoxic agents [6, 7, 10]. When tested chemicals have a phototoxic activity, the supercoiled CC form of plasmid DNA is changed into the open circular (OC) form that is electrophoresed differentially from the CC form. As shown in Fig. 2, almost all the plasmid DNA irradiated for 60 min in the absence of sparfloxacin remained in the CC form (lane 1). Irradiation of DNA for 60 min in the presence of 1 μ*M* sparfloxacin brought about partial conversion of the CC form to the OC form (lane 2). At a concentration of 10 μ*M* sparfloxacin completely converted the CC form to the OC form by a photodynamic action (lane 3) and a new band appeared in the high molecular mass area. With sparfloxacin at concentrations higher than 50 μ*M*, this new band disappeared and a band considered to be a double-stranded linear form appeared just below the OC band. However, irradiation in the presence of sparfloxacin at 500 and 1000 μ*M* for 30 or 60 min (lanes 6–8) completely fragmented the plasmid DNA which was observed only as a smear on the gel. On the other hand, the CC form was not converted to the OC form in the presence of 1000 μ*M* sparfloxacin without irradiation. As shown in Fig. 3, irradiation for 15 min was enough to convert the CC form to the OC form in the presence of 500 μ*M* sparfloxacin (lane 4). Further irradiation induced complete fragmention of the plasmid DNA (lanes 5–8).

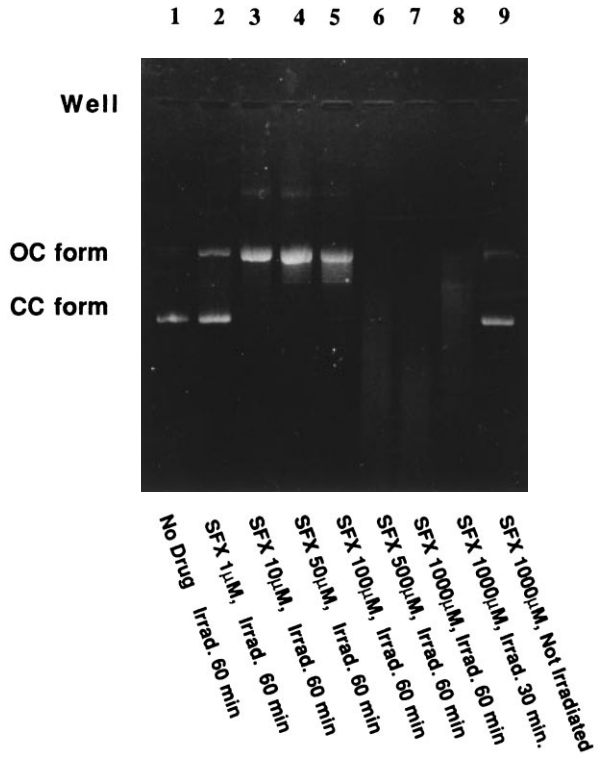

Fig. 2 Induction of DNA single-strand breaking by the photodynamic action of sparfloxacin. Supercoiled plasmid YRp7 DNA was photoirradiated in the absence or presence of various concentrations of sparfloxacin and electrophoresed (*SFX* sparfloxacin)

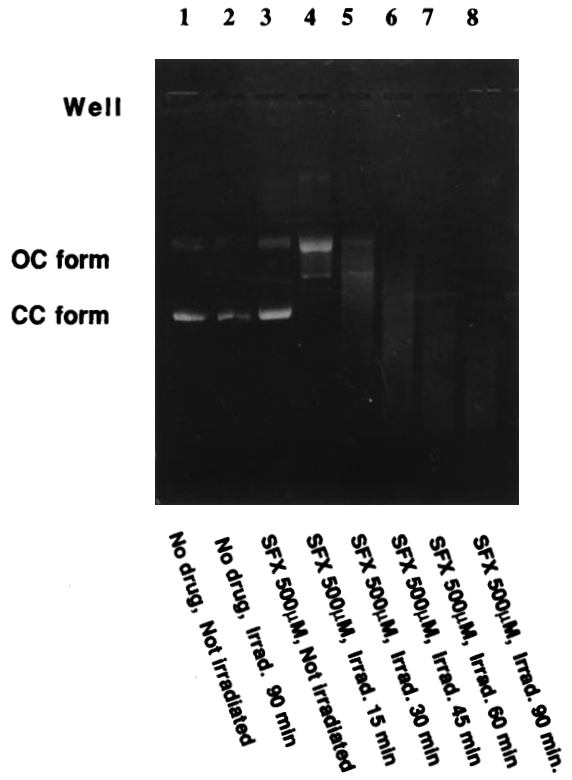

Fig. 3 DNA single strand breaking by 500 μ*M* sparfloxacin with various doses of irradiation (*SFX* sparfloxacin)

Murine cutaneous responses to sparfloxacin plus UV irradiation

The in vivo phototoxic effects of sparfloxacin plus UV were assessed by the primary irritant reaction in the murine earlobe. Mice were painted on the earlobes with 0.01 to 0.3% sparfloxacin in ethanol and irradiated with UVB and/or UVA. As shown in Fig. 4, the application of 0.3 or 0.1% sparfloxacin and irradiation with UVB (peak output, 200 mJ/cm^2 at 305 nm), but not UVA (peak output, 12 J/cm^2 at 365 nm), induced significant ear-swelling responses. When mice were treated with sparfloxacin plus UVB on two consecutive days, the cutaneous response was markedly enhanced compared with the response of those receiving one treatment. The ear-swelling responses to treatment with 0.1% sparfloxacin plus UVB depended on the fluence (Fig. 5). As shown in Fig. 6, sequential irradiation of sparfloxacin-painted earlobes with UVB (150 mJ/cm^2 at 305 nm) and UVA (12 J/cm^2 at 365 nm) or vice versa resulted in a significantly higher response than those irradiated with UVB. The ear-swelling response in the

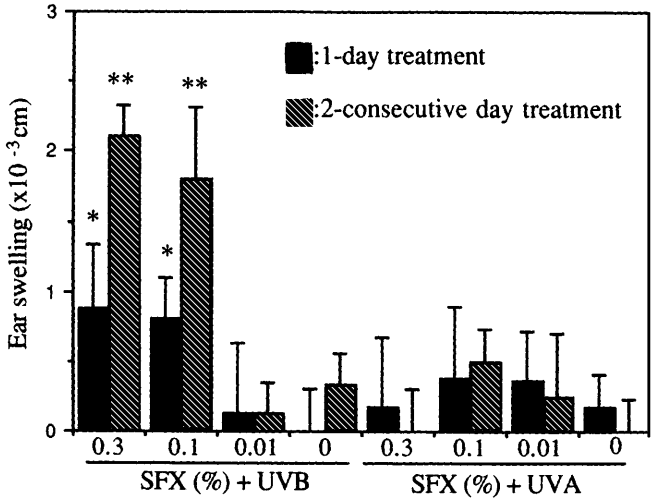

Fig. 4 Cutaneous responses to various concentrations of sparfloxacin plus UVB or UVA. Earlobes of mice were painted with the indicated percentage of sparfloxacin in ethanol and irradiated with UVB (200 mJ/cm^2 at 305 nm) or UVA (12 J/cm^2 at 365 nm). The *bars* represent SD. *P < 0.05, **P < 0.005, compared with the corresponding control group (0% sparfloxacin) (*SFX* sparfloxacin)

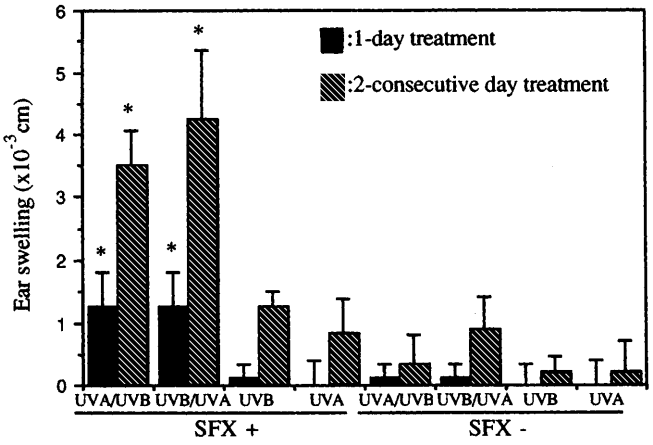

Fig. 6 Cutaneous responses to sparfloxacin plus UVA and/or UVB. The left earlobe of mice was painted with 0.1% sparfloxacin and irradiated with UVA (12 J/cm^2 at 365 nm) and then with UVB (100 mJ/cm^2 at 305 nm) *(UVA/UVB)* or the same process was performed with the order of wavebands reversed *(UVB/UVA)*. The right ear was irradiated with the same dose of UVB and/or UVA without the application of sparfloxacin. The *bars* represent SD. *P < 0.05, compared with the corresponding sparfloxacin + UVB group (*SFX* sparfloxacin)

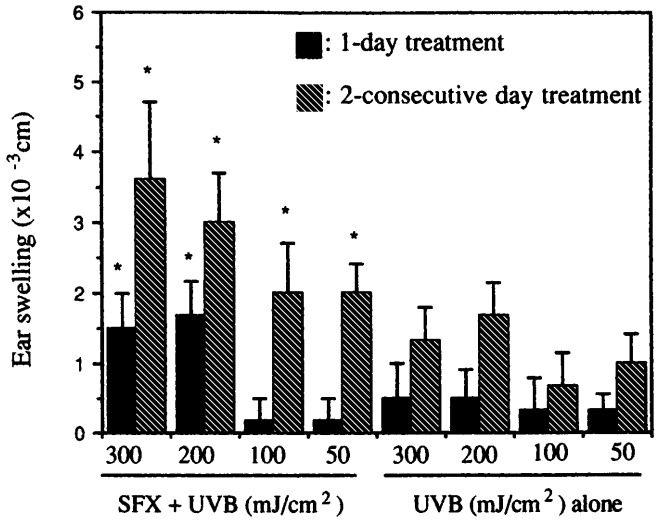

Fig. 5 Cutaneous responses to sparfloxacin plus various doses of UVB. The left earlobe of mice was painted with 0.1% sparfloxacin, whereas the right ear was not painted. Both earlobes were irradiated with the indicated doses of UVB (at 305 nm). The *bars* represent SD. *P < 0.05, compared with the corresponding UVB alone group (*SFX* sparfloxacin)

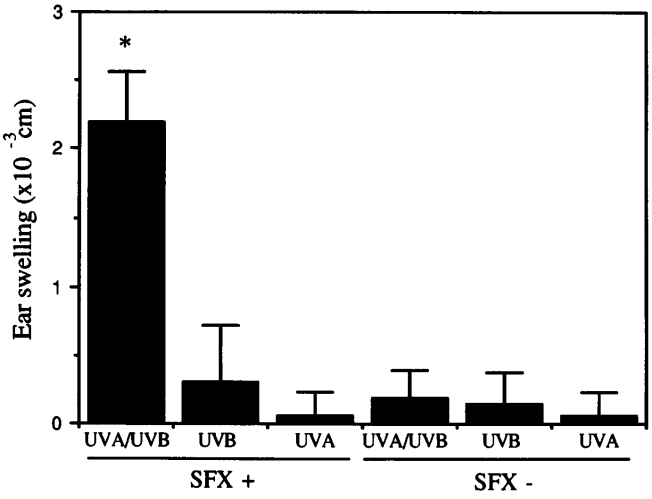

Fig. 7 Cutaneous responses to UVA and/or UVB in mice treated with sparfloxacin. Mice were treated orally with sparfloxacin (100 mg/kg). Both the earlobes were irradiated 1 h after treatment with UVA (12 J/cm^2 at 365 nm) and then with UVB (100 mJ/cm^2 at 305 nm), while control groups of mice were irradiated with UVA or UVB alone. The *bars* represent SD. *P < 0.01, compared with the UVB group (*SFX* sparfloxacin)

groups receiving both UVA and UVB seemed to be photoaugmentative rather than additive, in that the swelling values in these groups exceeded the values expected by addition of the swelling values in the UVA and UVB groups. Such synergism between UVB and UVA in the phototoxic action of sparfloxacin was also observed when mice were treated systemically with sparfloxacin and sequentially irradiated with UVA and UVB at doses based on the local elicitation response (Fig. 7). The photoaugmentative effect of UVA and UVB was more marked in mice treated orally with sparfloxacin than in those treated topically.

Histologically, the dermis of the earlobes that were painted with sparfloxacin and sequentially irradiated with UVB and UVA were more oedematous than those treated with UVA and/or UVB without the application of sparfloxacin. Inflammatory cell infiltration was sparse and there was no significant difference between treatments with UVB plus UVA and UVB alone in the number of mast cells, as assessed by toluidine blue staining.

Discussion

Sparfloxacin induced single strand breaks in DNA and converted the supercoiled CC form of plasmid DNA to the OC form by a photodynamic action. By using this highly sensitive method for evaluation of phototoxicity [10], we have shown phototoxicity of other new quinolone derivatives, including enoxacin [6] and tosufloxacin [7]. Enoxacin at 1 m*M* converts approximately 40% of the CC form to the OC form [6], while almost 100% of the CC form was converted to the OC form by sparfloxacin at concentrations as low as 10 μ*M* at the same radiation dose. Thus, the in vitro phototoxic potential of sparfloxacin is greater than that of enoxacin with regard to DNA breakage. This is in accordance with the clinical finding that the incidence of sparfloxacin photosensitivity is higher than that of other new quinolone derivatives [3–5, 9].

In a subject treated with sparfloxacin, UVB irradiation following suberythemogenic doses of UVA produces a marked erythematous response [9], indicating photosynergism between UVA and UVB in the in vivo action of sparfloxacin. Three possible ways by which UVA and UVB interact to produce a threshold erythema in normal subjects have been reported, including photoaddition, photoaugmentation (more than an additive effect), and photorecovery or photoprotection [14–16]. Since it is difficult to differentiate accurately the suprathreshold gradation of human skin erythema visually, we employed a mouse model in which in vivo cutaneous phototoxicity could be measured quantitatively to further characterize the interaction of UVA and UVB. The UV-induced erythematous change in the earlobes of mice treated with quinolones is a recognized method for evaluating the phototoxic potential of the drugs [8]. In this study, the in vivo phototoxic effect of sparfloxacin on skin was successfully evaluated and quantified by the ear-swelling response in mice which had received the drug topically or systemically. In our system, UVB, but not UVA, induced significant swelling responses. Furthermore, sequential irradiation of UVB and UVA or vice versa evoked greater ear-swelling responses than did UVB alone. The interaction between UVA and UVB in the production of ear swelling does not seem to be simply additive but rather augmentative. Thus, the results obtained from the murine experimental system also suggest photoaugmentation between UVA and UVB in in vivo sparfloxacin phototoxicity. Although several reports have suggested the presence of photoaugmentation between UVA and UVB [15, 16], an extensive study has demonstrated that UVA and UVB are simply photoadditive and there is no evidence of photoaugmentation in producing a threshold erythema on human skin [14]. Sparfloxacin photosensitivity suggests that a photoaugmentation occurs in subjects treated with a certain type of phototoxic agent.

The photoaugmentation was more marked in mice treated systemically than in those treated topically with sparfloxacin. One possible explanation for this difference is that the amount of sparfloxacin is higher in earlobe epidermis of mice painted topically with the drug and, therefore, the phototoxic response evoked by UVA or UVB alone is more inducible in these mice, resulting in a masking of the photoaugmentation of UVA and UVB. Alternatively, metabolite(s) of sparfloxacin may be produced after oral administration but not after topical application and such product(s) might have a stronger photoaugmentative activity than sparfloxacin itself.

Sparfloxacin has absorption peaks at both UVB (290 nm) and UVA (365 nm) wavelengths. In phototoxic responses, the action spectrum is thought to be nearly equal to the absorption spectrum, whereas the action spectrum in photoallergic responses is longer than the absorption spectrum [17]. Active oxygen intermediates, such as singlet oxygen, are involved in the DNA breaks induced by a quinolone plus UV [6]. It is possible that at least two species of oxygen intermediates and/or free radicals are differentially generated by UVA and UVB when sparfloxacin is irradiated. Cooperation of such chemical reactions might cause photoaugmentation in the in vivo cutaneous reaction. Alternatively, metabolite(s) of sparfloxacin might play a role in photoaugmentation. However, since not only systemically administered but also topically applied sparfloxacin induced cutaneous responses upon UVA irradiation, substantial involvement of metabolite(s) in photoaugmentation is unlikely. Further photochemical studies may clarify the photosynergism in this unique agent.

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