Title: Phototoxicity of new quinolones and its mechanisms

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Today's my presentation will consist of two parts, the first is a comparison of phototoxic potentials of quinolones, and the second is mechanism study.

Before explaining experimental data, let me show you incidence of phototoxic reactions among four quinolones on the US market.

The following data are cited from these documents. This table shows incidence of phototoxicity due to each quinolone. These data are summerized from FDA report issued in 1993. As you can see, ciprofloxacin, norfloxacin and ofloxacin induce phototoxic reaction at quite low percentages. Lomefloxacin shows very high percentage in occurrence of phototoxic reactions.

In Japan, almost the same tendency is appearing. That is, Lomefloxacin shows strong phototoxic reactions in patient who are in outside.

In addition we have to notice phototoxicity of enoxacin, sparfloxacin and fleroxacin. Enoxacin is the first quinolone antibacterial agent of which photosensitivity was reported in Japan. Until 1989 more than 15 cases are reported. Japanese dermatologists are unanimous that lomefloxacin is much stronger in photosensitivity than enoxacin. The remaining two are predicted to induce severe photosensitivity.

Today's one of topics, phototoxicity is one of mechanisms for photosensitivity and appears as primary reactions. Major symptoms of it are erythema and edema.

Levofloxacin, DR-3355, is the s-isomer of a racemic compound, ofloxacin and its pharmacokinetic profile is very close to that of ofloxacin. Phototoxicity is induced by interaction of light energy and chemical which can absorb light energy. Therefore, FDA report makes us estimate that incidence of photosensitivity due to levofloxacin is almost the same as that of ofloxacin.

Now, I would like to present our experimental data.

This slide shows an experimental method briefly. Mice are given oral administration of quinolone and then exposed to UVA, long-wave ultraviolet, ranged from 320 to 400 nm.

Ear thickness measurement was performed before and after the irradiation.

Please look at ears of two mice. Left mouse received UVA alone, and right mouse enoxacin plus UVA. UVA irradiation period is 4 hours and total energy is approximately 20 Joule/cm^2.

These irradiation conditions were nearly equivalent to sunbathing for 4 to 5 hours in the mid-summer in Tokyo. You can easily distinguish in auricle color between two.

This photo was taken 48 hours after the completion of the irradiation. I have to show you the effect of enoxacin alone. The result is almost the same as a case of UVA alone, that is, no change.

Then let me show you histological findings.

This specimen is a section of ear treated with UVA alone. Sample is collected 48 hours after the completion of the irradiation. There is No change.

This is a section from ear treated with enoxacin 200 mg/kg plus UVA irradiation. You can see difference between them. We can observe marked edematous changes, vascular ectasia

and considerable infiltration of inflammatory cells, especially neutrophils in the connective tissue surrounding the cartilage.

Then we investigated dose-responsibility of quinolone-induced phototoxicity.

We have two methods for phototoxicity evaluation, one is observation of ear, major phototoxic parameters we used is occurrence of erythema or edema. and the other is measurement of ear thickness.

This slide shows the results of the latter method. We found 24 hours after the irradiation was the best measurement point, since we could obtain linear dose-response curve for each quinolone and we can get 50% phototoxic dose of each quinolone. 50% value of Lomefloxacin is approximately 20 mg/kg, and those of ciprofloxacin, ofloxacin and DR-3355, it means levofloxacin, are 500 to 600 mg/kg.

The potentials from this method almost coincided with those seen on observation of erythema.

These results indicate that lomefloxacin has a very high phototoxic potential and ofloxacin and levofloxacin very low and that enoxacin is a middle class agent among them.

These results reflect well clinical incidences.

I would like to show you our studies on the cause of quinolone-induced phototoxicity and to explain different phototoxic potentials among quinolones.

We investigated pathways of inflammatory reactions induced by quinolones and proposed such mechanism using several scavengers and a quencher. This scheme was referred to a mechanism of intestinal ischemia proposed by Grisham. The key point of this mechanism is participation of xanthine oxidase.

Allopurinol is an inhibitor of xanthine oxidase, and act as suicide substrate. Pretreatment with allopurinol protected Phototoxicity induced by each quinolone. Xanthine oxidase is activated by serine protease, which act on conversion from xanthine dehydrogenase to xanthine oxidase. Pretreatment with protease inhibitor, STI, also protected. Thus, activation of xanthine oxidase is suggested.

To confirm the participation of xanthine oxidase, the effect of superoxid dismutase inhibitor, DDC, was investigated. Pretreatment with DDC showed augmentation of phototoxicity induced by each quinolone.

Superoxide dismutase generates hydrogen peroxide. and hydrogen peroxide and superoxide interact through the Harber-Weiss and Fenton reaction to generate hydroxyl radicals.

Catalase offered protection effect.

Superoxide anion radicals are autodismutated into singlet oxygen. Singlet oxygen quencher, b-carotene also gave protective effect.

From these results we proposed following scheme, that is, generation of reactive oxygens, mitochondria destruction, generation of substrates of xanthine oxidase and activation of xanthine oxidase, generation of superoxide and related toxic oxygen metabolites. These toxic reactive oxygen species cause tissue injury.

Then, a trigger of inflammatory reactions was investigated.

Phototoxic reactions are initiated by the absorption of photoenergy and occur through photochemical reactions. Four pathways are introduced photochemically in the cuase of phototoxicity.

One is generation of toxic photoproducts, 2nd is direct interaction to DNA, 3rd is generation of free radicals such as hydroxyl radicals. This reaction contain fragmentation steps in chemical structure.

The last is generation of singlet oxygen. Photoenergy absorbed is transferred to triplet oxygen which is stable, resulting in generation of highly reactive oxygen molecule, singlet oxygen.

We thought these toxic products including reactive oxygen would be a trigger of inflammatory reactions seen in skin tissues.

We measured difference absorption spectra to predict which possibility is the major.

These spectra are difference spectra of non-irradiated against irradiated. After determining UV absorption spectrum of quinolone solution in a cuvette, this absorption spectrum was then automatically replaced to baseline. After that, the cuvette was irradiated for desired period and difference spectrum was obtained.

Upper is lomefloxacin's difference spectrum irradiated for 10 min. There appeared peak and trough in UVA range. On the other hand, levofloxacin's result is shown below. 10 min's irradiation resulted in very small difference in height from trough to peak compared to that in lomefloxacin.

These data indicate that both of two quinolones are decomposed under UVA irradiation and that lomefloxacin is much more sensitive to UVA.

Then, the effect of photoproducts on ear inflammation was investigated.

UVA irradiated solution of each quinolone or chlorpromazine, CPZ was intradermally injected into auricle, and 24 hour later the alterations of ear thickness was determined. No quinolone-treated mice showed any change at any concentration, though CPZ-treated mice concentration-dependently developed ear swelling. Therefore, a possibility of photoproducts disappeared.

Using 3T3 cells, effects of scavengers on quinolone-induced phototoxicity were investigated. Quinolone concentrations were controlled so that almost the same phototoxic reactions could be appeared.

Addition of SOD, superoxide dismutase, decreased cell survival in each quinolone, but catalase protected it. DMTU, scavenger of hydroxyl radicals also enhanced cell survival, except for enoxacin.

These results suggested that generation of hydrogen peroxide and hydroxyl radicals were occurred during photochemical reactions.

Next levels of superoxide anion radicals, hydrogen peroxide and bleaching of p-nitrodimethylaniline in irradiated quinolone solution were determined.

Bleaching method was intended to determine hydroxyl radicals, but recently its specificity has been suspected. therefore it is better that this value reflect generation of free radicals.

Since superoxide and hydrogen peroxide were detectable in each quinolone solution and a very small amount of iron ions were present, all the values of reactive oxygens measured were considered as apparent levels.

Apparent levels of superoxide are not correlated with phototoxic potentials of these quinolone. On the other hand, hydrogen peroxide levels showed marked differences, reflected phototoxic potentials. Lomefloxacin shows very high level, ofloxacin and levofloxacin very low levels. Furthermore, bleaching levels also reflect phototoxicity in vivo.

Then the sources of toxic oxygens was sought in solvent, water. Dissolved oxygen was monitored during irradiation. Measurement of oxygen comsumption showed all 5 quinolones consumed dissolved oxygen in the course of time.

The chemial structure of major photodegradation products of ofloxacin is identified as that of dialdehyde form of methylpiperazine, and all the quinolones studied share piperazine ring.

These finding strongly suggested that dissolved oxygen was used mostly not only for oxidation of the parent compounds but also for the generation of toxic oxidants.

These results indicate that trigger of phototoxic inflammatory reactions is generation of toxic reactive oxygens and/or free radicals and that phototoxic potentials are dependent on the level of these toxic radicals.

The spread of the tissue damage caused by this phototoxicity probably follows the same inflammatory process as occurs in ischemic disease.

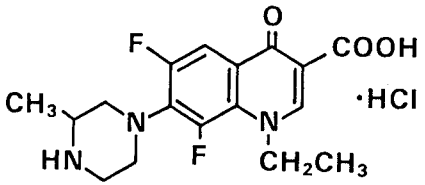
Table 1. Clinical Incidence of Photosensitivity due to New Quinolones.

Quinolone	No. of cases / No. of prescriptions	%	References
Lomefloxacin	182/259,000	0.07027	1), 2)
Ciprofloxacin	29/32,369,000	0.00009	1), 2)
Norfloxacin	19/10,226,000	0.00019	1), 2)
Ofloxacin	13/3,161,000	0.00040	1), 2)

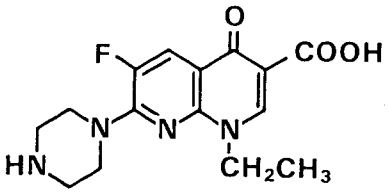
1) Echols RM et al. 18th International Congress of Chemotherapy, No. poster 27, Stockholm, 1993.

2) SCRIP, April 9th/13th, 1993, No 1810/11

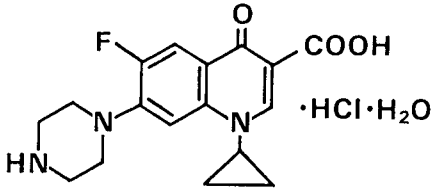
Chemical Structure



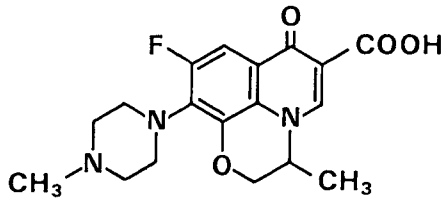
Lomefloxacin(LMFX)



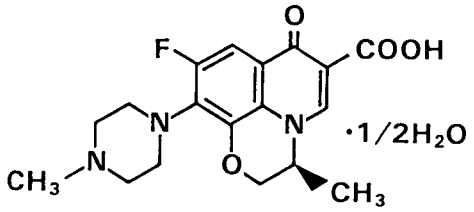
Enoxacin(ENX)



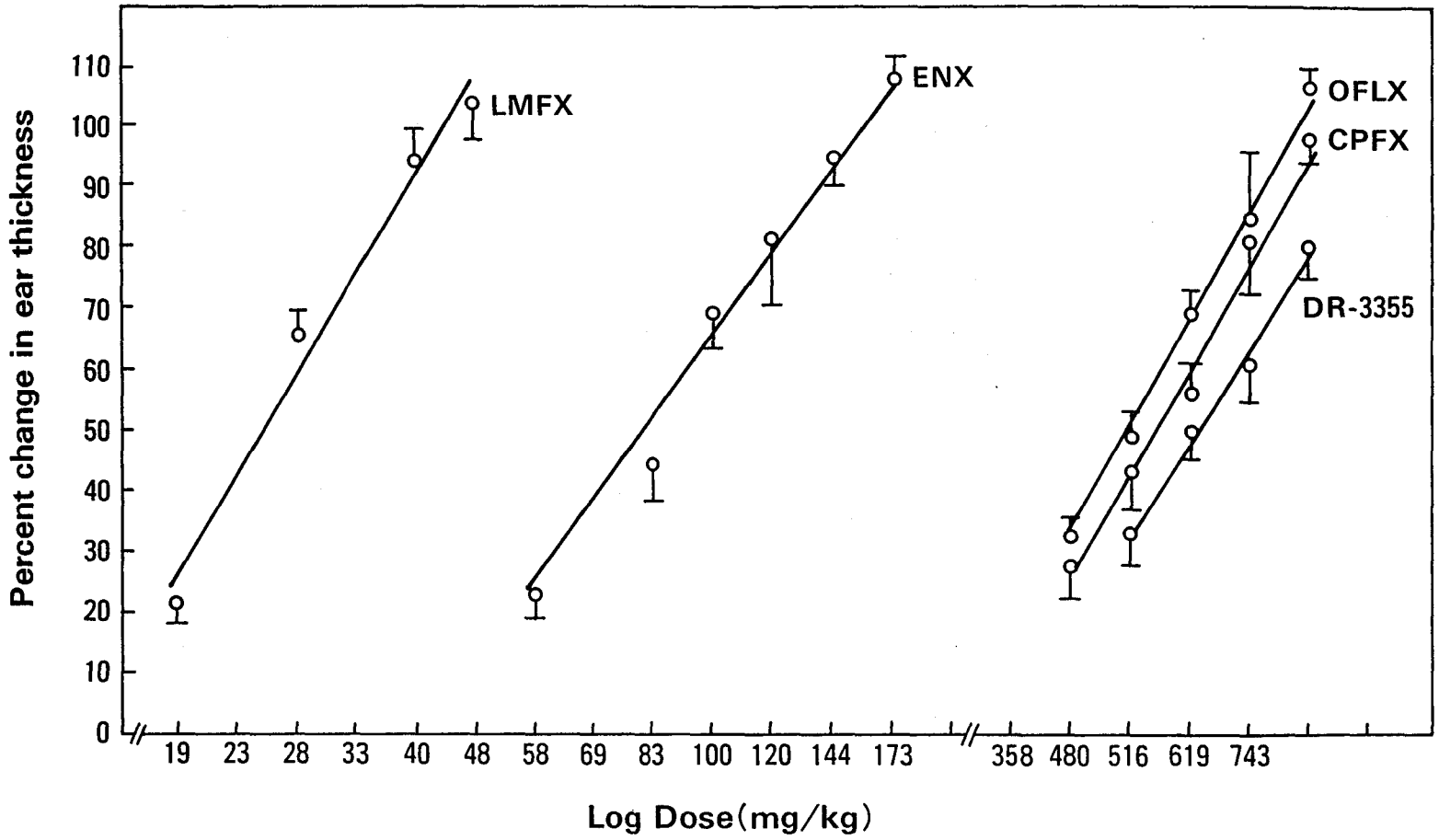
Ciprofloxacin(CPFX)



Ofloxacin(OFLX)



DR-3355


Percent change in ear thickness
110
100
90
80
70
60
50
40
30
20
10
0
LMFX
ENX
OFLX
CPFX
DR-3355
19 23 28 33 40 48 58 69 83 100 120 144 173
358 480 516 619 743
Log Dose(mg/kg)

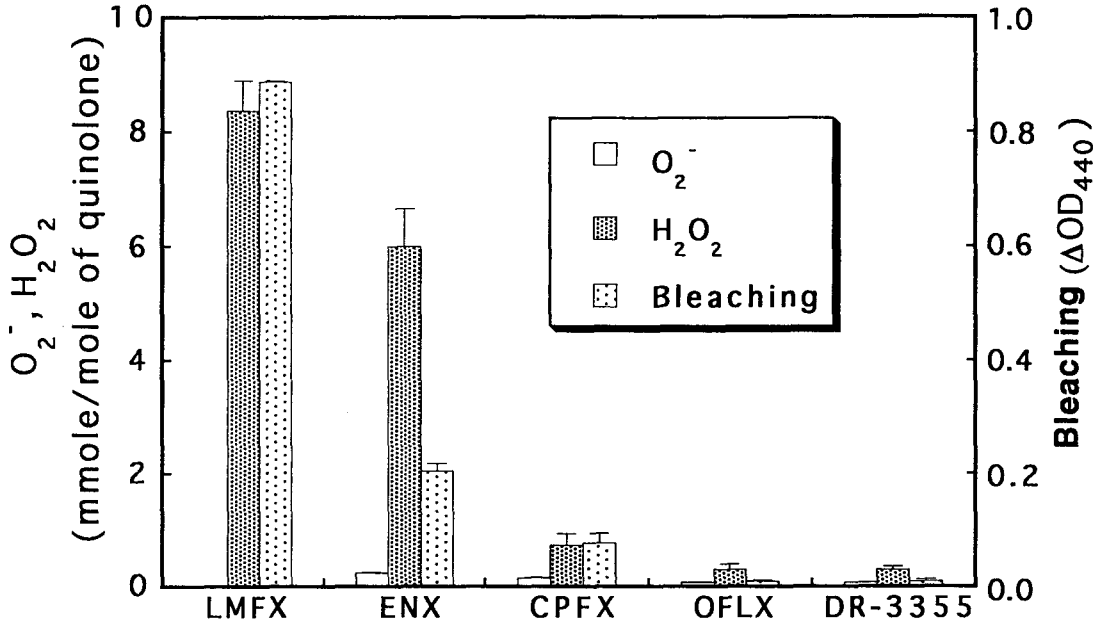


Fig. 20 Apparent levels of reactive oxygens generated and the level of the Bleaching of p-nitrosodimethylaniline during photochemical reactions between quinolones and UVA

Proposed Mechanism for Quinolone Phototoxicity in Skin