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A Quantitative in Vitro Determination of Relative Phototoxic Potential of Quinolone Antibiotics

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Abstract:

Quinolones are known to cause phototoxicity which is not readily predictable. Currently there is no dependable method to quantitate the phototoxic potential of these compounds. We constructed an in vitro model to determine phototoxic potential, using eleven quinolones, murine fibroblast cells (3T3) and UVA light. The quinolones included: naladixic acid (NDX), ciprofloxacin (CPX), fleroxacin (FLX), lomefloxacin (LMX), levofloxacin (LVX), ofloxacin (OFX), amifloxacin (AMX), norfloxacin (NFX), sparfloxacin (SPX), enoxacin (ENX), and Bay y3118. Minocycline (MCN) was used as a non-phototoxic standard. Test cells were exposed to quinolones in varying concentrations, followed by exposure to UV light (5-8 joules/cm^2). Drug and UV controls were treated without quinolone and UV exposure, respectively. Cells were incubated overnight; cellular integrity was measured by neutral red (NR) uptake. Cellular damage induced by test compounds compared with controls was measured, and the drug concentration (μg/ml) which caused a 50% decrease was designated as PTD$_{50}$. The PTD$_{50}$ values (in parentheses) for quinolones studied were as follows: ENX (2.5), NDX (5), BAY (7.5), SPX (20), NFX (25), LMX (30), LVX (35), OFX (40), CPX (40), FLX (45), MCN (50), and AMX (>50). This fibroblast-NR assay system may be a useful tool to estimate the relative phototoxic potential of quinolones.

Key words: Quinolone, Phototoxicity, Quantitation

Introduction:

Many pharmacologic agents, that reach the skin either by local absorption or through the systemic circulation, have been reported to cause a photosensitivity reaction when recipients are exposed to sunlight while taking the compounds. This reaction may lead to many deleterious effects in the skin, including sunburn, premature aging, and cancer (1, 2, 3, 4, 5). Tetracyclines are well known examples of phototoxic compounds (3, 4), and minocycline is known as a non-photosensitizing tetracycline (4). Recently quinolones have been implicated in a similar process (6, 7, 8, 9, 10, 11, 12). In fact, drug-induced phototoxic reactions due to nalidixic acid, a classic prototype quinolone compound, began appearing in the literature soon after its release for human use in the early 1960's (13, 14, 15). Since then, essentially every other quinolone in clinical use has been implicated in similar adverse reactions, and the reported incidence of photosensitivity reactions of quinolones ranges from less than 2.4% for ciprofloxacin (16) to as high as 10-15% for a high dose fleroxacin regimen (16, 17).

Other fluoroquinolones fall in between (18, 19, 20, 21, 22, 23). Chemical photosensitivity reactions, which represent adverse cutaneous reactions in response to simultaneous exposure to certain chemicals and sunlight (including UV and visible light) are commonly divided into two major types based on underlying mechanisms: phototoxicity and photoallergy. Phototoxicity represents an immediate and nonimmunologic reaction mimicking exaggerated sunburn, whereas photoallergy involves the participation of an immune mechanism and is usually a delayed type reaction. The majority of photosensitive reactions caused by the quinolones are predominantly phototoxic in nature, although typical photoallergic features have also been described with certain quinolones (16). Currently there is no dependable method by which one can quantitate the phototoxic potential of drugs in vitro. In this study, a series of eleven quinolones have been studied in an in vitro model for their phototoxic potential. In this model, mouse fibroblasts, UV lamp, and quinolones have been utilized to represent the skin tissue, light source and potential phototoxic

agents, respectively. The degree of metabolic derangements induced by the phototoxic reaction was quantitated by the neutral red (NR) uptake, and the minimum drug concentration to cause a 50% decrease in metabolic integrity was determined and expressed as the phototoxic dose (PTD_{50}).

Materials and Methods:

Quinolones

Eleven fluoroquinolones were obtained from their respective manufacturers or chemical suppliers as follows: Nalidixic acid from Sigma Chemical Co. (St. Louis, MO); enoxacin and sparfloxacin from Rhone-Poulenc Rorer (Collegeville, PA); lomefloxacin from G.D. Searle & Co. (Chicago, IL); ofloxacin and levofloxacin from Hoffman-La Roche Inc. (Nutley, NJ); ciprofloxacin and Bay y 3118 from Miles Pharmaceuticals (West Haven, CT); norfloxacin from Merck Sharp & Dohme Research Laboratories (Rahway, NJ) and amifloxacin from Sterling Winthrop Co. (Collegeville, PA). NR solution was obtained from Sigma Chemical Co. (St. Louis, MO). Antibiotic solutions were prepared according to the recommendations of manufacturers.

Cells:

ATCC CCL 163 Balb/3T3 (Mouse embryo fibroblasts) and ATCC 1475-CRL (normal control human skin) fibroblasts were cultured in 90% Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum and fetal bovine serum, respectively. Cells were cultured at 37°C in 10% CO_2 atmosphere. The cells were allowed to grow to confluence and were harvested by a trypsin digestion method. A homogeneous cell suspension was made in the medium and viable cell counts were made using a Trypan blue stain and hemocytometer. The cell concentration for the phototoxicity experiment was adjusted to be approximately 5 x10^4 cells/ml and an aliquot of 200 µL was used for each of the 96-wells of microtiter plates. This method usually results in 60 to 70% confluence by the next day (24 hours).

Light source and irradiation:

The light source consisted of two Sylvania F20 T12 350BL lamps installed on the inner surface of a light box lid. The light source could be turned on only when the lid was closed. A UVA (350nm, max.) flux was measured with a UVA-400 meter purchased from National Biological Corporation (Twinsburg, OH). The 96-well tissue culture plates, containing the fibroblasts (4 x10^4 cells/ml) and potential phototoxic agents in varying concentrations, were placed in the light box directly under the light source. The distance from the light source to the plates was 15 cm directly under the light source, and the uncovered plates were subjected to UVA irradiation for 40-45 min. to give a total of 6 to 8 joules/cm². The plates were incubated overnight at 37°C in 5% CO_2.

Neutral red (NR) solution preparation

The culture medium was removed by aspiration and was replaced with the NR solution, which was allowed to stand 18 to 24 hours before use to allow for precipitation of undissolved dye. NR solution was filtered through Whatman No.1 filter paper immediately before use. The NR solution was added to DMEM (50 µg/ml).

Measurement of phototoxicity:

The degree of cellular metabolic derangements induced by the exposure to UVA and drugs under investigation were estimated by an NR uptake method previously described (21). Quinolone concentrations ranging from 0 to100 µg/mL were tested in triplicate. The culture medium of the cell culture plates that had been UV irradiated and incubated overnight was aspirated and replaced with NR containing medium (200 µl) followed by further incubation for 3 hours at 37°C in 5% CO_2. At the end of this incubation period, the NR containing medium was aspirated and the wells were washed with an aqueous formol-calcium solution for 1 minute. An acetic acid-ethanol solution (1% acetic acid in 50% ethanol) was then added to extract the dye taken up by the cells and the test plates were gently agitated by hand; the light absorbance was measured at 540 nm using an enzyme-linked immunosorbent assay (ELISA) reader. The degree of relative phototoxicity was quantitated as follows: (NR uptake (test group)/Dark control value) x 100 was the phototoxicity (%); the dark control value was considered 100%, and phototoxicity was expressed as the per cent decrease compared with the dark control. The lowest quinolone concentration to cause 50% decrease in NR uptake was calculated from the drug concentration (µg/ml) - % decrease correlation curves for each compound tested and designated as the PTD_{50} expressed as µg/ml.

Statistical test:

The mean uptake inhibitions produced by each of the quinolone antibiotics were compared, pairwise, using the Tukey statistical procedure, which adjusts for multiple comparisons.

Results:

Three quinolones, BAY, NDX, and ENX caused significantly greater levels of inhibition than did all other quinolone antibiotics (p <0.002 for each pairwise comparison). PTD_{50}S for quinolones studied were as follows: Enoxacin, 2.5; nalidixic acid, 5.9; Bay y 3118, 7.5; sparfloxacin, 20; norfloxacin, 25; lomefloxacin, 30; levofloxacin, 35; ofloxacin, 40; ciprofloxacin, 40; fleroxacin, 45; minocycline, 50; and amifloxacin, >50. These results are presented in Fig. 1. Similar results (data not shown) were obtained when human skin fibroblasts were used. The initial cell concentration (5 x 10^4 cells/mL) was demonstrated to be critical in generating reproducible phototoxicity values.
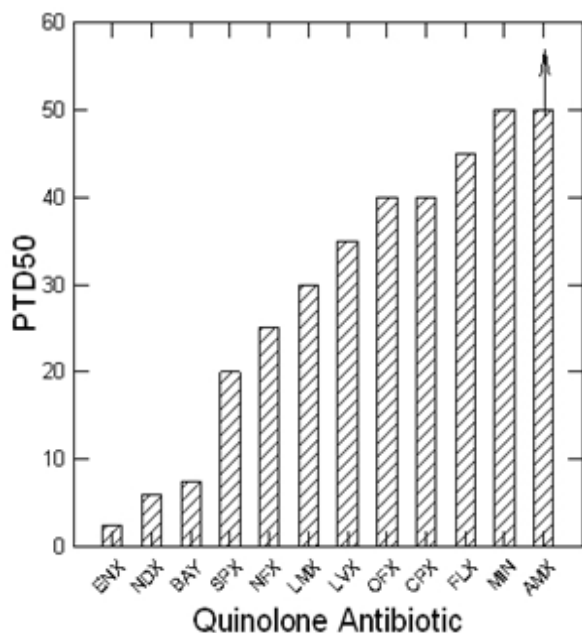
Figure 1. In vitro phototoxic potential of 12 antimicrobial agents expressed as the lowest quinolone concentration to cause a 50% decrease in neutral red solution uptake (PTD_{50}) expressed in µg/ml (see text). Antibiotics were ENX (enoxacin), NDX (nalidixic acid), BAY (Bay y3118), SPX (sparfloxacin), NFX (norfloxacin), LMX (lomefloxacin), LVX (levofloxacin), OFX (ofloxacin), CPX (ciprofloxacin), FLX (fleroxacin), MIN (minocycline), and AMX (amifloxacin). The arrow above the bar for AMX indicates that the PTD_{50} exceeded the highest concentration tested.

Discussion:

Quinolones are currently widely used antibiotics and have been in clinical use for the treatment of various infections including respiratory tract, urinary tract, skin and skin structure, and intraabdominal infections. Nalidixic acid was the first quinolone antibiotic introduced in 1962 (24) and it was reported to be associated with phototoxic reaction of the skin in the next few years (25, 26). Subsequently, in the mid-1980's, new fluoroquinolones with improved antibacterial activity and pharmacokinetic parameters were introduced. All these compounds showed varying degrees of phototoxicity and the incidence rates ranged from less than 2.4% for ciprofloxacin (16) to 10-15% for fleroxacin (16, 17). There have been many reports on incidence of quinolone phototoxicities in humans: temafloxacin, 0.1% (18); lomefloxacin, 2.4% (19); and sparfloxacin, 1.9% (20). Quinolone phototoxicity rates relative to one another from clinical studies approximately conform to the relative PTD_{50} values found in our study with the exception of fleroxacin which reportedly has a high incidence of phototoxicity (10%), but a relatively low in vitro phototoxic potency (10th lowest among 12 quinolones). This may be explained by unique circumstances in the cited clinical study which took place at a single center where patients with sexually transmitted diseases were given high daily doses of fleroxacin. In summary, using a unique in vitro method, we report a procedure for estimating the phototoxic potential of quinolones which correlates with relative photo toxicities found in clinical studies. This technique has promise in the screening of antimicrobial compounds for potential in vivo toxicity.

Transparency declaration

None of the authors has any financial conflicts of interest to declare.

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