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Fluoroquinolones Lower Constitutive H2AX and ATM Phosphorylation in TK6 Lymphoblastoid Cells via Modulation of Intracellular Redox Status

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Abstract

Accumulation of reactive oxygen species (ROS)-induced damage and mutations in genomic DNA is considered the primary etiology of aging and age-related pathologies including cancer. Strategies aimed at slowing these conditions often involve protecting against oxidative DNA damage via modulation of the intracellular redox state. Recently, a biomarker of DNA double-strand breaks (DSBs), *serine*-139-phosphorylated histone H2AX (γ H2AX), and its upstream mediator, activated PI-3-related kinase ATM (ATM^{P1981}), were shown to be constitutively expressed in cells and modulated by antioxidant treatment. Thus, both constitutive histone H2AX phosphorylation (CHP) and constitutive ATM activation (CAA) are thought to reflect a cell's response to endogenous ROS-induced DSBs. In the present study, we investigated the effects of a battery of fluoroquinolone (FQ) compounds, namely Ciprofloxacin, Enrofloxacin, Gatifloxacin, Lomefloxacin and Ofloxacin, on CHP and CAA in human TK6 lymphoblastoid cells. All FQs tested reduced CHP and CAA compared to controls following 6 and 24 h treatment, with CAA being more sensitive to their effects at both time points. In addition, intracellular ROS levels and mitochondrial activities were also lowered in FQ-treated cells at 6 and 24 h. We believe that FQs mediate this effect *via* a combination of ROS-scavenging and mitochondrial suppression, and therefore may protect against the onset or slow the progression of numerous oxidative pathophysiological conditions.

Keywords

Fluoroquinolones; DNA Double-strand breaks; Constitutive histone H2AX phosphorylation; Constitutive activation of ATM; ROS scavenging; Cell cycle

Introduction

In aerobic life, cells are continuously exposed to reactive oxygen species (ROS) formed during normal cell metabolism. These by-products, which are generated by the incomplete reduction of molecular oxygen to water, include superoxide and hydroxyl radicals and hydrogen peroxide, as well as the secondary radicals can 'leak' from the mitochondrial membrane into the intracellular environment [1]. Although the high reactivity of ROS does not permit a wide distribution, DNA is nonetheless a vulnerable biological target for oxidative damage.

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According to one approximation, during a single cell cycle of average duration (24 h), approximately 5000 DNA single-strand lesions (SSLs), e.g. 8-oxoguanine, thymine glycol and apurinic/apyrimidinic sites, per nucleus are generated by endogenous ROS [2]. It is estimated that 1% of these SSLs are converted to DNA double-strand breaks (DSBs) predominately during DNA replication; thus, on average, 50 DSBs per nucleus/day arise as a result of endogenous oxidative stress [2]. If not repaired efficiently, DSBs can induce mutations in DNA and/or cell death via apoptosis [3,4]. Importantly, accumulation of DNA damage and mutations has been proposed as a key factor in the development of pathophysiological conditions such as aging, cell senescence and age-related processes including cancer [5,6].

Phosphorylation of histone H2AX on *serine*-139 (defined as γ H2AX) by activated PI-3-related kinases, e.g. *serine*-1981-phosphorylated ATM ($\text{ATM}^{\text{P1981}}$), is known to occur following the formation of a DSB in nuclear chromatin [7,8]. γ H2AX acts as a molecular anchor, tethering broken DNA ends in close proximity and facilitates the retention of DNA repair factors at the damage site [9,10]. Interestingly, a basal level of γ H2AX and $\text{ATM}^{\text{P1981}}$ can be detected in unstressed cells, termed constitutive histone H2AX phosphorylation (CHP) and constitutive activation of ATM (CAA), respectively, and these are thought to reflect a cell's response to endogenous ROS-induced DSBs [11]. Indeed, several studies have reported that CHP and CAA can be modulated by antioxidants, the glucose anti-metabolite 2-deoxy-D-glucose (2-DG) and growth at different cell densities, all of which affect intracellular redox status [12,13]. Tumor protein p53 status has also been shown to influence the level of CHP in cultured cell lines [14]. The development of monoclonal antibodies specific for γ H2AX and $\text{ATM}^{\text{P1981}}$ has made the detection of these reporters of DNA damage very simple, yet highly sensitive. Furthermore, using immunocytochemistry (ICC) and a multiparameter flow cytometric (MFC) approach allows one to detect and quantify CHP and CAA in a high-throughput and rapid manner, and correlate them with DNA content, i.e. cell cycle phase, and level of apoptosis [15–17].

We recently showed that the fluoroquinolone (FQ) antibiotic ciprofloxacin (CPFX) lowered the level of CHP in a number of lymphoblast-derived cell lines, as well as confirming its specific cancer cell-killing properties (via apoptosis) [18]. Thus, CPFX may hold promise as a chemotherapeutic agent, as well as potentially delaying the onset of aging and protecting against neoplastic preconditioning in normal cells. In the present study, we examined the effects of CPFX and other FQ compounds, enrofloxacin (ENFX), gatifloxacin (GTFX), lomefloxacin (LMFX) and ofloxacin (OFX), on both CHP and CAA in TK6 lymphoblastoid cells. In addition, we assessed intracellular redox status, mitochondrial activity and cell cycle phase distributions as potential mechanisms of CHP and CAA modulation.

Materials and Methods

Cell Culture and Treatment

TK6 (wild-type p53) human lymphoblast cells were cultured in RPMI 1640 medium (GIBCO, OR, USA) supplemented with L-glutamine (2 mM) and fetal bovine serum (10%). Cells were treated with 20 $\mu\text{g}/\text{ml}$ CPFX, GTFX, ENFX, LMFX and OFX (all dissolved in 0.1 N HCl except ENFX, which was dissolved in DMSO; Bayer HealthCare, Germany) (Fig. 1), or vehicle alone for 6 or 24 h. All treatments were carried out at least in duplicate.

Immunofluorescence

Following treatment, cells were fixed in *p*-formaldehyde (1%) for 15 min at 4°C and post-fixed in ice-cold ethanol (70%) for at least 24 h at –20°C. Fixed cells were incubated with BSA (1%) containing either anti- γ H2AX antibody (diluted 1:100; BioLegend, CA, USA) or anti- $\text{ATM}^{\text{P1981}}$ antibody (diluted 1:100; Millipore Corporation, MA, USA) for 1 h at 25°C. Following washing in BSA (1%), cells were incubated with an Alexa Fluor 488 F(ab')₂ (diluted

1:100; Molecular Probes, OR, USA) for 30 min at 25°C. Cells were counterstained with propidium iodide containing RNase (both 10 µg/ml) and stored at 4°C overnight prior to flow cytometric analysis.

Fluorescence Measurement and Quantification

Cellular green (γ H2AX or ATM^{P1981}) and red (nuclear DNA) fluorescence were measured using a FACScan flow cytometer (Becton Dickinson, CA, USA) with the standard emission filters for green (FL1) and red (FL3) fluorescence as described in Halicka *et al* [17]. At least 10⁴ cells were counted per sample. To compare changes in γ H2AX and ATM^{P1981} fluorescence intensity in relation to cell cycle phase, mean values (integral values of individual cells) were calculated in each phase by gating G₁, S and G₂M sub-populations based on different DNA content. γ H2AX and ATM^{P1981} mean fluorescence derived from G₁, S and G₂M phases of FQ-treated cells was expressed as percent of control.

Assessment of Intracellular ROS

Intracellular ROS levels were measured using the fluorescent probe, H₂DCF-DA (Molecular Probes). Briefly, treated cells were washed twice in PBS, resuspended in pre-warmed PBS containing H₂DCF-DA (10 µM) and incubated at 37°C for 30 min. Cells were subsequently washed and resuspended in PBS, and their green (FL1) fluorescence was immediately measured using a FACScan flow cytometer.

Assessment of Mitochondrial Transmembrane Potential

Evaluation of accumulation of rhodamine 123 (Rh123) in TK6 cells during treatment with FQs at a concentration 20 µg/ml for 1 to 24 h has been used according to Darzynkiewicz *et al*. [19]. 1 ml of cell suspension was equilibrated with 1 µg/ml of Rh123 for 30 min in the dark. Cellular green (Rh123) fluorescence was measured using FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) with the standard emission filters. Mean fluorescence in arbitrary units (AU) was evaluated and compared between non treated control cells and cells incubated with FQs for 6h and 24 h

Results

FQ-Mediated Lowering of CHP

In control TK6 cells, CHP was detected in all phases of the cell cycle (Fig. 2A left panel). A representative plot of FQ-mediated lowering of CHP is shown in Fig. 2A (right panel); cells treated with 20 µg/ml CPFQ for 6 h clearly exhibited reduced CHP compared to controls (left panel). Treatment of TK6 cells for 6 h with 20 µg/ml of all FQ compounds tested resulted in the lowering of CHP in all phases of the cell cycle (Ranges: G₁; 7–22%, S; 12–19%, G₂M; 0–22%) (Fig. 2B left panel). Similarly, TK6 cells incubated for a further 18 h in the presence of 20 µg/ml FQs also had a reduced CHP level, however, this was less pronounced than at 6 h (Ranges: G₁; 15–17%, S; 12–17%, G₂M; 2–10%) (Fig. 2B right panel).

FQ-Mediated Lowering of CAA

In control TK6 cells, CAA was detected in all phases of the cell cycle (Fig. 3A left panel). In a similar manner to CHP, treatment with 20 µg/ml CPFQ for 6 h resulted in a lowering of CAA compared to controls but this effect was more pronounced than the effect on CHP (Fig. 3A right panel). Again, 20 µg/ml FQ treatment for 6 h resulted in marked lowering of CAA in all phases of the cell cycle (Ranges: G₁; 11–37%, S; 13–38%, G₂M; 10–34%) (Fig. 3B left panel). After 24h, CAA remained lowered in TK6 cells treated with 20 µg/ml FQ, however, this was less pronounced than at 6 h (Ranges: G₁; 12–22%, S; 14–27%, G₂M; 8–26%) (Fig. 3B right panel).

Effects on Intracellular ROS

Intracellular ROS levels were assessed in TK6 cells treated with 20 µg/ml FQ for 6 and 24 h using the fluorescent probe H₂DCF-DA. All FQ compounds tested markedly reduced (Range: 23–39%) the levels of intracellular ROS compared to controls following 6 h treatment (Fig. 4 left panel). Intracellular ROS levels remained lower (Range: 9–27%) than controls after 24 h treatment, however, this was less pronounced than at 6 h (Fig. 4 right panel).

Effects on Mitochondrial Transmembrane Potential

Mitochondrial transmembrane potential was estimated in TK6 cells exposed to 20 µg/ml FQ for 6 and 24 h using the fluorescent probe Rh123 [19]. All FQ compounds tested lowered (Range: 6–26%) mitochondrial potential compared to controls following 6 h treatment (Fig. 5 left panel). Mitochondrial potential remained reduced in cells treated with CPFX (6%), ENFX (18%) and OFX (3%) for 24 h (Fig. 5 right panel). In contrast, the activity of mitochondria in cells following 24 h treatment with GTFX (107%) and LMFX (102%) was slightly increased (Fig. 5 right panel).

Effects on Cell Cycle

Cell cycle phase distributions were evaluated following 6 and 24 h treatment with 20 µg/ml FQs. Treatment for 6 h with all FQs tested resulted in no change in cell cycle phase distribution compared to controls (no change in relative cell counts was observed either; data not shown). In contrast, 24 h FQ treatment induced a slight G₂M phase cell cycle accumulation (Range: 5–6%) compared to controls, without having detectable effects on G₁ and S phases (Table 1). Importantly, this was reflected in relative cell counts (% control) determined after 24 h treatment (CPFX; 60%, ENFX; 81%, GTFX; 80%, LMFX; 69% and OFX; 70%).

Discussion

Oxygen is an absolute requirement for energy production in eukaryotic cells and low levels of intracellular ROS are known to be essential for normal physiological function [20]. On the other hand, ROS production by endogenous processes, e.g. oxidative metabolism, and the accumulation of oxidative damage is considered the primary etiology of aging and associated pathologies [1,2]. Many strategies aimed at slowing the aging process and cancer prevention focus on protecting against oxidative DNA damage, primarily via the scavenging of endogenous ROS.

It is postulated that CHP reflects ongoing DNA damage caused by ROS produced during cell metabolic activity. Quiescent cells, e.g. peripheral blood lymphocytes (PBLs), that reside in G₀ phase of the cell cycle show minimal levels of CHP, however, it is increased many-fold on stimulation with the polyvalent mitogen phytohaemagglutinin (PHA) [21]. We recently reported that the FQ antibiotic CPFX reduced the level of CHP in various cultured cells, including PHA-stimulated PBLs [18]. The aim of the present study was to investigate further the effects of CPFX and other FQ compounds on CHP in TK6 cells, as well as on one of its upstream mediators, ATM^{P1981} (as CAA). In addition, we sought to elucidate the mechanism underpinning this apparent effect by investigating intracellular redox status as well as aspects of cell metabolic activity and cell cycling.

Compared to control cells, CHP was reduced following treatment with 20 µg/ml CPFX, ENFX, GTFX, LMFX and OFX for both 6 and 24 h. Generally, each FQ compound lowered γH2AX by a similar degree in each phase of the cell cycle, i.e. there was no cell cycle phase-specificity. We next examined the effects on CAA in the same cells; a more pronounced reduction in ATM^{P1981} compared to CHP in all phases of the cell cycle was observed, a phenomenon that has been reported before [22,23]. Activation of ATM, followed by phosphorylation of histone

H2AX is a rapid cellular response to the generation of a DSB in nuclear chromatin [7–10]. Since our studies were conducted with purportedly non-genotoxic FQ concentrations, individual treatments clearly resulted in a decline in the response to endogenous ROS-induced DSBs. This finding suggests that either FQs interfere with the machinery that elicits this DNA damage response, thus leaving many DSBs unidentified by the cell, or, more likely, that they directly modulate the level of damaging intracellular ROS.

Antioxidant treatment (N-acetyl-L-cysteine) has previously been shown to reduce both CHP and CAA in A549, TK6 and normal bronchial epithelial cells [11,22]. Moreover, A549 cells growing for 24 h in the presence of buthionine sulfoximine, a glutathione (GSH)-depleting agent, displayed highly elevated levels (~60%) of CHP in S and G₂M phases, compared to controls [11]. In the present study we utilized the fluorogenic probes, H₂DCF-DA and Rh123, to assess intracellular ROS levels and mitochondrial potential, respectively, in FQ-treated TK6 cells. A similar approach was recently used in conjunction with ICC and MFC to study the effects of 2-DG on CHP and CAA in PHA-stimulated PBLs [13] or TK6 cells with the biscoclaurine alkaloid cepharanthine [23]. Following 6 h FQ exposure, we observed that both ROS- and mitochondrial activity-associated fluorescence detected by each probe was markedly lower in treated cells than controls. Thus, it appears that intracellular status was modulated by FQs *via* an inhibitory effect on mitochondrial activity. However, the discordance between CHP/CAA status which remained lowered compared to controls, and increased mitochondrial potential following 24 h treatment with GTFX and LMFX suggests another mechanism may also play a role. An antioxidant-related effect, i.e. intracellular scavenging of endogenously-generated ROS, is plausible based on the evidence that rebamipide, a quinolone derivative used in gastric ulcer therapy, is a potent ROS scavenger and reported to attenuate levels of oxidative DNA damage in human gastric mucosal cells [24]. It should also be noted that the level of mitochondrial transmembrane potential may not necessarily be correlated with the overall metabolic activity and production of ROS [25]. Interestingly, the cell cycle progression as reflected by the DNA content frequency histograms was only disrupted >6 h treatment, indicating that these redox effects impacted upon cell proliferation rather than cell stasis being responsible for altering the redox balance.

Our findings differ from a number of studies described in the published literature. It is well known that FQs stimulate hydroxyl radical production inside bacteria, ultimately contributing to their death [26]. With a mechanism distinct to its well understood phototoxic effects, CPFX was reported to alter GSH redox status in rat liver and brain tissue, and, furthermore, its metabolism by a hepatic microsomal system resulted in ROS generation [27–30]. CPFX has also been shown to have pro-oxidant effects in primary cultures of rat astrocytes, producing DNA damage that can be partially modulated by vitamin E [31]. However, concentrations used in this *in vitro* study were at least 7.5-fold higher than in our model, and end points, e.g. DNA damage, were measured after both 24 and 48 h treatment. It is possible that such DNA damage was induced as part of an apoptotic or cytotoxic response, as an earlier study reported significant cytotoxicity at ≥50 mg/l [32].

We believe that, at low concentrations, CPFX as well as GTFX, ENFX, LMFX and OFX may attenuate mitochondrial activity as well as function as antioxidants, thus offering protection against the DNA damaging effects of endogenous ROS. CPFX in particular appears to hold promise as an anti-tumor drug as it specifically induces apoptosis in cancer-derived cells, in addition to potentially slowing the aging process, e.g. cell senescence, and protecting against neoplastic preconditioning in normal cells.

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Abbreviations

H₂DCF-DA	2',7'-Carboxyl-dichlorodihydrofluorescein diacetate
CPFX	Ciprofloxacin
CAA	Constitutive activation of ATM
CHP	Constitutive histone H2AX phosphorylation
2-DG	2-Deoxy-D-glucose
DSBs	DNA Double-strand breaks
ENFX	Enrofloxacin
FQs	Fluoroquinolones
GTFX	Gatifloxacin
ICC	Immunocytochemistry
LMFX	Lomefloxacin
MFC	Multiparameter flow cytometry
OFX	Ofloxacin
PBLs	Peripheral blood lymphocytes
PHA	phytohaemagglutinin
γH2AX	Serine-139 phosphorylated H2AX
ATM^{P1981}	Serine-1981-phosphorylated ATM
ROS	

Reactive oxygen species

GSH

Reduced glutathione

SSLs

DNA Single-strand lesions

References

1. Kohen R, Nyska A. Oxidation of biological systems, oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 2002;30:620–50. [PubMed: 12512863]
2. Vilenchik MM, Knudson AG. Endogenous DNA double-strand breaks, production, fidelity of repair, and induction of cancer. *Proc Natl Acad Sci U S A* 2003;100:12871–6. [PubMed: 14566050]
3. Rich T, Allen RL, Wyllie AH. Defying death after DNA damage. *Nature* 2000;407:777–83. [PubMed: 11048728]
4. Lips J, Kaina B. DNA double-strand breaks trigger apoptosis in p53-deficient fibroblasts. *Carcinogenesis* 2001;22:579–85. [PubMed: 11285192]
5. Beckman KB, Ames BN. Oxidative decay of DNA. *J Biol Chem* 1997;272:19633–6. [PubMed: 9289489]
6. Gorbunova V, Seluanov A. Making ends meet in old age, DSB repair and aging. *Mech Ageing Dev* 2005;126:621–8. [PubMed: 15888314]
7. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* 1998;273:5858–68. [PubMed: 9488723]
8. Sedelnikova OA, Pilch DR, Redon C, Bonner WM. Histone H2AX in DNA damage and repair. *Cancer Biol Ther* 2003;2:233–5. [PubMed: 12878854]
9. Rogakou EP, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in DNA double-strand breaks in vivo. *J Cell Biol* 1999;146:905–16. [PubMed: 10477747]
10. Bassing CH, Alt FW. H2AX may function as an anchor to hold broken chromosomal DNA ends in close proximity. *Cell Cycle* 2004;3:149–53. [PubMed: 14712078]
11. Tanaka T, Halicka HD, Huang X, Traganos F, Darzynkiewicz Z. Constitutive histone H2AX phosphorylation and ATM activation, the reporters of DNA damage by endogenous oxidants. *Cell Cycle* 2006;5:1940–5. [PubMed: 16940754]
12. Huang X, Tanaka T, Kurose A, Traganos F, Darzynkiewicz Z. Constitutive histone H2AX phosphorylation on Ser-139 in cells untreated by genotoxic agents is cell-cycle phase specific and attenuated by scavenging reactive oxygen species. *Int J Oncol* 2006;29:495–501. [PubMed: 16820894]
13. Tanaka T, Kurose A, Halicka HD, Traganos F, Darzynkiewicz Z. 2-deoxy-D-glucose reduces the level of constitutive activation of ATM and phosphorylation of histone H2AX. *Cell Cycle* 2006;5:878–82. [PubMed: 16628006]
14. Tanaka T, Kurose A, Huang X, Traganos F, Dai W, Darzynkiewicz Z. Extent of constitutive histone H2AX phosphorylation on Ser-139 varies in cells with different TP53 status. *Cell Prolif* 2006;39:313–23. [PubMed: 16872365]
15. Tanaka T, Kurose A, Huang X, Dai W, Darzynkiewicz Z. ATM activation and histone H2AX phosphorylation as indicators of DNA damage by DNA topoisomerase I inhibitor topotecan and during apoptosis. *Cell Prolif* 2006;39:49–60. [PubMed: 16426422]
16. Kurose A, Tanaka T, Huang X, Halicka HD, Traganos F, Dai W, Darzynkiewicz Z. Assessment of ATM phosphorylation on Ser-1981 induced by DNA topoisomerase I and II inhibitors in relation to Ser-139-histone H2AX phosphorylation, cell cycle phase, and apoptosis. *Cytometry A* 2005;68:1–9. [PubMed: 16184611]
17. Halicka HD, Huang X, Traganos F, King MA, Dai W, Darzynkiewicz Z. Histone H2AX phosphorylation after cell irradiation with UV-B: relationship to cell cycle phase and induction of apoptosis. *Cell Cycle* 2005;4:339–45. [PubMed: 15655354]

18. Smart DJ, Halicka HD, Traganos F, Darzynkiewicz Z, Williams GM. Ciprofloxacin-induced G₂ arrest and apoptosis in TK6 lymphoblastoid cells is not dependent on DNA double-strand break formation. *Cancer Biol Ther* 2008;7:113–119. [PubMed: 18059176]
19. Darzynkiewicz Z, Staiano-Coico L, Melamed MR. Increased mitochondrial uptake of rhodamine 123 during lymphocyte stimulation. *Proc Natl Acad Sci USA* 1981;78:2383–2387. [PubMed: 6941298]
20. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002;82:47–95. [PubMed: 11773609]
21. Tanaka T, Kajstura M, Halicka HD, Traganos F, Darzynkiewicz Z. Constitutive histone H2AX phosphorylation and ATM activation are strongly amplified during mitogenic stimulation of lymphocytes. *Cell Prolif* 2007;40:1–13. [PubMed: 17227291]
22. Zhao H, Tanaka T, Halicka HD, Traganos F, Zarebski M, Dobrucki J, Darzynkiewicz Z. Cytometric assessment of DNA damage by exogenous and endogenous oxidants reports the aging-related processes. *Cytometry A* 2007;71A:905–914. [PubMed: 17879239]
23. Halicka HD, Ita M, Tanaka T, Kurose A, Darzynkiewicz Z. The biscoclaurine alkaloid cepharanthine protects DNA in TK6 lymphoblastoid cells from constitutive oxidative damage. *Pharmacol Rep* 2008;60:93–100. [PubMed: 18276990]
24. Hahm KB, Lee KJ, Kim JH, Cho SW, Chung MH. *Helicobacter pylori* infection, oxidative DNA damage, gastric carcinogenesis, and reversibility by rebamipide. *Dig Dis Sci* 1998;43:72S–7S. [PubMed: 9753230]
25. Darzynkiewicz Z, Traganos F, Staiano-Coico L, Kapuscinski J, Melamed MR. Interactions of rhodamine 123 with living cells studied by flow cytometry. *Cancer Res* 1982;42:799–806. [PubMed: 7059978]
26. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* 2007;130:797–810. [PubMed: 17803904]
27. Becerra MC, Paez PL, Larovere LE, Albesa I. Lipids and DNA oxidation in *Staphylococcus aureus* as a consequence of oxidative stress generated by ciprofloxacin. *Mol Cell Biochem* 2006;285:29–34. [PubMed: 16541200]
28. Schmidt U, Schluter G. Studies on the mechanism of phototoxicity of BAY y 3118 and other quinolones. *Adv Exp Med Biol* 1996;387:117–20. [PubMed: 8794202]
29. Gurbay A, Hincal F. Ciprofloxacin-induced glutathione redox status alterations in rat tissues. *Drug Chem Toxicol* 2004;27:233–42. [PubMed: 15478945]
30. Gurbay A, Gonthier B, Daveloose D, Favier A, Hincal F. Microsomal metabolism of ciprofloxacin generates free radicals. *Free Radic Biol Med* 2001;30:1118–21. [PubMed: 11369501]
31. Gurbay A, Gonthier B, Signorini-Allibe N, Barret L, Favier A, Hincal F. Ciprofloxacin-induced DNA damage in primary culture of rat astrocytes and protection by Vitamin E. *Neurotoxicology* 2006;27:6–10. [PubMed: 16122804]
32. Gurbay A, Garrel C, Osman M, Richard MJ, Favier A, Hincal F. Cytotoxicity in ciprofloxacin-treated human fibroblast cells and protection by vitamin E. *Hum Exp Toxicol* 2002;21:635–41. [PubMed: 12540033]

Figure 1.
Molecular structures of CPFX (A), ENFX (B), GTFX (C), LMFx (D) and OFx (E).

Figure 2.

Effect of FQ treatment (20 μ g/ml) on CHP in TK6 cells. **A.** Representative plot of γ H2AX expression (as CHP) in control cells (left panel). CHP in cells treated for 6 h with CPFX (right panel). A skewed solid line which represents the maximal level of IF for 97% of the control cells is included in each plot for comparing the apparent attenuation of the maximal IF of the treated cells vs control. **B.** CHP level in different phases of cell cycle following 6 h FQ treatment relative to control (left panel). CHP level in different phases of cell cycle after 24 h FQ treatment relative to control (right panel).

Figure 3.

Effect of FQ treatment (20 $\mu\text{g/ml}$) on CAA in TK6 cells. **A.** Representative plot of ATM^{P1981} expression (as CAA) in control cells (left panel). CAA in cells treated for 6 h with CPFX (right panel). A skewed solid line which represents the maximal level of IF for 97% of the control cells is included in each plot for comparing the apparent attenuation of the maximal IF of the treated cells vs control. **B.** CAA level in different phases of cell cycle following 6 h FQ treatment relative to control (left panel). CAA level in different phases of cell cycle after 24 h FQ treatment relative to control (right panel).

Figure 4. Effect of 20 $\mu\text{g/ml}$ FQ treatment on intracellular ROS levels after 6 h (left panel) and 24 h (right panel). Data expressed as percent of controls ($100\% \pm \text{SEM}$).

Figure 5.
Effect of 20 $\mu\text{g/ml}$ FQ treatment on mitochondrial activity after 6 h (left panel) and 24 h (right panel). Representative data expressed as percent of controls (100%).

Table 1
Effect of 20 µg/ml FQ treatment on cell cycle phase distribution in TK6 cells after 24 h. Data expressed as percent of cells ± SEM.

	CTRL	CPFX	ENFX	GTFX	LMFX	OFX
G ₁	36±1	34±0.5	33±1	33±1	34±2	31±2
S	53±3	50±0.5	50±0.5	51±0.5	50±0.5	52±1
G ₂ M	11±4	16±0.5	17±0.5	16±0.5	16±1	17±1