ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1990, p. 8–12
0066-4804/90/010008-05$02.00/0

Vol. 34, No. 1

Inhibitory Effects of Quinolone Antibacterial Agents on Eucaryotic Topoisomerases and Related Test Systems

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INTRODUCTION

Two earlier reviews discussed the biochemical characteristics and physiological importance of topoisomerases (3, 46). Details of various laboratory assays measuring the effects of quinolones on both procaryotic and eucaryotic topoisomerases were also reviewed. Biochemical and genetic studies have linked the antibacterial effect of quinolones to inhibition of the A subunit of DNA gyrase (46). Much of the knowledge that has been gained about bacterial gyrase as a target for quinolones has been used in attempts to discover novel agents with improved antibacterial activity (10, 15, 19, 28, 29, 43). Assays measuring the DNA supercoiling and cleavage activity of bacterial gyrase in the presence of quinolones have been used to find quinolones with greater inhibitory activity against this enzyme (10). Although it is generally accepted that quinolone antibacterial agents are selective for DNA gyrase, there are relatively few reports investigating the potential effects of these agents against enzymes involved with eucaryotic DNA replication and those maintaining DNA topology in cells. In addition, there are reports of in vitro cytogenetic abnormalities associated with some quinolones as well as inhibitory effects on DNA replication in lymphocytes (21, 24). The purpose of this review is to analyze some of the reported effects of quinolones in these test systems and to examine the evidence for potential quinolone involvement with eucaryotic topoisomerases.

REPORTED EFFECTS OF QUINOLONES ON EUCARYOTIC TOPOISOMERASES AND DNA POLYMERASE α

Although the current quinolones are not considered to be potent inhibitors of eucaryotic topoisomerases, some effects on these and other enzymes involved with DNA replication have been observed (7, 37, 42). The laboratory assay methods used to measure the activity of topoisomerases have been reviewed elsewhere (3). Miller et al. found that nalidixic and oxolinic acids had 50% inhibitory concentrations (IC_{50}s) of 500 and 100 μg/ml, respectively, for topoisomerase II isolated from Hela cell nuclei in a decatenation activity assay (37). A similar degree of inhibition was found against topoisomerase II isolated from calf thymus nuclei by Hussy et al. (27). These investigators determined IC_{50}s in the catenation reaction for ciprofloxacin, norfloxacin, ofloxacin, and nalidixic acid of 150, 300, 1,300, and 1,000 μg/ml, respectively (27). Such studies have also indicated that there is no correlation between the potency of quinolone inhibition of bacterial DNA gyrase and their relative inhibition of eucaryotic topoisomerase II (27). Using an assay for measuring the relaxation activity of topoisomerase II isolated from *Drosophila melanogaster* nuclei, Osheroff et al. found K_is for nalidixic and oxolinic acids of 625 and 340 μg/ml, respectively (41). These relatively high drug levels required for inhibition may explain the failure of previous studies to demonstrate inhibition of *D. melanogaster* and rat liver topoisomerase II with low levels of nalidixic acid analogs (16, 26, 27, 41). In contrast, coumermycin A1 and novobiocin, two DNA gyrase B-subunit inhibitors that act at the ATP-binding site of DNA gyrase, were found to be 10- to 100-fold more potent inhibitors of eucaryotic topoisomerase II relaxation activity in vitro than were quinolones (41). This result is consistent with the observation that the sequence homology at the ATP-binding sites between eucaryotic and procaryotic type II topoisomerases is greater than that observed in the corresponding consensus cleavage sites of the enzymes, where quinolones exert their effects against DNA gyrase (46, 49).

A limited number of studies that evaluate the interactions of quinolones with eucaryotic topoisomerase I are available. Quinolones have been shown to be less inhibitory for *Escherichia coli* topoisomerase I than for DNA gyrase. Tabary et al. (47) found that the IC_{50}s of pefloxacin, ciprofloxacin, norfloxacin, and ofloxacin against procaryotic topoisomerase I ranged between 35 and 50 μg/ml in the relaxation assay. This level of relaxation inhibition was approximately 10-fold lower than what was observed against DNA gyrase supercoiling activity (47). It is not surprising, therefore, that little inhibition of eucaryotic topoisomerase I has been observed with quinolones (47). In one study (27), nalidixic acid and ofloxacin were shown to have no inhibitory activity against calf thymus nuclear topoisomerase I relaxation activity at concentrations of up to 1,000 μg/ml. Norfloxacin and ciprofloxacin exhibited limited inhibition in these tests, with IC_{50}s between 300 and 400 μg/ml (27).

Other enzymes involved in DNA replication are somewhat inhibited by nalidixic acid analogs. Nalidixic acid and 4-quinolones have been shown to alter the chain length distribution of replication products synthesized by eucaryotic DNA polymerase α (13, 42). Yeast leucyl- and glycyltransfer RNA synthetases are also inhibited by high concentrations of nalidixic and oxolinic acids (51). All of these inhibitory activities were only detected at drug levels 100- to 1,000-fold higher than that required to inhibit bacterial DNA gyrase (13, 16, 37, 41, 51). Hussy et al. (27) examined the effects of the newer 4-quinolones on enzymes involved in eucaryotic DNA replication. When activated DNA was used as a template primer, DNA synthesis by calf thymus 9S DNA polymerase α primase complex was reversibly inhibited at concentrations above 100 μg/ml (27). At a concentration of 1,000 μg/ml, the activity of DNA polymerase α was reduced 20% by ofloxacin, 60% by nalidixic acid, and greater than 80% by ciprofloxacin (27). In contrast, the 4-quinolones

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tested at these concentrations did not increase the error rate of the DNA polymerase α primase complex (27).

The available in vitro data indicate that of the quinolones tested so far, none appear to be potent inhibitors of eucaryotic topoisomerases or DNA polymerase α. The selectivity of quinolones for bacterial DNA gyrase is substantiated by the fact that the drug levels required for inhibition of eucaryotic topoisomerases are 100 to 10,000 times higher than those required for inhibition of bacterial DNA gyrase (41). It should be noted, however, that the majority of in vitro results with the eucaryotic enzyme report drug concentrations required to inhibit 50% of topoisomerase activity in a given laboratory assay. It is not known what level of topoisomerase inhibition may be relevant in vivo. In this sense, levels of quinolones significantly below the calculated IC_{50} have been shown to produce some inhibition of eucaryotic topoisomerases (27, 47).

ABNORMAL CELLULAR RESPONSES ASSOCIATED WITH QUINOLONES IN VITRO

Despite the reported in vitro selectivity of quinolones for bacterial DNA gyrase, a number of abnormal eucaryotic cellular responses have been observed with these compounds. Oomori et al. (40) found a good correlation between the cytotoxic effects of quinolones in vitro against HeLa cells and their ability to inhibit the relaxation activity of topoisomerase II purified from these cells. Other effects of quinolones have been manifested as decreased growth rates observed in mitogen-stimulated lymphocytes (21, 27). For example, ofloxacin at 10 μg/ml delayed the onset of growth of cultured lymphoblasts by 1 to 2 days, while ciprofloxacin at 100 μg/ml was found to inhibit cell growth completely (27). Forsgren et al. (21) described a marked stimulation of [^{3}H]thymidine incorporation into T lymphocytes incubated with phytohemagglutinin and a quinolone. Stimulation of [^{3}H]thymidine uptake was significantly above the response obtained with phytohemagglutinin treatment alone and occurred with ciprofloxacin, norfloxacin, ofloxacin, amifloxacin, enoxacin, and pefloxacin at physiological concentrations between 1.56 and 6.25 μg/ml (21). Nalidixic acid and cinoxacin exhibited no effect in these studies at levels of up to 25 μg/ml (21). Ciprofloxacin at 20 μg/ml was shown to inhibit the progression of mitogen-stimulated lymphocytes through the S and G_2/M stages of the cell cycle and to decrease the secretion of immunoglobulins G and M in pokeweed mitogen-stimulated B cells (21). The stimulation of [^{3}H]thymidine uptake in human lymphocytes by quinolones suggests an increase in DNA synthesis or inhibition of de novo nucleotide biosynthesis. However, the lack of progression of cells incubated with ciprofloxacin through the S phase in cell cycle analysis indicated that DNA synthesis was being inhibited at the drug concentrations tested (21). Subsequent studies indicated that ciprofloxacin and other quinolones do not directly inhibit pyrimidine nucleotide biosynthesis in peripheral blood lymphocytes or deplete pyrimidine nucleotide pools in cells (6). The mechanisms responsible for the stimulation of [^{3}H]thymidine uptake in lymphocytes incubated with quinolones and the cell cycle inhibition effects are not understood at this time (6, 20, 21).

Several other in vitro and in vivo tests have been used to identify potential genotoxic (DNA-damaging) effects of quinolones in preclinical studies. Since quinolones inhibit bacterial DNA gyrase and a related topoisomerase is found in eucaryotes, negative genotoxicity test results provide a level of confidence that the experimental quinolone will not inhibit DNA metabolism in vivo. Some genotoxicity tests are designed to identify mutagenic activity in specific genes or more general chromosomal changes. Others are designed to detect drug-associated DNA strand breakage and inhibition of DNA replication. The most frequently cited mutagenicity tests include the in vitro Ames test, Chinese hamster V79 cell test, mouse lymphoma cell test, plasmid shuttle vector assays, and the in vivo micronucleus and dominant lethal tests in mice (1, 11, 12, 18, 32, 34, 50). Additional tests monitoring DNA strand breakage include the alkaline elution procedure (30) and the unscheduled DNA synthesis (UDS) test, the latter of which is run with quinolones incubated in rat hepatocyte cell cultures and also with rat hepatocytes removed from animals dosed with the test quinolone and analyzed directly (38). A number of quinolones are inhibitory in some of these in vitro genotoxicity tests, although positive in vivo tests are rare (Table 1). It was reported that ciprofloxacin, norfloxacin, ofloxacin, and pefloxacin were mutagenic in the mouse lymphoma assay and also induced DNA damage in the in vitro rat hepatocyte UDS assay (44, 45). These quinolones, however, did not show abnormal effects in either the Ames or the V79 test for gene mutagenicity or in the micronucleus and dominant lethal tests in mice (44, 45). The activity of ciprofloxacin in the UDS assay

TABLE 1. Inhibition by quinolones in eucaryotic test systems

Test	Drug (inhibitory level[a] or response)	Reference
In vitro		
Topoisomerase II		
D. melanogaster	Nalidixic acid (625)	41
	Oxolinic acid (340)	41
Calf thymus	Ofloxacin (1,300)[b]	27
	Norfloxacin (300)[b]	27
	Ciprofloxacin (150)[b]	27
	CP-67,015 (70)[c]	
Topoisomerase I, calf thymus	Ciprofloxacin (400)	27
	Norfloxacin (300)	27
DNA polymerase α	Ofloxacin, nalidixic acid, ciprofloxacin (1,000)	27
UDS	Ciprofloxacin (positive)	44
	Norfloxacin (positive)	44
	Ofloxacin (positive)	44
	Pefloxacin (positive)	44
Alkaline elution	Ciprofloxacin (10)	6
	Ofloxacin (80)	6
Cell cycle progression	Ciprofloxacin (20)	21
Mouse lymphoma cell	Ciprofloxacin (positive)[d]	44, 45
	Norfloxacin (positive)[d]	44, 45
	Ofloxacin (positive)[d]	44, 45
	Pefloxacin (positive)[d]	44, 45
	CP-67,015 (100)[d]	24
In vivo: mouse bone marrow cell	CP-67,015 (500)	24

[a] Values reported in the literature as inhibitory concentrations (micrograms per milliliter) for in vitro assays or as the effective dose (milligrams per kilogram) in animals for the in vivo assay.
[b] IC_{50} in catalytic catenation assays.
[c] IC_{50} in DNA cleavage reactions (Barrett et al., 27th ICAAC).
[d] A positive mutagenic response was reported.

was confirmed in a separate study in which the compound, when tested at 5 μg/ml, induced a positive UDS response in human lymphocytes (6). A similar evaluation of norfloxacin and fleroxacin (AM-833) showed that they were not mutagenic, did not induce DNA strand breakage, and did not elicit a UDS response in either human or mouse skin fibroblasts (25).

As mentioned above, the alkaline elution test measures DNA strand breakage (30) and has been used to test quinolones for this activity, as described by Bredberg et al. (6). The cellular DNA is prelabeled with [^{3}H]thymidine and, after incubation with a quinolone for 24 h, the cells are washed and lysed on a 2-μm-pore-size filter. The double-stranded DNA is unwound in alkaline buffer, and the single strands are eluted through the filter. When the test drug induces DNA strand breakage, the smaller DNA species generated elute more freely through the filter, as detected by increased counts of radioactivity in the eluate. With this method, significant DNA breakage was obtained in lymphoblastoid cells incubated with ciprofloxacin, ofloxacin, and norfloxacin at test levels of 10, 80, and 160 μg/ml, respectively. The DNA breakage effect observed with ciprofloxacin was dose dependent, and at 80 μg of drug per ml, the breakage approximated that obtained from exposure to 1,000 rads of X rays (6). Bredberg et al. described some potential technical problems encountered in the alkaline elution procedure that can drastically affect the degree of DNA breakage observed (6). As an example, cells on a filter washed with room temperature buffer exhibited less DNA breakage than did cells kept on ice during the procedure. A significant finding in this study (6) was that treated cells have the ability to rapidly reverse DNA breakage when incubated at 37°C for only 15 min in quinolone-free medium. This result suggests that some eucaryotic cells have the ability to rapidly reverse the DNA damage associated with exposure to quinolones, and this ability may increase the chance of obtaining a false-negative result with this in vitro assay. This result also suggests that more quinolones may have the ability to induce DNA breakage in cell cultures (6). It has also been noted that in vitro genotoxicity test results can be significantly influenced by variations in the ionic strength of the test medium (45).

CORRELATIONS OF IN VIVO EFFECTS WITH IN VITRO GENOTOXICITY TEST RESULTS

A major issue surrounding quinolone research involves the relevance of positive in vitro genotoxicity tests for measuring the safety of these compounds. There is currently a good deal of debate in the literature concerning the interpretation of positive in vitro genotoxicity results observed in a single test system, because of the finding that quinolones do not induce the same responses in in vivo tests (9, 36, 39). McQueen and Williams (36) found that norfloxacin, ofloxacin, pefloxacin, and ciprofloxacin induced DNA breakage and repair in the in vitro rat hepatocyte assay at levels between 300 and 500 μg/ml. No in vivo response was obtained in lymphocytes taken from rats given single subcutaneous doses of ciprofloxacin at 30 or 190 mg/kg (36). These authors contend that the negative in vivo results rendered the positive in vitro test results physiologically irrelevant, given the relatively high concentrations of test substance required to elicit the in vitro response (36). Furthermore, in a published clinical study, no cytogenetic abnormalities were observed in peripheral blood lymphocytes isolated from adults receiving 500 to 2,000 mg of ciprofloxacin daily for 1 to 10 weeks or with 200 mg of ofloxacin daily for 1 week (39).

An interesting relationship between in vitro and in vivo genotoxicity test results has been described by Holden et al. (24). These investigators described a new 6,8-difluoro-7-pyridyl 4-quinolone, CP-67,015, that was active in genotoxicity tests performed both in cultured cells and in animals (24). Although CP-67,015 was a directly acting mutagen at ≥100 μg/ml in the mouse lymphoma cell assay, the Chinese hamster ovary cell–hypoxanthine-guanine phosphoribosyl transferase gene (HGPRT) assay, and the V79 cell-HGPRT forward mutation assay (24, 32), it was not mutagenic in the Ames test (24). The compound also induced chromosome aberrations in cultured human lymphocytes at levels of ≥50 μg/ml and produced genetically abnormal bone marrow cells in mice given five daily parenteral doses of 500 mg/kg per day (24). Furthermore, CP-67,015 was found to be at least 10-fold more potent than nalidixic acid, norfloxacin, oxolinic acid, or ciprofloxacin at enhancing eucaryotic topoisomerase II-mediated DNA cleavage in vitro (J. J. Barrett, T. D. Gootz, C. A. Farrell, S. A. Sokolowski, and M. Frescura, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 249, 1987). CP-67,015 is a 4-quinolone that is inhibitory to both procaryotic and eucaryotic type II topoisomerases and clearly induces genotoxic changes in standard in vitro and in vivo tests at drug levels that approach physiological relevance (Table 1). This is particularly true since it has been observed that peak levels of quinolones in the urinary tract range from 100 to 650 μg/ml (2) and can be concentrated 4- to 20-fold above levels in serum in lymphocytes and to variable degrees in other tissues such as kidney, liver, and intestine (4).

CONCLUSIONS

In summary, the available data indicate that most quinolones examined are not highly inhibitory for eucaryotic topoisomerases or other enzymes involved in DNA replication. Although a number of positive in vitro genotoxicity test results have been documented with several different 4-quinolones, the effective concentrations would be clinically achieved predominantly in the urinary tract (4, 5). It has been suggested that in vitro genotoxicity test results should not be of concern, since corroborating positive in vivo test results have not been observed with these antimicrobial agents (36, 44, 45). There is also disagreement in the literature concerning which genetic toxicology tests are relevant for testing quinolones and more general disagreement over the ability of a single positive test to predict the performance of compounds in long-term carcinogenicity studies in rodents (9, 12, 14, 18, 22, 23, 31, 33, 35, 36, 48, 50, 52). Given the multiple physiological activities of topoisomerases (46) and the diverse methods that have been developed to measure their activity (3), it will be interesting to see whether all of the many new and structurally diverse 4-quinolones retain their selectivity for bacterial DNA gyrase and remain nontoxic for eucaryotic cells. In this regard, it is important to note that not all eucaryotic topoisomerases have been routinely tested with 4-quinolones in published studies. The topoisomerase II found in mitochondria, for example, has been shown to be biochemically distinct from the commonly studied enzyme found in the nuclei of eucaryotic cells (7, 8, 17). It appears that future research in the area of topoisomerases will be helpful in understanding these issues.

ACKNOWLEDGMENTS

We thank Henry Holden and John Williams for a critical review of the manuscript.

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