Abatement by naringin of lomefloxacin-induced genomic instability in mice

Sabry M. Attia
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt

Lomefloxacin is a difluorinated quinolone antibacterial drug. It is widely used against infectious diseases including meningitis, those of the genitourinary and upper respiratory tracts, and skin infections. Lomefloxacin, like other fluoroquinolones, is mutagenic and the formation of reactive oxygen species appears to be responsible for this genomic instability. The anti-mutagenic effects of naringin, a grapefruit flavonone, against lomefloxacin-induced genomic instability in vivo were evaluated in mouse bone marrow cells by chromosomal aberration and micronucleus (MN) assays. Naringin was neither genotoxic nor cytotoxic in mice at doses equivalent to 5 or 50 mg/kg. Pretreatment of mice with naringin significantly reduced lomefloxacin-induced chromosomal aberrations and the MN formation in bone marrow. The protective effect of naringin was found to be stronger at the higher dose, indicating the dose-dependent effect of naringin. Lomefloxacin induced marked biochemical alterations characteristic of oxidative stress, including enhanced lipid peroxidation and reduction in the reduced glutathione level. Prior administration of naringin ahead of lomefloxacin challenge ameliorated these biochemical markers. It is concluded that naringin has a protective role in the abatement of lomefloxacin-induced genomic instability that resides, at least in part, in its anti-radical effects. Thus, naringin might be a good alternative to reduce genotoxic risks associated with lomefloxacin therapy.

Introduction
Many mutagens have been identified and are known to be potentially deleterious to human health. Two general strategies have been suggested to cope with this problem: (i) to reduce the exposure of an individual to known mutagens as much as possible and (ii) to take advantage of inhibitors of mutagenesis with the final purpose of their eventual application as anti-mutagenic agents (1). The latter field has been widely explored with several components of the diet. Grapefruit is part of the diet in most countries, where it is often consumed regularly as juice. The chemical responsible for the characteristic sour flavour of the fruit is naringin, a flavonone that is rapidly transformed into naringenin by the action of the enzymes α-rhamnosidase and β-glucosidase (2). Naringin exhibits various pharmacological and therapeutic properties including action as an antimicrobial, anti-mutagenic, anti-cancer, anti-inflammatory, cholesterol lowering and cardioprotective agent (3–12). Moreover, naringin has a strong liver-protective and preventive activity against hepatic diseases (13). Like most flavonoids, naringin has metal chelating, antioxidant and free radical scavenging properties (14–16) and has been reported to offer some protection against lipid peroxidation (17–20).

Quinolone antibacterial agents have been widely used in numerous diseases because of their broad-spectrum activity against both gram-negative and gram-positive pathogens (21,22). The primary target of quinolone antibacterial drugs is bacterial DNA gyrase, which is similar to DNA topoisomerase II of eukaryotic cells (23). They inhibit the resealing of DNA strand breaks by the A subunit of DNA gyrase following supercoiling. This inhibition results from saturation of binding sites on bacterial DNA and subsequent structural changes in DNA. There are few reports that make an adequate classification of carcinogenic risks due to the therapeutic use of quinolones (24). With respect to the genotoxicity of quinolones, in general, certain quinolones have been reported to be associated with ultraviolet-A irradiation-induced gene mutation in bacteria and cultured mammalian cells (25,26), chromosomal aberration or micronucleus (MN) induction in cultured mammalian cells (25,27,28) and DNA strand breakage in in vitro and in vivo comet assays (25,29–31), causing genomic instability that may lead to mutagenesis or carcinogenesis. In photocarcinogenicity studies using Skh-1 hairless mice with ultraviolet-A irradiation, lomefloxacin, a difluorinated quinolone, has been reported to cause cystic squamous cell carcinoma in the majority of animals treated (32,33).

An in vivo genotoxicity study has shown that lomefloxacin induced a statistically significant reduction in mitotic index and an increase in chromosomal aberrations and per cent abnormal metaphase in mouse bone marrow (34). Moreover, in an in vivo study carried out by Itoh et al. (35), in which single doses of 25 or 50 mg/kg lomefloxacin were given by oral intubations followed by light irradiation and the mice were killed on days 2, 3, 4, 5 or 8 after treatment, lomefloxacin at either dose caused significant increases in MN frequency, which peaked on day 4. Nevertheless, no significant increase in MN frequency was observed after treatment without light irradiation at these doses. Lomefloxacin was previously tested in our laboratory with the mouse bone marrow MN assay (S.M. Attia, unpublished data); the MN frequency was significantly increased in polychromatic erythrocytes (PCEs) following dosing with 320 mg/kg at both 24 and 48 h. Together, these results demonstrate that lomefloxacin has genotoxic activity.

The cause of genotoxicity induced by quinolones is thought to be the formation of reactive oxygen species, such as superoxide anion, hydrogen peroxide, hydroxy radical and singlet oxygen (36–40). These reactive oxygen species have been associated with cancer, aging and many other degenerative diseases. Therefore, it is advisable to avoid or reduce use of quinolones or to propose alternative DNA-damage reduction
methods (41). In order to reduce the genotoxic damage caused by exposure to free radicals due to chemical compounds, therapeutic drugs, air pollutants and metabolic procedures, the use of some anti-mutagens found in the normal diet has been studied as a possible DNA-damage reduction method (41,42). On the other hand, flavonoids, such as naringenin, are extremely useful therapeutic drugs, whose use cannot be avoided, despite them being reactive oxygen species generators. Consumption of a diet rich in antioxidants, which are required as micronutrients in the human diet, is perhaps a good alternative to improve health and reduce the risks associated with reactive oxygen species exposure (43).

Flavonoids have been shown to possess antioxidant properties and may reduce the risk of developing diseases associated with oxidative stress. The free radical scavenging and anti-mutagenic properties of naringenin have been investigated in previous studies (44). Naringenin, a flavonoid found in citrus fruits, has been shown to have antioxidant and anti-inflammatory properties. It has been studied as a possible DNA-damage reduction method (41,42).

**Materials and methods**

**Animals**

Adult male white Swiss albino mice weighing 20–25 g (10–12 weeks old) were obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA, Giza, Egypt). The animals were maintained under standard conditions of humidity, temperature (25 ± 2 °C) and light (12-h light/12-h dark). They were fed with a standard pellet mouse diet (El-Nasr Co., Cairo, Egypt) and had free access to water. The animal experiments were conducted according to the regulations of the Committee on Bioethics of the College of Pharmacy, Al-Azhar University, Cairo, Egypt. The total number of animals was 60 treated mice and 10 vehicle control mice.

**Drugs and chemicals**

Naringenin, lomefloxacin, and cyclophosphamide (Sigma-Aldrich, St Louis, MO, USA) were dissolved in phosphate-buffered saline immediately before use. Naringenin was administered at the dose levels of 5 and 50 mg/kg. The anti-mutagenic doses for naringenin were chosen by reference to earlier studies. Naringenin was administered at the dose levels of 5 and 50 mg/kg. The anti-mutagenic doses for naringenin were chosen by reference to earlier studies. Naringenin was administered at the dose levels of 5 and 50 mg/kg.

**Experimental protocol**

The animals were randomly divided into seven groups consisting of 10 mice each. Three groups were administered lomefloxacin [320 mg/kg, intraperitoneally (i.p.)], two groups received a single i.p. injection of naringenin at a dose of either 5 or 50 mg/kg body weight 1 h prior to lomefloxacin administration. A vehicle-treated control group and naringenin (5 and 50 mg/kg) groups were also included. The experiment included a positive control group administered cyclophosphamide at the dose of 40 mg/kg. The animals were killed by cervical dislocation at 24 h after lomefloxacin treatment.

**Chromosome analysis**

Five mice in each group were separated and injected i.p. with colchicine at 4 mg/kg body weight, 90 min before sacrifice. The slides were prepared essentially as per the modified method of Adler (48). In brief, both femurs were dissected and bone marrow was flushed out from the femoral cavity with foetal calf serum. The cells were dispersed by gentle pipetting and collected by centrifugation at 1100 rpm; the harvested bone marrow cells were incubated in 10 ml of 0.075M KCl for 20 min at 37 °C. At the end of the incubation period, the cell suspension was centrifuged (3000 rpm at 4 °C) for 5 min and then resuspended in 3 ml of fresh KCl solution for smear preparation. An aliquot of bone marrow cells was transferred into test tube and cells were resuspended in a small volume of foetal calf serum for smear preparation. Two to three changes of fixative were included. The experiment included a positive control group administered cyclophosphamide at the dose of 40 mg/kg. The animals were killed by cervical dislocation at 24 h after lomefloxacin treatment.

**The MN assay**

The remaining five animals from each group were employed for the bone marrow MN test. The animals were sacrificed and bone marrow collected in tubes containing 3 ml of foetal calf serum and centrifuged for 10 min at 1100 rpm. An aliquot of bone marrow cells was transferred into test tube and cells were resuspended in a small volume of foetal calf serum for smear preparation. Two to three changes of fixative were included. The experiment included a positive control group administered cyclophosphamide at the dose of 40 mg/kg. The animals were killed by cervical dislocation at 24 h after lomefloxacin treatment.
One hundred metaphases were scored for chromosomal aberrations per mouse, for a total of 500 metaphases per treatment. A G animal was evaluated by calculating the number of dividing cells in a population of 1000 cells. The results are expressed as mean ± standard deviation. Data on oxidative damage parameters were analysed using analysis of variance, followed by Tukey–Kramer for multiple comparisons. Results were considered significantly different if the P-value was <0.05.

**Results**

**Effect of naringin on lomefloxacin-induced chromosomal aberrations**

The results of the chromosomal aberrations are presented in Table I. As expected, animals treated with the positive control cyclophosphamide showed a high frequency of structural and numerical chromosomal aberrations in mouse bone marrow cells after treatment in comparison with the negative control [P < 0.01 (Mann–Whitney U-test)]. Naringin treatment did not exhibit a significant difference in the frequency of structural and numerical chromosome aberrations compared to the solvent control at either dose tested. Lomefloxacin treatment caused a significant increase in total frequency of chromosomal aberrations and abnormal metaphases (P < 0.01). The major two types of aberrations observed in the present study were gaps and breaks. Cells with fragments or polyplody were also observed frequently in the lomefloxacin group but not statistically significant in comparison to the solvent control. Naringin pre-treatment reduced the total frequency of chromosomal aberrations and abnormal metaphases in lomefloxacin-treated animals in comparison to those treated with lomefloxacin alone, and the higher dose of naringin gave the more effective reduction in the abnormal metaphases and total chromosomal aberrations [P < 0.05 (Kruskal–Wallis test followed by Dunn’s multiple comparisons test)].

**Effects of naringin and/or lomefloxacin on the mitotic activity at metaphase**

Mitotic index data recorded in the bone marrow cells at metaphase stage are also presented in Table I. Drastic inhibition in the mitotic activity of bone marrow cells was recorded following cyclophosphamide administration [P < 0.05 (Mann–Whitney U-test)]. Treatment of mice with naringin did not affect the mitotic activity as compared to the control value. Treatment with lomefloxacin induced a decrease in the mitotic index of bone marrow cells. However, this reduction was not statistically significant in comparison to the control (P > 0.05). Pre-treatment with naringin (50 mg/kg) elevated the reduced mitotic indices to nearly normal level.

**Effect of naringin on lomefloxacin-induced MNPCe**

The results of the conventional MN test are presented in Figure 1. The frequency of MNPCe with cyclophosphamide was significantly higher than in the solvent control group. At a dose of 40 mg/kg cyclophosphamide, the frequency of MNPCe was 1.56% compared to 0.32% in the solvent control [P < 0.01 (Mann–Whitney U-test)]. The frequency of MN in both negative and positive control is consistent with other published studies and confirmed the sensitivity of the experimental protocol followed in the detection of genotoxic effects. Similarly, lomefloxacin at a dose of 320 mg/kg significantly increased the frequency of MNPCe from 0.32% in the control to 0.84% (P < 0.01). Naringin treatment did not exhibit a significant difference in the frequency of MNPCe compared to the solvent control at either dose tested. With regard to the animals treated with naringin plus lomefloxacin, a weak protection was observed with 5 mg/kg of naringin. However, this protection was not statistically significant in comparison to the lomefloxacin alone (P > 0.05). With 50 mg/kg, however, naringin produced a clear significant inhibitory effect on the MNPCe induced by lomefloxacin alone (0.42% compared with the lomefloxacin value of 0.84%, P < 0.05) and not statistically significant in comparison to the solvent control (Kruskal–Wallis test followed by Dunn’s multiple comparisons test).

**Effects of naringin and/or lomefloxacin on the mitotic activity at interphase**

The results for the ratio of PCE:NCE are presented in Figure 2. The positive control cyclophosphamide significantly decreased the per cent PCE from 48.4% in the control to 42.8% [P < 0.05 (Mann–Whitney U-test)] indicating a reduction in erythroblast proliferation most likely by mitotic arrest. Naringin did not modify the ratio in comparison with the one observed in the control group. Lomefloxacin treatment caused a significant

### Table I. Distribution of the different types of chromosomal aberrations, percentage of total aberrations, abnormal metaphases and mitotic index in bone marrow of mice after treatment with cyclophosphamide (CP), naringin (Nar) and/or lomefloxacin (Lomo)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chromosomal aberrations</th>
<th>Percentage of total aberrations (mean ± SD)</th>
<th>Abnormal metaphases (mean ± SD)</th>
<th>Mitotic index (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>B</td>
<td>F</td>
<td>R</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CP 40 mg/kg</td>
<td>14</td>
<td>53</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Nar 5 mg/kg</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nar 50 mg/kg</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Lomo 320 mg/kg</td>
<td>7</td>
<td>17</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Lomo and Nar 5 mg/kg</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Lomo and Nar 50 mg/kg</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

One hundred metaphases were scored for chromosomal aberration per mouse, for a total of 500 metaphases per treatment. G = gaps, B = breaks, F = fragments, R = rings, P = polyplody and SD = standard deviation. Cells with gaps were not included in the total aberrations or abnormal metaphases. The mitotic activity per animal was evaluated by calculating the number of dividing cells in a population of 1000 cells. **P < 0.01 versus control and *P < 0.05 versus lomefloxacin alone (Kruskal–Wallis test followed by Dunn’s multiple comparisons test). **P < 0.05 and *P < 0.01 versus control (Mann–Whitney U-test).
decrease in the per cent PCE from 48.4% in the control to 44.0% \( P < 0.05 \) (Mann–Whitney U-test). The combination of 50 mg/kg naringin with lomefloxacin produced a response which was close to that observed with the solvent control.

**Effect of naringin on lomefloxacin-induced reactive oxygen species production**

The effect of naringin on lomefloxacin-induced oxidative stress in mice was assessed by measuring serum GSH and MDA levels. The serum level of GSH in the solvent control is consistent with other published studies. Serum GSH level did not show significant variation in naringin-treated animals compared to the solvent control. The GSH level observed in lomefloxacin-treated animals was significantly decreased compared to the solvent control \( (P < 0.01) \). Animals pre-treated with naringin showed a significant increase in GSH level over the lomefloxacin-treated group and the maximum increase in GSH was observed in animals pre-treated with naringin 50 mg/kg (Figure 3). In the control group, the value of 1.2 nmol/ml MDA is relatively low; however, it is in agreement with the 2.54 nmol/ml of serum MDA in the control mice reported by Alesa et al. (51). The treatment with naringin failed to induce any significant changes in the serum levels of MDA at both doses. A significant rise in serum MDA level was observed in the lomefloxacin group \( (P < 0.01) \). Pre-treatment with naringin was found to significantly \( (P < 0.01) \) decrease the MDA concentrations only at the higher dose (50 mg/kg) relative to the values obtained after treatment with lomefloxacin alone (Figure 4).

**Discussion**

It has been reported that naringin has various pharmacological and therapeutic actions. In spite of its potent efficacies, naringin shows little toxicity or mitogenicity in mice. More specifically, naringin exhibits no toxicity when administered orally at 1000 mg/kg, which corresponds to a human dose of 81 mg/kg body weight of naringin (13). Since naringin is a safe and readily available antioxidant, one needs to examine its interaction with drugs. The current study demonstrates that naringin was neither genotoxic nor cytotoxic at the doses...
tested. Moreover, it is able to protect mouse bone marrow cells against the lomefloxacin-induced genomic instability and produce a decline in cell proliferation as observed by the reduction in chromosomal aberrations, MN frequencies and increase in mitotic activities, respectively. An in vivo genotoxicity study has shown that lomefloxacin induced statistically significant increase in chromosomal aberrations, per cent abnormal metaphase and reduction in mitotic index in mouse bone marrow after treatment with lomefloxacin (34). In agreement with this report, the present study showed the exposure to lomefloxacin caused significant increase in the total chromosomal aberrations and abnormal metaphases. The treatment of mice with naringin before exposure to lomefloxacin resulted in significant reduction in both the total number of chromosomal aberrations and abnormal metaphases induced by lomefloxacin. A similar effect has been also reported for radiation-induced chromosomal aberrations in the mouse bone marrow. Irradiated mice pre-treated with naringin had significantly fewer chromosomal aberrations in bone marrow than those pre-treated with double-distilled water (7).

Lomefloxacin was previously tested in our laboratory with the mouse bone marrow MN test. At 24 and 48 h after treatment with 320 mg/kg, the MN showed significant increase in PCE. Furthermore, lomefloxacin caused a significant depression of erythroblast proliferation indicating an inhibition of erythroblast proliferation most likely by mitotic arrest. The current study showed a significant inhibitory effect of naringin on the MN produced by lomefloxacin. However, in regard to this response, it is worthwhile to emphasize the observed dose-dependent effect of naringin, with the stronger inhibitory effect produced with 50 mg/kg: this suggests the possibility of using an even higher amount of the chemical with good results. The genotoxicity protection was also directly correlated with mitotic activity as more bone marrow protection was noted with animals pre-treated with 50 mg/kg pre-treated animals when mitotic activity was examined at interphase stage (PCE:NCE ratio). Alvarez-González et al. (10) observed a reduction in ifosfamide-induced MN and mitotic activity in mouse bone marrow cells pre-treated with naringin. Similar anti-mutagenic effects have been also reported for radiation-induced MN in the mouse bone marrow and bleomycin-induced MN in V79 cells (5,8).

The exact mechanism by which naringin protected against lomefloxacin-induced genomic instability in the form of chromosomal damage and MN is not well understood. One possible explanation is that simultaneous treatment with naringin would allow interception of free radicals generated by lomefloxacin before they reach DNA. In the present work, in order to evaluate whether the observed anti-mutagenic effect was due to an enhancement of the scavenger of free radicals generated by lomefloxacin, oxidative stress markers such as lipid peroxidation and GSH were measured after the animals were treated with lomefloxacin, compared with the simultaneous treatment with naringin and the solvent control animals. The results demonstrate that naringin pre-treatment reduced the lomefloxacin-induced lipid peroxidation and prevented the reduction in GSH significantly. The increased GSH level suggests that protection by naringin may be mediated through the modulation of cellular antioxidant levels. These observations confirm earlier studies in which naringin was reported to scavenge free radicals and lipid peroxides (14–20,52).

It is well known that quinolones produce superoxide anion, hydrogen peroxide, hydroxy radical and singlet oxygen, which cause damage to cellular genome and also the cell membrane leading to lipid peroxidation (37–40). MDA, the product of lipid peroxidation also interacts with DNA causing strand breaks that in turn develop into chromosomal breaks. These chromosome breaks may appear as MN in the daughter cell after the first cell division. Earlier studies have shown that naringin scavenged peroxyl, superoxide and hydroxyl radicals in a concentration-dependent manner. Moreover, naringin has been reported to elevate glutathione peroxidase, glutathione reductase, glutathione, superoxide dismutase and catalase and to reduce lipid peroxidation and iron-induced toxicity (6,7,20,52). Scavenging of these free radicals by naringin seems to be an important mechanism against the lomefloxacin-induced genomic instability.

It is, however, worth noting that combination therapy with antioxidants and quinolone provided protection against the quinolone’s genotoxicity without interfering with its bactericidal efficacy (44–46). These results might be explained if it is considered that the bactericidal effect of this antibiotic group is due to inhibition of DNA gyrase, which is the result of the covalent link of the 3-carboxyl and 4-carbonyl groups with the -NH2 terminal group of the bacterial DNA gyrase, and not by free radical production, which can be inhibited by antioxidants (44). Moreover, as mentioned in the Introduction, naringin also possesses antimicrobial and anti-fungal effects (3,53,54). Other studies have reported that antioxidants are able to reduce the development of antibiotic resistance among pathogenic bacteria, because oxygen-free radicals generated by antibiotic metabolism may also be a major cause of antibiotic resistance development (55). Therefore, the simultaneous use of antioxidants with quinolone therapy is advisable to reduce the genotoxic risk of reactive oxygen species generated by quinolone.

In conclusion, prior administration of naringin ahead of lomefloxacin challenge to male mice ameliorated all the cytogenetic parameters altered by lomefloxacin. Apart from the regulatory role of naringin on MDA production observed in the current work, the anti-mutagenic effects of the flavonoid could possibly reside for the most part on its antioxidant effects. Thus, naringin might be a good alternative to reduce genotoxic risks associated with lomefloxacin therapy.

Funding
The Faculty of Pharmacy, Al-Azhar University.

Acknowledgements
The author thank Professors A. A. Abdel-Aziz and F. M. Hamada for their helpful discussion and critical reading of the manuscript.

Conflict of interest statement: None declared.

References


Received on April 19, 2008; revised on July 20, 2008; accepted on July 28, 2008