EXPERIMENTAL BIOLOGY

A Chronobiological Study of Cell Multiplication in Esophageal Epithelium of Mice Given Lomefloxacin at Different Times of the Day

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The diurnal rhythmic pattern of cell division is preserved after both afternoon and night injection of lomefloxacin, but a rightward phasic shift of the diurnal rhythm is observed, the amplitude of rhythmic fluctuations being unchanged. In both cases the mesor of the mitotic index virtually does not differ from that of the control, and the pool of dividing cells over 24 hours and throughout the follow-up period is even larger than the control values, which attests to a high adaptive potential of the proliferative system of esophageal epithelium.

Key Words: *mitotic activity; lomefloxacin; biological rhythms*

Chronopharmacology involves studies of the biological activity of drugs with respect to phases of biological rhythms and the effect of preparations on the temporal organization of biological systems [2]. Studies in this direction are aimed at devising optimal pharmacotherapeutic schemes in order to enhance the effect of treatment, to mitigate side effects, and to minimize the toxic effect of drugs. To date, a considerable body of data on the chronopharmacology of different drugs, including antibacterial drugs, has been accumulated [1,2]. There are reports on the effect of antibacterial preparations on cell multiplication in animal tissues and cell cultures [4-6]. However, the effect of these preparations on the biological rhythms of cell proliferation is yet to be studied.

The 3rd generation quinoline derivatives (fluoroquinolines) are antibacterial drugs which are widely used in modern clinical medicine [7,14,15]. These preparations are frequently administered once a day [14], which requires a knowledge of their chronopharmacology.

The objective of the present study was to investigate the effect of lomefloxacin (LF), a representative of the fluoroquinolines, on the biological rhythm of cell multiplication. Stratified squamous epithelium of the esophagus, which is a fast-regenerating tissue and exhibits a clearly pronounced diurnal rhythm of proliferation [3,8,9,11-13], served as the object of investigation. We are grateful to Prof. T. A. Gus'kova for her gift of lomefloxacin.

MATERIALS AND METHODS

One hundred thirty-two male albino nonpedigree mice weighing 15-18 g were used in the experiments. The animals were maintained under a 12-hour light regime (light from 8:00 to 20:00 h). Food and water were given ad libitum. The experiments comprised three series. The animals of series I served as the control, comprising an afternoon and

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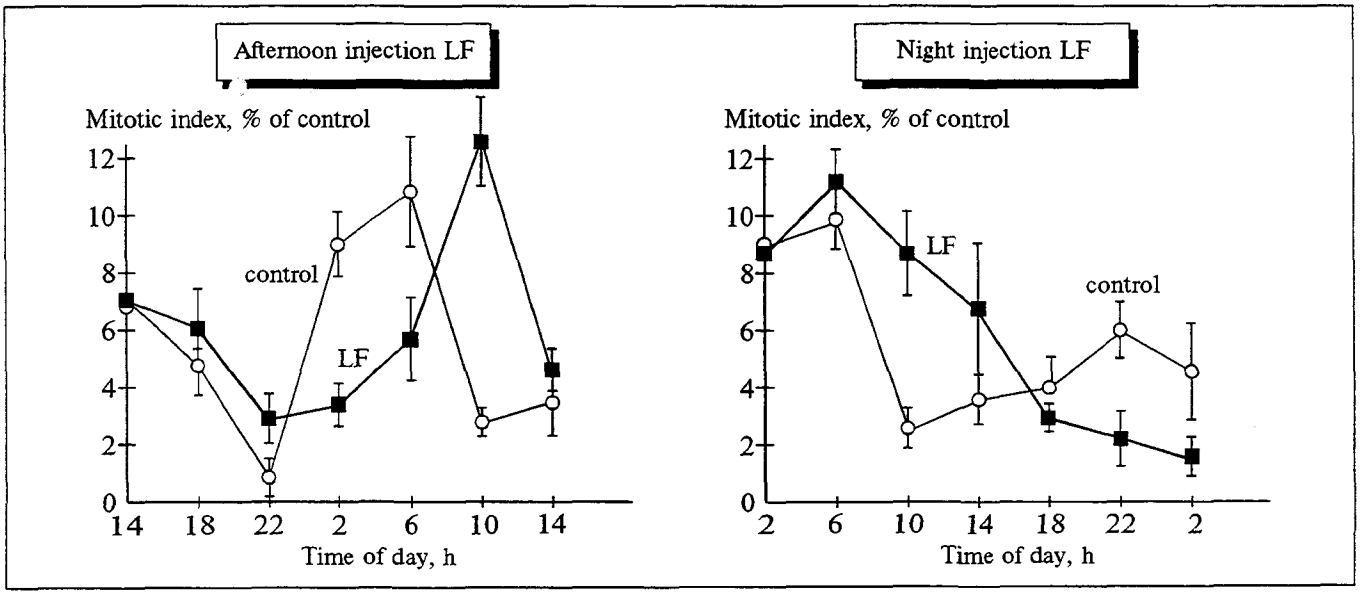

Fig. 1. Variation of MI of esophageal epithelium in mice.

a night group. The animals of series II received LF in a dose 0.5 mg/kg subcutaneously at 14:00 h, and the animals of series III at 2:00 h. The mice of series I were sacrificed every 4 hours over 36 hours, from 14:00 h on day 1 to 2:00 h on day 2. The mice of series II and III were sacrificed every 4 hours over 20 hours, from 18:00 h of day 1 to 14:00 h of day 2 (series II), and from 6:00 h on day 1 to 2:00 h on day 2 (series III). Each experimental point comprised 6 animals. The esophagus was excised for examination. After the standard histological procedure, sections 5 μ thick were prepared and stained with hematoxylin after Mayer. In sections derived from each animal the mitotic index (MI) was calculated as the fraction of dividing cells among the examined 10,000 cells of the basal epithelial layer and expressed in pro mille. The numerical data were statistically processed using the Fisher-Student test; the differences were regarded as reliable at $p \leqslant 0.05$. The plotted curves of MI variation over 24 h were used in graphic-parametric analysis [10] of the diurnal biological rhythms of cell proliferation. The curves were analyzed by taking segments 24 h long (between 14:00 on day 1 and 14:00 on day 2 for LF injection in the afternoon and between 2:00 h on day 1 and 2:00 h on day 2 for LF injection at night). The values of MI at 14:00 and 2:00 h on day 1 were taken to be the

TABLE 1. Results of Graphic–Parametric Analysis of Diurnal Rhythms of Cell Division in the Esophageal Epithelium of Control Mice and of Mice Given LF

Parameter	Control (day)	LF (day)	Control (night)	LF (night)
Acrophase, time of day	6:00	10:00	6:00	6:00
AA, ‰	10.1	9.8	8.6	7.5
RA	12.2	4.4	4.6	4.8
Mesor, ‰	5.9	6.3	6.1	6.5
AP, time of day	24:30–8:30	6:30–17:00	22:00–8:00	24:30–14:30
Duration of AP, h	8	10.5	10	14
Midpoint of AP, time of day	4:30	12:00	3:00	7:30
SC, 1/h	1.5	0.4	0.2	0.6
P_m, ‰	140.8	151.2	147.2	156.4
P_{map}, ‰	73.2	96.1	94.6	119
P_{map}/P_m, ‰	52	63.6	64.3	76.1
P_{mt}, ‰	140.5	153.6	144.7	167.4

Note. P_{mt}: pool of dividing cells over the follow–up period; other notation mentioned in the text (AA: absolute amplitude; RA: relative amplitude; AP: active phase of rhythm; SC: synchronization coefficient of MI over rhythm; P_m: pool of dividing cells over one day; P_{map}: pool of dividing cells over AP).

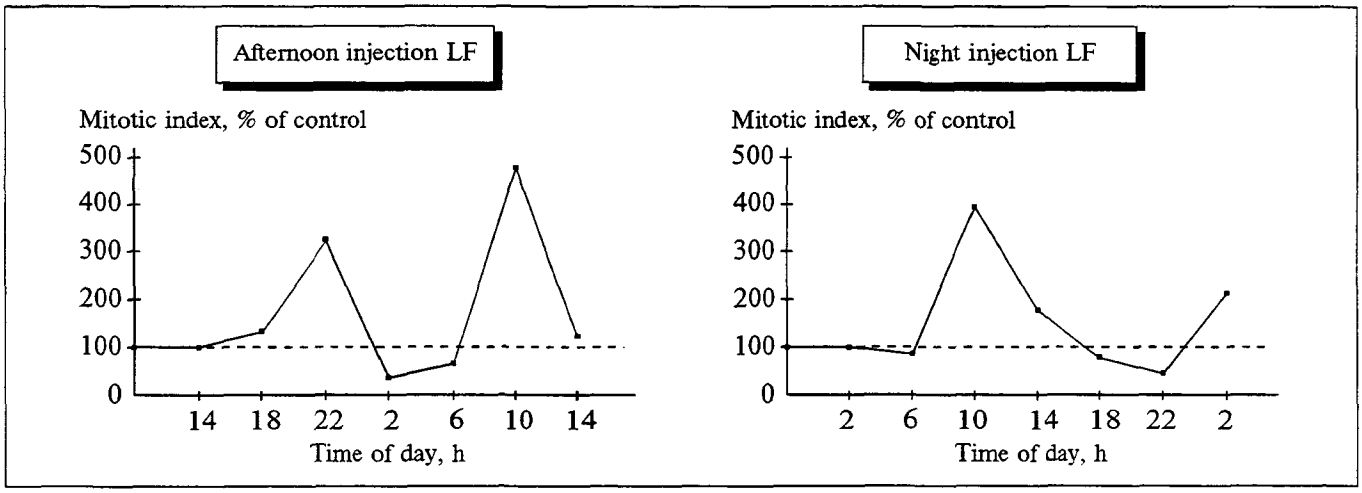

Fig. 2. Variation of MI of esophageal epithelium in mice given LF.

MI values for the same hours on day 2. In calculations of the diurnal pool of dividing cells the duration of mitosis was taken to be 1 hour [9].

RESULTS

As is seen from Fig. 1, *a*, a monophasic diurnal rhythm of MI with the maximum of dividing cells at 6:00 h and the minimum at 22:00 h ($p<0.001$) is exhibited by the esophageal epithelium of the control mice of the afternoon group. The same pattern of changes is characteristic of the rhythmic fluctuations of MI in the esophageal epithelium of the control animals in the night group (Fig. 1, *b*), in which the maximum MI is also observed at 6:00 h and the minimum at 10:00-18:00 h ($p<0.002$). The parameters of diurnal rhythm of MI of epithelial cells of the esophagus of the control animals from both groups are presented in Table 1. As is seen from the table, the majority of parameters differ little between the two groups. Meanwhile, in the animals of the night group the values of absolute amplitude (AA), relative amplitude (RA), and synchronization coefficient (SC) are 15, 62, and 87%, respectively, lower than in the animals of the afternoon group, which demonstrates that synchronization of the involvement of cells in mitosis is attenuated in the night group. It should be mentioned that in this group the duration of the active phase (AP) is 25% longer than in the afternoon group, this evidently resulting in a 29% increase in the pool of dividing cells over the AP (P_{map}). The temporal correlation between the diurnal dark-light cycle and the phasic structure of the diurnal rhythm of MI of cells of the esophageal epithelium in mice of the two control groups is similar to that reported in the literature [3,8,13]. An increased mitotic activity in the morning (when the dark gives way to the light) is typical of this rhythm.

When the mice were injected with LF in the afternoon, 8 hours later (at 22:00 h) the MI of the esophageal epithelium increased as compared to the control (by 222%, $p<0.05$). After 12 and 16 hours (at 2:00 and 6:00 h) MI dropped 60 and 50% vs. the control ($p<0.001$ and $p<0.02$, respectively), but 20 hours after the injection of the preparation (at 10:00 h) it rose sharply (429%, $p<0.003$). Finally, after 24 hours (at 14:00 h), MI virtually normalized. Thus, the afternoon injection of LF to mice caused changes in the proliferative system of the esophageal epithelium, which had a phasic dynamic pattern (first an increase, next a decrease, and then an increase followed by normalization of MI by the end of the experiment) over the follow-up period

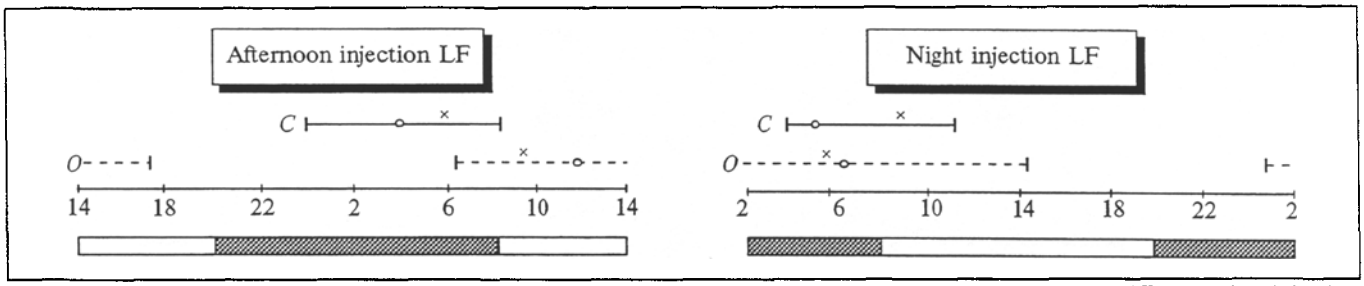

Fig. 3. Phasogram of biological rhythms of cell division in esophageal epithelium of mice in the control (*C*) and after injection of LF (*E*). Midpoint of AP marked by a circle and acrophase by a cross.

(24 hours). The time when MI declines is the same as the time when MI increases. This is reflected by the value of P_{mt} (the pool of dividing cells over the follow-up period), which differed little between the experimental animals and the controls.

On the whole, the nonuniform (phasic) changes of MI of the esophageal epithelium of animals given LF in the afternoon exhibit a pattern which may be regarded as a monophasic diurnal rhythm of MI (between 14:00 and 10:00 h) with the maximum at 10:00 h and the minimum at 22:00-2:00 h ($p<0.005$) (Fig. 1, *a*). Thus, a rightward phasic shift of the diurnal rhythm of MI is observed in this case. The acrophase, the onset of AP, and the end of AP are shifted 4, 6, and 8.5 hours, respectively, to the right (Table 1 and Figs. 1, *a* and 3, *a*). Certain variations (as compared to the control) are observed in the parameters of the diurnal rhythm of MI. The afternoon injection of LF causes a decrease of RA of the rhythm (by 64%) and to an even more marked drop of SC (73%), which provides evidence of attenuated synchronization of the time course of cell multiplication. However, AA, the mesor, and P_m differ little from the control. Hence, after injection of LF in the afternoon, the overall result of the proliferative activity is unchanged, which manifests itself in preservation of the values of the mesor and of the diurnal proliferative pool. The system has an appreciable compensatory potential. P_{map} is 31% higher in the experimental mice than in the controls, which may be due to the increased duration of AP (by 31%). In the control and experimental groups more than one-half of the daily volume of cell proliferation is provided for by cell multiplication during AP of the diurnal rhythm of MI.

Eight hours after night injection of LF to mice (at 10:00 h), a 250% increase of MI in comparison with the control ($p<0.001$) is observed (Figs. 1, *b* and 2, *b*), and after 12-16 hours (at 14:00-18:00) MI is virtually unchanged from the control values. Twenty hours after the injection (at 22:00 h) a drop of MI (66%, $p<0.001$) and 24 hours after it (at 2:00 h on day 2) its rise (120%, $p<0.05$), as compared to the controls, were observed in the experimental animals.

Thus, after injection of LF at night, the MI of the esophageal epithelium of mice exhibits a monophasic rhythm over 24 hours (from 2:00 to 22:00 h) with the maximum at 6:00 h and the minimum at 22:00 h ($p<0.001$) (Fig. 1, *b*). In this case a rightward phasic shift occurs vis-a-vis the control rhythm. The acrophases coincide, while the onset of AP is shifted 2.5 hours and the end of the AP 6.5 hours to the right (Table 1, Figs. 1, *b* and 3, *b*). Following either the night or afternoon injection of LF, the mesor and P_m in the experimental animals are virtually unchanged from the control, and P_{mt} even exceeds the control value (by 16%) in this case. Hence, the result of proliferative activity is unaltered. On the other hand, AA slightly (13%) drops, RA is unchanged, and SC sharply rises (200%). P_{map} in the experiment exceeds the control value (by 26%), which may be due to the increased duration of AP (by 40%). In both the experimental and control mice more than one-half of the diurnal pool of dividing cells multiply during AP of the rhythm.

These findings suggest that both afternoon and night injection of LF to mice cause certain changes in the proliferative system of the esophageal epithelium. In either case the rhythmic pattern of cell division is preserved over 24 hours, but a rightward phasic shift of the diurnal rhythm occurs in relation to the control rhythm. The amplitude of rhythmic fluctuations changes little. After either an afternoon or a night injection of LF, the proliferative system of esophageal epithelium exhibits a high adaptive potential. This manifests itself in the fact that in the experimental animals the mean diurnal MI is virtually unchanged from the control, and the pool of dividing cells over 24 hours and over the entire follow-up period is even slightly larger than in the control. Thus, the use of the fluoroquinoline lomefloxacin does not cause marked disturbances in the activity of the proliferative system of the esophageal epithelium.

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