MODIFICATIONS IN THE MITOTIC AND AMITOTIC DIVISION OF TETRACYCLINE TREATED LYMPHOCYTES, IN VITRO

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The peripheral blood lymphocytes in man, subjected to short-term cultivation, are reproduced through mitotic and amitotic division (6, 8, 9). The latter form of division is more rarely encountered than the former, and is most frequently accomplished through constriction (fission) or gemmation of the nucleus.

There is an opinion according to which the various forms of cell division are the result of specific peculiarities in the living conditions (5, 8). Having in mind the latter considerations and the data concerning the inhibitory effect of tetracyclines (1, 2, 3), we made it our aim to study the changes in the mitotic and amitotic activity of lymphocytes treated with tetracycline of varying concentrations and duration.

Material and Method

The mitotic and amitotic activity of lymphocytes from the peripheral blood of man, subjected to short-term cultivation, is studied (7).

Lymphocytes underwent treatment with 5, 50 and 250 gamma/ml tetracycline for a duration of 24 and 48 hours. The control culture was not treated with antibiotics.

All experiments were repeated five times using blood obtained from different, clinically healthy individuals.

Determination was made separately of mitoses and amitoses through fission and gemmation (cells with 1, 2 and more buds).

A total of 175 000 cells were employed in the analysis of mitotic and amitotic division of lymphocytes.

All data were statistically elaborated after the method of alternative analysis, and disclosed a reliability degree within the limits of $p<0.1—0.0001$.

Results and Discussion

Tables 1, 2, 3 and 4 illustrate the results of the studies carried out under the experimental conditions outlined. The absolute values of the data obtained in the control experiments are reduced to a single level — 100 per cent — for all the indicators (mitotic activity and amitotic division — fission, gemmation). Thus, the changes in individual indicators under the different experimental conditions are more clearly illustrated. On the

Table 1

Percentage of Mitoses

Duration in hours	Control	5 gamma/ml	50 gamma/ml	250 gamma/ml
24	100	92	80	46
48	100	72	76	23

Table 2

Percentage of Fissions

Duration in hours	Control	5 gamma/ml	50 gamma/ml	250 gamma/ml
24	100	86	79	82
48	100	85	95	48

Table 3

Percentage of Buddings

Duration in hours	Control	5 gamma/ml	50 gamma/ml	250 gamma/ml
24	100	105	81	74
48	100	89	81	58

Table 4

Total Percentage of Amitoses

Duration in hours	Control	5 gamma/ml	50 gamma/ml	250 gamma/ml
24	100	105	81	76
48	100	89	84	58

other hand, comparison of the individual indicators is rendered more demonstrative.

Upon analysis of the amitotic division in the lymphocytes cultivated with 5 gamma/ml tetracycline, a difference is established between the 24- and 48-hour effect of the antibiotic. Accordingly, at 24-hour-long treatment, the total quantity of amitoses rises to 105 per cent as compared to the control. Parallel to the increase of this particular indicator, the cells

entering in mitotic division, under analogical experimental conditions, decrease to 92 per cent of the starting level. In the cultures treated for 48 hours with 5 gamma/ml tetracycline, the amitotic and mitotic division of lymphocytes is simultaneously inhibited. The inhibitory effect of the antibiotic is rather weakly pronounced in terms of amitotic activity, and amounts to 89 per cent of the value of untreated culture, while lymphocytes undergoing mitotic division after 48-hour-long treatment reach 72 per cent of the control values.

A more detailed tracing of the various types of amitotic division in the cells treated with 5 gamma/ml tetracycline showed that amitotic division through fission (constriction) is respectively 86—85 per cent in the 24- and 48-hour cultures. The values of amitotic division through budding do not show difference in their percentage ratio to the control as compared to the total amounts of cells undergoing amitotic division (see Table 3). In 24-hour treatment the budding cells reach 105 per cent, and in 48-hour treatment — 89 per cent of the control level. Consequently, at 5 gamma/ml antibiotic, the longer duration of cultivation exerts a different effect upon the quantity of lymphocytes undergoing division through gemmation. This is an issue worthy of notice, since according to some authors (4, 8) there is a parallelism in the intensity and dynamics of development of mitoses and amitoses through fission, whereas gemmation shows an inverse dependence. In the experiment described, at 5 gamma/ml concentration, inhibition of the amitotic division through fission is pronounced in both treatment intervals. Gemmation of lymphocytes at the stated antibiotic concentration intensifies upon treatment lasting for 24 hours.

The increase of tetracycline concentration up to 50 gamma/ml leads to a reduction of the total amount of amitoses in either of the treatment intervals. Thus, in cultures treated for 24 hours, the amitoses are 81 per cent, whilst in those treated for 48 hours — 84 per cent. From Table 2 and 3 it becomes obvious that the values of budding and constricting cells alike decrease under the experimental conditions outlined. However, as in 5 gamma/ml concentration, in the latter case too the inhibition of mitotic activity is more strongly manifested. After 24-hour treatment the mitoses are 80 per cent, and after 48-hour treatment — 76 per cent in comparison with the controls (Table 1).

Treatment of lymphocytes with 250 gamma/ml tetracycline for a duration of 24 and 48 hours leads to inhibition of amitotic division to 76 and 58 per cent respectively. Along with that, although to a higher degree, the mitotic activity of the cells is inhibited to 46 and 23 per cent respectively upon explantation with the antibiotic for 24 and 48 hours. At the indicated tetracycline concentration and following 24-hour treatment, fissions are 82 per cent, and buddings — 74 per cent of the starting level. Increasing the antibiotic treatment to 48 hours brings about a reduction of fissions to 48 per cent, and of buddings — to 58 per cent in comparison with the untreated culture.

The results of the analysis of mitotic and of the various forms of amitotic division of lymphocytes, treated for 24 and 48 hours with 5, 50 and 250 gamma/ml tetracycline, warrant the assumption that:

1. Parallel to increasing the treatment time and the concentration of the antibiotic, the inhibitory effect of tetracycline on mitotic and amitotic division of lymphocytes, in vitro, is intensified.

2. Amitotic division is inhibited less than mitotic division under the respective experimental conditions. At concentration 5 gamma/ml tetracycline and 24 hours duration of the treatment, a slight inhibition of mitotic division, simultaneously with stimulation of amitotic division is observed. It is quite probable that it is a matter of a compensatory phenomenon (8).

3. The gemmation values in the lymphocytes are closer to the summed up results for amitotic division in the treated cells. This is due to the fact that gemmation is encountered ten times more frequently than fission in lymphocytes undergoing short-term cultivation. Consequently, buddings reflect more confidently the changes in amitotic activity of lymphocytes subjected to tetracycline treatment. The latter circumstance is quite significant owing to the fact that statements have been made to the effect that changes in amitotic division, gemmation in particular, reflect qualitative characteristic features of the immune process (8).

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ИЗМЕНЕНИЯ МИТОТИЧЕСКОГО И АМИТОТИЧЕСКОГО ДЕЛЕНИЯ ТРЕТИРОВАННЫХ IN VITRO ТЕТРАЦИКЛИНАМИ ЛИМФОЦИТОВ

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РЕЗЮМЕ

При анализе митотического и разных форм амитотического деления (почкования и перетяжки) у третированных в течение 24 и 48 часов 5, 50 и 250 гамма/мл тетрациклином лимфоцитов было установлено, что нарастание длительности воздействия и концентрации антибиотика увеличивает ингибиторный эффект тетрациклина. Амитотическое деление подавляется в более слабой степени чем митотическое в соответствующих опытных постановках. При концентрации 5 гамма/мл тетрациклина и продолжительности воздействия в течение 24 часов наблюдается слабое подавление митотического, при одновременной (вероятно компенсаторной) стимуляции амитотического деления.