# COMPARATIVE STUDY ON THE EFFECT OF TETRACYCLINE AND TETRAOLEANE ON CULTIVATED HUMAN LYMPHOCYTES

L. N. Vasileva, G. G. Nikolova

Nowadays, antibiotics are considered as an essential and indispensable factor in the treatment of acute and chronic bacterial infections. The incessant and rapid mutability of microorganisms greatly interferes with the process of preparing drugs against which it is rather difficult to develop resistance. Along with that, a further by no means less important problem emerges, e. g. minimum toxicity and lack of effect on the reactivity of microorganisms (11).

The combination of broad spectrum antibiotics in many instances appears to be a feasible solution of the above mentioned problem (4). The results of numerous comparative clinical and experimental studies on the combined antibiotic tetraoleane and its basic component tetracycline are unequivocally in support of the combination in question (1, 5, 6, 7).

In a number of clinical researches reference is made to an inhibition of the immunologic response of the organism upon administration of tetracycline preparations (8). In earlier works by one of the authors (3, 10) an inhibitory effect of tetracycline upon lymphocyte cultures has been established.

Since the small lymphocyte proves to be an essential linkage in the realization of the overall and complex process of immunologic reactivity in the organisms, we made it our aim to study comparatively the action exerted by tetracycline and tetraoleane on lymphocyte cultures.

## Material and methods

Lymphocyte cultures from the peripheral blood of clinically healthy subjects, cultivated according to the method of Moorhead et al (9), as modified by Tzoneva, were used. The substance for venous application, produced and obtained from the plant for antibiotics in Razgrad, underwent treatment with 5 and 250 gamma/ml tetracycline and tetraoleane. In the controls of the various experimental conditions no antibiotics were added.

Following 72-hour-long explantation, preparations stained according to Ramanowsky-Giemsa were made.

The experiments were repeated ten times for the cultures treated with tetracycline, and three times — for those treated with tetraoleane.

The mitotic activity and blast transformation were investigated in a total of 177,000 lymphocytes at $p<0.001$.

## Results and discussion

At equal duration of the explantation of human lymphocytes (72 hours) with 5 and 250 gamma/ml tetracycline and tetraoleane, changes in their mitotic activity and blast transformation were established as compared to control cultures (Fig. 1). In lymphocytes treated with 5 and 250 gamma/ml tetracycline for a duration of 72 hours, a lowering of the amount of dividing cells was noted to 67 and 8 per cent respectively, relative to the control level, admitted for 100 per cent. In tetraoleane-treated cultures at concentration 5 and 250 gamma/ml, similary a reduction of the mitotic activity of cells was observed. It was found that mitoses in them were 93 and 19 per cent respectively of the lymphocytes which had already initiated division in the untreated culture.

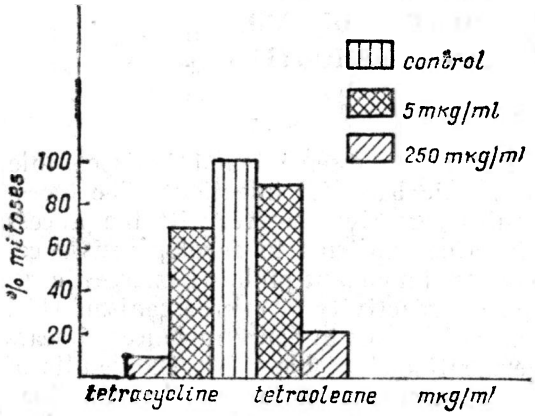


Fig. 1. Percentage values of mitoses upon human lymphocytes treatment, in vitro, with 5 and 250 gamma/ml tetracycline and tetraoleane, relative to the control level.

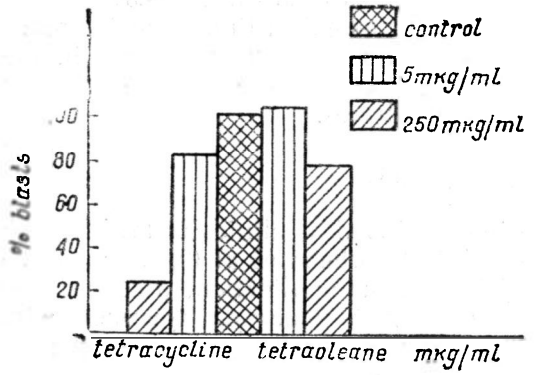


Fig. 2. Percentage values of cells undergoing blast transformation in lymphocytes treated, in vitro, with 5 and 250 gamma/ml tetracycline and tetraoleane, compared with the control (accepted for 100).

From the above data it is evident that the inhibitory effect of tetraoleane with reference to the mitotic activity of lymphocytes is less manifested compared to tetracycline, under analogical experimental conditions. Such a phenomenon is manifested both at the low and high concentrations of the two antibiotics (5 and 250 gamma/ml resp.). Since the beginning of mitosis in cultivated lymphocytes is preceded by their blast transformation, the correlation of blast cells in the experimental conditions (tetracycline and tetraoleane treatment) was considered as an expression of their readiness to step in mitosis. It was furthermore established that while at 5 gamma/ml tetracycline the blasts are 77 per cent of the cells undergoing transformation at analogical tetraoleane concentration, at 250 gamma/ml tetracycline they amount to 42 per cent.

In the study of blast transformation of cultures treated with tetracycline and tetraoleane, we calculated the per cent correlation between lymphocytes undergoing blast transformation from each experiment and from the control

setting (Fig. 2). Here too, the inhibitory action was more strongly manifested in lymphocytes explanted with tetracycline where, yet at 5 gamma/ml concentration, the blasts were 90 per cent of the cells, whereas at the same tetraoleane concentration their values remained unaltered. The difference in the degree of blast transformation inhibition between the antibiotics under study referred to is very strongly pronounced at 250 gamma/ml concentration. Only 22 per cent of the tetracycline explanted lymphocytes in this particular experiment were blasts, whilst with tetraoleane they were 79 per cent.

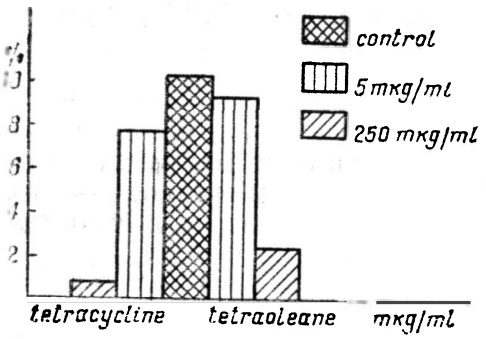


Fig. 3. Percentage of cells undergoing blast transformation with mitotic activity in human lymphocytes treated, in vitro, with 5 and 250 gamma/ml tetracycline and tetraoleane, and in untreated with antibiotic culture.

Under the experimental conditions outlined above, we established apart from quantitative, also some morphological changes in the blast transformed cells, manifested with vacuolization of the protoplasm, total reduction of its volume and loosening of chromatin at the higher tetraoleane and tetracycline concentrations. The finding of disturbances in the transformation of lymphocytes in our experiment is furthermore supported by an additional indicator — the percentage of dividing blasts under identical experimental conditions in comparison with the control (Fig. 3). At 5 gamma/ml tetracycline, the percentage of blasts initiating mitosis is 7.4, and at 250 gamma/ml — barely 0.3 against 10 per cent in the untreated culture. In this case too, the inhibitory effect of tetraoleane under analogical conditions of the experiment (5 and 250 gamma/ml) is less marked in comparison with tetracycline, and the percentage of blast-transformed lymphocytes displaying mitotic activity is 8.6 and 2.1 respectively. The differences are rather pronounced at the higher concentration of the antibiotics used in the experiment, and might be due to a certain incompleteness of the lymphocytes' blast transformation in the conditions of the experiment.

With a view to the extensive practical utilization of antibiotics of the tetracycline group, and recently, of tetraoleane as well (2, 5, 8), we feel that their comparative study is not merely of theoretical, but also of practical interest.

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## СРАВНИТЕЛЬНЫЕ ИССЛЕДОВАНИЯ ДЕЙСТВИЯ ТЕТРАЦИКЛИНА И ТЕТРАОЛЕАНА НА КУЛЬТИРОВАННЫЕ ЛИМФОЦИТЫ ЧЕЛОВЕКА

*Л. Н. Василева, Г. Г. Николова*

РЕЗЮМЕ

Проведены сравнительные исследования над действием тетрациклина и тетраолеана в отношении бластной трансформации и пролиферативной активности лимфоцитов человека in vitro. В опытных постановках в течение 72 часов на клетки оказывалось воздействие 5 и 250 гаммами/мл указанных антибиотиков. Контроли не третированы тетрациклином и тетраолеаном.

Установлено, что тетраолеан подавляет слабее митотическое деление и бластную трансформацию лимфоцитов, в сравнении с тетрациклином при аналогических условиях эксплоатации. Полученные результаты вызывают не только теоретический, но и практический интерес в связи с ингибирующим действием антибиотиков в отношении иммунологической реактивности организмов.