NOTES ON SOME DISORDERS IN THE DIVISION OF PARACHLORANILINE-TREATED HUMAN LYMPHOCYTES IN VITRO

L. Vassileva, M. Kazakova

Parachloraniline is a substance endowed with a high toxicity, utilized in the curing of rubber, as well as in dye and pharmacologic productions. It represents an intermediate by-product in the manufacturing of selective action herbicides, and a disintegration product of some of the latter (M. Kazakova — 1968). This is the reason to study its action, in vitro, exerted on human lymphocytes subjected to short-term cultivation.

Material and Method

The research was conducted in vitro, on cultivated lymphocytes from five clinically healthy individuals, using the method of Moorhead et al, as modified by M. Tzoneva (1967).

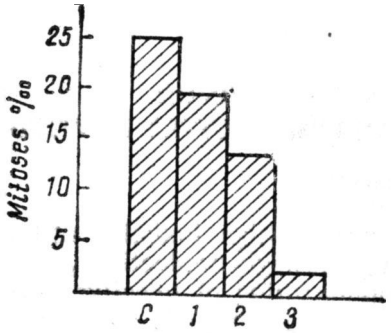
In the beginning of cultivation, parachloraniline was introduced at three different concentrations — 20, 100 and 500 gamma/ml. We proceed from MAC for parachloraniline equal to 0.01 mg/m^3 in the atmosphere, and LD_{50} for white mice — 228 mg/kg (M. Kazakova — 1968; V. Kondrashev — 1965). During the explantation period (72 hours), the control culture was not treated with parachloraniline. The mitotic and blastic index was determined per 100,000 cells. Along with that recordings were made of the amitotic division (through budding and fission) under analogical experimental conditions. Statistical elaboration of the data was made after the method of alternative analysis.

Results and Discussion

After following the mitotic activity of parachloraniline-treated lymphocytes, accordingly 19.32, 13.4 and 1.44 per cent mitoses were established at dose 20, 100 and 500 gamma/ml parachloraniline, against 24.9 per cent in the control culture (Diagr. 1) ($p>0.001$).

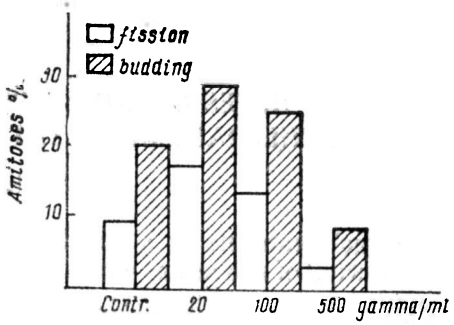
It can be seen from the results that the inhibitory effect of parachloraniline is clearly manifested already at the low concentration of the substance. Inhibition of the lymphocytes' blast transformation activity, assumed as an expression of the cells' preparation for mitotic division, is not recorded at 20 and 100 gamma/ml parachloraniline (88 per cent in either of the concentrations), whilst it is strongly pronounced at 500 gamma/ml (27 per cent against 89 per cent in the control, $p>0.001$). It is quite likely that the factor under study disturbs the transition from blast cells to division at rather low concentrations. The latter period coincides with G_2M of the explanted lymphocytes' life cycle. At high parachloraniline concentration (500 gamma/ml) an inhibition of either

of the processes occurs — blast transformation and mitotic division. However, here too, it should be stressed that the process of mitotic division of cells is more strongly affected, i. e. G_2M transition occurs earlier and exhibits heavier changes following treatment with the substance. Analogical results, pointing



Diagr. 1. Comparative representation of the results of mitotic activity of human lymphocytes treated in vitro with 20, 100 and 500 gamma/ml parachloraniline

c — controls; *1* — 20 gamma/ml; *2* — 100 gamma/ml; *3* — 500 gamma/ml



Diagr. 2. Values of amitoses through fission and budding in human lymphocytes treated with 20, 100 and 500 gamma/ml, as compared to control experiments

to a higher sensibility of the G_2M period in the life cycle of explanted lymphocytes, were established in a separate research using tetracycline treatment with varying duration and concentration (L. Vassileva — 1970).

Upon analysis of the various forms of amitoses in our experiment, it was established that unlike mitoses, a certain degree of stimulating effect of parachloraniline was present at the two lower concentrations. Moreover, 500 gamma/ml of the substance inhibited simultaneously the mitotic division, and the cells with already initiated amitosis. Although according to literature data a parallelism is observed in the tissue cultures in terms of intensity and dynamics of development of mitoses and amitoses through fission, with budding showing an inverse dependence (M. Tzoneva — 1970), we failed to establish a similar dependence in parachloraniline-treated lymphocyte cultures. Thus, in cultures untreated with parachloraniline 23.2 per cent budding cells were found, whilst among those cultivated with 20 and 100 gamma/ml parachloraniline their number augmented accordingly to 30.1 and 25.7‰ ($p>0.05$). Since the latter phenomenon was recorded simultaneously with a ceratin degree of inhibition of the mitotic division in analogical experimental conditions, it is quite possible to be considered as a manifestation of a compensatory ability of the cells to proliferate. On the other hand, an increase in the cells with more than one bud was also noted which, in turn, leads to a total increase in the number of buds to 38.42 and 28.72‰ (at 20 and 100 gamma/ml parachloraniline) against 25.2‰ in the control ($p>0.1$). In lymphocytes treated with 50 gamma/ml parachloraniline we found a simultaneous reduction of the total number of budding cells, and of the cells with one, two and more buds as well (7.7 budding cells and a total of 9.8‰ buds, $p>0.001$). On the other hand, an increase in the quantity of cells reproduced through fission was likewise observed among lymphocytes treated with 20 and 100 gamma/ml (from 9.96 in the control to 17.28 and 14.18‰ resp., $p>0.001$) (Diagr. 2).

The stimulating parachloraniline effect on the amitotic division of lymphocytes is manifested, under the experimental conditions described, both in terms of budding and fission. On its part, the high parachloraniline concentration inhibits simultaneously either of the processes, with fission being established in 3‰ of the cells. To assay the parachloraniline effect on the division processes in human lymphocytes, treated in vitro with various concentrations of the substance, we recorded and made a general comparison of the data from the two basic types of division (mitotic and amitotic one). In the control setting of the experiment the lymphocytes undergoing division amount to 60.06‰, at 20 gamma/ml parachloraniline — 70.02‰, and at 100 and 500 gamma/ml — 56.3 and 13.52‰ respectively ($p > 0.001$). It is evident from the data submitted that the lowest concentration of the substance used exerts an overall stimulating effect on the division of cells, mainly at the expense of amitoses. Since it is a well known fact that the cells may occasionally undergo division through amitosis whenever a change in their physiological state occurs, while in tissue cultures division through amitosis in completely vital cells has been also observed, it is quite possible that at 20 gamma/ml parachloraniline concentration the lymphocytes respond with division through fission and budding because of the inhibition of mitotic activity. According to M. Tzoneva (1970) this particular type of cultivated lymphocytes' proliferation should be interpreted as a probable compensatory mechanism in the production of cell populations relative to the active antigen or allergen without interrupting the cell function and antibody production. Parachloraniline at 100 gamma/ml concentration affects in a different way the two types of division — it inhibits mitoses and slightly stimulates amitoses, but on the whole, the dividing activity of the treated cells is inhibited. Lymphocytes treated with 500 gamma/ml parachloraniline are with impaired ability to accomplish division, regardless of its type. Hence, parachloraniline proves a factor which upon getting in contact with cells, in vitro, alters their capacity for plastic transformation and proliferation. The possibility of cultivated lymphocytes for mitosis is strongly inhibited, and for amitosis — rather slightly. The increase in parachloraniline concentration enhances its inhibitory effect.

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О НЕКОТОРЫХ НАРУШЕНИЯХ ДЕЛЕНИЯ ЛИМФОЦИТОВ ЧЕЛОВЕКА ПОД ВОЗДЕЙСТВИЕМ ПАРАХЛОРАНИЛИНА ИН ВИТРО

Л. Василева, М. Казакова

РЕЗЮМЕ

Изучено действие 20, 100 и 500 гамм/мл парахлоранилина на деление (митотическое и амитотическое) культивированных лимфоцитов человека.

Самая меньшая из примененных концентраций парахлоранилина оказывает стимулирующее воздействие на клеточное деление, преимущественно за счет амитозов в сравнении с контрольной, не подвергавшейся воздействию парахлоранилина культурой. При применении 100 гамм/мл парахлоранидина слабо ингибируются митозы и слегка стимулируются амитозы, при общем угнетении деления клеток, подвергающихся этому воздействию. Концентрация парахлоранилина в 500 гамм/мл сильно угнетает митотическое и слабее амитотическое деление лимфоцитов.