Bulgarian Academy of Sciences

Acta cytobiologica et morphologica, 2
Sofia • 1992

Cell Biology

Immunomodulating influence of cyclophosphamide and biocarbazine on antitumour immunity of hybridoma bearing mice

Tz. Mihova, D. Nikolova, I. Goranov**

Department of Pathology, Medical Academy, Sofia
** Institute of Cell Biology and Morphology, Bulgarian Academy of Sciences, Sofia*

A great number of experimental data show that neoplastic growth causes an activation of T-suppressor cells, which does not permit the development of an effective antitumour immune response [4, 8]. On the other hand, the precursors of the T-suppressor cells show a much higher sensitivity to cyclophosphamide (CY) in comparison with the other T-subpopulations and B-cells [5, 6]. T-suppressor cells can be eliminated with relatively low single doses of CY-20 mg/kg – to which the other cells are resistant [7].

Present investigations is an attempt to study the influence of CY and biocarbazine (BC) on the immune response of hybridoma bearing mice.

Key words: antitumour immunity, hybridoma bearing mice, cyclophosphamide (CY), biocarbazine (BC), hybridoma (H).

Material and methods

Animals: BaLb/c mice (240), 3 months old, males and females.
Experimental scheme: The cytostatics have been applied interperitonelly in single doses of 20, 50, 100 and 200 mg/kg 48 hours before tumour transplantation. Cell suspension (10^6 tumour cells/0,1 ml), about 96% vitality has been injected subcutaneously in the abdominal area to each experimental animal. The control group has been injected with tumour cells only.
Animals examination: The latent period (in days) – from implantation to the appearence of a palpable tumour and percentage of "tumour bearing" has been followed.

Tumour growth inhibition test (TGIT) in the percent: *Wc* – means weight of the tumour in the control group on mg; *We* – means weight in experimental animals: $TGIT = \frac{Wc - We}{Wc} \times 100$.

Immunological tests: Quantitative indicate of plaque-forming cells (PFC) in spleen [3] and PFC/10^8 cells.

Indicate of delayed type hypersensitivity on method of Cluman, modified of Bratanov et al. [1].

The histological slides from tumours have been done with hematoxylin and eosin, PAS (Periodic acid-Schiff)-reaction, Gomori and Mallory.

The statistical study has been performed with *t*-criteria of Student-Fisher.

Results and discussion

"Tumour bearing" groups from 90% in control animals to 24% in the pretreated with 20 mg/kg BC and to 38% – with CY animals. This percent is lower with 50 mg/kg dose – for BC is 12% and 15% for CY. Percentage of "tumour bearing" with the highest doses of both cytostatics reaching 40%, but in the animals with 100 mg/kg CY it is lower – 33% (Fig. 1).

The latent period between tumour inoculation and the appearance of a palpable tumour is maximally prolonged by 50 mg/kg dose BC (25 ± 1 day). The latent period is shortened by increasing the dose to 200 mg/kg and for BC it is equal to this in the control group, while for CY it is with two days longer (Fig. 2).

TGIT is highest for both cytostatics with doses of 50 and 100 mg/kg in contrast to the dose of 200 mg/kg, where the influence of the tumour inhibition is minimal (Fig. 3).

Histologically – the tumours have well marked stroma with a reduction in the number of tumour cells. Doses of 50 mg/kg (Fig. 4) and 100 mg/kg BC and CY cause wide field of necrosis.

Data from PFC – test in the spleen disclose, that antibody cells are more markedly increased in animals, prior to treatment with the lower doses of 20 mg and 50 mg/kg BC and CY. Primary humoral immune response to sheep red blood cells (SRBC) is reduced with dose of 100 mg/kg in comparison to the control group (bearing the tumour but not treated). This inhibition is more strongly expressed with the highest dose of 200 mg/kg BC. The statistical study shows a significant difference between the different groups, treated with the same cytostatic. By injecting animals with 20 mg/kg CY the number of PFC in spleen is higher than that of PFC in animals with 100 mg/kg, as is the same for animals treated with 200 mg/kg ($P < 0{,}001$). Similar results have been obtained by PFC/10^8 spleen cells and this indicates reliable difference between the group with 50 mg/kg CY, which shows a higher number of PFC, than this treated with 100 mg/kg ($P < 0{,}05$) and 200 mg/kg ($P < 0{,}001$). In animals treated with lower doses BC the number of PFC is significantly greater than in those treated with high doses. There is statistically a high difference in the number of PFC between the animals with 20 mg/kg BC compared to PFC – with 100 mg/kg and 200 mg/kg (Fig. 5). The number of PFC with a dose of 50 mg/kg BC is higher than that with doses of 100 and 200 mg/kg BC ($P < 0{,}001$).

Micrometric measurement on the induction of contact dermatitis in the control animals and the pretreated mice with BC and CY are introduced in Fig. 6.

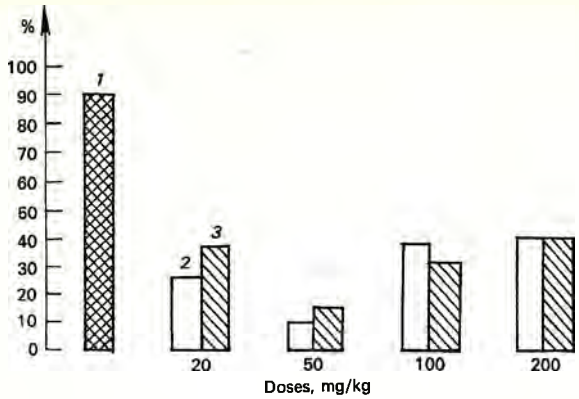

Fig. 1. Effect of cyclophosphamide and biocarbazine on the "tumour bearing" of hybridoma (%)
1 – H; *2* – H+BC; *3* – H+CY

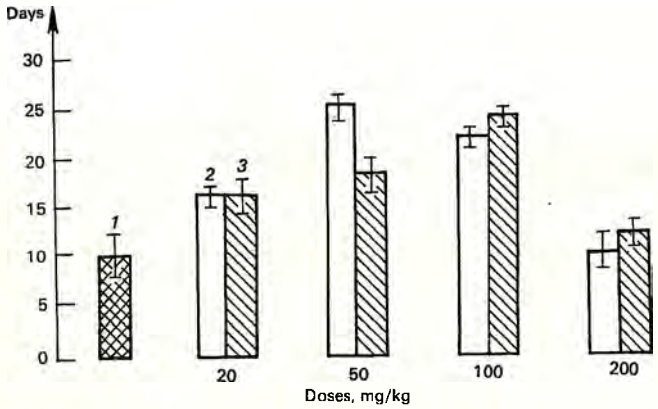

Fig. 2. Effect of cyclophosphamide and biocarbazine on the latent period of hybridoma (days)
1 – H; *2* – H+BC; *3* – H+CY

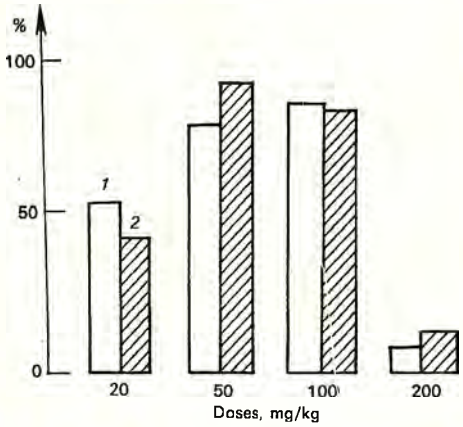

Fig. 3. Effect of cyclophosphamide and biocarbazine on the inhibition of tumour growth (%)
1 – CY; *2* – BC

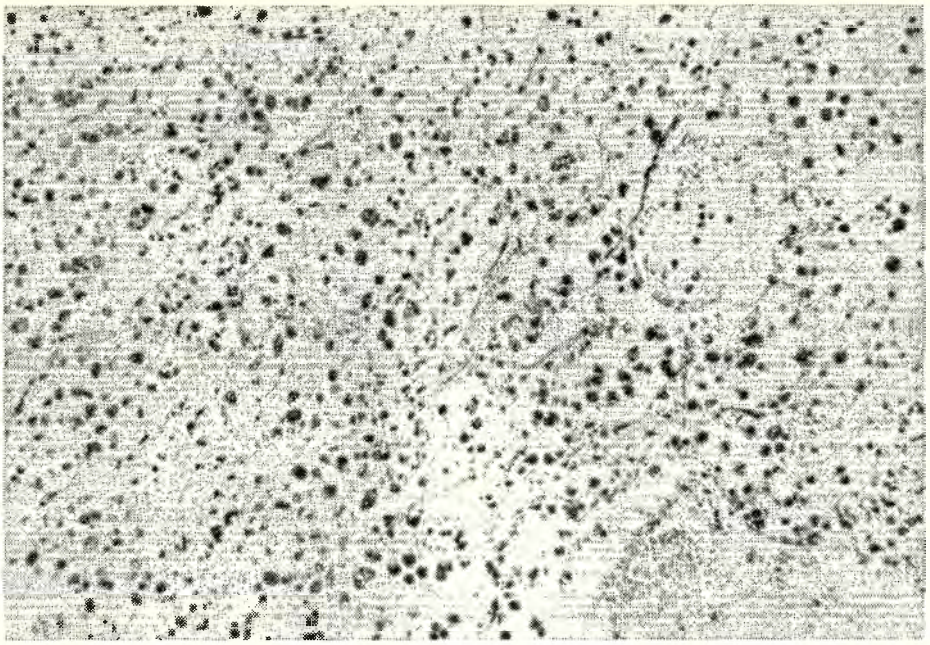

Fig. 4. Hybridoma histological picture demonstrates a reduction of tumour cell number. Necrotic alteration are observed (after a 50 mg/kg BC pretreatment). × 120

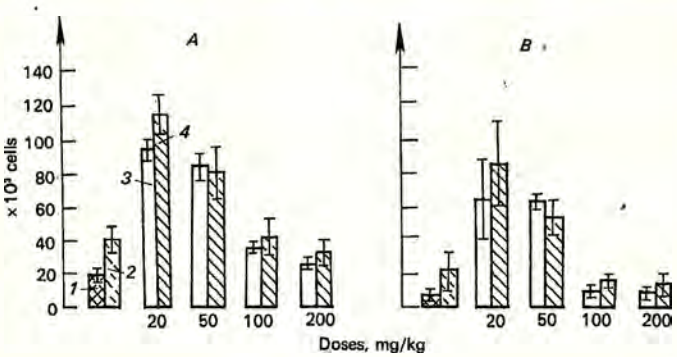

Fig. 5. Influence of cyclophosphamide and biocarbazine on the plaque-forming cells by hybridoma bearing mice

A – PFC per spleen; *B* – PFC per 10^5 spleen cells
1 – control (H); *2* – control (SRBC); *3* – BC+H; *4* – CY+H

It is obvious that all BC and CY doses have an enhancing effect on the delayed type cell-mediated reaction in comparison to the control group. This supports the statistical evaluation for the dose 20 and 50 mg/kg BC and CY and by 200 mg/kg CY. The statistical comparative analysis shows that there is a reliable difference in the values of the low and high doses of the cytostatic (for BC 20 mg/kg with $P<0{,}01$) to 100 mg/kg BC and with $P<0{,}05$ to 200 mg/kg BC. Even with the

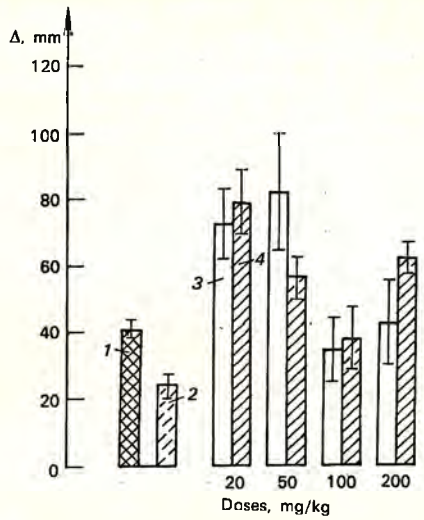

Fig. 6. Effect of cyclophosphamide and biocarbazine on the induction of contact dermatitis by hybridoma bearing mice
1 – control (DNFB); *2* – control (H); *3* – BC; *4* – CY

highest BC and CY doses the cell-mediated immune reaction is more strongly expressed in comparison with the hybridoma control group.

The cytostatics CY and BC are metabolized and are eliminated from organism in the first 5-6 hours. In the present study BC and CY are applied 48 hours before tumour cell inoculation. This totally excludes the immediate influence on tumour cells of the applied cytostatics. Our data for the inhibition effect of CY and BC on the "tumour bearing" and the prolongation of the latent period of hybridoma suggest and induction of the immune response. The low doses – 20 and 50 mg/kg CY and BC, respectively, are more effective, as shown by our results. Similar findings have been reported by Schwartz et al. [7], Capetola et al. [2] for 20 mg/kg CY. They permit the assumption that T-suppressor cell precursors have different sensitivity to different doses CY.

Our results are especially significant for the influence of low doses CY and BC on the primary humoral immune response and the reaction of contact dermatitis in hybridoma bearing mice. These doses of CY and BC possibly inhibit T-suppressor cell activity and in such a way the T-helpers are free from the comparative influence. This gives a possibility for proliferation of cytotoxic T-lymphocytes and B-cells. All this leads to an enhancement the antibody synthesis. However, the high BC and CY doses stimulate only the cell-mediated immune response, while PFC are lowered. This fad is probably due to their B-cell-cytotoxicity.

References

1. Bratanov, M., S. Dimitrova, A. Neronov, I. Goranov. Experimental induction contact dermatitis – morphometricity and micrometricity investigation. – Comp. Rend. Acad. Bulg. Sci., 36, 1981, 385-389.

2. Capetola, J., D. Argertieri, R. Culver. The effect of Concavalin A., Cyclophosphamide and Splenectomy in DTH to SRBC in mice. – Fed. Procc., 39, 1980, No 3, 479-480.
3. Dresser, D., G. Greaves. Assays for antibody producing cell. – Cell Immunol., 2, 1973, Ch. 72.
4. Gerlier, D., C. Rabourdin-Combe. Antigen processing from cell biology to molecular interactions. – Immunology Today, 10, 1989, No 1, 3-5.
5. Gill, H., Y. Liew. Effect of cyclophosphamide on the T-suppressor cells for DTH to SRBC in mice. – Eur. J. Immunol., 8, 1978, No 3, 172-176.
6. Goranov, I., D. Nikolova, M. Dimitrova. Effect of cyclophosphamide sensitive of guinea pigs to experimental nephritis. – Acta morphol., 4, 1983, 33-40.
7. Schwartz, A., S. Orbach-Arbouys, P. Askenase, R. Gershon. Cyclophosphamide sensitive T-cells as modulators of the immune response. – Fed. Proc., 35, 1976, 571-572.
8. Smith, S., C. Terminelli, C. Kipilman, Y. Smith. Comparative effects of Azatioprine, Cyclophosphamide and Frentizole on cellular immunity in mice. – J. Immunopharmacol., 3, 1981, No 2, 133-170.