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ARTICLE

A Trace Element Preparation Increases Antitumor Activity in Mice

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A trace element preparation (Béres Drops Plus, BDP) produced immunomodulatory effects in previous in vitro and in vivo experiments. Here, C57B1/6 inbred mice were transplanted with either Lewis lung tumor or with B16 melanoma. BDP was given intraperitoneally a. before transplantation; b. after transplantation or c. after the removal of the primary tumor. As a result, BDP pretreatment could slow down the tumor progression by decreasing the number and the volume of metastases as well as the proportion of mice with metastases without influencing the growth of the primary tumors. Furthermore, BDP treatment improved the immunological activity of the tumor-bearing host, too. These preliminary data suggest that the parenteral administration of the practically non-toxic BDP could help to control tumor progression in experimental models. (Pathology Oncology Research Vol 3, No 1, 34–37, 1997)

Key words: trace elements, antimetastatic, immunomodulation

Introduction

The growth and progression of malignant tumors clearly indicate the failure of host defense. Therefore, any approach, which can increase or promise the improvement of host capacity to control malignancies, deserves attention. There are continuous attempts to take advantage of the biological activity of natural products, including trace elements. Béres Drops Plus (BDP), a composition of different inorganic and organic compounds (*Table 1*), produced immunomodulatory effects in previous experiments.[1-4] It was assumed that the trace elements activate the phagocytic cells of the RES weakened by X-ray irradiation.[1] In another experiment, BDP increased the function of phagocytes in the RES of ethanol-intoxicated animals.[2] BDP increased the *in vitro* production of interleukin-6 (IL-6) in human monocytes and glial cells.[3] Corticosteroid induced inhibition of IL-6 production could be reversed by BDP, which has been regarded as an immunostimulatory effect.[3] Since tumor progression in almost all instances is accompanied by a failed immunological defense, here, we used two metastatizing murine tumors to check the potential antitumor value of BDP, *in vivo*.

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Abbreviations: BDP: Béres Drops Plus® 3LL: Lewis lung tumor; MM: B16 melanoma; AFC: antibody forming cells; LGL: large granular lymphocytes; RES: reticuloendothelial system; TSF: thymic serum factor; NK cells: natural killer cells.

Materials and methods

Tumors and mice

Altogether 286 C57B1/6 inbred mice (from the breeding stock of A A Bogomolets Inst Physiol, Natl Acad Ukraine, Kiev) of 18-25 g body weight, and of both sexes were transplanted either with Lewis lung tumor (3LL) or with B16 melanoma (MM). Tumor cell suspension (2.0-2.5x 10^5 tumor cells per mice) was given subcutaneously into the femoral part of the hind leg (in a volume of 0.1 ml) or into the foot pad (in 0.05 ml). The mice were kept in conventional cages, on standard mouse pellet and on tap water *ad libitum*.

Table 1. **Composition of trace element mixture BDP^R**

Inorganic components					
Iron	2.0	Zinc	1.1	Sodium	0.64
Magnesium	0.4	Manganese	0.31	Potassium	0.28
Copper	0.25	Molybdenum	0.19	Vanadium	0.12
Nickel	0.11	Boron	0.1	Fluorine	0.09
Chlorine	0.03	Cobalt	0.025		
Organic components					
Glycerol	6.0			EDTA	2.4
Glycine	2.3			L–(+)– tartaric acid	1.6
Succinic acid	0.5			L–(+)–ascorbic acid	0.3

Data are in mg/ml; solvent: distilled water. Trace elements are present in partly complexed ionic forms.

Treatments

In each treatment schedule, the single dose of Béres Drops Plus (BDP; composition see in *Table 1*) contained 0.7 µl of undiluted stock BDP in 100 µl distilled water and was injected intraperitoneally daily. This dose was based on our previous experiments where thymic serum activity was efficiently induced in thymectomized mice.[4] The control – tumorous but non-treated – mice received distilled water. Healthy mice were included as "intacts".

The following experiments were performed:

Exp.1. BDP was given after transplantation: treatment started on the transplantation day, followed daily for 5 days; and this 5-day course was repeated leaving a 3 day interval between the courses.

Exp. 2. BDP was given before transplantation for 5 days.

Exp. 3. BDP was given after the removal of the primary tumor: tumor cells were transplanted into the foot pad; 7 days later the hind leg was amputated (under anesthesia with hexenal, i.p., 80 µg/g body weight); treatment with BDP started on the day after amputation and continued for 5 days.

Table 2. **Effects of different BDP treatments on the body weight as well as on the weight of the lymphoid organs of mice C57Bl/6 with 3LL**

Groups of mice	*Number of mice*	*Body weight (g)*	*Thymus (mg)*	*Spleen (mg)*
Exp 1				
Intact	8	19.80±1.67	44.50±5.70	163.00±33.12
Control	13	18.60±0.40	24.50±8.70	116.90±15.70
BDP treated	14	19.20±0.54	10.00±1.88	130.30±18.96
Exp 2				
Intact	8	22.35±2.11	36.50±3.17	112.75±9.01
Control	8	23.30±1.36	31.90±5.09	139.25±11.9
BDP-treated	9	22.40±6.70	39.20±4.38	203.60^{x,xx}±11.40
Exp 3				
Intact	8	21.13±1.70	40.50±3.61	137.90±9.62
3LL	6	19.60±1.40	35.40±9.60	133.80±13.7
3LL + AMP	10	22.90±0.83	48.90±7.60	168.30±7.60
3LL +AMP + BDP	10	20.50±0.60	52.10±6.50	127.80^{o}±12.80

Note: Significant difference ($p<0.05$) from indices in intact (^{x}), control (^{xx}) mice and in group 3LL+AMP (^{o});
AMP – amputated

Table 3. **Effect of BDP on primary humoral immune response in mice with 3LL**

Groups of mice	*Number of AFC per 10^6 splenocytes*	*Absolute content of AFC in spleen*	*Number of hemolysines (unit of optical density)*
Intact*	116.3 ± 24.5	5580.7 ± 1028.8	0.216 ± 0.012
Control*	107.5 ± 22.7	4823.4 ± 915.3	0.102 ± 0.028
Treated*	203.7 ± 38.1^x	16876.0 ± 3575.8^{x,xx}	0.322 ± 0.041^{x,xx}

Note: ^{x} – significant difference ($p<0.05$) from indices in intact mice
^{xx}– significant difference ($p<0.05$) from indices in mice of control group
* number of mice: intact (6), control (8) treated (6)

Evaluation

Parameters estimating antitumor activity – 1. life span; 2. body weight; or 3. irrespective of the treatment schedule the mice were sacrificed on the 22nd-24th posttransplantation day and the weight of thymus and spleen was measured, the number of lung metastases was counted under stereomicroscope, and the total volume of the metastases was calculated.

Parameters estimating immune status – The number of antibody forming cells (AFC) in the spleen was studied using the method of local hemolysis in gel[5] and by the hemolytic activity of splenocytes.[6] Mice were given heterologue erythrocytes (sheep erythrocytes were injected i.p. in a dose of 10^8 per mice) for 4 days before collecting the blood at the 24th posttransplantation day. The number of large granular lymphocytes (LGL) was also counted.[7] Thymic serum factor (TSF) was measured according to Bach et al.[8]

The data were statistically evaluated by Student's "t" test.

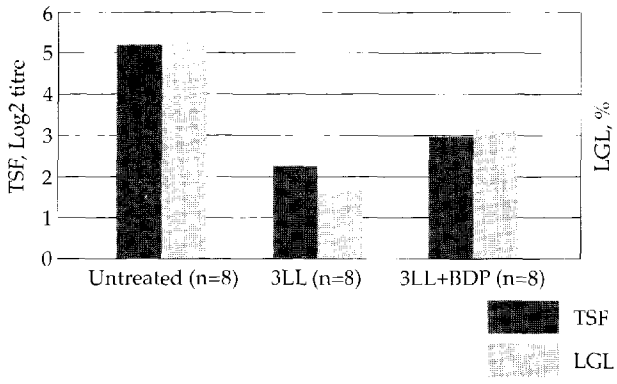

Figure 1. *The effect of BDP on TSF titre and on the quantity of LGL in C57B1/6 mice with 3LL*

Results

Effect of BDP on lymphoid organs in tumor bearing animals

The changes in the weight of the thymus and spleen in the tumor-bearing mice was considered as an indication on the antitumor activity of the host. The growth of 3LL tumor is usually accompanied by the loss of thymus weight. In Exp 1 BDP treatment decreased the thymus weight even further *(Table 2)*, without affecting the weight of the spleen. However, the pretreatment with BDP (Exp 2) produced an opposite effect: the weight of the spleen increased, but the weight of the thymus remained unchanged. In Exp 3, when BDP was given after the removal of the primary tumor, only the weight of the spleen decreased slightly ($p<0.05$).

Pretreatment with BDP (Exp 2) increased not only the weight of the spleen but also the number of AFCs among the splenocytes *(Table 3)*. The relative number of LGLs and the level of TSF decreased substantially in 3LL-bearing mice and this negative change was partly prevented by BDP pretreatment *(Fig. 1)*.

Effect of BDP on tumor growth and metastatization

BDP did not alter the growth of the primary 3LL tumor and the proportion of mice with lung metastases (Exp 1) was again similar: 17/20 without BDP; 11/14 with BDP. However, pretreatment with BDP (Exp 2) resulted in a slight decrease both in the number and volume of lung metastases.

Although the tendency was the same after amputation, and the differences were statistically significant, the number of metastases was too low to reach a biologically relavant conclusion. At the same time, the proportion of mice with metastases also decreased (5/8 without BDP, 3/9 with BDP) suggesting that BDP can at least delay metastasis formation. *(Table 4)*.

Table 4. **Effect of Béres Drops Plus on the growth and metastatization of 3LL and MM in C57B1/6 mice**

Groups (number of mice)	*Tumor weight (g)*	*Number of metastases mean ± S.D. (range)*	*Volume of metastases (mm^3)*
3LL			
Exp 1			
Control (20)[+]	3.43±0.27 (0.5-5.0)[++]	3.95±0.85 (1-14)	3.06±1.70 (0.06-28.54)
BDP-treated (14)	3.53±0.30 (1.0-5.0)	3.30±0.95 (1-11)	3.78±2.00 (0.06-21.12)
Exp 2			
Control (8)	2.77±0.53 (1.7-5.0)	8.0±4.22 (1-34)	10.64±2.80 (0.06-70.16)
BDP-treated (9)	2.92±0.34 (1.5-4.5)	4.56±1.23 (0-11)	4.99±2.34 (0.12-20.98)
Exp 3			
3LL (7)	1.60±0.15 (0.90-2.0)	11.60±3.31 (1-23)	36.4±15.2 (0.06-114.16)
3LL+AMP (10)	0	1.40±0.37 (0-6)	1.52±0.78 (0-8.2)
3LL+AMP+BDP (10)	0	0.54±0.15[x,xx] (0-3)	1.09±0.30 (0-8.0)
MM			
Control (16)	5.8±0.74 (2-8)	7.68±1.2 (0-27)	12.17±1.67 (0-64.94)
BDP-treated (12)[#]	5.3±0.49 (3.5-7)	3.16±0.89[xxx] (0-10)	3.57±1.66[xxx] (0-18.78)

Note: The volume (V) of metastatic colony was calculated: $V = 0.52\ d^3$, d = diameter

[+] variations of limits in parentheses

AMP – amputation

[#] BDP was given before transplantation: daily for 5 days

[x] significant difference ($p<0.05$) from indices in mice of group 3LL and control group

[xx] – significant difference ($p<0.05$) from indices in group 3LL + AMP

[xxx] – significant difference ($p<0.05$) from indices in mice of control group

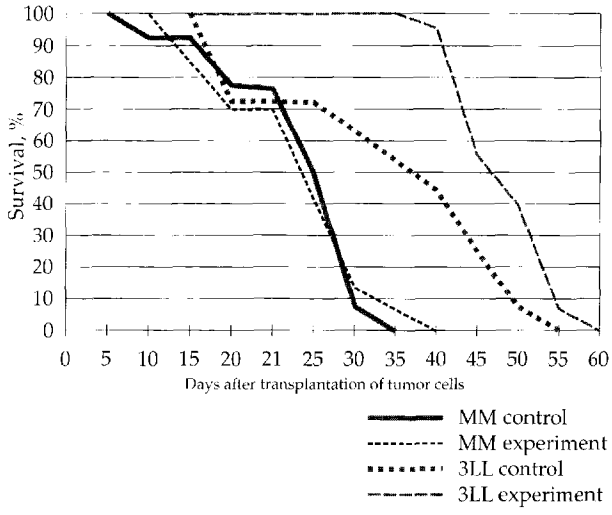

Figure 2. Survival curves of BDP-treated and non-treated tumor-bearing mice. Note the increased life span in BDP-treated 3LL bearing mice. BDP was given before transplantation (daily for 5 days).

In B16 melanoma, the BDP pretreatment did not change the weight of the primaries, but the number and volume of lung metastasis decreased ($p<0.05$) *(Table 4)*.

Since pretreatment with BDP caused some inhibitory effect on lung metastases (decrease in the number and volume of metastases, as well as the incidence of metastasis) the life span of mice was recorded. *Fig. 2* shows that there was no difference in the treated and untreated groups in case of B16 melanoma, however, the life span of 3LL bearing mice increased (ILS_{50} = 126%).

Discussion

Two sets of data proved the biological activity of BDP. The first is related to the improved immunological activity of the tumor-bearing host (considering the production of specific antibodies, large granular lymphocytes [presumably NK cells], as well as certain thymic functions). The second set of results indicate that, although BDP is not able to influence the growth of the primary tumors, given either before or after tumor transplantation, it can slow down the tumor progression by decreasing the number and volume of metastases. Given as pretreatment the proportion of mice with metastases decreased, as well. It would be too early to suggest a link between the two sets of data, but it can not be ruled out.

These preliminary results suggest that the practically non-toxic BDP could be valuable in an attempt to influence tumor progression. Obviously, BDP alone has limited value, but in combination, could be an important supportive component of strategies for cancer management.

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