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Chemotherapy and Immunotherapy of Diabetic and Non-Diabetic Mice Bearing Fibrosarcoma

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Abstract—*Cells of methylcholanthrene-induced fibrosarcoma were transplanted into normal mice, diabetic mice and into diabetic mice treated with insulin. The tumour growth rate and the effects of the therapy with drugs (cyclophosphamide, methotrexate) and* Corynebacterium parvum *were followed. Tumours grew more slowly in diabetic than in non-diabetic or insulin-treated diabetic hosts. Chemotherapy prolonged the survival equally in all experimental groups of mice, but immunotherapy was effective only in non-diabetic and insulin-treated diabetic fibrosarcoma-bearers. If the malignant cells from the diabetic donor were transplanted into secondary diabetic recipient, the tumour growth rate was again the same as in nondiabetic hosts. Chemotherapy was even more effective in these tumour-bearers, but the immunotherapy did not show any antimalignant effect. This discrepancy was attributed to different modes of action of cytostatic drugs and of bacterial immunostimulant.*

INTRODUCTION

IT HAS been observed many times that transplantable animal tumours grow more slowly in diabetic than in non-diabetic hosts [1–3]. This retarded growth rate could be attributed to the lack of insulin in diabetic tumour-bearers, since proliferation of malignant as well as normal tissues needs this hormone [4, 5]. However, at least some tumours can 'adapt' themselves to the diabetic environment. Namely, we have shown that Ehrlich tumour and an aplastic mammary carcinoma, if transplanted from diabetic to secondary diabetic recipients, will grow again as fast as in non-diabetic hosts [5, 6]. All that clearly shows that the growth characteristics of experimental tumours change in diabetic organism. This fact might influence the effectiveness of antimalignant treatment of diabetic tumour-bearers. The question is not only a theoretical one, since malignant tumours appear and have to be treated in diabetic patients as well [7, 8].

To get some information about the possibilities of treating tumour-bearing diabetic animals, we have applied standard chemotherapy and immunotherapy in diabetic mice with methylcholanthrene-induced fibrosarcoma. Some groups of animals received simultaneously insulin to manage their diabetes. Here we report the data obtained.

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MATERIALS AND METHODS

Female CBA/H mice from the "Ruder Bošković" breeding colony were used when 5 months old. They were kept not more than five to a plastic cage and were provided with food and water *ad libitum*.

Experimental diabetes was induced by single i.v. injection of alloxan (Merck-Darmstadt) at 75 mg/kg or by injection of streptozotocin (Calbiochem, San Diego) at 225 mg/kg.

Fibrosarcoma, induced 2 yr ago in a CBA/H mouse by methylcholanthrene (0.1 mg in 0.1 ml of olive oil injected s.c.), has been maintained by serial transplantations. In the experiments described herein the 17th to 19th tumour transplantation generations were used. Mice received 10^6 viable malignant cells i.m. into the right leg.

Chemotherapy consisted of a single i.p. injection of cyclophosphamide (Endoxan, Bosnalijek-Sarajevo) at the dose of 200 mg/kg. or of methotrexate (Zdravlje-Leskovac) at the dose of 50 mg/kg. The drugs were administered on the 3rd day following tumour transplantation.

For immunotherapy, the suspension of *Corynebacterium parvum* (Wellcome, Beckenham) was used. Animals received i.v. 300 μg of *C. parvum* dissolved in 0.2 ml of Hanks solution 3 days after fibrosarcoma transplantation.

For treatment of diabetes, 4 i.u. of crystalline insulin (Pliva, Zagreb) was injected s.c. into diabetic mice each day starting 1 day after alloxan or streptozotocin injection.

The level of blood glucose was determined by the method of Hyvärinen and Nikkila [9].

The fibrosarcoma growth rate was followed by measuring the diameter of tumour nodule with caliper several times during the experiment. Three opposite diameters were determined and the mean of these values was taken as a measure of the tumour size.

The results are presented as the mean ±standard deviation (S.D.), and the significance of the differences observed were checked by the Student's *t*-test. Probabilities of significance above 95% ($P<0.05$) were accepted as significant.

RESULTS

Growth of fibrosarcoma in diabetic mice

In the first experiment malignant cells were transplanted into normal and into diabetic mice 7 days after injection of alloxan. Figure 1 shows that the growth rate of fibrosarcoma, as judged by measuring the diameter of the tumour nodule, was much slower in diabetic than in non-diabetic hosts. However, in the insulin-treated diabetic mice, as well as in the diabetic mice receiving tumours from the diabetic donors, the fibrosarcoma growth rate was the same as in non-diabetic animals. In accordance with slower tumour enlargement in diabetic hosts the survival of these animals was significantly longer than the survival of animals in other experimental groups.

In the second experiment the diabetes was induced *after* tumour transplantation. The growth curves and the survival of mice injected with alloxan or streptozotocin when the tumours were approximately 5–7 mm in diameter are shown in Fig. 2, and the effects

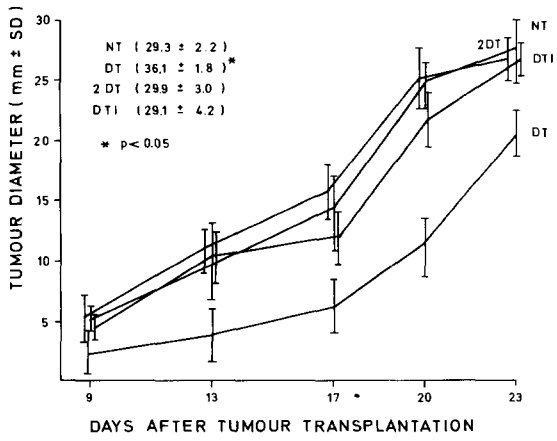

Fig. 1. Fibrosarcoma growth curves and the survival times (in parentheses) of the following groups of mice: non-diabetic (NT), diabetic (DT), diabetic treated with insulin (DTI), diabetic receiving fibrosarcoma from the diabetic donor (2DT). Seven to nine mice per group. Bars indicate ±S.D. from the mean.

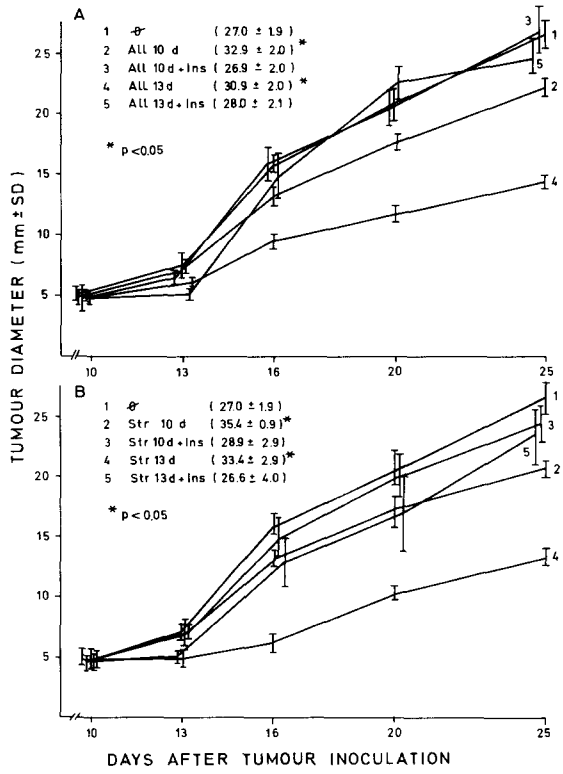

Fig. 2. Fibrosarcoma growth curves and the survival times (in parentheses) of non-diabetic and diabetic tumour-bearers. Diabetes was induced by alloxan (All—part A) or streptozotocin (Str—part B) injected 10 days *(curves 2 and 3) or* 13 days *(curves 4 and 5) after tumour transplantation. Insulin (Ins) was administered daily starting* 1 day *after diabetes induction. Seven to nine mice per group. Bars indicate* ±S.D. *from the mean.*

of the diabetes induced when the tumours were 14–16 mm in diameter are shown in Fig. 3. Diabetes induced when the tumours were small retarded the malignant growth evidently and prolonged the survival of these animals significantly (Figs. 2A and B, curves 2 and 4). On the other hand, in the diabetic tumour-bearers treated with insulin the growth rate and the survival were practically the same as in non-diabetic animals with fibrosarcoma (Figs. 2A and B, curves 3 and 5).

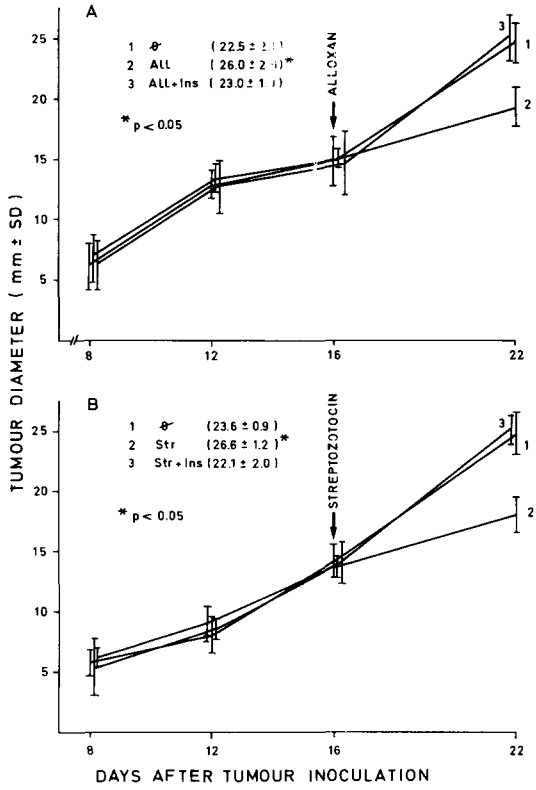

Fig. 3. Fibrosarcoma growth curves and the survival times (in parentheses) of mice injected with alloxan (part A) or streptozotocin (part B) 16 days after fibrosarcoma transplantation. Seven to nine mice per group. Bars indicate ±S.D. *from the mean.*

The same was observed in the tumour-bearing mice injected with alloxan or streptozotocin as late as 16 days after tumour transplantation, when the fibrosarcoma nodules were approximately 15 mm in diameter. In these mice, again, induction of diabetes retarded the malignant growth and prolonged the survival, while the antidiabetic therapy with insulin "normalized" the fibrosarcoma growth and the survival times (Figs. 3A and B).

Treatment of fibrosarcoma in diabetic mice

Standard cytostatic drugs—cyclophosphamide and methotrexate—as well as the immunostimulating agent *C. parvum*, were used to treat diabetic mice with fibrosarcoma. The effects were compared with those in non-diabetic and in insulin-treated diabetic tumour-bearers. As visible from the Table 1, chemotherapy significantly prolonged the survival of all groups of animals (non-diabetic, diabetic, and diabetic treated with insulin). However, immunotherapy was only effective in non-diabetic and insulin-treated diabetic mice whereas in diabetic tumour-bearers it was not effective.

In the repeated experiment, one group of diabetic animals received malignant cells from diabetic donor ('adapted' tumours with the same growth rate as in non-diabetic hosts). In these mice chemotherapy also exerted an evident antimalignant effect. If the percentage prolongations of the survival times are compared, it even appears that the cytostatics exerted stronger anti-tumour effect in the diabetic mice bearing 'adapted' fibrosarcoma than in the animals bearing tumours from non-diabetic donors. Immunotherapy, however, did not influence the survival of diabetic mice with 'adapted' tumours, although it was effective in non-diabetic tumour-bearers (Table 2).

Table 1. Effect of chemotherapy and immunotherapy on the survival of non-diabetic and diabetic mice bearing fibrosarcoma

	Tumour-bearers					
	Non-diabetic		Diabetic		Diabetic treated with insulin	
Treatment*	Survival†	%	Survival	%	Survival	%
None	30.1 ± 2.2	100	36.3 ± 1.9	100	31.2 ± 2.0	100
Cyclophosphamide	35.3 ± 1.9‡	117	45.4 ± 2.4‡	125	36.0 ± 2.9‡	115
Methotrexate	34.5 ± 0.9‡	115	43.2 ± 4.9‡	119	35.1 ± 0.9‡	113
C. parvum	36.6 ± 1.8‡	122	35.1 ± 4.5	97	37.1 ± 2.5‡	119

*One injection 3 days after tumour transplantation.
†Mean survival time of 6–8 individual values (days ± S.D.).
‡$P < 0.05$ in comparison to non-treated mice.

Table 2. Effect of chemotherapy and immunotherapy on the survival of diabetic mice bearing fibrosarcoma from normal and diabetic donors

	Tumour-bearers					
	Non-diabetic		Diabetic		Diabetic with 'adapted' tumours	
Treatment*	Survival†	%	Survival	%	Survival	%
None	28.1 ± 0.9	100	35.0 ± 2.8	100	27.4 ± 2.2	100
Cyclophosphamide	34.4 ± 1.9‡	122	39.0 ± 2.4‡	112	39.4 ± 4.5‡	144
Methotrexate	35.1 ± 2.9‡	125	38.8 ± 1.4‡	111	40.1 ± 5.0‡	146
C. parvum	36.0 ± 3.9‡	128	34.0 ± 2.7	97	29.1 ± 3.6	106

*One injection 3 days after tumour transplantation.
†Mean survival time of 6–8 individual values (days ± S.D.).
‡$P < 0.05$ in comparison to non-treated mice.

DISCUSSION

The data on the inhibited growth rate of methylcholanthrene-induced fibrosarcoma in diabetic mice reported here, accord with our previous findings that transplanted Ehrlich tumour and aplastic carcinoma grow more slowly in diabetic than in non-diabetic recipients [5, 6]. This could be connected with the shortage of insulin, since the growth rate of transplanted fibrosarcoma is 'normal' in diabetic recipients treated with insulin. The phenomenon of 'adaptation' of the malignant tissue has been already described and discussed [5, 6], and here some comments will be given on the data obtained with the antimalignant therapy.

Cyclophosphamide and methotrexate are equally effective in the treatment of the tumours growing in diabetic as well as in non-diabetic hosts. Immunotherapy, on the other hand, is not effective in the diabetic tumour-bearers. This discrepancy could be explained by the different mode of action of these two therapeutic procedures. Both the drugs used directly destroy proliferating malignant cells. Because the tumour growth fractions i.e., the percentages of proliferating malignant cells (but not the absolute numbers) are very similar in the diabetic and in non-diabetic hosts (our data, not published), it is conceivable that the cytostatic drugs have a similar antimalignant effect in diabetic and in non-diabetic tumour-bearers. But, in diabetic recipients of 'adapted' fibrosarcoma cells, the growth fraction (the proliferative pool) of malignant cells is much greater (our data), and this may probably account for a stronger antimalignant effect of chemotherapy. Thus, if the sensitivity of the 'adapted' tumours to the chemotherapy changes, it would rather increase than decrease.

Treatment with *C. parvum* presumably exerts its effect indirectly through activation of the host's immune system in general, and of macrophages in particular [10]. Because diabetic animals have diminished numbers of lymphocytes and macrophages [11, 12], we suggest that therapy with *C. parvum* cannot activate enough cells to combat the tumour. If so, the treatment with bacterial immunostimulator cannot suppress the growth rate of fibrosarcoma in diabetic hosts. In diabetic mice treated with insulin, however, the number of lymphocytes and the immune reactivity are normal [12], and this could explain why the therapy with *C. parvum* in diabetic tumour-bearers treated with insulin was effective, like that in non-diabetic tumour-bearers.

Diabetes mellitus in humans, especially the juvenile type, is often connected with depressed immune functions [13, 14]. The immune reactivity of these patients could be restored if they are treated with insulin [14]. Therefore, if our experimental data could be extrapolated to humans, our conclusion would be the following: in patients suffering from diabetes and malignant disease, immunotherapy should be introduced only when the diabetes is well controlled by insulin administration.

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