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Growth of a Methylcholanthrene-Induced Fibrosarcoma in Mice with Diabetes Mellitus

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Abstract—*Growth of a methylcholanthrene-induced fibrosarcoma was retarded in diabetic mice. Tumors maintained in diabetic mice grow faster after each subsequent transplantation into diabetic mice. The observed proliferation enhancement of fibrosarcoma maintained in diabetic mice is caused by insulin synthesis, apparently by the tumor cells themselves. Hypoglycemia accompanied intramuscular growth of fibrosarcoma. In diabetic hosts, the tumor decreased the blood glucose level almost to the level seen in non-diabetic mice.*

INTRODUCTION

MANY tumors are accompanied by hypoglycemia caused apparently by their intensive glucose consumption, production of insulin or an 'insulin-like' substance by the tumor, deficient hepatic gluconeogenesis of release of an 'insulinoid' from the tumor [1–7]. The metabolism of glucose in tumor cells differs from that in normal cells [4, 8]. Due to intensive glycolysis, tumor cells consume more glucose than cells of normal tissue [9–11]. This excessive gylcolysis has been seen in many tumors and it can lower the level of the blood sugar [1–7]. The greater permeability of the membrane of some tumor cells (often virus-induced) for glucose, or enhanced transport of glucose into tumor cells, might also be a factor in inducing hypoglycemia in malignancy [12–14].

In diabetic animals a large number of tumors grow much slower than in normal animals. Hypoinsulinemia might be an important reason for this impaired growth [15–22]. Indeed, insulin therapy of diabetic mice bearing thymoma, caused faster development of the tumor [6].

The aim of these experiments was, firstly, to follow the growth of methylcholanthrene-induced fibrosarcoma in diabetic mice. Secondly, by transplanting tumor cells from primary diabetic recipients into secondary diabetic recipients, it was tested whether the tumor habituates itself to the diabetic condition. Third, tumors maintained in diabetic mice, grew faster after each subsequent transplantation into diabetic mice. Here we present evidence that these effects are caused by the appearance of insulin in diabetic animals and that fibrosarcoma cells cultivated in diabetic mice during several generations release insulin.

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MATERIALS AND METHODS

Mice

Male CBA mice, 6 months old, were used. The mice were kept in plastic cages and were provided with food (Pliva, Zagreb, Yugoslavia) and tap water *ad libitum*.

Tumor

Fibrosarcoma was induced by methylcholanthrene (0.1 mg in 0.1 ml of olive oil injected s.c.) and the 14th to 17th transplantation generations were used in these experiments. Tumor was transplanted i.m. into the right leg (1×10^6 cells per mice).

Experimental diabetes

Alloxan (Merck, Darmstadt, Federal Republic of Germany) was injected i.v. as a 0.5% solution in Hanks. The doses were 75,

100 and 120 mg/kg. In all experimental groups the mice received alloxan 7 days before tumor cells.

Blood glucose

The level of blood glucose was determined by the method of Hyvarinen and Nikkila [23]. The level was expressed as mg/100 ml of the blood.

Tumor growth

The basic criteria for tumor growth was the survival of the recipients. We also measured the diameter of the tumor node.

Tissue extract

Excised solid tumor tissue was homogenized in physiological saline (50 mg/ml of medium) using an all-glass Potter–Elvehjem homogenizer. The homogenate was centrifuged at 75,000 ***g*** for 1 hr at 4°C. The supernatant was decanted and used for insulin determination.

Radioimmunoassay for insulin

Immunologically reactive insulin (IRI) in the sera and in extracts of the tumor was determined by the method of Morgan and Lazarow [24], using ^{125}I-insulin and the crystalline rat insulin standards (Sorin, Saluggia, Italy).

Statistical analysis

The data are presented as the arithmetical mean $\pm$ standard deviation (S.D.), and the significance between the results was checked by Student's *t*-test. The level of significance was put at the level of $P<0.05$ [25].

RESULTS

Table 1 shows the influence of the number of injected tumor cells on the length of survival of diabetic mice and on the rate of tumor growth. Diabetic mice lived longer than non-diabetic mice injected with an equal number of tumor cells.

Table 2 shows the dependence of the growth rate of fibrosarcoma on the dose of alloxan which caused the diabetes. There was no clear correlation between the drug dose and the cell number. Diabetic mice always lived longer than non-diabetic animals. A conclusion from this experiment is that diabetes hinders the growth of this tumor.

Figure 1 show levels in the blood glucose of

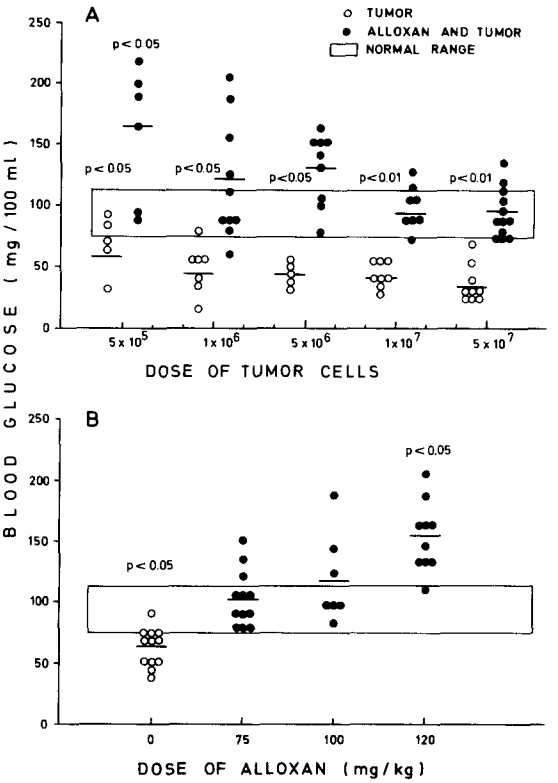

Fig. 1. (A) Effect of the initial tumor cell load on blood glucose levels in alloxan-diabetic and non-diabetic mice with fibrosarcoma. Glucose was determined 25 days *after inoculation of tumor cells and* 32 days *after injection of alloxan* (100 mg/kg). *(B) Effect of alloxan dose on blood glucose levels in mice subsequently inoculated with fibrosarcoma. Glucose was determined* 25 days *after inoculation of* 1×10^6 *tumour cells and* 32 days *after injection of alloxan.*

diabetic and non-diabetic mice with fibrosarcoma. As expected, alloxan causes hyperglycemia, and the effect is clearly dose-dependent; larger doses of the drug cause more severe diabetes. Tumor cells, on the contrary, lower the blood glucose, both in the non-diabetic mice and in mice with alloxan-induced diabetes. This effect is also dose-dependent and it reflects the tumor cell load; the greater number of tumor cells inoculated into diabetic or normal mice, the lower the glucose level in their blood. Inoculation of many tumor cells into diabetic mice even abolished the alloxan-induced hyperglycemia and keeps the blood glucose level within the normal range.

The 'antihyperglycemic' effect of fibrosarcoma is evident from data in Fig. 1B. In mice with alloxan-induced diabetes tumor decreased the blood sugar, so that in mice with terminal tumors the glucose levels approached the normal range. In non-diabetic animals, terminal tumors caused hypoglycemia.

In next experiments animals were divided

Table 1. Dependence of the growth of fibrosarcoma in diabetic mice on the number of injected tumor cells

No. of tumor cells inoculated	Alloxan (100 mg/kg)	Tumor diameter in mm on day 24 (mean ± S.D.)	Survival of mice in days (mean ± S.D.)
5×10^5	No	16.3 ± 1.9	45.1 ± 3.8
	Yes	9.1 ± 0.5*	56.1 ± 4.9*
1×10^6	No	25.4 ± 3.1	32.1 ± 2.8
	Yes	20.4 ± 2.9*	36.4 ± 1.9*
5×10^6	No	27.4 ± 3.3	29.1 ± 2.0
	Yes	20.5 ± 1.9*	33.4 ± 1.9*
1×10^7	No	29.4 ± 1.8	26.4 ± 0.8
	Yes	26.1 ± 0.8*	28.3 ± 0.9*
5×10^7	No	30.4 ± 1.8	25.0 ± 1.3
	Yes	27.1 ± 2.0*	24.0 ± 0.9

Eight mice per group.
*$P < 0.05$ compared to the corresponding control group not receiving alloxan.

Table 2. Dependence of the growth of fibrosarcoma in diabetic mice on the diabetogenic dose of alloxan

Alloxan (mg/kg)	No. of mice	Tumor diameter in mm on day 24 (mean ± S.D.)	Survival of mice in days (mean ± S.D.)
0	10	29.3 ± 1.4	31.5 ± 2.0
75	8	26.2 ± 1.8*	34.0 ± 0.8*
100	12	23.6 ± 3.0*	37.1 ± 2.9*
120	11	20.0 ± 1.5*	40.2 ± 1.9*

All mice received 1×10^6 tumor cells on day 0.
*$P < 0.05$ as compared to the group of tumor-bearing mice not receiving alloxan.

into 3 experimental groups. The first group consisted of mice with alloxan-induced diabetes. Animals were inoculated with 1×10^6 tumor cells from normoinsulinemic mice. Tumors developing in diabetic mice were considered here to be first-generations tumors (DT). Tumor cell suspensions from these animals were used for inoculation into other diabetic mice to produce the second generation tumor (2DT). Third-generation tumors (3DT) were induced.

In Table 3 are displayed the tumor diameter, mean survival times, glucose levels and levels of immunoreactive insulin in diabetic mice bearing tumors of successive generations. Comparison of the first-generation tumor diameters in normoinsulinemic (T) and diabetic (DT) shows that tumors grow slower in the latter group. This is in accordance with the longer mean survival time of diabetic mice with the first-generation tumor (Table 3). Successive transplantation of tumor cells results in enhanced growth of tumor in each further generation. The enhanced tumor growth is accompanied by the significant shortening of the mean survival time. Glucose level in the blood of mice with fibrosarcoma (T) is lower, while in diabetic mice with first-generation tumors (DT) it is higher than in healthy animals (N). In subsequent generations of tumors glucose levels are decreased, so the higher the generation of the tumor in diabetic mice, the lower the glucose level. The levels of immunoreactive insulin (IRI) in peripheral blood (Table 3) in diabetic mice (D) are considerably lower than in normal mice (N). The same is valid for diabetic mice into which a tumor from normoinsulinemic donor is transplanted (DT). But, by transplanting a first-generation tumor from a diabetic

Table 3. Mean tumor diameter, mean survival time, IRI and glucose in blood of non-diabetic and diabetic mice after transplantation of fibrosarcoma

Treatment	Tumor diameter in mm on day 23 (mean ± S.D.)	Mean survival time in days (± S.D.)	IRI on day 24 (μU/ml ± S.D.)	Glucose in blood (mg/100 ml ± S.D. on day 24)
N	—	—	27.9 ± 1.8 (5)	97.1 ± 12.8 (6)
T	28.4 ± 1.9 (8)	31.4 ± 2.8 (8)	24.1 ± 4.8 (6)	67.1 ± 12.0 (8)
D	—	—	4.6 ± 0.9*† (5)	391.0 ± 32.3*‡ (5)
DT	25.8 ± 3.1‡ (9)	37.9 ± 0.9‡ (9)	6.1 ± 0.4* (6)	129.6 ± 13.0 (5)
2DT	28.6 ± 4.5 (8)	29.6 ± 3.0 (8)	20.1 ± 6.7 (6)	98.5 ± 17.0 (5)
3DT	32.5 ± 0.7‡§ (11)	25.0 ± 2.0‡§ (11)	29.1 ± 0.8 (6)	76.1 ± 10.1* (6)

Numbers in parentheses denote No. of animals in the respective group.
N = untreated mice; T = tumor-bearing mice; D = alloxan-treated diabetic mice; DT = tumor-bearing D; 2DT and 3 DT = D bearing tumors transplanted from DT and 2 DT, respectively.
*$P < 0.05$ for group N.
†Determined 8 days after alloxan injection.
‡$P < 0.05$ for group T.
§$P < 0.05$ for group DT.

mouse to another diabetic mouse, insulin is raised towards normal levels.

We have monitored also the insulin-content in tumor-extracts from diabetic and hyperglycemic mice. The changes of the hormone levels in the tumor tissue in all the cases closely resembled those observed in peripheral blood.

DISCUSSION

The results have indicated that methylcholanthrene-induced fibrosarcoma grew faster in non-diabetic mice. This effect has been noticed by several workers with respect to tumor-growth, in diabetic mice [6, 7, 15–22]. This means that diabetes and its secondary ketonemia and/or acidosis provides no suitable environment for the tumor growth. Acidosis itself is a well-known factor in retardation of the tumor growth [4, 8, 26, 27], and this is slightly related to the inhibitory effects of acidosis on tumor glycolisis.

Fibrosarcoma adjusts itself however, to adverse metabolic conditions. As soon as 2 weeks after transplantation into diabetic hosts tumor cells develop 'resistance' toward diabetes, and grow at normal rate in secondary diabetic hosts.

Another important finding is the enhancement of the growth of the third generation of tumor in diabetic mice; an effect not observed in hyperglycemic non-diabetic animals [7]. The enhanced growth of the third generation of tumor in diabetic mice was accompanied by increased levels of insulin in blood and in the tumor tissue. Since recipient mice of this generation of tumor were not able to produce their own insulin, appearance of insulin in these mice must be connected with the presence of the tumor.

Many authors have shown that tumor cells secreted various factors [1, 5, 6, 28–32]. Our finding that fibrosarcoma cells might secrete insulin-like substances points to the another possible secretory ability of the tumor cells. It might however be possible that it is a question of substances like NSILA, somatomedin and 'insulinoids'.

We showed that fibrosarcoma caused a fall of the blood glucose in the host. Hypoglycemia was proportional to the number of tumor cells inoculated into the host. One reason for hypoglycemia could be an augmented consumption of glucose by the cells of the tumor [33–36]. A faster transport of glucose through the membrane of tumor cells could be an important factor since transport of 3-*O*-methylglucose is faster in Rous sarcoma cells than in fibroblasts [12]. Facilitated transport of glucose is attributed to the changes of the membrane of virus-transformed cells [13].

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