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CONCISE REPORT

Somatostatin Suppresses Growth of Murine Myeloid Leukemia In Vivo

By K. Pavelić, J. Gabrilovac, V. Božikov, J. Pavelić, M. Petek, and M. Boranić

Daily injections of somatostatin into mice with myeloid leukemia retarded the tumor growth. This myeloid leukemia is an insulin-dependent tumor (in that it grows more slowly in hypoinsulinemic diabetic mice than in nondiabetic animals). Since somatostatin decreased the level of immunoreactive insulin in mice with myeloid leukemia, and since the treatment with insulin abrogated the antileukemic effect of somatostatin, we attribute retarded growth of this leukemia to decreased secretion of insulin, caused by somatostatin.

MANY TUMORS are insulin-dependent in that they grow slowly in hypoinsulinemic hosts.[1-4] Since one of the effects of somatostatin is suppression of insulin secretion,[5-9] it would be of interest to test whether the treatment of hosts bearing insulin-dependent tumors with somatostatin would cause retardation of growth of the tumor.

Experiments presented in this article show that somatostatin significantly suppressed growth of an insulin-dependent murine myeloid leukemia.

MATERIALS AND METHODS

Animals. Male RF/O inbred mice from our conventional colony were used. They were 12 wk of age at the initiation of experiments. The animals were kept in groups of five or less in plastic cages and were provided with standard pelleted food and tap water ad libitum.

Leukemia. Strain-specific, transplantable myeloid leukemia of RF/O mice was induced by irradiation at the Radiobiological Institute TNO in Rijswijk in 1963 and brought to Zagreb in 1968. The tumor invades spleen, bone marrow, and liver. Injection of 10^6 tumor cells causes death after 10 days with multiple thromboemboli in the lungs and brain (due to the massive numbers of circulating leukocytes). Characteristics of this leukemia have been outlined elsewhere.[10]

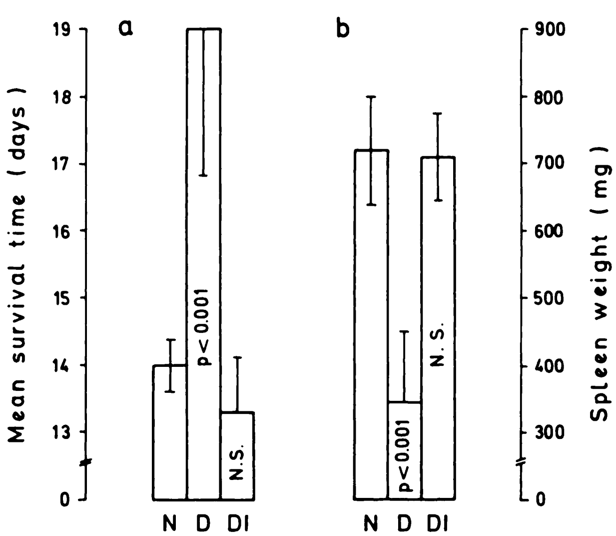

Fig. 1. Survival times (A) and spleen weight (B) of mice inoculated with 10^3 leukemia cells: N, nondiabetic; D, diabetic; DI, diabetic treated with insulin (2 IU/mouse/day, each day after tumor transplantation). Spleen weights were determined in mice sacrified 13 days after inoculation of leukemia. The data are expressed as means ± standard deviation (7–10 mice per group). Groups D and DI were compared with the control group N.

Cell suspensions. These were prepared aseptically from spleens of moribund leukemic mice. The spleens were minced and passed through nylon gauze, and the resulting cell suspension was washed 1–3 times in Hanks' solution. Number of viable cells was determined by using Trypanblue exclusion test.

Somatostatin. Synthetic somatostatin used in these experiments was a linear peptide obtained from Biodata S.P.A., Italy. It was injected intraperitoneally in 3 daily portions.

Insulin. Following transplantation of leukemia, diabetic mice were daily given subcutaneous injection(s) of insulin (Pliva, Zagreb, Yugoslavia).

Diabetes. Diabetes was induced by an intravenous injection of alloxan (Merck, Darmstadt, F.R. Germany), 100 mg/kg, 7 days before tumor transplantation, as described by Pavelić.[4] This dose induces diabetes in all mice. The diabetic state of alloxan-diabetic mice was checked by determination of immunoreactive insulin (IRI) and glucose levels. IRI level was significantly decreased (from 20.0 μU/ml ± 4.0 to 4.7 μU/ml ± 2.0), and the glucose level was significantly increased (from 104 ± 30 mg/dl to 380 ± 95).

Biochemical analysis. The sera for biochemical analysis were obtained from axillar vessels at various times after tumor transplantation. Immunologically reactive insulin (IRI) in the sera was determined by the method of Morgan and Lazarow[11] using ^{125}I-insulin and the crystalline rat insulin standards (Sorin, Saluggia, Italy). The blood glucose level was determined by the O-toluidine method of Hyvarinen and Nikkila.[12]

Therapeutic parameters. Criteria for estimation of leukemia growth were the survival time and the spleen weight of the animals.

Statistics. The results were statistically evaluated by Student's t test. Differences between groups were considered significant if p values were below 0.001.

RESULTS

Evidence for Insulin Dependence of Murine Myeloid Leukemia

This leukemia is strongly insulin-dependent as judged by our standard model of tumor transplanta-

From Ruder Bosković Institute, Department of Experimental Biology and Medicine, Zagreb, Yugoslavia.

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Address reprint requests to Dr. K. Pavelić, Ruder Bošković Institute, P.O.B. 1016, Bijenička c. 54, 41000 Zagreb, Yugoslavia.

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tion in alloxan-diabetic mice.[4] The survival time was significantly prolonged (for 5 days) and the spleen weight was markedly decreased (50%) in diabetic mice in comparison with normal nondiabetic mice with leukemia (Fig. 1). Daily injections of insulin into diabetic mice accelerated the tumor growth to the level seen in nondiabetic mice (Fig. 1).

Antileukemic Effect of Somatostatin

The effect of daily injections of somatostatin (2.0 μg/mouse/day in 3 portions) on the survival time was assessed in mice injected with decreasing numbers of leukemia cells. Somatostatin significantly prolonged mean survival time in all experimental groups, but the effect was most pronounced in mice injected with the lowest dose of leukemia cells (Fig. 2A). The dose of 2.0 μg somatostatin was chosen because in preliminary experiments it was the minimal one causing significant decrease of IRI.

In the next set of experiments, dose-response effect of somatostatin was tested in mice that received inocula of 10^3 leukemia cells. Retardation of leukemia growth was observed with all doses, except for the lowest one (0.5 μg; Fig. 2B).

The Mechanism of Antileukemic Effect

The observed inhibitory effect of somatostatin on the leukemia growth could be due to *direct* cytotoxicity of somatostatin for tumor cells. In order to test this possibility, leukemia cells were incubated with somatostatin over a broad range of its concentrations (8192–32 ng/ml), and the number of dead cells was

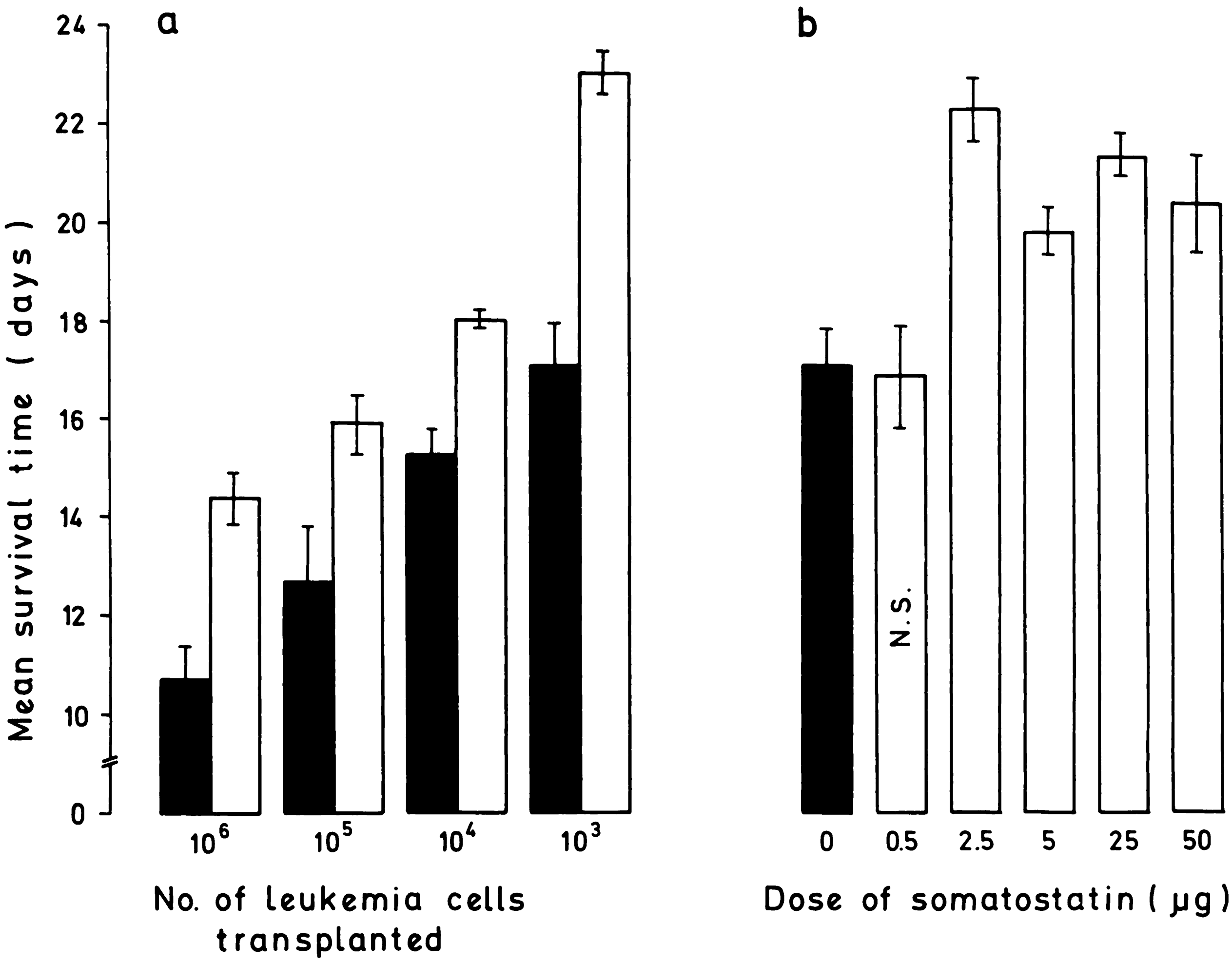

Fig. 2. The effect of somatostatin on the survival of mice inoculated with myeloid leukemia. (A) Dependence on the number of transplanted leukemia cells, applying a fixed dose of somatostatin (2.0 μg/day/mouse); (B) dependence on the dose of somatostatin in mice inoculated with a fixed number leukemia cells (10^3). Seven to ten mice per column. The results are expressed as means ± standard deviation. Full columns indicate control, i.e., nontreated mice. All values, except for that indicated "NS," are significantly different from their respective controls at the level of $p < 0.001$.

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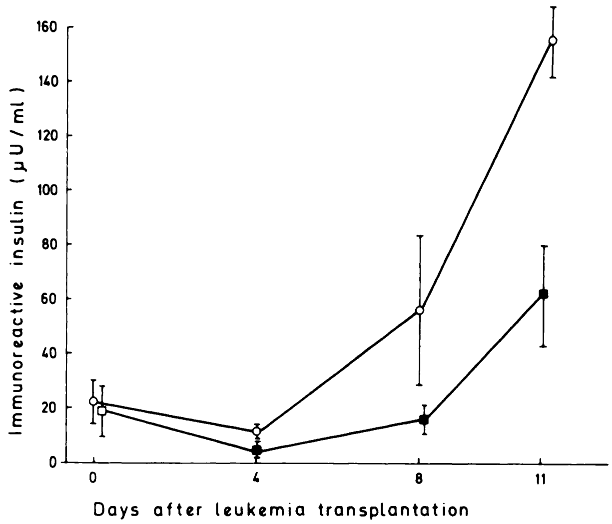

Fig. 3. The effect of somatostatin (2.0 μg/mouse/day) on the IRI level in serum of mice bearing myeloid leukemia. Nine to 12 mice per group. The results are expressed as mean ± standard deviation. ○, nontreated mice; □, mice treated with somatostatin. Full symbols represent values significantly different from those in nontreated leukemic animals.

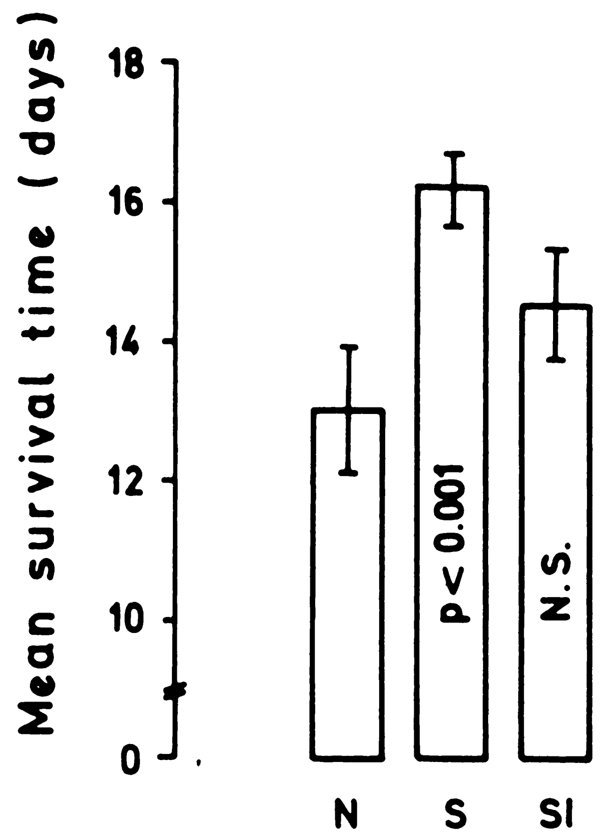

Fig. 4. Survival times of mice with myeloid leukemia: N, nontreated; S, somatostatin-treated; SI, somatostatin- and insulin-treated. Seven mice per group. The data are expressed as means ± standard deviations. Hormone-treated groups (S and SI) were compared with the nontreated control (N).

estimated at various intervals thereafter (5–120 min). No cytotoxic effect of somatostatin was observed by any of the concentrations used (data not shown). Thus, the action of somatostatin on tumor cells seems to be *indirect*.

Injection of somatostatin into leukemic mice suppressed secretion of insulin (Fig. 3). Therefore, we supposed that somatostatin acts *indirectly* on leukemia cells by decreasing the level of insulin, which is necessary for leukemia growth. Indeed, when the decreased level of (endogenous) insulin, caused by somatostatin, was restored to normal values by injections of (exogenous) insulin, the antileukemic effect of somatostatin was abrogated (Fig. 4).

DISCUSSION

Daily treatment of mice bearing leukemia with somatostatin significantly suppressed growth of leukemia. This leukemia was shown to be an insulin-dependent tumor. As somatostatin decreased the level of insulin, retardation of leukemia growth might be due to the shortage of insulin. In fact, restoration of insulin level to normal values by exogenous insulin resulted in "normalization" of leukemia growth.

There is little evidence about antitumor effects of somatostatin. It is known that somatostatin suppresses growth of insulinomas in human and experimental animals[9,13,14] and decreases insulin secretion from these tumors.[13,14] On the other hand, insulin is important for growth of tumors in experimental animals[1,4,17,18] and human beings.[15,16] For example, there is positive correlation between the stage of development of Hodgkin's disease and the level of insulin.[16] Inhibition of insulin secretion (alloxan or streptozotocin injection, pancreatectomy) suppressed tumor growth.[1,15,18] Thus, inhibition of insulin secretion induced by somatostatin could explain described retardation of leukemia development.

Another possible mechanism of antileukemic effect of somatostatin might be suppression of secretion of the growth hormone,[19] which, like insulin, is needed for growth of some human and animal leukemias.[20,21] Addition of growth hormone into the culture of leukemia cells accelerates their proliferation,[21] while hypophysectomy slows down development of experimental leukemias.[22,23]

Thus, somatostatin might have abated progression of this murine leukemia through endocrine mechanisms—either via insulin or via the growth hormone. Together with our previous data,[2,4,17,18] this stresses the importance of hormonal regulatory mechanisms for the growth of malignant cells.

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REFERENCES

1. Jehl JA, Mayer J, McKee RW: Influence of the hereditary obese hyperglycemic syndrome and of alloxan-diabetes on the survival of mice with Ehrlich ascites carcinoma. Cancer Res 15:341, 1955

2. Pavelić K, Slijepčević M: Growth of a thymoma in diabetic mice treated with insulin. Eur J Cancer 14:675, 1978

3. Pavelić K: Aplastic carcinoma in diabetic mice: Hyperglycemia-suppressed proliferation rate and insulin synthesis by tumor cells. J Natl Cancer Inst 62:139, 1979

4. Pavelić K, Slijepčević M, Pavelić, J, Ivić J, Audy-Jurković S, Pavelić ZP, Boranić M: Growth and treatment of Ehrlich tumor in mice with alloxan-induced diabetes. Cancer Res 39:1807, 1979

5. Alberti KGM, Christensen A, Prange-Hansen A, Iversen J, Lundback K, Seyer-Hansen K, Ørskov H: Inhibition of insulin secretion by somatostatin. Lancet 2:1299, 1973

6. Efendić S, Luft R, Grill V: Effect of somatostatin on glucose induced insulin release in isolated perfused pancreas and isolated rat pancreatic islets. FEBS Lett 42:169, 1974

7. Kwan CW, Karam JH, Guillemin R, Forsham PH: Inhibition of pancreatic glucagon response to arginine by somatostatin in normal man and insulin-dependent diabetics. Diabetes 23:876, 1974

8. Gerich JE, Lorenzi M, Schneider V, Forsham PH: Effect of somatostatin on plasma glucose and insulin responses to glucagon and tolbutamide in man. J Clin Endocrinol Metab 39:1057, 1974

9. Lorenci M, Gerich JE, Karam JH, Forsham PH: Failure of somatostatin to inhibit tolbutamide induced insulin secretion in patients with insulinomas: A possible diagnostic tool. J Clin Endocrinol Metab 40:1121, 1975

10. Boranić M, Dominis M, Pavelić Z, Blaži-Poljak M: Colony-forming ability of a lymphoid and a myeloid murine leukemia. J Natl Cancer Inst 51:275, 1973

11. Morgan CR, Lazarow A: Immunoassay of insulin: Two antibody systems, plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes 12:115, 1963

12. Hyvarinen A, Nikkila BA: Specific determination of blood glucose with O-toluidine. Clin Chim Acta 7:140, 1962

13. Scuro LA, Lo Cascio V, Adami S, Galvanini G, Bianchi I, Cominacini L, Corgnati A: Somatostatin inhibition of insulin secretion in insulin-producing tumors. Metabolism 25:603, 1976

14. Luyckx AS, Lefebvre PJ: Effect of somatostatin in the Syrian hamster bearing a transplantable islet-cell tumor. Horm Metab Res 10:105, 1978

15. Ingle DJ: Comparison of pancreatic and steroid diabetes in respect to tumor growth and glycosuria. Diabetes 14:93, 1965

16. Pavelić K, Pekić O, Slijepčević M, Popović M: Insulin levels in Hodgkin's disease. Br J Haematol 46:133, 1980

17. Pavelić J, Benković B, Pavelić K: Growth and treatment of B-16 melanoma in hyperglycemic mice. Res Exp Med 177:71, 1980

18. Pavelić K: Growth of a methylcholanthrene-induced fibrosarcoma in mice with diabetes mellitus. Eur J Cancer 16:279, 1980

19. Hall R, Besser GM, Schally AV, Coy DH, Evered D, Goldie DJ, Kastin AJ, McNeilly AS, Mortimer CH, Phenekos C, Tunbridge WMG, Weightman D: Action of growth hormone in healthy men and in acromegaly. Lancet 2:581, 1973

20. Golde DW, Bersch N, Li CH: Growth hormone modulation of murine erythroleukemia cell growth in vitro. Proc Natl Acad Sci USA 75:3437, 1978

21. Desai LS, Lazarus H, Li CH, Foley GE: Human leukemic cells. Effect of human growth hormone. Exp Cell Res 81:330, 1973

22. Bentley HP, Hughes ER, Peterson RDA: Effect of hypophysectomy on a virus-induced T-cell leukemia. Nature 252:747, 1974

23. Huggins C, Oka H: Regression of stem-cell erythroblastic leukemia after hypophysectomy. Cancer Res 32:239, 1972

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