CONCISE REPORT

Somatostatin Suppresses Growth of Murine Myeloid Leukemia In Vivo

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Many tumors are insulin-dependent in that they grow slowly in hypoinsulinemic hosts. Since the effects of somatostatin are suppression of insulin secretion, 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<sup>6</sup> 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.

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 a Trypan blue 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. 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 axillary vessels at various times after tumor transplantation. Immunologically reactive insulin (IRI) in the sera was determined by the method of Morgan and Lazarow using 125I-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.

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 transplantation.

Fig. 1. Survival times (A) and spleen weight (B) of mice inoculated with 10<sup>6</sup> 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 sacrificed 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.

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Submitted November 13, 1980; accepted December 11, 1980.

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tion in alloxan-diabetic mice. 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...
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 and decreases insulin secretion from these tumors. On the other hand, insulin is important for growth of tumors in experimental animals and human beings. For example, there is positive correlation between the stage of development of Hodgkin’s disease and the level of insulin. Inhibition of insulin secretion (aloxan or streptozotocin injection, pancreatectomy) suppressed tumor growth. 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, which, like insulin, is needed for growth of some human and animal leukemias. Addition of growth hormone into the culture of leukemia cells accelerates their proliferation, while hypophysectomy slows down development of experimental leukemias.

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, this stresses the importance of hormonal regulatory mechanisms for the growth of malignant cells.
REFERENCES

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