In Vivo and in Vitro Effect of Progesterone on the Growth of Some Mouse and Human Tumours

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Summary. The effect of progesterone on the growth of tumours of different morphological and cytokinetic characteristics and origin has been investigated, such as mammary aplastic carcinoma, Ehrlich ascitic and solid tumour, fibrosarcoma, melanoma B-16, myeloid leukaemia and three cell lines, HeLa, HEp-2 and L929. The administration of progesterone has been proved to stimulate the growth of aplastic carcinoma of the breast in vivo. Directly implemented into cell culture of the same tumour it increased the incorporation of 3H-thymidine into DNA. In HeLa and HEp-2 cell cultures progesterone stimulated the population growth of these cells. However, progesterone has shown no effect at all on the growth of fibrosarcoma, melanoma, Ehrlich tumour, myeloid leukaemia and L929 cells, fibroblasts of C3H mice.

Key words: Progesterone – Tumours – Growth

Introduction

Progesterone stimulates the growth of dimethylbenzanthracene-induced breast cancer in mice [6]. The stimulating effect depends on the estrogen most probably because estrogen helps to increase the number of progesterone receptors in breast cancer cells [6]. Given to mice and rats with developed breast cancer, progesterone stimulates the growth of these tumours [17,18]. Progesterone has no significant effect on the growth of some other tumours or, on the contrary, shows even a suppressive effect [11]. The metabolism of progesterone in breast cancer cells is somewhat different from that in normal breast tissue [7].

In the literature the information on the in vivo effect of progesterone is rather scarce and often quite controversial. The purpose of this study is to investigate on a greater number of samples in vivo and in vitro the effect of progesterone on the growth of the tumour and the mechanism underlying this activity. In this way, a better understanding of the effect of progesterone on tumour growth, which is
important in view of the relatively frequent use of progesterone in combination with other chemotherapeutics for malignant tumours, will, hopefully, be achieved. Various samples of mouse and human tumours of different histological and kinetic characteristics and origin were used for these analyses.

**Materials and Methods**

**Mice**

Highly inbred 2- to 3-month-old female mice (CBA, C57BL and RF strain) raised at the Ruder Bošković Institute Breeding Center, Zagreb, Yugoslavia, were used. Mice were housed in plastic cages, four mice per cage, and were fed with standard pellets (Sljeme, Zagreb, Yugoslavia).

**Tumours**

Mammary aplastic carcinoma [10], Ehrlich ascitic and solid carcinoma and 3-methylcholanthrene-induced fibrosarcoma were maintained in CBA mice; myeloid leukaemia in RF mice [2] and melanoma B-16 in C57BL mice. With the exception of Ehrlich tumour, all others were strain-specific and, with the exception of fibrosarcoma, all were maintained in our laboratory for several years by serial transplantations. Fibrosarcoma was induced by s.c. injection of 3-methylcholanthrene (0.1 mg) in 0.1 ml olive oil, and the eighth transplantation generation was used in these experiments.

The dosages selected for cell transplantation were slightly above the threshold for induction of tumours in all recipients [2, 9, 10]. The numbers of cells transplanted were $1 \times 10^6$, $5 \times 10^6$, $1 \times 10^7$ and $2 \times 10^7$ for Ehrlich carcinoma, $1 \times 10^6$ for fibrosarcoma, mammary aplastic carcinoma, melanoma, and myeloid leukaemia. Fibrosarcoma, Ehrlich solid carcinoma, melanoma, and mammary aplastic carcinoma cells were injected i.m. into the right leg. Ehrlich ascitic carcinoma cells were transplanted i.p., and leukaemia cells were injected i.v.

**Progesterone**

Progesterone (medroxyprogesterone acetate, Depo-Provera, Galenika, Beograd, Yugoslavia) was given s.c. twice a week. The experimental mice received various doses of progesterone dissolved in Hanks solution. Control animals received 0.2 ml of Hanks physiological solution.

**Tumour Growth**

The basic criterion for the growth of both tumour forms (i.e. solid or ascitic) was the survival of the recipients. In the mice bearing ascitic tumour, the number of cells was also counted. It was investigated morphologically and cytochemically that most of the cells in ascites were tumour cells and the rest (approximately 5–8%) were macrophages and polymorphonuclear leucocytes [1]. In mice bearing solid tumour, either the tumour diameter was measured or the weight of the tumour was determined some time after transplantation. For determination of the tumour size three perpendicular tumour diameters were measured by a caliper every 2–3 days. The tumour volumes were calculated by the formula $abc \times 0.5236$, were $a$, $b$ and $c$ are opposite diameter of the tumour.

The number of cells in the peritoneal cavity of killed mice was counted as follows: 3 ml sterile 0.9% NaCl solution was injected i.p. The abdomen was massaged for 1 min, and five drops of the ascitic fluid were pipetted. The number of cells was expressed per milliliter of this abdominal washing without attempting to calculate the total cell population in the abdominal cavity.

The weight of the solid tumour was determined on a torsion balance (acuity 0.1 mg) after careful dissection of the tumour node from tissues of killed mice.