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Effects of progesterone on ovarian tumorigenesis in xenografted mice☆

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Abstract

Circumstantial evidence indicates that progestins reduce the risk of epithelial ovarian cancer. We report that the tumorigenic capacity of human ovarian carcinoma (SKOV-3) cells inoculated into the peritoneal cavity of athymic mice is suppressed by pretreatment with subcutaneous progesterone-releasing pellets. Numbers of tumor implants on the intestines/mesentery and invasiveness into underlying host tissues were reduced at 6 weeks following exposure to progesterone. Progesterone prevented tumors from forming on the liver. Life spans of progesterone-treated animals were prolonged. There was no beneficial effect of administration of progesterone if initiated after ovarian tumors had become established on organ surfaces. Our findings implicate a role for progesterone in ovarian cancer prophylaxis.

Keywords: Progesterone; Epithelial ovarian cancer; Athymic mice

1. Introduction

The majority (>90%) of cancers of the ovary are thought to originate by clonal expansion of a surface epithelial cell that was mutated by genotoxins generated during the mechanics of ovulatory follicular rupture [1]. There are four basic stages of advancement in common epithelial ovarian cancer. Stage I is defined by the formation of an epithelial inclusion cyst that invades the ovarian cortex. Cancerous cells are extruded into and seed the abdominal cavity when an inclusion cyst ruptures. Pelvic spread and generation of ascites fluid are the hallmarks of Stage II disease. Stage III is characterized by tumor implants involving the small intestine, mesentery, and superficial liver. Distant disseminated metastasis (e.g. to the parenchymal liver) occur in Stage IV. Death usually results from intestinal (e.g. bowel obstruction) or edematous (e.g. due to recurrent ascites and pleural effusion) complications [2,3].

The sequences of events that lead to common epithelial ovarian cancer are multifactorial. Several aberrant steps are undoubtedly required to yield a phenotype with distinct growth and metastatic advantages. The initiation and progression of ovarian cancer is generally considered to have some level of hormonal

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involvement. It appears that progestins protect against ovarian cancer development [4].

Transformed ovarian epithelial cells hypersecrete urokinase plasminogen activator (uPA) from exocytotic vesicles—which mediates tissue degradation and metastasis [5]. Relatively high doses of progesterone, by perturbing (due to its lipophilicity) plasma membrane fluidity and uPA secretion, inhibited the invasive potential (gel infiltration) of malignant ovarian epithelial cells [6,7]. There also is in vitro evidence that apoptosis of ovarian cancer cells is induced by progesterone [8–10]. The objectives of the following investigations were to assess the putative antitumorigenic effects of progesterone in immunocompromised mice bearing an intraperitoneal (ip) injection of human ovarian adenocarcinoma cells.

2. Materials and methods

Experiments were conducted with the approval of the University of Wyoming Animal Care and Use Committee. Reagents were purchased from Sigma Chemical Co. (St Louis, MO) unless indicated otherwise.

Six- to eight-week-old nu/nu BALB/c athymic mice were maintained in a pathogen-free environment under controlled temperature (24 °C) and lighting (12L:12D) conditions. Sterilized rodent chow and water were supplied ad libitum.

Animals were implanted subcutaneously (sc, dorsal neck) with two control or progesterone-releasing pellets (SC/P-131; 2×3 mm, 25 mg, 60-day; Innovative Research of America, Sarasota, FL). Serum samples obtained from tail bleeds were analyzed for progesterone using a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA). The assay was sensitive to 0.0156 ng progesterone. Dilutions of mouse serum were parallel to the standard curve. Progesterone recoveries from spiked samples were $>$ 98%. Assay coefficients of variation were $<$7%.

The epithelial ovarian cancer cell line SKOV-3 (American Tissue Culture Collection, Rockville, MD) was propagated to confluence in T-75 flasks (Corning Costar, Cambridge, MA) at 37 °C under an atmosphere of 5% CO_2 in 15 ml RPMI-1640 (R6504) medium supplemented with 10% charcoal-stripped/-heat-inactivated fetal calf serum (Atlanta Biological, Norcross, GA), 10 μg/ml insulin (I6634), and 1% antibiotic/antimycotic solution (A9909). A trypsin (0.25%)/EDTA (0.03%) solution was used to harvest cells ($>$95% viabilities as indicated by trypan blue exclusion). Ten million cells suspended in 0.1 ml of culture medium were injected ip.

In Experiment 1, five mice per group were implanted with control or progesterone-releasing pellets and injected the following day with SKOV-3 cells. Serum progesterone determinations were made on Days 0, 1, 2, 7, 14, 21, 28, 35, and 42 post-implant. Animals were killed by cervical dislocation on Day 42. Tumor nodules on the external surfaces of the duodenum/mesentery (#/cm length) and liver (#/cm^2) were counted (three sites per organ) and their diameters were measured with precision calipers. Samples of intestine and liver were excised for light microscopic examination (hematoxylin/eosin-stained, paraffin-embedded sections); tumors were categorized (three per organ) as invasive (i.e. had penetrated the parenchyma) or superficial. Sections of kidney, lung, heart, brain, adrenal, pancreas, thyroid, stomach, and skeletal muscle were assessed for histopathological evidence of hormonal side-effects.

A follow-up study (Experiment 2) was conducted to determine if exposure to progesterone as indicated above translates into a life-sparing effect. Circulatory progesterone (until Day 42) and survival days post-implant were monitored in 27 control and 31 principal mice. Abdominal organs were inspected for tumors at necropsy.

An additional study (Experiment 3) was conducted to determine if administration of progesterone has a prospective therapeutic impact after ovarian cancer cells have colonized onto organ surfaces. Mice were implanted with control ($n=5$) or progesterone-releasing ($n=6$) pellets at 3 weeks post-inoculation of SKOV-3 cells. Sera collected on Days 0, 1, 2, 7, 14, and 21 post-implant were assayed for progesterone. Animals were killed at 6 weeks following xenograft (i.e. at 3 weeks $-/+$ exogenous progesterone) and tumor dynamics monitored as described previously (Experiment 1).

Animals were assigned to treatments at random. Serum progesterone profiles were contrasted using a split-plot analysis of variance procedure for repeated measures. Morphometric subsample values were averaged. Treatment effects on tumor parameters and life spans were compared by Student's *t*-test.

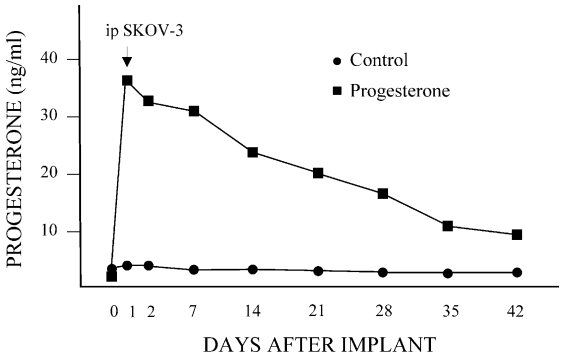

Fig. 1. Mean circulatory progesterone concentrations in control and progesterone-treated mice (combined data from Experiments 1 and 2). Pooled standard error = 1.8. Time × treatment, $P < 0.01$.

3. Results

3.1. Experiments 1 and 2

Progesterone profiles in mice implanted with placebo (blank) or progesterone-releasing pellets are illustrated in Fig. 1. Data on abdominal tumors and survival are shown in Fig. 2.

Fewer intestinal/mesentery tumors were present in progesterone-treated compared to control animals (Fig. 2A). Progesterone also inhibited infiltration of tumor cells into the intestinal wall (Fig. 2B–D) and prevented tumor implants from forming on the surface of the liver (Fig. 2E). Diameters of intestinal/mesenteric tumors tended to be smaller in the progesterone than control group (Fig. 2F); however, this contrast was not statistically significant ($P < 0.1$). There was no difference due to organ location in tumor diameters of controls (liver = 1.7 ± 0.2 mm). Liver tumors had not (on Day 42) pervaded the organ capsule. Morphological manifestations of apoptosis (nuclear pyknosis, cytoplasmic condensation, fragmentation/residual bodies [11]) among tumor cells were low (<5%) and not different between groups. No untoward side-effects of progesterone were noted.

Life spans of mice treated with progesterone were extended (Fig. 2G). An intestinal obstruction caused

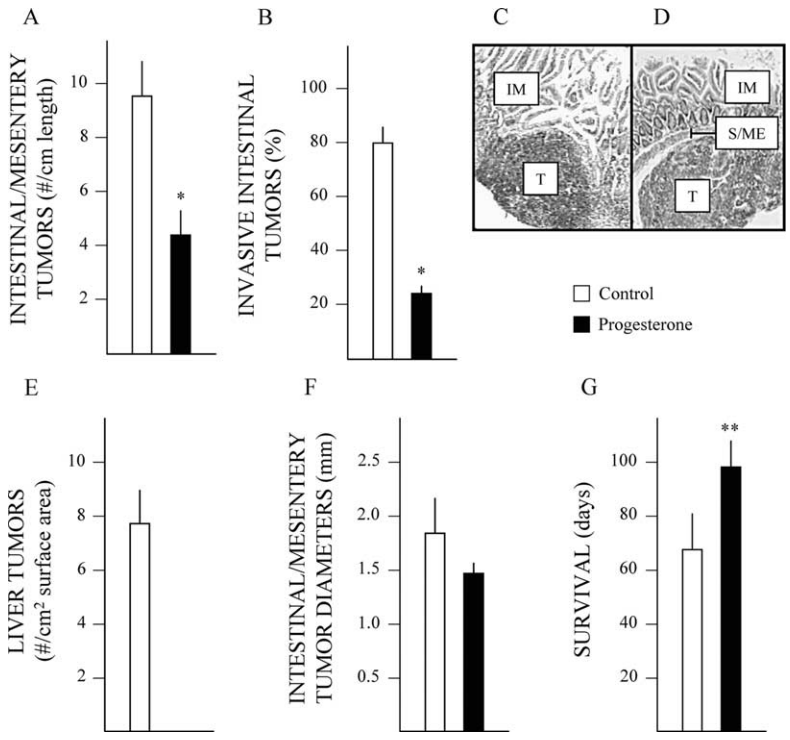

Fig. 2. Tumor burdens (Day 42 post-implant) and life spans of control and progesterone-treated mice. Treatment means + standard errors are plotted. Asterisks indicate differences: *$P < 0.01$, **$P = 0.05$. Representative photomicrographs of invasive and superficial SKOV-3 tumors (T) are shown in panels C and D, respectively (IM, intestinal mucosa; S/ME, serosa/muscularis externa).

by an ovarian tumor was evident upon death in all cases. Again, tumors on the liver were observed only in the control group.

3.2. Experiment 3

Serum progesterone concentrations during the 3 weeks after treatment (data not shown) were similar to those depicted in Fig. 1. Nevertheless, there was no suggestion of tumor apoptosis or retrogression elicited by progesterone. Tumor indices of control and principal groups were not significantly different; data (not presented) were essentially identical to those of controls shown in Fig. 2A, B, E, and F.

4. Discussion

Results of our initial study demonstrate that prior exposure to progesterone suppresses tumorigenesis in immunodeficient mice challenged ip with a (large) SKOV-3 burden. This finding, and the observation that progesterone enhances the DNA repair capability of ovarian surface epithelial cells associated with post-ovulatory follicles [12], indicate that the prophylactic/anticancer effects of progesterone extend beyond its ovulation-inhibiting property. Actions of progesterone on the tumorigenic competence of SKOV-3 cells were presumably mediated by a diminution in plasma membrane fluidity—thus affecting apposition, infiltration, and colonization onto host tissues. That progesterone completely circumvented the formation of tumors on the liver could be reflective of the fact that it is an organ in which steroid hormones are sequestered for metabolism [13].

Unfortunately, it does not appear that progesterone was of any benefit (e.g. by promoting apoptosis) once ovarian tumors became well established. This is consistent with the general consensus that progestins are of limited efficacy in the treatment of Stage III/IV disease [14,15]. It is possible that objective apoptotic responses are dependent on level of progesterone receptor expression and (or) the spatial circumstance of cells (e.g. suspended or assimilated into a tumor). Progesterone receptors were undetectable in SKOV-3 cells [16].

Epithelial ovarian cancer is a deadly insidious affliction because it typically remains asymptomatic until it has expanded into the abdominal cavity [17]. If early diagnoses come to fruition, and there are several promising proteomic screening techniques on the horizon [18], then progesterone could be used as an interceptive strategy. Treatment of advanced ovarian cancer generally involves debulking surgery used in combination with platinum-containing drugs, alkylating agents, and (or) taxol; not withstanding, most patients become refractory to chemotherapy and relapse [17]. Progesterone might be of some utility in attenuating disease reoccurrences from residual cells following cytoreductive surgery. Perhaps ip delivery of progesterone, with the intention of elevating local/abdominal concentrations, would optimize therapeutic outcomes.

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