[CANCER RESEARCH 53, 5374–5376, November 15, 1993]

Advances in Brief

Synthetic Retinoid Fenretinide Is Effective against a Human Ovarian Carcinoma Xenograft and Potentiates Cisplatin Activity[1]

Franca Formelli[2] and Loredana Cleris

Division of Experimental Oncology B, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, 20133 Milan, Italy

Abstract

Fenretinide or *N*-(4-hydroxyphenyl) retinamide (4HPR) is a synthetic retinoid currently being tested clinically, which can inhibit the development and the growth of breast and prostate cancers in rodents. The efficacy of 4HPR alone and in combination with cisplatin was tested against the human ovarian carcinoma IGROV-1 xenograft i.p. Administration p.o. of 4HPR was not effective, whereas intracavitary treatment significantly increased the survival time of treated mice. It also enhanced the antitumor activity of cisplatin. These findings suggest that 4HPR may be an active agent against epithelial ovarian tumors.

Introduction

Vitamin A is essential for growth, vision, and the maintenance of the differentiated state of various tissues and of normal reproductive function in both males and females. Retinoids, *i.e.*, natural and synthetic derivatives of vitamin A, are being clinically evaluated both as chemopreventive and therapeutic agents (1). These compounds have also been shown to inhibit experimental carcinogenesis and to induce differentiation and/or growth inhibition of fully transformed malignant cells (1).

4HPR[3] is a synthetic retinoid demonstrated to be effective and relatively nontoxic in preclinical tests and is being assessed as a chemopreventive agent in randomized clinical trials (2, 3). In rodents, 4HPR is less toxic than other retinoids when administered at equimolar concentrations and is effective in preventing breast, urinary bladder, skin, lung, and prostate tumors (4, 5). Some evidence has also been reported on the therapeutic effects of 4HPR when administered alone and in combination with tamoxifen (6) and glucarate (7) on established carcinogen-induced mammary tumors in rats. Recently, it has also been reported that this retinoid is active against prostate tumor cells transplanted in rats (8).

Ovarian cancer is a major cause of female cancer death. The high mortality associated with this tumor is due in part to its occult nature as manifested by frequent extension into the peritoneal cavity at the time of diagnosis. Treatment includes maximal surgical resection and postoperative chemotherapy. DDP-containing regimens are the most effective against this tumor, but they are rarely curative. Remissions that result from treatment are often short and after relapses, patients are usually resistant to subsequent chemotherapy (9).

Limited information is available on the effects of retinoids on ovarian tumor cells and no data have been reported on the *in vivo* effects of these agents against this tumor, either as chemopreventive or therapeutic agents.

The objectives of the present study were to investigate the efficacy of 4HPR treatment against ovarian carcinoma and to assess whether the combination of 4HPR with cisplatin, a cytotoxic drug with established activity against ovarian tumors, might result in increased activity. The human epithelial ovarian carcinoma IGROV-1 (10), which grows as an i.p. ascitic carcinomatosis, was chosen as an experimental model for assessing the clinical potential of 4HPR against ovarian carcinomas.

Materials and Methods

Materials. 4HPR was kindly provided by the R. W. Johnson Pharmaceutical Research Institute (Spring House, PA); 4HPR capsules of 100 mg each were also provided by R. W. Johnson. When administered i.p., 4HPR was dissolved in absolute ethanol and then diluted in sterile 0.9% NaCl solution, containing 1.65 mg/ml bovine serum albumin (Sigma, Milan, Italy), at a final ethanol concentration of 5%. When administered p.o., the content of each 4HPR capsule was diluted in sesame oil. 4HPR was freshly prepared once a week, always protected from light and kept at 4°C. DDP (Platamine; Farmitalia Carlo Erba, Milan, Italy) was dissolved in sterile distilled water, diluted in sterile 0.9% NaCl solution, and administered immediately after preparation.

Animals, Tumor, and Treatment. Seven-to-9-week-old female Swiss *nu/nu* mice were obtained from Charles River (Calco, Italy) and kept under standard laboratory conditions according to the guidelines of our Institute. They were maintained in laminar air flow rooms in sterilized cages with sterilized bedding and food and acidified water. The IGROV-1 tumor, a human epithelial ovarian adenocarcinoma line (10), kindly supplied by Dr. J. Benard (Institut Gustave Roussy, Villejuif, France), was established to grow as ascites and was maintained as i.p. transplants by Dr. G. Pratesi in our laboratory (11). Mice were given injections i.p. of 2.5×10^6 tumor cells in 0.2 ml phosphate-buffered saline. Six animals per group were used in each experiment which was performed at least twice. Drug treatment started 1 day after tumor cell inoculation. 4HPR was administered i.p. or p.o. 5 days/week until the onset of ascitic tumor in 2–3 treated mice/group (for 3 weeks when given alone and 5 weeks when associated with DDP). DDP was administered i.p. on a weekly schedule for a total of 3 injections. Both drugs were delivered at a volume of 10 ml/kg of body weight. Control mice were similarly treated i.p. with 5% ethanol in 0.9% NaCl solution containing 1.65 mg/ml bovine serum albumin or p.o. with sesame oil. The median survival time of control mice ranged from 18 to 24 days in the different experiments.

Statistical Analysis. The two-tailed Student's *t*-test was used for statistical analysis of the survival times.

Results

The antitumor activity of 4HPR against ascitic growing IGROV-1 tumor cells was tested by administering the drug i.p. or p.o. When administered i.p., 4HPR was delivered (5/week for 3 weeks) at daily doses of 60 and 120 mg/kg, the latter dose being the highest one soluble in 5% ethanol. In Fig. 1 the results of two experiments with homogeneous results are plotted together. A significant increase in the survival time ($P < 0.005$) was caused by both doses. No statistically significant difference in activity was observed between the 2 doses in all the experiments performed (5 of 5). A lower 4HPR dose, 1 mg/kg,

Received 8/9/93; accepted 10/5/93.

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[1] This work was supported in part by the Istituto Superiore della Sanità (Italy-USA Program on New Therapies of Neoplasia), by Associazione Italiana per la Ricerca sul Cancro and by Ministero della Sanità.

[2] To whom requests for reprints should be addressed, at the Istituto Nazionale Tumori, Via Venezian 1, 20133 Milan, Italy.

[3] The abbreviations used are: 4HPR, fenretinide [or *N*-(4-hydroxyphenyl)-all-*trans*-retinamide]; DDP, cisplatin; TGF, transforming growth factor.

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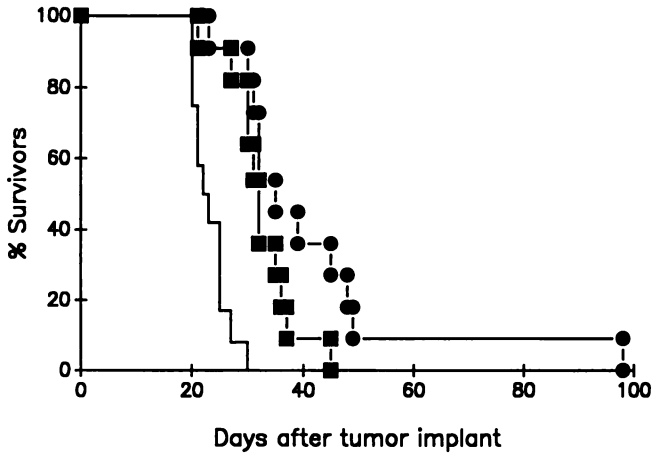

Fig. 1. Activity of 4HPR against the human ovarian carcinoma IGROV-1. Female Swiss *nu/nu* mice, inoculated i.p. with 2.5×10^6 IGROV-1 cells, were treated starting 1 day after tumor implant with 4HPR i.p. 60 (●) and 120 (■) mg/kg, 5 days/week for 3 weeks. Control mice (——) were similarly treated i.p. with the same solvent used for 4HPR (see "Materials and Methods"). Two experiments, each one with 6 mice/group, with homogeneous results are plotted together. $P < 0.05$ Student's t test for 60 and 120 mg/kg *versus* controls.

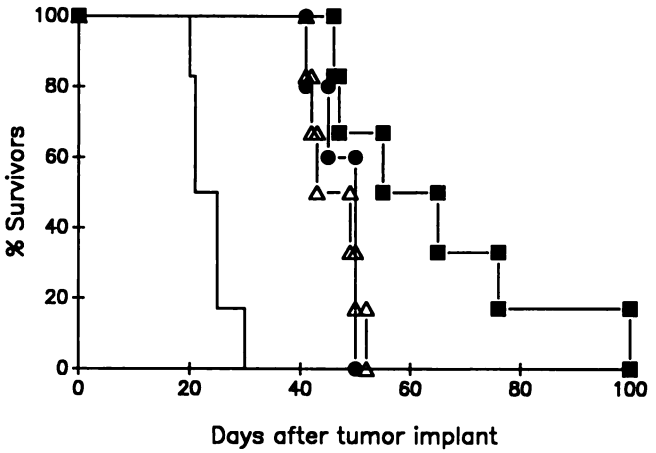

Fig. 2. Activity of DDP alone and combined with 4HPR against the human ovarian carcinoma IGROV-1. Female Swiss *nu/nu* mice inoculated i.p. with 2.5×10^6 IGROV-1 cells were treated starting 1 day after tumor implant with DDP alone, *i.p.* 6 mg/kg, 1/week for 3 weeks (△), or with DDP associated with 4HPR, i.p. 60 (●) and 120 (■) mg/kg, 5/week for 5 weeks. 4HPR was administered 3 h after DDP. Control mice (——) were similarly treated i.p. with the same solvent used for 4HPR (see "Materials and Methods"). $P < 0.05$ Student's t test for DDP associated with 4HPR 120 mg/kg *versus* DDP alone.

was also tested in one experiment and it did not cause any increase in the median survival time, even though one long-term survivor (>4 months) was observed (data not shown). 4HPR administered p.o. (5/week for 3 weeks) at daily doses of 60, 120, and 240 mg/kg neither increased survival time nor produced long-term survivors (data not shown). There was no evidence of local or systemic toxicity after i.p. or p.o. treatment with the doses administered.

Following the observation of antitumor activity of 4HPR against the IGROV-1 ovarian carcinoma, we tested the efficacy of the combination of this retinoid with DDP, a cytotoxic drug with established activity against this tumor. DDP was administered i.p. at the dose of 6 mg/kg (every 7 days for 3 doses). When 4HPR was combined with DDP, it was administered i.p. 3 h after the cytotoxic drug at doses of 60 and 120 mg/kg (5/week for 5 weeks). The results are reported in Fig. 2. DDP alone was effective against this tumor, causing a 119% increase in the median survival time (46 *versus* 21 days for DDP-treated and control mice, respectively). The combination of DDP with 4HPR at the higher dose of 120 mg/kg was significantly more effective than DDP alone ($P < 0.05$).

Discussion

In this paper we report, for the first time, that a synthetic retinoid, 4HPR, has a therapeutic effect against a human ovarian carcinoma. To our knowledge, there are no other data on the therapeutic effect of retinoids against ovarian tumors or on their chemopreventive efficacy in ovarian carcinogenesis. In fact, in spite of the well-established preventive effect of retinoids in experimental carcinogenesis of different organs, including the mammary gland, urinary bladder, prostate, lung, skin, liver, colon, and esophagus (4, 5) these agents have never been tested in carcinogen-induced ovarian tumors. This is probably due to the lack of appropriate models for ovarian cancer or to the complexities of the existing models (12). On the other hand, there is evidence for an important role of retinol and of its derivatives in ovary growth and functions. Vitamin A is essential for the maintenance of normal reproductive functions in both female and male rats (13), and retinol and retinoic acid modulate follicle-stimulating hormone action on ovarian cells (14). Among the investigated human organs, the ovary has the highest concentration of cellular retinol binding protein (15) and an average level of expression of nuclear retinoic acid receptorβ (16). It is known that the growth of ovarian follicle is stimulated by TGFα and is inhibited by TGFβ, both factors produced and secreted by theca cells within ovarian follicles (17). It has also been reported that the growth of human ovarian carcinoma cell lines may be inhibited by TGFβ (18). There is now substantial evidence that the growth inhibitory effect of retinoids on some cells is mediated by induction of secretion of specific isoforms of TGFβ (19). However, it seems to be unlikely that the induction of TGFβ is the mechanism for the antitumor effects observed here, as the IGROV-1 cell line is not sensitive to TGFβ *in vitro* (18).

The lack of activity of 4HPR when administered p.o. might be due to the fact that the 4HPR levels required in the peritoneum for cell killing are achieved only after intracavitary administration. Similarly, systemic administration of doxorubicin, a well-established cytotoxic drug, was poorly effective on the same tumor, whereas its i.p. administration greatly affected mice survival time (11). Another interesting finding of the present study is the increased antitumor efficacy of the intracavitary administration of DDP when combined with 4HPR. Using *in vitro* assays, we are currently evaluating whether this effect is additive or synergistic and whether it occurs in other human ovarian tumors.

In conclusion, our results show that 4HPR has an influence on the proliferation of ovarian carcinoma cells and suggest the importance of further research in order to understand the role of 4HPR as well as of other retinoids on ovarian carcinogenesis and on the growth of established ovarian tumors. Since 4HPR is currently being evaluated for prevention of breast cancer in a large number (3000) of women (2) it is likely that, in the same study, the efficacy of this retinoid on the incidence of ovarian tumors will also be assessed.

Acknowledgments

We thank Monica Tortoreto for excellent technical help and Laura Zanesi for editorial assistance.

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Cancer Res 1993;53:5374-5376.

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