Thymidine Phosphorylase Activity and Prodrug Effects in a Three-Dimensional Model of Angiogenesis

Implications for the Treatment of Ovarian Cancer

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Platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP) is associated with angiogenesis and the progression of human ovarian cancer. The enzyme converts thymidine to thymine and 2'-deoxyribose-1-phosphate and can also metabolize the prodrug 5'-deoxy-5-fluorouridine (Furtulon) to 5-fluorouracil and 5'-deoxy-D-ribose-1-phosphate. The aim of this study was to obtain information about the activities of Furtulon in an established three-dimensional model of angiogenesis. The plan was to study partial and complete effects of Furtulon (in the absence and presence of PD-ECGF/TP or ovarian cancer cyst fluids) on the formation and destruction of microvessels from cultured segments of rat aorta in serum-free media. The endpoint was the number and form of microvessels compared with controls after 4, 7, 11, and 14 (and sometimes 17) days in culture. Furtulon (10 μ mol/L) gradually reduced the size and number of microvessels over 17 days of culture (100 μ mol/L significantly reduced the number by day 4). PD-ECGF/TP (10 ng/ml) and ovarian cancer cyst fluids (2% in medium, v/v) stimulated the production of microvessels. The culture of explants with Furtulon and PD-ECGF/TP or ovarian cancer cyst fluids (from day 1 or day 11 of culture) enhanced the vasoclastic activity of the drug. The effect of Furtulon at the highest dose (1000 μ mol/L) or at a lower dose (100 μ mol/L) in the presence of ovarian cancer cyst fluid was not reversible after culture day 11. (*Am J Pathol* 1998, 153:1573-1578)

Angiogenesis is associated with the progression of hyperplasia to neoplasia,¹ the growth of malignant tumors *in*

situ, and metastasis.² In particular, it has been shown that the density of microvessels within malignant tumors of the breast³ or ovary⁴ is inversely related to the survival time of patients. The use of transvaginal color Doppler imaging and pulsed Doppler spectral analysis to study pelvic masses before surgery has revealed evidence suggestive of disordered angiogenesis⁵ and an association between high-velocity blood flow within the tumor and the presence of transformed cells in the excised tissues.⁶ The analysis of RNA for putative angiogenic factors in ovarian malignancies revealed the overexpression of platelet-derived endothelial cell growth factor (PD-ECGF) compared with tissue from benign tumors and the normal ovary.⁷ Subsequently, the concentration of PD-ECGF in ovarian tumor tissue was shown to be positively correlated with the peak systolic blood velocity.⁸

There is good evidence that PD-ECGF is identical to thymidine phosphorylase (TP)⁹⁻¹⁴; both preparations catalyze the reversible phosphorolysis of thymidine to thymine and 2'-deoxy-D-ribose-1-phosphate. PD-ECGF/TP has been shown to possess angiogenic activity *in vivo*¹⁵ and chemotactic activity *in vitro*.¹⁶ The enzyme activity appears to be essential for the stimulatory effect of PD-ECGF on angiogenesis.^{12, 17} We have shown that thymidine and 2'-deoxy-D-ribose-1-phosphate inhibit microvessel formation in a three-dimensional, serum-free model of angiogenesis, whereas PD-ECGF/TP and β -amino-iso-butyric acid (a metabolite of thymine) have a stimulatory effect.¹⁸ Thymidine phosphorylase (sometimes called pyrimidine phosphorylase) has also been shown to convert an anti-cancer prodrug (Furtulon, 5'-deoxy-5-fluorouridine) to 5-fluorouracil and 5'-deoxy-D-ribose-1-phosphate.¹⁹ Increased PD-ECGF/TP expression has been shown to potentiate the anti-tumor activity of Furtulon in a breast cancer cell line.²⁰ Conversely,

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resistance to fluoropyrimidine nucleoside treatment might result from the loss of thymidine phosphorylase activity.²¹

We have investigated the activity of Furtulon in a three-dimensional, serum-free model of angiogenesis in the presence of PD-ECGF/TP or selected human ovarian cancer cyst fluids known to contain low or high concentrations of the enzyme. The aim was to obtain information about the potential activity of the drug on microvessel formation and disintegration, which might help the development of an alternative new adjuvant therapy for early ovarian cancer. The experiments were designed to study the activity of the drug at different concentrations and defined periods of culture and to determine whether the effects were reversible.

Materials and Methods

Recombinant human PD-ECGF was purchased from R&D Systems Europe Ltd., Abingdon, UK. All other reagents were obtained from Sigma Chemical Co. Ltd., Poole, UK. Furtulon (5'-deoxy-5-fluorouridine) was a gift to K. Hata from the Nippon Roche Research Centre, Kamakura City, Kanagawa, Japan. Two cyst fluids from patients at King's College Hospital with histological evidence of ovarian cancer were selected from previously collected samples on the basis of 1) high intratumor peak systolic blood velocity (PSV) as determined by color Doppler imaging and pulsed spectral analysis before surgery and 2) the level of TP activity as determined by a radioenzymatic assay. Cyst fluid 1 was taken from a 43-year-old premenopausal woman with stage III clear-cell adenocarcinoma (PSV of 33 cm/second and TP activity by radioenzymatic assay of 76 pmol of thymine produced/ μ l/hour). Cyst fluid 2 was taken from a 53-year-old postmenopausal woman with stage 1a serous cystadenocarcinoma of borderline malignancy (PSV of 24 cm/second and TP activity of 21 pmol of thymine produced/ μ l/hour).

Isolation of Rat Aorta

The protocol of Nicosia and Ottinetti²² was used with minor modifications.²³ Female Wistar rats (1 to 3 months old) were killed by asphyxiation with carbon dioxide followed by cervical dislocation. The full length of the thoracic aorta was removed under aseptic conditions after partial dissection of the fibroadipose tissue *in situ*. The aorta was held at the cut ends of the tissue to avoid damaging the wall and immediately transferred to a tissue culture dish containing cold Dulbecco's modified Eagle's medium/Ham F12 medium (DMEM/HAM F12, 1/1 v/v). Any remaining adherent periadventitial fat and connective tissue was removed, and the aorta was cut into ring segments (1 mm wide); the proximal and distal 2-mm pieces were discarded. The rings were bisected to give half-ring explants, which were transferred to fresh DMEM/HAM F12.

Agarose rings were used as culture wells to set the collagen gels. Briefly, a sterile 1.5% (w/v) solution of agarose (type VII) was poured into 10-cm tissue culture dishes and, once set, agarose rings were obtained by

punching two concentric circles in the agarose gel with specifically designed aluminum punches with diameters of 10 mm and 17 mm, respectively. The agarose rings were transferred to 10-cm culture dishes; each dish contained five rings. Collagen (50 mg of type I from rat tail) dissolved overnight in 2 ml of sterile 0.1% (v/v) glacial acetic acid was diluted to a final concentration of 4.3 mg/ml with 10% (v/v) DMEM/HAM F12. The ionic strength and pH of the solution were raised simultaneously to induce gel formation. The final collagen gel solution consisted of 4.3 mg/ml collagen, NaHCO₃ (11.7 mg/ml in DMEM/HAM F12) and 10X Eagle's minimal essential medium. These solutions were stored on ice, and aliquots from each were rapidly removed and mixed in the ratio 7:2:1 (v/v/v), respectively. The pH was adjusted to physiological levels with 0.1 mol/L sodium hydroxide using phenol red as a visual indicator. The bottom of each agarose well was coated with 200 μ l of collagen solution. Collagen gel formation occurred rapidly at 37°C. The agarose wells were completely filled with collagen solution (~600 μ l in total), and the aortic explant was transferred to the agarose well. The explant was positioned in the center of the collagen gel facing downward; 20 ml of serum-free medium was added to each culture dish, and the dishes were stored at 37°C overnight.

On day 1, gels were removed from the surrounding agarose, randomly allocated to test or control groups, and allowed to float in 1 ml of test or control medium. Explant gels were incubated in a humidified incubator at 37°C (5% CO₂/95% O₂), and the medium was changed every 2 to 3 days in all cultures. DMEM/HAM F12 was used as the serum-free growth medium. All media were supplemented with glutamine (2 mmol/L), penicillin (100 U/ml), streptomycin (100 μ g/ml), and amphotericin B (2.5 μ g/ml). The form of the microvessel outgrowths and the endothelial nature of component cells has been reported by Nicosia and Ottinetti.^{22, 24} Although the spontaneous regression of microvessels after 14 days of culture has been reported,²⁵ this phenomenon was not observed in our laboratory. An example of a control explant on culture day 11 from our studies has been published.¹⁸

Reagent Preparation

A stock solution of 5'-deoxy-5-fluorouridine (100 mmol/L) was prepared in sterile filtered phosphate-buffered saline and stored at 4°C. Aliquots of the stock solutions were added to culture medium to give final concentrations of 1 to 1000 μ mol/L. For dose-response studies, serial dilutions of the stock solutions were prepared in culture medium. PD-ECGF/TP was dissolved in DMEM/HAM F12 to give a concentration of 10 ng/ml, the minimum required to stimulate microvessel formation in the assay.¹⁸ The ovarian cancer cyst fluids were added to culture medium to give a 2% mixture (v/v).

Microvessel Quantitation

Each explant was examined on days 4, 7, 11, and 14 (and sometimes day 17) of culture. The number of mi-

Table 1. Dose Response of Furtulon at Four Concentrations on Microvessel Formation by Day of Culture

	Number of explants	Microvessel number (mean $\pm$ SD)			
		Day 4	Day 7	Day 11	Day 14
Control	7	23.4 $\pm$ 2.7	22.6 $\pm$ 2.7	23.3 $\pm$ 2.5	21.3 $\pm$ 1.9
Furtulon					
1 μ mol/L	5	23.6 $\pm$ 2.1	21.5 $\pm$ 3.8	22.3 $\pm$ 2.2	20.3 $\pm$ 2.3
10 μ mol/L	6	22.5 $\pm$ 4.3	17.0 $\pm$ 3.0	12.0 $\pm$ 3.1	7.2 $\pm$ 1.9
100 μ mol/L	5	15.2 $\pm$ 3.7	9.8 $\pm$ 2.7	2.0 $\pm$ 0.6	1.5 $\pm$ 0.5
1000 μ mol/L	7	10.3 $\pm$ 3.0	2.7*	0.1*	0.0*

*Data not normally distributed due to zero values for individual replicates.

crovessels was counted by direct observation of live explants under phase-inverted optics according to established criteria.²² Microvascular sprouts were distinguished from fibroblasts by their distinct morphology (microvessels were thicker and more uniform in width along the whole length of the vessel). Single microvessels often produced two new sprouts, which were counted as two microvessels (because of probable anastomosis between converging vessels). The number of microvessels in cultures with a large number of sprouts was determined at the outer perimeter of the growth area. A hand-held tally counter was used to determine the number of microvessels. The time required for scoring each explant ranged from 1 to 3 minutes.

Thymidine Phosphorylase Radioenzymatic Assay

The phosphorylation of thymidine by ovarian cancer cyst fluid was measured with a radioisotopic technique based on the method of Sumizawa et al²⁶ with minor modifications. The reaction mixture had a total volume of 80 μ l and consisted of 10 μ l of 10 mmol/L TrisHCl, pH 7.6, 10 μ l of 50 mmol/L sodium phosphate, pH 7.0, and 10 μ l of thymidine to have a final concentration of 0.1 mmol/L, containing 2 μ Ci of [6-³H]thymidine (2 Ci/nmol; 1 mCi/ml), and 50 μ l of cyst fluid (1:2 dilution v/v). The reaction was started by the addition of the enzyme source; distilled water was used as replacement for the enzyme source in negative controls. The reaction was incubated at 37°C for 1 hour and terminated by the addition of 80 μ l of 20% trichloroacetic acid. The precipitated proteins were removed by centrifugation at 10,000 $\times$ g for 20 minutes.

Aliquots (2 μ l) of the supernatant were transferred, in duplicate, to cellulose-F chromatography plates at locations that had been previously impregnated with thymidine (0.2 μ l of 80 mmol/L thymidine) and thymine (0.4 μ l from a saturated solution in warm distilled water) as reference markers. The plates were developed by one-dimensional ascending chromatography with sterile water at room temperature for 1.5 hours. Areas containing thymine and thymidine were identified under ultraviolet light, removed with scissors, and placed in scintillation fluid. The amount of radioactivity was determined by liquid scintillation counting. The amount of thymine produced per hour per microliter of sample was calculated.

Statistical Analysis

The distributions of values for microvessel number from each set of replicate determinations (control and experimental) were assessed. The coefficient of variation (CV%) for the normally distributed control replicates (between 18 and 51 microvessels) ranged from 5.2 to 11.6. The corresponding CV% for TP cultures (over the range 1 to 100 ng/ml) ranged from 5.7 to 11.0, and for Furtulon cultures (over the range 1 to 10 μ mol/L) from 6.3 to 9.9 (100 μ mol/L produced a 90% inhibition of microvessel formation). The value for the effect of each compound tested per explant was expressed as a percentage of the mean of the control replicates for that experiment, and the data are shown as the mean $\pm$ SE or SD. Student's *t*-test was used to evaluate the significance of differences between the results from test cultures and untreated controls and between endpoints at different times in culture. A *P* value of < 0.05 was considered to be significant.

Results

Control Tissues

Microvessels sprouted from the edges of explants in control groups by day 4 of culture. The number and length of the vessels increased with time until the experiments were terminated on culture day 14 or 17.

Effects of Furtulon

The effect of Furtulon at four concentrations (1, 10, 100, and 1000 μ mol/L) on microvessel number at days 4, 7, 11, and 14 of culture is shown in Table 1. There was a significant reduction in microvessel number at the higher concentrations of drug (100 and 1000 μ mol/L) by day 4 of culture and a gradual reduction throughout the culture period at the level of 10 μ mol/L. Vasodegradation was characterized by a reduction in the width of vessels with subcellular fractionation followed by cellular dissolution (suggestive of death by necrosis).

Effects of Thymidine Phosphorylase

Explants were cultured in a medium containing Furtulon (10 μ mol/L) and authentic PD-ECGF/TP (10 ng/ml) or

Table 2. Effect of Platelet-Derived Endothelial Cell Growth Factor/Thymidine Phosphorylase (PD-ECGF/TP), Ovarian Cancer Cyst Fluids 1 and 2, and Furtulon on Microvessel Number in a Three-Dimensional Serum-Free Model of Angiogenesis

Test substances	Microvessel number (% of control, mean $\pm$ SD)			
	Day 4	Day 7	Day 11	Day 14
PD-ECGF/TP	134.8 $\pm$ 15.2	121.6 $\pm$ 29.9	121.5 $\pm$ 26.4	136.9 $\pm$ 25.1
Cyst fluid 1	188.4 $\pm$ 33.2	139.6 $\pm$ 36.8	155.0 $\pm$ 39.3	170.9 $\pm$ 18.4
Cyst fluid 2	185.2 $\pm$ 35.7	150.9 $\pm$ 37.9	275.0 $\pm$ 60.1	240.5 $\pm$ 21.4
Furtulon	106.3 $\pm$ 28.5	80.1 $\pm$ 14.2	50.3 $\pm$ 12.8	36.0 $\pm$ 2.3
Furtulon plus PD-ECGF/TP	95.2 $\pm$ 25.3	32.6 $\pm$ 7.7	7.5*	3.6*
Furtulon plus cyst fluid 1	114.3 $\pm$ 25.2	49.9 $\pm$ 15.5	20.0 $\pm$ 5.0	7.9*
Furtulon plus cyst fluid 2	169.3 $\pm$ 35.9	59.7 $\pm$ 7.7	25.0*	9.5*

There were 7 to 10 explants per experiment. The following concentrations were used: PD-ECGF/TP, 10 ng/ml; ovarian cancer cyst fluids, 2% v/v; Furtulon, 10 μ mol/L. Number of control vessels used were as follows: 20 on day 4, 18 on day 7, 20 on day 11, and 21 on day 14; 28 explants (7 per day) were used.

* Data not normally distributed due to zero values for individual replicates.

ovarian cancer cyst fluids 1 and 2 (2% v/v). These results together with the corresponding values for the explants cultured with the individual additives are shown in Table 2. The values are reported as a percentage of the controls (ie, explants cultured in media alone). PD-ECGF/TP and both cyst fluids increased the number of microvessels over the culture period (from 137% to 241% of the control mean at day 14). Furtulon reduced the number of microvessels at days 7, 11, and 14 of culture. PD-ECGF/TP enhanced the inhibitory effect of Furtulon over the culture period. Both cyst fluids increased the number of microvessels in the presence of Furtulon at day 4 of culture, but thereafter the vasoclastic activity of the drug was increasingly apparent.

Effects on Established Microvascular Networks

Control and PD-ECGF/TP-treated explants were allowed to develop until day 11 of culture when a well established microvascular network had been established. One-half of the explants were transferred from their respective media to culture medium containing 100 μ mol/L 5'-deoxy-5-fluorouridine (Furtulon). In both control and PD-ECGF/TP-treated explants there was a rapid decrease in the number of microvessels after day 11. The decrease continued until day 17 of culture when the experiment was terminated. The data are summarized in Figure 1.

The vasoclastic effect of Furtulon was also studied in cultures stimulated by cyst fluids 1 and 2. 5'-Deoxy-5-fluorouridine (10 μ mol/L) was added to the respective cyst fluid medium of one-half of the explants on day 11 of culture. The effect on the number of microvessels seen on culture days 14 and 17 is shown in Figure 2. The addition of Furtulon produced a significant decrease in the level of microvessel formation from day 11 of culture onwards ($P < 0.01$). The inhibition was more pronounced with cyst fluid 1, which had higher TP activity. The level of thymidine phosphorylase activity in the cyst fluid correlated with the level of decrease in the number of microvessels ($P < 0.0001$) on day 14 of culture.

Reversibility of Vasoclastic Effect

The ability of Furtulon to completely destroy microvessels was examined further by exchanging medium containing

5'-deoxy-5-fluorouridine (100 μ mol/L and 1 mmol/L) for a medium containing cyst fluid 1 for one-half of the explants on day 11 of culture. A small but not significant recovery was seen with explants cultured in the 100 μ mol/L concentration of 5'-deoxy-5-fluorouridine, whereas no recovery was seen with explants exposed to the 1 mmol/L concentration (data not shown). The recovery from the lower dose involved the inhibition of existing microvessels breaking down and new vessels sprouting from the wall of the explant.

Finally, one-half of the explants cultured in medium containing either cyst fluid 1 or cyst fluid 2 and 5'-deoxy-5-fluorouridine were transferred on day 11 of culture to medium containing the corresponding cyst fluid alone. The change in medium produced a small but not significant increase in the number of microvessels from day 11 to day 14 of culture with both fluids (data not shown). The increase failed to continue to day 17 of culture. At this time the number of microvessels had reduced to the level observed for explants cultured in the respective cyst fluid and 5'-deoxy-5-fluorouridine. This finding suggests that

Figure 1. Number of microvessels produced from rat aorta explants cultured in control and thymidine phosphorylase media for 11 days followed by media containing 5'-deoxy-5-fluorouridine. —○—, control; ---○---, control plus 5'-deoxy-5-fluorouridine (100 μ mol/L); —□—, thymidine phosphorylase (10 ng/ml); ---□---, thymidine phosphorylase (10 ng/ml) plus 5'-deoxy-5-fluorouridine (100 μ mol/L). Results are expressed as means $\pm$ SE (12 explants).

Figure 2. Microvessel growth from rat aorta explants cultured in the presence of ovarian cancer cyst fluid (2% v/v) for 11 days followed by the addition of 5'-deoxy-5-fluorouridine (10 μ mol/L) to the medium (means $\pm$ SE, 9 explants). —○—, cyst fluid 1; ---○---, cyst fluid 1 plus 5'-deoxy-5-fluorouridine at day 11; —□—, cyst fluid 2; ---□---, cyst fluid 2 plus 5'-deoxy-5-fluorouridine at day 11. Control mean was 19 vessels on day 4, 18 on day 7, 20 on day 11, and 21 on day 14; 28 explants (7 per day) were done.

the recovery from 5'-deoxy-5-fluorouridine in combination with cyst fluid was not sustainable.

Discussion

The results using the rat aorta assay are the first to show that Furtulon can inhibit formation of microvessels or initiate their breakdown *in vitro* in a dose-dependent manner. The effect on microvessel number is most likely due to the endothelial cells expressing PD-ECGF/TP, which converts the drug to 5-fluorouracil leading to cell death through the inhibition of thymidylate synthetase and incorporation of fluoronucleotides into DNA and RNA.²⁷ This hypothesis is consistent with the finding that 5-fluorouracil was a potent inhibitor of microvessel formation over the whole period of culture (data not shown). Furthermore, 5'-deoxy-D-ribose-1-phosphate, the other immediate product of PD-ECGF/TP activity on Furtulon, may also inhibit cellular proliferation by binding to other bases and inhibiting DNA synthesis by chain termination. However, the fact that 5-fluorouracil (1 mmol/L) was more effective than 5'-deoxy-5-fluorouridine (1 mmol/L) at inhibiting microvessel formation suggests that not all of the prodrug was metabolized. The proposed mechanism of action (by the production of cytotoxic metabolites) is supported by the following data: 1) Furtulon was minimally toxic to Chinese hamster ovary cells *in vitro*,²⁸ 2) the prodrug did not have any severe cytotoxic effects on normal human cells from tissue extracts,²⁹ and 3) the prodrug was selectively activated by tumor cells.³⁰ Moreover, it has been demonstrated that Furtulon and 5-fluorouracil kill P338 leukemia cells in a similar manner.³¹

The probability that Furtulon mainly acts as a prodrug is further supported by the finding in the present study of increased vasostatic activity after the addition of exogenous PD-ECGF/TP or aliquots of ovarian cancer cyst fluid (shown to possess PD-ECGF/TP activity). The inhibitory

effect of the prodrug on microvessel formation, however, did not seem to be related to the level of TP activity within the ovarian cancer cyst fluid. This finding may be because 1) the cyst-fluid-stimulated endothelial cells are highly susceptible to the effect of Furtulon at the early stages of microvessel formation, 2) the amount of Furtulon converted by the low level of PD-ECGF/TP in cyst fluid 2 (21 pmol of thymine produced/hour/ μ l) may be sufficient to inhibit microvessel formation, or 3) the extra protein associated with the cyst fluid may enhance the inhibitory effect of the drug in a specific manner.

This is also the first report to our knowledge that Furtulon is potentially vasoclastic, ie, the drug may also be capable of destroying established microvascular networks. This effect is important because a malignant ovarian tumor is likely to have a complex microvascular network by the time that it is detected and treated. The vasoclastic effect of Furtulon was enhanced by the addition of PD-ECGF/TP to the culture medium. This finding suggests that the effect of Furtulon could be increased by promoting the expression of PD-ECGF/TP within the tumor or endothelial cells (and it is likely that the toxic effects of the prodrug metabolites would affect all local cell types). Our results also demonstrate that Furtulon is vasoclastic in cyst-fluid-stimulated cultures although the cellular origin of the PD-ECGF/TP is unknown. The initial degradation effect of the prodrug would appear to be related to the level of thymidine phosphorylase activity within the cyst fluid. Cyst fluid 1, which had high TP activity, caused more microvessels to disintegrate in combination with 5'-deoxy-5-fluorouridine in established cultures than cyst fluid 2 (with a low level of TP activity) on day 14 of culture.

It would also appear that the recovery of endothelial cells in the assay system was dependent upon the dose of Furtulon, a low level of recovery being observed with 100 μ mol/L, whereas no recovery was seen with 1 mmol/L. This finding suggests that a high dose would be required to prevent a significant recovery of the microvasculature in human ovarian cancers. The level of tumor expression of PD-ECGF/TP is likely to play a role in determining the amount of Furtulon that would be required. A similar conclusion can be drawn from the results of the experiment involving the removal of 5'-deoxy-5-fluorouridine from cyst-fluid-treated cultures. However, unlike control and PD-ECGF-treated cultures there was not a significant regrowth of the microvasculature at a lower dose. This difference is likely to be due to other factors (possibly angiogenic inhibitors) present in the cyst fluids that have yet to be identified. It is also possible that the exposure to a 10 μ mol/L dose of Furtulon, a realistically clinically achievable dose in tumor tissue,³² in combination with cyst fluid was more effective at inhibiting microvessel formation than the 100 μ mol/L concentration used in control and PD-ECGF-treated cultures. This explanation would correspond with the fact that a recovery experiment without cyst fluid at a 1 mmol/L concentration of Furtulon failed to produce any regrowth of microvessels.

The preliminary data presented in this paper suggest that the use of TP activity in combination with Furtulon

might provide a potentially highly effective method by which to reduce the level of inherent or tumor-stimulated angiogenesis in pathological conditions, thereby limiting disease progression. One possible drawback of the effectiveness of this approach is the nonspecific nature of the inhibition. However, the local administration of an appropriate dose should limit this potentially adverse effect, and any nonspecific action may aid in the reduction of the excessive stimulation of angiogenesis seen after surgery to remove ovarian tumors. Furthermore, it is tempting to speculate that the clinical use of Furtulon, or related prodrugs, may be useful for the pretreatment of stage I tumors (as detected by ultrasonography with color Doppler imaging) before surgical removal.

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