Final Report

DOE Grant DE-FG02-ER62793

Title of Project: Auger Electron-emitting Estrogens for Treatment of Peritoneal Micrometastases

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Grants ID no. 18042

May 1, 1999 – April 30, 2003

DISCLAIMER

Executive Summary: This research was intended to evaluate the potential of Auger electron-emitting estrogens to treat estrogen receptor positive (ER+) micrometastases in the peritoneal cavity. Initial efforts required the re-establishment of the ER+, intraperitoneal (IP) micrometastasis model in the immunodeficient mouse, since initial attempts to reproduce the previous model in the nude mouse did not lead to IP tumors. Subsequent studies showed that by using the SCID mouse, increasing the number of tumor cells injected, now mixed with Matrigel, and waiting a longer time for tumor development provided a consistent ER+ IP micrometastasis model. During the time required for re-establishing the mouse tumor model, studies were conducted using direct injection of radioiodinated estrogen (17α-iodovinylestradiol, IVME2) into subcutaneous MCF-7 tumors in SCID mice to determine both the retention of the iodoestrogen injected directly in the tumor and the distribution of the iodoestrogen to normal target tissues in the animal. These studies showed that while such administration gave heterogeneous distribution within the tumors, once the iodoestrogen was located in the tumor it showed prolonged (up to 24 hour) retention in the tumor with only low levels of uptake by other ER+ normal tissues. Results from in vitro/in vivo studies designed to relate the uptake of radioiodinated estrogen by ER+ tumor cells (in vitro) with tumor cell viability and tumor yield in vivo demonstrated that incorporation of about 5,000 iodoestrogen molecules per cell was equivalent to about a 90% cell kill, about 9,000 iodoestrogen molecules per cell resulted in about a 99% cell kill whereas 24,000 iodoestrogens per cell gave a greater than 99% and no evidence for any tumors. Results from a number of in vivo studies on the uptake of iodoestrogen injected i.p. on ER+ tumor cell uptake in the peritoneal cavity were conducted, showing there was a volume, and concentration-dependent uptake by the tumor cells but that for a given amount of iodoestrogen higher uptake was seen using larger volume of lower concentrations of the iodoestrogen injected into the peritoneal cavity rather than a smaller volume with a higher concentration Finally, several actual tests of therapy of intraperitoneal tumor cells with [^{123}I]-IVME2 were carried out with ER+ cells in the i.p. cavity of the SCID mouse. The first experiment showed that although only about 500 molecules per cell of the iodoestrogen were taken up by the ER+ tumors cells in the peritoneal cavity, it was sufficient to reduce the viability of the tumor cells, resulting in reduced tumor yields and tumor weights. While two injections of [^{123}I]-IVME2 a week apart were more effective, the higher the specific activity the more effective the cell kill. However, a second experiment in which the actual specific activities of the [^{123}I]-IVME2 could not be definitively determined due to technical problems showed that treatment at the day of tumor implantation reduced the number and size of tumors, and subsequent treatments did not significantly reduce the tumor numbers or average tumor wt per animal. While difficulties in the synthesis of the new, high affinity nonsteroidal iodoestrogen, I-THPE could not be tested because of difficulties in the synthesis of the precursor for its radio iodination, the method of preparation was completed just at the end of the grant period and will be reported in the literature.

Detailed summary of work performed.

1) Tumor Model

While research in our laboratory prior to the initiation of this grant established a micrometastases model for ER+ cancer in the peritoneal cavity by injecting 10^6 to 10^7 MCF-7 human breast cancer cells into nude mice, attempts to reproduce the results with this model after initiation of this grant showed that with nude mice IP tumors were not obtained and even injection of cells subcutaneously resulted in variable tumor yields and tumor growth. Several in vivo tumor induction studies were carried out, first using nude mice with increased numbers of cells injected into the peritoneal cavity, and longer times allowed for tumor induction, then using subcutaneous injection of the cells to be able to assess whether the tumor cells themselves, the intraperitoneal site or the nature of the mouse was the cause of the problem. Eventually the research resulted eventually in determining that using SCID, rather than nude, mice and co-injection of larger numbers of the tumor cells with Matrigel were required to obtain consistent intraperitoneal tumors, usually found under the liver near the bile duct. The time to measurable tumors also was significantly longer than seen earlier, about 4 months. Thus the therapy studies were delayed substantially because either the nature of the tumor cells had changed or the immune competence of the nude mice had varied over those used in the preliminary studies.

2) Retention of estrogen receptor (ER) capacity in tumors growing in immunodeficient mice.

As we were able to induce subcutaneous tumors during the sequence of experiments described above these tumors were assayed to determine the amount of estrogen receptor in the tumors to make sure that they continued to express ER. Immunohistochemical assay for ER confirmed the consistent expression of ER in the cells of the tumor, and quantitative assays indicated an average of about 26,000 ER sites per cell, a high average for ER positive human cancers. Intraperitoneal injection of tumor cells mixed with Matrigel, the method that gave the most uniform tumor yield of i.p. tumors resulted in early micrometastases that included Matrigel, but by the time the tumors reached 20 mg or larger, the tumor histology was similar to subcutaneous tumors without residual Matrigel.

3) Retention of radioiodoestrogen following intratumoral injection.

During the time that was required to re-establish a useable tumor model we had animals with subcutaneous tumors available that allowed us to determine what the retention of radioiodoestrogen could be in tumors that had significant amounts of radioiodoestrogen. To do this we conducted several experiments in which the [^{125}I] radioiodoestrogen was injected directly into the tumors followed by sacrifice of the mice at various times thereafter to determine not only the retention pattern of the injected radioiodoestrogen, but also to assay other ER positive normal tissues in the body for estimation of the possible deleterious effect of radioiodoestrogen released by tumor tissue. Although we had a modified needle constructed with multiple holes to try to provide more uniform distribution of the radioiodoestrogen within the tumor, the heterogeneity within the tumors was evident with the modified as well as usual needles. The conclusions from these studies were as follows:

a) The largest tumors had the greatest percent retention of radioiodoestrogen
b) As expected the highest concentration was in the smallest tumors, and this was seen even at longer times, up to 16 hours.
c) All tumors showed significant variation in radioiodoestrogen concentration within the tumors, suggesting that there was limited ability of the radioiodoestrogen to diffuse within each tumor. This suggests that initial uptake needs to be uniform within small tumors to be effective, again emphasizing the benefit to treat micrometastases as initially proposed.
d) The highest non-tumor concentrations were found in the uterus of animals with the smallest tumors, consistent with the observation of the largest percent retention in the larger tumors
e) There was a trend for increases in uterine concentration with time, even up to 20 hours, indicative of continued uptake and retention by normal ER-containing tissues in the animal.
f) The long term retention of radioiodoestrogen in the larger tumors where as much as 50% of the injected radioactivity was found at both 2 and 6 hours, suggests that if one is able to deliver the radioiodoestrogen to the tumor, the ER content is sufficient to effect its retention.
g) The ratio of the average tumor radioiodine concentration to that in the uterus (indicative of recirculation) ranged from 2.7 to 27. The general preponderance of tumor over uterine radioiodoestrogen is consistent with a greater effectiveness in the tumor over normal tissue via this mode of injection.

As part of the studies with available subcutaneous tumors during the tumor model development we also injected several of these tumors with the short half-life I-123 radioiodoestrogen (IVME2) to try to assess if direct injection into larger tumors would show any radiolytic effects on the tumors. We compared rumors injected with 0.5, 1.0, 2.0 and 4.0 mCi of [^{123}I] 17α-iodovinylestradiol with control tumors looking at the histology of the tumors at 7 days after injection. There was no evidence for any differences in the tumor histology related to radioiodoestrogen or dose of radioiodoestrogen. This is probably not unexpected if one considers that with tumors that averaged several hundred milligrams in weight, even the high dose of [^{123}I] 17α-iodovinylestradiol there would be about 10 molecules per cell, and our previous research established that the mean lethal dose of [^{123}I] 17α-iodovinylestradiol is about 500-800 decays per cell.

4) In Vitro/In Vivo Tumor Experiment

In an attempt to estimate the relative efficacy of incorporated [^{123}I]-IVME2 in reducing the viability of ER+ tumor cells, an experiment was conducted to evaluate the tumor potential of MCF-7 ER+ tumor cells implanted subcutaneously in the SCID mouse comparing different numbers of control tumor cells with tumor cells treated with [^{123}I]-IVME2 in vitro at one of three concentrations. Groups of 5 SCID mice were used in the following conditions:

Groupnumber of cells		treatment
A1	100×10^5	control
A2	10×10^5	control
A3	1×10^5	control
B1	100×10^5	600 pM [^{123}I]-IVME2
B1	100×10^5	170 pM [^{123}I]-IVME2
B1	100×10^5	40 pM [^{123}I]-IVME2
C	100×10^5	600 pM [^{123}I]-IVME2 + excess unlabeled E2

The cells were incubated in vitro at 37°C for 30 minutes, followed by washing with medium to remove unbound [^{123}I] -IVME2, mixing with an equal volume of cold Matrigel and subcutaneous injection into the backs of the mice. The animals were followed using electronic caliper measurement of two diameters of the tumors, and after subtraction of the skin thickness, used to calculate tumor volumes, using the formula for an ellipsoid of revolution. The results at 11 weeks were as follows.

Group	Mice w/tumors (%)	Av tumor vol (mm^3)	million cells injected	Mol/cell
A1	5 (100)	722	10	
A2	4 (80)	133	1	
A3	2 (20)	55	0.1	
B1	0	0	10	23,900
B2	4 (80)	38	10	9215
B3	4 (80)	224	10	5546
C	5 (100)	320	10	400

The molecules per cell was determined by assaying the amount of [^{123}I]-IVME2 associated with the washed, resuspended cells that were then injected into the mice. Hence, it appears that when about 24,000 molecules per cell of [^{123}I] -IVME2 are incorporated more than 99 % of the cells are killed, since a dose of 1% of that number of control cells did form some tumors. Interestingly, in vitro this only required a concentration of less than 1 nM [^{123}I] -IVME2. However, when half this number of molecules per cell were incorporated the results appeared to be between that of 1 and 10% of the number of treated cells used. Finally, when 5500 molecules per cell of [^{123}I] -IVME2 were incorporated, the results were consistent with about a 10% or less cell kill. The specificity of the [^{123}I] -IVME2 effect is seen in group C, where even with the highest concentration of [^{123}I] -IVME2, when unlabled estradiol was used to inhibit the binding to ER, all the animals had tumors. The fact that the average tumor size was smaller than group A1 might suggest that the 400 molecules per cell that were incorporated may have had a small effect on tumor cell viability. Following autopsy of the tumors from the group B3 assays showed that the tumors contained about 14,000 ER molecules per cell similar to the 14-21,000 ER per cell of the control tumors, suggesting that the treatment with the [^{123}I] -IVME2 did not cause the selection of ER negative tumor cells for growth. The cells incubated in vitro were also assayed for in vitro viability, in collaboration with Dr. Linda Yasui, at Northern Illinois University, who calculated a mean leathal dose of under 200 decays per cell, somewhat less than we had previous estimated from in vitro studies of ~500 decays per cell.

5) Uptake of radioiodoestrogen by intraperitoneal tumor cells.

In an effort to understand the kinetics of uptake and retention of radioiodoestrogen by ER+ tumor cells that are in the peritoneal cavity, several experiments were conducted to assay the levels of radioiodine in the cells at various times after treatment. In most cases we used [^{125}I] -IVME2, because of its lower cost and because the longer half-life of Iodine-125 relative to the actual therapy isotope, Iodine-123 simplifies calculations of retention. Since with these experiments we were not interested in assessing cell viability after treatment, and there was no reason to expect an isotope effect on uptake and retention, the longer half-life isotope was chosen. In these experiments either nude mice or later SCID mice were used and the amount of radioiodine in tumor cells in the peritoneal cavity were measured as a function of time, comparing cells labeled with [^{125}I] -IVME2 in vitro and injected i.p. with that in cells that were injected unlabeled, followed shortly thereafter by i.p. administration of 0.5 ml of 4 nM [^{125}I] -IVME2. In each case about 13 million cells were injected at the indicated time point, the animals were sacrificed and immediately thereafter 1 ml of PBS was injected into the peritoneal cavity. This fluid was distributed throughout the peritoneal cavity and used to aspirate cells, followed by two additional PBS washes of the peritoneal cavity. The collected fluid was centrifuged to collect the cells, the number of which were counted and then assayed for radioiodine. In the first experiment a relatively low incorporation of [^{125}I]-IVME2 in the MCF-7 cells was effected in vitro, ~2400 molecules per cell. The results below are given as numbers of molecules of iodoestrogen per cell.

	In vitro labeled cells		unlabeled cells + [^{125}I]-IVME2 in vivo	
Time point	tumor cells	uterus	tumor cells	uterus
2 hr	1640	10	1241	180
24 hr	384	7	913	61
48 hr	104	6		

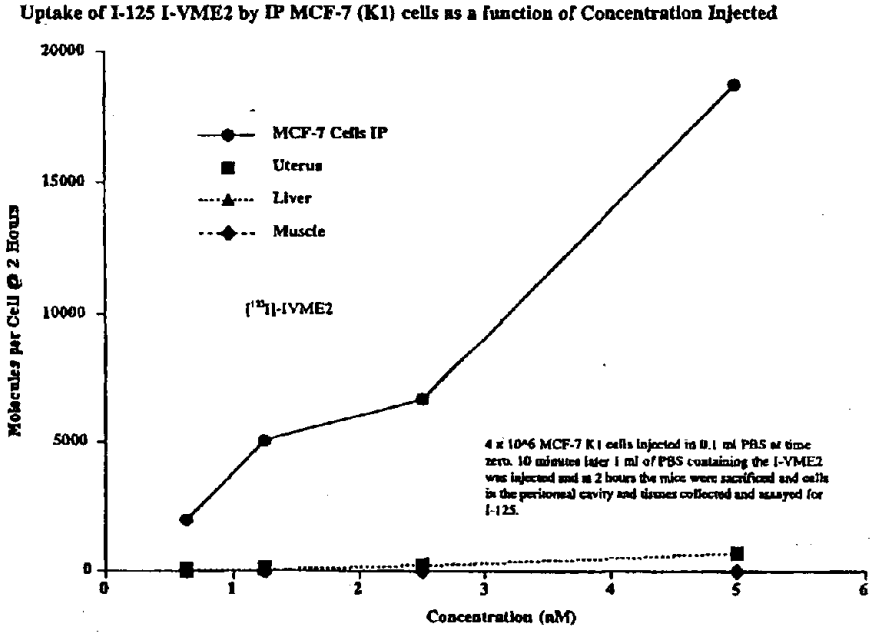
While the recovery of cells at 2 hours was excellent, averaging about 40-60 % of injected cell numbers, by 24 hours only 2.5 to 6% of the initially injected cells could be recovered. Furthermore, it can be seen that at 2 hours the radioactive cells still retained about two-thirds of their initial activity, and still had about 16% at 24 hours. In this experiment the labeling of the cells when they were in the peritoneal cavity compared favorably with that of the pre-labeled cells, although clearly the latter were only labeled to a low number of molecules per cell. It is also of interest that while, as expected, the uterine content of radioiodine was higher when the [^{125}I]-IVME2 was injected into the animal, the actual number of molecules per cell in the uterus was below the mean lethal dose, and therefore would not be expected to give rise to significant cell killing in this normal tissue.

Since other experiments suggested that in SCID mice we were able to recover more cells injected into the peritoneal cavity with Matrigel, this experiment was repeated, in this case using pre-labeled cells with a higher iodoestrogen content to start with. In this case the in vitro incubation with [^{125}I]-IVME2 gave a content of 37,500 molecules per cell. For comparison after the cells were injected i.p. this was followed with 0.5 ml of 1.25 nM [^{125}I]-IVME2.

	In vitro labeled cells		unlabeled cells + [^{125}I]-IVME2 in vivo	
Time point	tumor cells	uterus	tumor cells	uterus
2 hr	11,251	48	2093	138
24 hr	1713	65	1763	68
48 hr	208	44	162	57

While at 2 hours substantially more radioiodoestrogen was associated with the cells pre-labeled in vitro than those labeled in vivo, by 24 hours this difference had disappeared and by 48 hours less than 10 % of the radioiodine remained. Interestingly, in this case where the there was initially a higher labeling in vitro, relatively more radioactivity reached the uterus from the in vitro pre-labeled cells than previously seen when the pre-labeled cells had less iodoestrogen. Thus in this case the uterine radioactivity was not very different between the two groups. Consistent with the earlier preliminary results, more cells could be recovered, up to 20% at 24 hours.

Recognizing that most likely for therapeutic purposes in humans, the significant cost of I-123 at therapeutic doses would limit the total amount of I-123 iodoestrogen that could be administered, we conducted several experiments to look at the uptake of iodoestrogen by ER+ tumor cells in the peritoneal cavity as a function of concentration or volume of the injectant



As seen in the following table, comparing the uptake by injected MCF-7 cells in the peritoneal cavity when different volumes of 1 nM [^{125}I]-IVME2 were injected injected it was clear that larger volumes gave significantly better cell uptake, even though theoretically one would expect that the uptake by the cells should relate to the concentration rather than the volume.

Volume (ml)	Median Molecules per Cell
0.25	1341
0.5	1675
1.0	2967
1.5	5556

It may be that with the smaller volumes the intraperitoneal fluid already present in the animal dilutes the radioactive estrogen.

However, if the total amount of radioactivity will be limiting, due to cost, it would be important to know whether small amounts of a high concentration or a larger volume of a lower concentration could give rise to better uptake by tumor cells in the peritoneal cavity. To answer that question we looked at the two hour and 6 hour retention of [^{125}I]-IVME2 in i.p. tumor cells when the same total amount of [^{125}I]-IVME2 radioactivity was injected. Again the results are presented as average molecules per cell.

Injectant	2 hour time point	6 hour time point
0.25 ml of 20 nM	19,004 ± 3,006	6850 ± 3,170
0.5 ml of 10 nM	19,656 ± 8,468	6934 ± 6,080
1.0 ml of 5 nM	33,149 ± 7,460	10,894 ± 2,989
1.5 ml of 3.3 nM	26,296 ± 12,734	7,255 ± 3,853

These data suggest that at least up to a point a larger volume of a lower concentration is more effective at labeling cells than is a smaller volume of a higher concentration.

6) Intraperitoneal Therapy of ER+ Tumor Cells with [^{123}I]-IVME2

Having established a useable i.p. tumor model and studied a number of factors that bear on the potential efficacy of intraperitoneal lavage therapy, such as the uptake and retention of radioiodoestrogen by i.p. tumor cells in vivo, we proceeded to set up a large therapy trial in the SCID mouse. In this experiment we hoped to compare the tumor yields and tumor weights of control mice injected with 10 million MCF-7 tumor cells (mixed with an equal volume of Matrigel) with mice also injected with [^{123}I]-IVME2 at several times and also with mice injected with similar numbers of cells incubated with [^{123}I]-IVME2 in vitro. The groups of animals were as follows:

[^{123}I] IVME2 day zero - Sp. Act. 93,000 Ci/mmole (~40% theory)
day 7 - Sp. Act. ~240,000 Ci/mmole (100% of theory)

Group A - Control - only 10^7 MCF-7 K1 tumor cells
Group B - 10^7 MCF-7 K1 tumor cells incubated in vitro with [^{123}I]-IVME2
Group C - 10^7 MCF-7 K1 tumor cells + 0.5 ml 1 nM [^{123}I]-IVME2 IP day 0
Group D - 10^7 MCF-7 K1 tumor cells + 0.5 3 nM [^{123}I]-IVME2 IP day 0
Group E - 10^7 MCF-7 K1 tumor cells + 0.5 3 nM [^{123}I]-IVME2 IP day 0 & day 7
Group F - 10^7 MCF-7 K1 tumor cells + 0.5 3 nM [^{123}I]-IVME2 IP day 7

Group G - 10^7 MCF-7 K1 tumor cells + 0.5 ml 5 nM [^{125}I]-IVME2 IP 2 & 24 hr assay
Group H - no tumor cells - 0.5 3 nM [^{123}I]-IVME2 IP day 0 (2 mice) & day 7 (5 mice)
Group I - No Treatment

For group B, in which the cells were pre-labeled in vitro the cells were incubated with 1.87 nM [^{123}I]-IVME2 for 30 minute s at 37°C, washed with media, resuspended and counted. The cells were found to incorporate~40 DPM per cell, equivalent to 44,700 molecules per cell. In addition to injecting 10 million of these cells into each of the 10 SCID mice in group B, additional cells were plated in vitro for colony assay to assess cell survival. Those results showed less than 0.1% survival in vitro.

In addition, to estimate the relative uptake of radioiodoestrogen in the tumor cells in the mice, additional mice, group G, that also received the 10 million control MCF-7 cells, were injected i.p. with 0.5 ml of 5 nM[^{125}I]-IVME2, animals sacrificed and the i.p. cells and various other normal tissues assayed for radioactivity at 2 and 24 hours.

	2 Hours		24 Hours	
	DPM/mg*	Molecules/cell**	DPM/mg*	Molecules/cell**
Tumor Cells	42,575	5250	1,648	203
Uterus	4,281	528	2,289	282
Vagina	1,031	127	711	88
Liver	761	94	202	25
Muscle	66	8	8	0.9

* DPM per 10^6 cells for tumor cells
** based on 10^6 cells per mg of tissue

These results showed that there was a very good retention of iodoestrogen in the tumor cells, with about 30% of the 2 hour content of [^{125}I]-IVME2 remaining at 24 hours. While normal ER+ tissues showed a significant uptake from this higher than usual (i.e. 5 nM) concentration of iodoestrogen, the level even in the uterus was only about 10% of that found in the tumor cells at 2 hours. However, it appeared that the retention in the uterine cells was better with time, indicative of the higher ER content of these cells compared with the tumor cells. Other normal tissues showed even lower levels of retained [^{125}I]-IVME2.

The control animals showed a rather uniform production of i.p. tumors, generally appearing under the liver near the bile duct as shown in the photograph above. Less consistently there were also tumors at various other locations, usually associated with the intestines or abdominal fat. Rarely were the lesions found to invade the adjacent tissue.

In addition, in most of the mice, with the exception group B, injection site tumors appeared. These tumors were either attached to the peritoneal muscle or the skin at the site in which the tumor cells were injected into the animal. The generally uniform occurrence of these tumors in animals injected with untreated tumor cells attested to the viability of the tumor cells. It is most likely that small numbers of these cells were trapped in the muscle or skin at the time of injection, and not being affected by the subsequent i.p. treatment with iodoestrogen since they were not exposed to the peritoneal cavity, were not affected by treatment. Thus they served more or less as an internal control for the viability of the tumor cells. This can be seen by the fact that animals in group B, which had lower viability due to treatment with the radioiodoestrogen in vitro, did not show such tumors.

The animals were autopsied after 16 weeks with the following results:

Mouse Experiment 12 - Analysis of tumor yields and tumor weights

	A Control	B In vitro	C Low day 0	D high day 0	E high day 0 & 7	F high day 7
Injection Site						
% w/tumors	80	0	90	100	90	80
Av wt (mg)	112.7	0	425.8	233.6	234.5	161.5
Under Liver						
% w/tumors	90	0	90	80	40	40
Av wt (mg)	37.6	0	17.5	7	2.6	10.3
Other Sites						
% w/tumors	80	0	40	30	30	30
Av wt (mg)	31.4	0	2.1	2	2	2.3

The results at 16 weeks showed that injections of [^{123}I] I-VME2 into the peritoneal cavity of mice previously injected with ER+ MCF-7 tumor cells can reduce the number of intraperitoneal tumors formed and the weight of the tumors, thereby demonstrating a radiolytic effect of the I-123 labeled estrogen. Although a single assay of the I-123 in tumor cells in the peritoneal cavity showed less than 20% of the incorporated radioiodine of the higher dose of the [^{125}I] I-VME2 studied more extensively (shown above), the viability of the tumor cells in the peritoneal cavity after administration of [^{123}I] I-VME2 were significantly reduced. In this experiment generally larger tumors were found at the injection site. Since the cells deposited in the muscle or skin at the time of tumor cell injection would not be reached by the i.p. infusion of [^{125}I] I-VME2, there was no decrease in the number or size of these tumors in the treated animals. The mice receiving two doses of [^{125}I] I-VME2, a week apart, showed a greater reduction of internal tumors than those receiving only one dose. Animals receiving only one dose of [^{123}I] I-VME2 a week after receiving the tumor cells still showed a reduction of tumor number and weight, compared with controls. However, it is likely that the improved specific activity of the [^{123}I] I-VME2 obtained at day 7 contributed to this efficacy and that if the initial [^{123}I] I-VME2 had been of a higher

specific activity even better tumor control may have been possible. Unfortunately, because other projects ongoing in our laboratory involve the handling and study of various estrogen receptor ligands, the minute contamination from these substances very likely contribute to our recent difficulty in obtaining near theoretical specific activities for radio-iodoestrogens. Also, the incorporation of [^{125}I] I-VME2, as molecules of iodoestrogen per cell from the i.p. injection in vivo, was substantially less (i.e. less than 2% of) that taken up by similar cells incubated with an even lower dose of [^{123}I] I-VME2 in vitro for 30 minutes suggesting that the iodoestrogen does not stay in the peritoneal cavity very long. This is probably one important respect in which the mouse model differs from teh human disease we hope to treat. With micro metastatic human ovarian cancer the peritoneal fluid movement from the peritoneal cavity is often blocked, which would provide more optimal conditions for incorporation of radio-iodoestrogen into intraperitoneal micrometastases in the human. Finally, the animals in Group H were used to study whether the administration of [^{123}I] I-VME2 would cause any radiolytic effects in normal mice. Extensive gross examination as well as detailed histochemical evaluation of sections of the uterus, vagina, ovary and liver did not provide any evidence for damage to these normal tissues after one or two treatments with [^{123}I] I-VME2.

In an effort to confirm and extend the promising results from the mouse therapy experiment shown above, a second experiment was designed to extend the number of treatments, as well as hopefully the amount of [^{123}I] I-VME2, to effect more extensive tumor inhibition. Unfortunately, as a result of a combination of technical and instrumental problems this experiment was compromised insofar as we were unable to obtain accurate specific activity determinations on the [^{123}I] I-VME2 injected into the mice, and low yields from the radioiodinations led to lower than the desired 5 nM of [^{123}I] I-VME2 for administration, generally in the 1 – 2 nM range. As in the previous experiment essentially all the mice had injection site tumors, again an internal control that the tumor cells injected were viable. The mice were sacrificed at 18 weeks with the following results when considering only the non-injection site tumors:

Group	Av # of tumors/mouse	Av wt of tumors/mouse (mg)
A) Control	2.7	58.6
B) Treat day 0 only	0.75	27.0
J) Treat day 0 & 28	1.25	24.7
C) Treat day 0 & 7	2.16	24.1
D) Treat day 0, 7, & 14	1.5	21.7
E) Treat day 7 only	2.75	19.1
F) Treat day 7 & 14	2.57	40.0
G) Treat day 14 only	2.71	21.5

All treatment groups appeared to reduce the average tumor weight per mouse, but only animals treated on day zero appeared to reduce the average number of tumors per animal. Since we suspected from the previous experiment and other in vitro studies that the specific activity was important as related to efficacy, and we were unable to document the specific activities for the various injectants, it is difficult to make more detailed interpretation of the results. Although the results are consistent with the results of the earlier experiment, indicating that i.p. infusion of [^{123}I] I-VME2 can treat ER+ tumor cells in the peritoneal cavity, they do not provide the hoped for insight into how one might increase the efficacy of such therapy or completely prevent tumor

formation. It is nonetheless likely that to accomplish this will require high specific activity [^{123}I] I-VME2 and probably multiple therapies. However, it is reassuring that assays of the ER content of tumors that were excised from [^{123}I] I-VME2-treated mice retained significant ER content, suggesting that multiple treatments with [^{123}I] I-VME2 could be effective.

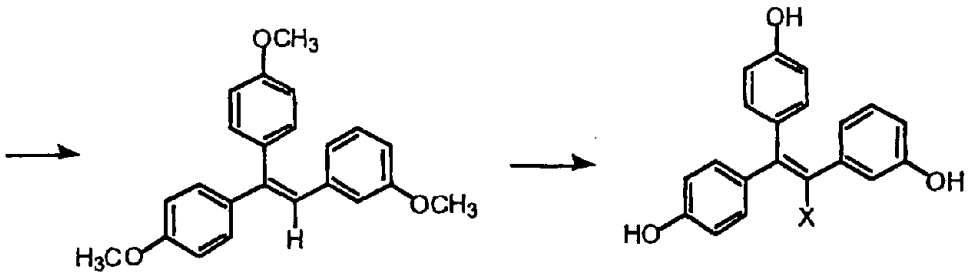
7) Synthesis of haloestrogens and stannyl precursors for the radio-iodinations

This part of the research was conducted under a subcontract with Professor Robert Hanson, Professor of Chemistry at Northeastern University. The objectives for this portion of the project were twofold. The first was to prepare the stannylvinyl analog of moxestrol (11β-methoxy-17α-ethynyl estradiol) for radio-iodination. The second objective was to prepare the stannyl derivative of tris(hydroxyphenyl)ethylene [THPE] for radio-iodination.

The first objective was readily achieved using the procedures that we had developed in our laboratory. Beginning with 11β-methoxy-estrone we added lithium acetylide to obtain the moxestrol. Hydrostannation with tri-n-butyltin hydride then gave, after column chromatography, the pure 17α-tri-n-butylstannylvinyl-11β-methoxy estradiol. The pure material was then submitted to Dr. DeSombre for the radio-iodination studies.

The second objective involved significantly more developmental work. There was a suggested method of synthesis based upon previous studies, however, the procedures were not well defined. The method involved the synthesis of the bromo-derivative of THPE which would be protected as the tris-(trimethylsilyl) ether, lithiation with phenyllithium followed by addition of tri-n-butyltin chloride. The synthesis of the parent THPE tris(methyl) ether was uneventful. Bromination (or iodination) with a variety of halogenating agents under different conditions gave mixtures of mono- and di-halogenated products plus unreacted starting material. The separation was non-trivial, requiring careful chromatographic techniques as well as multiple separations to achieve pure mono-brominated material. Demethylation of the brominated tris-ether also was non-trivial as the products are sensitive to acid and light. Again, careful chromatographic separations were necessary to isolate the pure bromo-THPE from THPE (formed during the demethylation reaction) and other by-products. The pure material was immediately protected as the tris-silyl ether to prevent oxidation of the sensitive phenolic compound. Metallation of the tris-(trimethylsilylated) material with phenyllithium using the recommended conditions was unsuccessful. Desilylation occurred and the bromine was not exchanged. We undertook the metallation reaction using the tris-methyl ether and found that the exchange required 0°C, not -40°C, while the stannylation occurred at -40°C. The entire process could be achieved at the lower temperature if we employed sec-butyllithium instead of phenyllithium. Therefore, we protected the phenolic groups of bromo-THPE as the tert-butyl-dimethylsilyl ethers and metallated with sec-butyllithium at -40°C. The product was formed, however, the chromatography conditions resulted in a partial desilylation of the product and only a small quantity was isolated. This material was characterized and a small quantity was submitted to Dr. DeSombre for radio-iodination. A small quantity of the stannylated tris-methyl ether of THPE was also submitted for radio-iodination.

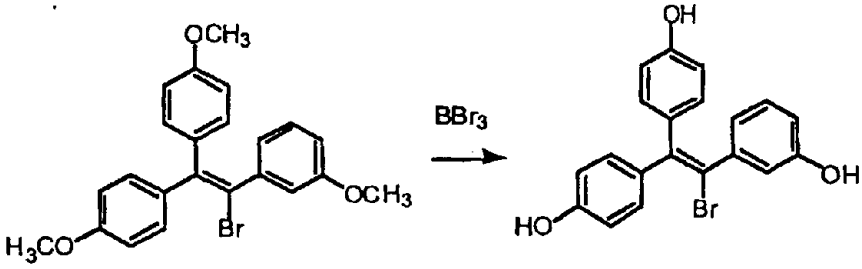
As a companion to this work, samples of THPE, bromo-THPE and iodo-THPE were prepared as standards and as materials for receptor binding studies.



THPE-tris-methyl ether

X = H, THPE
X = Br, Br-THPE
X = I, I-THPE

NBS or Br_2

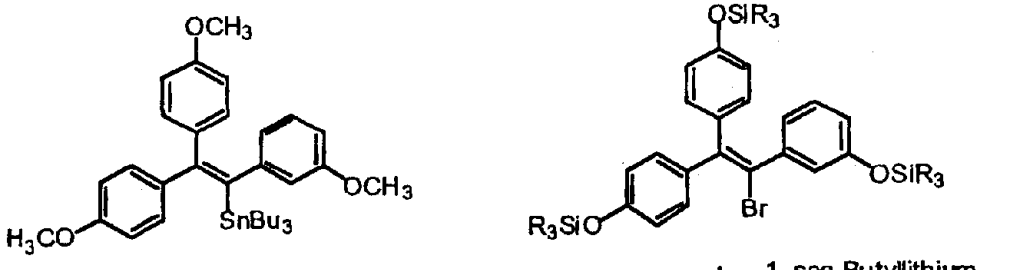


Mono-, di, and starting material

Br-THPE plus THPE

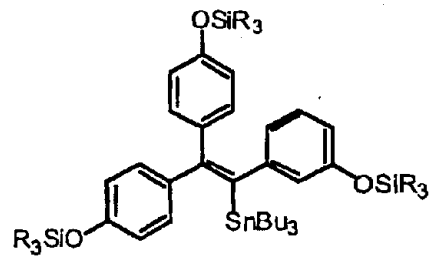
1. Phenyllithium/ sec-Butyllithium
2. Tributyltin chloride

R_3SiCl, Imidazole



Stannyl THPE-tris methyl ether

1. sec-Butyllithium
2. Tri-n-butyltin chloride



Stannyl THPE tris-silyl ether

Preparation of stannyl THPE precursor for radio-iodination studies.

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1. T. Kearney, A. Hughes, R. N. Hanson and E. R. DeSombre. Radiotoxicity of Auger electron-emitting estrogens for MCF-7 spheroids: A potential treatment for estrogen receptor-positive tumors. Radiat. Res. 151: 570-579 (1999).

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3. L. S. Yasui, A. Hughes and E. R. DeSombre. Production of clustered DNA damage by ^{125}I decay. Acta Oncol. 39:739-749 (2000).

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5. L. S. Yasui, A. Hughes, and E. R. DeSombre. Relative biological effectiveness of accumulated ^{125}IUdR and ^{125}I-estrogen decays in estrogen receptor expressing human breast cancer cells, MCF-7. Radiat. Res. 155: 328-334 (2001).

6. L. S. Yasui, A. Hughes and E. R. DeSombre. Estrogen receptors (ER) in ^{125}I-estrogen decay-mediated cell killing: comparison of non-ER-expressing human breast cancer cells, MDA-231 and ER-expressing MCF-7 cells. Int. J. Radiat. Biol., 77: 955-962 (2001).

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8. R. H. Hanson, A. Hughes and E. R. DeSombre. Synthesis and estrogen receptor binding of tris-hydroxyphenyl ethylenes. In preparation

9. A. Hughes, R. H. Hanson, and E. R. DeSombre. Therapy of estrogen receptor positive micrometastases in the peritoneal cavity with the Auger Electron-emitting Estrogen, 17α-[^{123}I] iodovinylestradiol. In preparation