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A COMPARISON OF ESTROGEN AND ANDROGEN RECEPTOR LEVELS IN HUMAN PROSTATIC TISSUE FROM PATIENTS WITH NON-METASTATIC AND METASTATIC CARCINOMA AND BENIGN PROSTATIC HYPERPLASIA

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Summary—Estrogen receptors (ER, $N = 72$) and androgen receptors (AR, $N = 33$) were determined by high pressure liquid chromatography (HPLC) in 72 human prostatic tissues obtained at prostatectomy, and exploratory statistical analyses of the resulting data were performed. To facilitate use of these data as well as other pertinent information from the patient charts, a program for a comparatively large data base was implemented on a Wang minicomputer.

The median values of cytosolic AR in the four cancer stages examined were statistically different from each other ($P = 0.01$), with AR increasing from stages A through D. Even though ER differences between the four stages were not significant ($P = 0.13$), there was a trend, in the data examined, for median ER values to decrease with stages B through D. On the other hand, median BPH values for both ER and AR were found to lie mid-scale compared with the respective cancer stages, leading to the conclusion that receptor measurements probably cannot distinguish between CA and BPH in human prostatic tissue, at least as measured by competitive binding techniques.

INTRODUCTION

We have previously reported [1–4] results from our laboratory which strongly support the concept that estrogen receptors (ER) are present in human prostatic tissue. These data were obtained using gel permeation columns in high pressure liquid chromatographs (HPLC), a technique which several other laboratories have also found [5–8] to offer advantages in quantitating steroid hormone receptors.

We report here on a series of exploratory statistical analyses in which 72 ER and 33 androgen receptor (AR) determinations were performed using the HPLC method on 72 prostatic tissues from 49 patients who had undergone open prostatectomies at this Institute.

To support these statistical analyses, we have developed software for a minicomputer of relatively small storage capacity for the documentation and retrieval of receptor data. We report here on this software because it is of interest from the following points of view: (a) Our data base and program are specifically targeted towards receptor determination in the prostate, and the items/questions depicted in the appendix may in part or totally be of use to other workers to implement their own retrieval programs, and (b) Comparatively little programming was needed to implement it, since a software package from Wang Laboratories was used, known as Inquiry Data Entry Access System (IDEAS); and the changes we made in the IDEAS package are documented and can also be made by others interested in this area.

EXPERIMENTAL

Determination of receptors

With the exception of cytosol preparation for needle biopsy-size tissue, all methods and materials used in this work have been published previously [1–4]. For the preparation of cytosol from needle biopsies, the appropriate volume of homogenization buffer was pipetted into a pre-chilled tube of a 0.1 ml all-glass homogenizer (Pierce Chemical Co., Rockford, IL). Needle biopsy tissue (about 20 mg per biopsy, total 50–100 mg) was allowed to thaw on ice. After a piece of tissue was added to the homogenizer tube, homogenization was performed by hand by rotating the pestle of the homogenizer between the index finger and thumb, keeping the tube immersed in ice. After homogenization of one piece was complete, the next one was added, and so on. The total volume of homogenate was about 0.2 ml and an aliquot was transferred to the cellulose propionate tube (capacity 175 μl) of an 18° rotor of a Beckman Airfuge installed in a cold room (4°C) and supplied with compressed air from tanks which were also placed in the cold room. Centrifugation for 30 min at maximum speed (95,000 rpm) gave about 100 μl of clear cytosol.

Documentation, retrieval and statistical analysis

A Wang Laboratories 2200VP minicomputer was used for documentation and retrieval of data. The Wang "IDEAS" software package is a series of programs written in Wang Basic II which can be accommodated by a 32K CPU and which will produce data entry screen masks and retrieval programs primarily targeted towards business needs, such as inventories and/or payrolls. During information retrieval it is possible to manipulate the output data using simple arithmetic functions only. It was of interest to modify the programs to accomodate our needs of documentation and retrieval of receptor data and of data from patient charts. Three modifications were implemented which are not part of the normal use of the program; two are unique changes in the BASIC source code. In the original form, the IDEAS program would produce only one screen of questions or items for each record (data for one sample). The number of questions or information items required by our needs necessitated six screens appearing consecutively per record (see the appendix for the questionnaire in each of the six screens). The second modification involved the insertion of a subroutine which computes days elapsed between surgery (obtaining the tissue) and the date the assay was performed, or days the tissue was kept in storage. The last modification was made to maximize use of the limited storage capacity of the hardware, *viz.* three floppy drives of $\frac{1}{4}$ mbyte each. After the IDEAS program had been used to implement our documentation program, only the program files that were necessary to execute the data entry and retrieval program were kept on one diskette, and parts of that one and the two others were used for storage of information. We were thus able to store about 500 records each on two diskettes and 176 records on the "program" diskette. Each record used 647 bytes of mixed alphanumeric and numeric storage, but the record length was "packed" or compressed to 473 bytes/record. Thus, our total storage capacity for the three diskettes (which have to be on line during execution of the program) was 1176 records. Because of storage limitation, the program files on the first diskette enable data entry and retrieval using only one type of report generator. Therefore if another form of the report was needed, another program diskette had to be used, and so on. As in most retrieval programs, all data stored can be printed, or total or partial data from those records (samples) that are selected for a particular characteristic can be printed.

Retrieval of all the information stored per record tended to diffuse the data since the print-out per record occupied 40 lines of 132 characters each. Although this "total" print-out was important, another retrieval program was implemented with only 13 items in the print-out for each record. This allowed focusing on the particular items that were of interest.

The changes made to implement our retrieval program will be made available upon communication with the principal author.

The exploratory statistical analyses were conducted using Minitab [9] and Statistical Package for Social Sciences (SPSS) [10] on the Institute's Univac 90/80 mainframe computer.

RESULTS

Tissue was categorized according to patient chart pathology reports of the prostatic area surrounding that from which the sample was excised. The weight of tissue received ranged from less than 40 mg for needle biopsies to greater than 1.0 g; tissue obtained by biopsy was designated as "needle" and the large pieces of tissue as "large". In order to compare receptor values in human prostatic cancer and BPH tissues following prostatectomy of patients with localized prostatic carcinoma (stages B1 and B2), the urologist removed tissue from the lobe which upon examination was ascertained to contain cancer [2]. Another piece was excised from an area distal to the first one and assumed to be histologically compatible with BPH. All tissue was coded in a blind study. We also measured receptors in tissue from prostatic cancer staged A through D. ER determinations were performed on all 72 tissues; however, since the quantity of tissue was often limited and our priority was to study ER, it was not possible to determine both AR and ER in all tissues. Thus, AR determinations were made on only 33 of the specimens.

The information in each of the categories could be retrieved from data inputted and stored by means of our Documentation and Retrieval program implemented for the Wang minicomputer in our laboratory. As can be seen in screen 1 (Fig. 3), items 13 and 14 classify the specimens in question as large or needle biopsy pieces and as single piece or pieces that are part of a pair for comparison. In screen 4 (Fig. 6), item 63 is the numerical equivalent of the disease stage. Item 111 of screen 6 (Fig. 8) gives the days elapsed from surgery (this is not an inputted value; it is only printed in the output since it is computed at that time). Item 112 gives estradiol (E_2) incubated. It was of interest to us to compare receptor values determined with three other factors associated with the methods used in this work, *viz.* amount of protein under the HPLC peak, days elapsed between surgery and assay (days tissue stored) and amount of E_2 incubated. No trends or any consistent correlations were found in the three comparisons.

Figure 1 is a dot diagram of the 72 ER and 33 AR values measured in the present work. Data were converted to log (1 + value) to accommodate the wide range of AR values. ER values ranged from a low of 0.0 to a high of 58 femtomoles E_2 per mg protein in HPLC binding peak, with a mean of 13.2 and a median of 9.22 femtomoles. Figure 1 also gives the dot diagram of AR values measured in 33 of the tissues. They range from 0 to 1344 femtomoles DHT

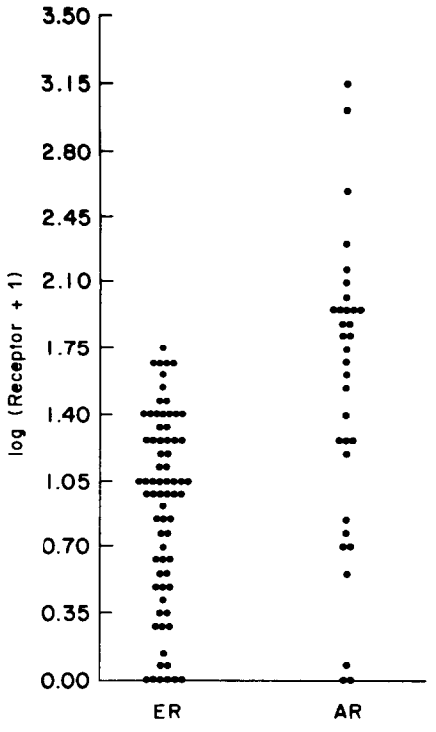

Fig. 1. Dot diagrams of the 72 ER and 33 AR values used in the present work.

per mg protein in the HPLC peak, with mean and median values of 134 and 55 femtomoles, respectively.

Table 1 lists means, standard deviations, medians and p-values generated by Mann–Whitney rank comparisons of CA and BPH specimens. In the case of comparisons among the categories of ER only, in five of the seven categories no significant difference was found (i.e. $P > 0.10$). Of particular importance to receptor use as markers in prostatic cancer is that there were no significant differences in ER values when all BPH specimens were compared with all CA specimens and when "Needle" BPH specimens were compared with "Needle" CA specimens. Marginal differences were found when ER values in "Large" pieces of BPH and CA were compared ($P = 0.10$) and when all BPH and Stage B CA were compared ($P = 0.08$).

In the categories of AR (lower section of Table 1) there was no overall difference in BPH and CA, but there were differences when BPH was compared with CA stages A and D.

Figure 2 is a dot diagram of all ER and AR CA data plotted by stage as well as the corresponding BPH values. It can be seen that with the exception of stage A CA, the median AR values for the three CA stages and BPH are higher than those of the corresponding ER values. A Kruskal–Wallis test showed that for AR, there was a significant difference ($P = 0.01$) between the four CA stages. In Fig. 2 it can be seen that in the data examined, there is an ascending order for the median AR values, with stage D being highest. A Kruskal–Wallis test showed that there were no significant differences between the ER values of the four cancer stages ($P = 0.13$); nevertheless, it can be seen (Fig. 2) that there is a descending order of medians from stage B through D.

The median values of both the ER and AR BPH samples were at or around the values for stage C cancer. It should be mentioned that BPH samples for this study were obtained mostly from cystoprostatectomy patients who were undergoing surgery because of cancer of the bladder.

DISCUSSION

Receptor measurement in human prostatic tissue may (a) help define the approx. 20% prostate CA patients who do not respond to hormonal treatment at diagnosis, (b) give early warning to the oncologist that the patient is becoming refractory to hormonal treatment, (c) help define the hormonal/chemotherapy ratio applicable to a particular patient if mixed regimens were adopted at diagnosis and (d) indicate that a patient may be amenable to treatment with certain anti-androgenic compounds.

Even though the prostate as a male accessory gland is under the influence of androgens, compared with ER studies in breast cancer, studies of AR in the human prostate have proven far more complicated

Table 1. Means (x), Standard Deviations (S), Medians (M) and Mann–Whitney test results

Tissue compared	n_1	n_2	x_1	x_2	S_1	S_2	M_1	M_2	P
		Estrogen receptor							
All BPH specimens vs all CA specimens	24	48	9.7	15.0	9.6	15.4	7.6	9.4	0.30
All BPH specimens vs Stage A Ca		9		16.1		14.9		9.4	0.24
All BPH specimens vs Stage B CA		21		20.3		18.4		16.6	0.08
All BPH specimens vs Stage C CA		14		9.0		8.9		7.0	0.73
All BPH specimens vs Stage D CA		4		5.5		5.9		4.3	0.49
Single "large" BPH vs Single "Large" CA	14	12	13.1	8.1	10.2	8.5	10.1	5.4	0.10
Single "needle" BPH vs Single "Needle" CA	6	2	1.3	1.8	0.9	1.0	1.3	1.8	0.40
		Androgen receptor							
All BPH specimens vs all CA specimens	8	25	80.1	152.1	68.8	332.0	68.5	47.6	0.54
All BPH specimens vs Stage A CA		4		5.6		8.9		1.9	0.02
All BPH specimens vs Stage B CA		9		45.7		40.5		31.1	0.36
All BPH specimens vs Stage C CA		9		93.4		123.0		71.3	0.96
All BPH specimens vs Stage D CA		3		842.5		659.0		1087.0	0.05

[11]. Recently [12], it was shown that nuclear AR data measured in needle biopsies give a better correlation with hormonal response than do AR measured in the cytosol. On the other hand, Blankenstein *et al.*[13] found no significant correlation between the results obtained for individual human prostates when AR values obtained from large (500 mg) samples were compared with those from needle biopsy size samples (25 mg). The lack of correlation could not be attributed to variations in the assay nor to differences in percentage of epithelium in the samples. Although the existence of ER in human prostatic tissue has been somewhat controversial, it appears that there is now acceptance of their presence as evidenced by recently published work, including studies done in this laboratory [1–4, 13–15].

Ekman *et al.*[14] have recently shown that human prostatic ER cytosolic receptors exhibit the same characteristics described for other tissues [14, 16–18] *viz.* the existence of multiple binding sites. They stated that in human prostatic tissue single point analysis of ER performed with 5–20 nM E_2 will overestimate the high affinity receptor. This undesirable co-estimation of the lower affinity receptor can be minimized by adding a reducing agent to the cytosol preparation, and by using a ligand concentration of 0.5 nM E_2. Since our data were already on file in our Documentation and Retrieval program, we performed correlations to determine if ligand concentration significantly affected the ER values measured. The results showed that with incubated E_2 concentrations ranging from 0.608 to 12.28 nM there was no significant correlation between E_2 and the values of ER determined. This may in part be due to the added reducing agent in cytosol preparations [2].

The amount of protein in the HPLC was correlated with results of ER and AR determinations. Since the amount of protein is already a denominator in the receptor dimension, the correlations were performed to point out any non-linearity in the experimentally generated ratio of E_2 specifically bound and the amount of protein. There were no consistent correlations or trends in these comparisons.

The analysis of the relationship between receptor value and the number of days the tissue was stored in the deep freeze was important because of the wide range of days (6–624 days with an average of 80.7). Again there was no consistent correlation in all categories of comparisons performed, and probably indicated that there was no consistent deterioration of tissue, if any, with time.

The five plots on the right-hand side of Fig. 2 of AR data CA stages A through D and of the BPH specimens in which AR were determined, and the Kruskal–Wallis test show that data for the four cancer stages were significantly different; further, it can be seen that in the data examined, the median values of the AR increased from stage A through D. It can be speculated that this increase in the median receptor value may be due to changes in the histological make-up of the tissue, i.e. cell differentiation or initiation or modification of hormonal treatment

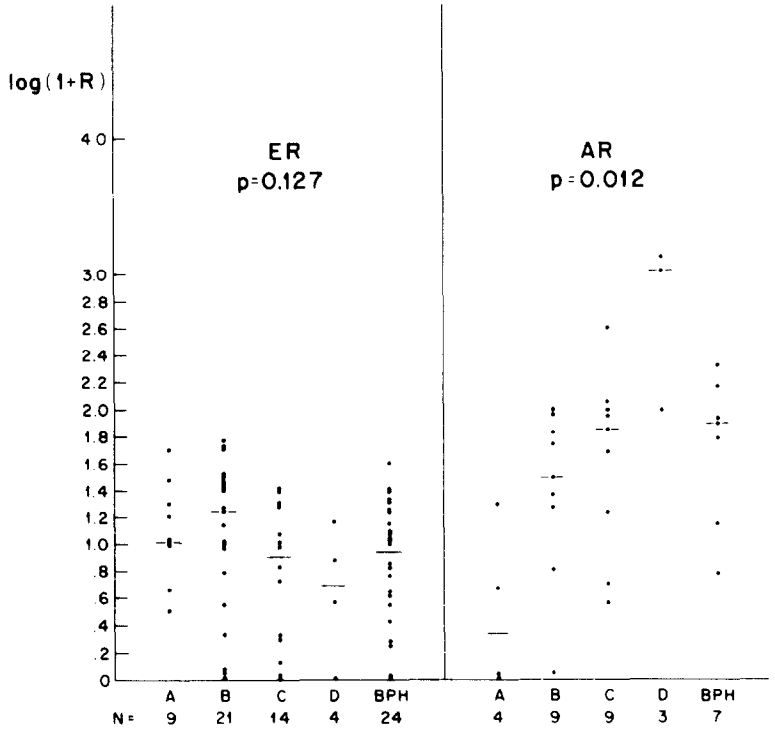

Fig. 2. Dot diagram of ER and AR in prostatic cancer stages A through D and in BPH tissue. Horizontal lines are median values for the receptor samples.

General Patient Information

1. Serial Number of Specimen.
2. Source of Tissue (RPMI, VA, MF, NPCP, Other).
3. Chart or Other Hospital Identification Number.
4. Date Tissue Obtained (MMDDYY).
5. Sample Obtained from: 1. Prostatectomy, 2. Biopsy, 3. TUR, 4. Metastases, 5. Lymph Node(s), 6. Separated Epithelium, 7. Separated Stroma, 8. Other.
6. Histology in Report Accompanying Tissue: 1. Normal, 2. BPH, 3. Cancer, 4. Other.
7. Was Tissue Obtained at Surgery (S) or Autopsy (A)?
8. Subject's Name.
9. Age of Subject at Time of Obtaining Tissue Sample.
10. Race of Subject (Caucasian, Negro, Oriental, American Indian, Other).
11. Diagnosis of Subject's Condition (Normal, BPH, CA of the Bladder, CA of the Prostate, Other).
12. Approximate Duration of Disease (MMYY).

13. 1 = single piece, 2 = two pieces comparison
14. 1 = large piece, 2 = needle biopsy

Clinical Information

1. Serial Number of Specimen.

If Diagnosis of Item 11 on Previous Screen was Cancer of the Prostate,

15. Histological Grade of Tissue.
16. Clinical Stage of Disease.
17. Pathological Stage.

Metastatic Involvement

18. Metastatic Involvement? (1=Yes, 2=No, 3=Unknown, 4=Reported).
19. Lung (Y/N)?
20. Liver (Y/N)?
21. Central Nervous System and/or Brain (Y/N)?
22. Bone Marrow (Y/N)?
23. Regular Lymph Nodes (Y/N)?
24. Distant Lymph Nodes (Y/N)?
25. Other (Y/N)?

26. Float-03
27. Float-04.

Treatment and Medication at the Time of Obtaining Tissue Specimen

1. Serial Number of Specimen.
28. Current Treatment.
29. Response to Treatment: 1. Progression, 2. Stable, 3. Partial Regression, 4. Complete Regression.

Indicate Weeks Elapsed Since:

30. Last Radiation
31. Cystectomy
32. Last TUR
33. Last Biopsy
34. Orchiectomy
35. Prostatectomy
36. Hypophysectomy
37. Adrenalectomy
38. Lymph Node Dissection

Indicate Weeks of: 39. Hormonal Therapy 40. Chemotherapy

Was the Subject at the Time of Obtaining the Sample Receiving:

41. Analgesics?
42. Phenothiazines?
43. Antibiotics?
44. Steroids?
45. Barbituates?

46. Float-05
47. Float-06

Blood and Tissue Biochemistry Parameters

1. Serial Number of Specimen
48. Total Alkaline Phosphatase.
49. Bone Alkaline Phosphatase.
50. Liver Alkaline Phosphatase.
51. Intestinal Alkaline Phosphatase.
52. Acid Phosphatase by CIEP.
53. Acid Phosphatase by SPIF.
54. Blood Estrogens.
55. Blood 17-Ketosteroids.
56. Other Blood Steroid (Specify).
57. Testosterone Binding Globulin.
58. Prostatic Tissue Estrogens.
59. Prostatic Tissue 17-ketosteroids.
60. Other Prostatic Tissue Steroids (Specify).
61. Prostatic Tissue 5-alpha Reductase.

62. Date PAP
63. Numerical Equivalent of Disease Stage (A=1, B=2, C=3 and D=4)
64. Float-09

Receptor Determination Part 1

1. Serial Number of Sample
65. Date Assay Performed
66. Weight of Tissue mg.
67. Number of TUR Pieces Used
68. Number of Biopsy Pieces Used

CYTOSOL			NUCLEAR				
Singl=1 Scat=2	ug. Prot.	R/mg Prot.	Singl=1 Scat=2	ug. Prot.	R/mg Prot.	mg. DNA	R/mg DNA

By High Pressure Liquid Chromatography

Estrogen Receptor
Anderogen Receptor
Progestin Receptor

By Dextran-Coated Charcoal

Estrogen Receptor
Androgen Receptor
Progestin Receptor

Receptor Determination Part 2

1. Serial Number of Specimen.

SUCROSE DENSITY GRADIENT CENTRIFUGATION

ER in the Cystol 4S Peak is , and in the 8S Peak is fmole/mg Protein.
ER in the Nuclear Peak is fmole/mg Protein.

AR in the Cytosol 4S Peak is , and in the 8S Peak is fmole/mg Protein.
AR in the Nuclear Peak is fmole/mg Protein.

PR in the Cytosol 4S Peak is , and in the 8S Peak is fmole/mg Protein.
PR in the Nuclear Peak is fmole/mg Protein.

108. Histology of Immediate Area of Tissue in Which Receptors Were Determined

109. Amount of Radiation Received, if any, Rads.
110. REMARKS

111. Days Elap
112. ER incub.
113. Float-12
114. Float-13
115. Float-14
116. Float-15

Figs 3–8. Screens 1 through 6 of the Documentation and Retrieval Program.

which diminishes endogenous androgen levels prior to prostatectomy, thus increasing "available" receptor. Even though the Kruskal–Wallis test for the ER data did not show a significant difference between the CA stages, there appears to be a decreasing trend among the median values of stages B through D cancer (Fig. 2). The same arguments for increase of AR values with cancer stage could also be applied to the decrease of ER but in reverse, e.g. the effect of estrogen and/or hormonal treatment prior to the prostatectomy may cause a decrease in the ER values with progression of the disease. It is possible that future work on correlations of receptor levels with other parameters stored in our Documentation and Retrieval Program will shed more light on this subject.

The median values for BPH specimens for both AR and ER (Fig. 2) were similar to the corresponding values for stage C CA, and thus any attempted correlation of receptor values with health state of the prostate (normal/BPH/CA) would have been obscured.

There has been a trend in the literature to show that prostatic receptor values in CA and BPH tissue are significantly different and therefore measurements of these values could define the presence of cancer in that tissue by comparing the receptor value with an average representing BPH. It is possible to conclude from the present study that receptor measurements cannot distinguish between CA and BPH in human prostatic tissue, at least as measured by methods which have competitive binding as their basis. In addition to the results presented above, Mobbs *et al.*[19] have recently reported similar ranges of cytosol AR concentrations in BPH and untreated prostatic carcinoma. This lack of difference should not, however, detract from use of receptor measurements as markers in prostatic cancer in one or more of the four possible applications described at the beginning of this section.

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