Virchows Arch [Pathol Anat] (1984) 402:353–359

Virchows Archiv A

Histochemical demonstration of endogenous estrogen in breast carcinomas: biochemical and clinical correlation*

Isao Katayama[1], Masaki Shimizu[1], Myota Miura[1], Masanobu Maruyama[2], Masayuki Kobayashi[2], Yuuichi Iino[3], Masaru Izuo[3], and Susumu Wakatsuki[1]

[1] Department of Pathology, Saitama Medical School, Saitama, 350-04
[2] Department of Surgery, Saitama Medical School, Saitama
[3] Department of Surgery, Gunma University School of Medicine, Gunma, Japan

Summary. In a study of 277 patients with breast carcinomas, the PAP immunoperoxidase method for demonstrating endogenous estrogen was correlated with the sucrose density gradient (SDG) assay and with histologic and clinical features. The results from the PAP method and SDG assay agreed in 59 of 84 patients (82.1%) on whom both methods were performed. Histologically, the PAP method was positive in 7 of 7 patients with non-invasive carcinomas, in 164 of 233 patients (70.4%) with common invasive ductal carcinoma, and in 21 of 22 of those with special histological types of invasive carcinomas not including Paget's disease, medullary or apocrine carcinoma, where only 5 of 14 were positive. Clinically, 15 of 18 patients with positive endogenous estrogen showed a response to endocrine therapy as opposed to 1 of 9 patients with a negative endogenous estrogen. The mean survival was 31.2 and 15.6 months, respectively for patients with positive and negative endogenous estrogen. Remission for longer than 2 years was seen more often in patients with positive endogenous estrogen. These results suggest a clinical utility of the present PAP method which, therefore, deserves a further trial as an alternative to histochemical methods aiming at the estrogen receptors.

Key words: Breast neoplasms – Estrogen receptors – Immunoperoxidase technique – Sucrose density gradient assay

The estrogen receptor assay has been found to assist in prognosis as well as in determining the response to endocrine therapy of individual patients

* This work was supported by Grants-in Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan (No. 56480119).

This paper was presented at the 72nd Annual Meeding of International Academy of Pathology (United States-Canadian Division), Atlanta, Georgia, March 1, 1983.

Offprint request to: I. Katayama at the above address

with breast carcinomas. For this purpose, biochemical methods using tissue homogenates have been widely employed since their introduction in 1970 (Howanitz 1981). In more recent years, various histochemical methods for demonstrating receptors on tissue sections were advocated because of the potential advantages over biochemical methods. However, histochemical methods in general have still had to overcome several technical problems before they could be accepted as theoretically valid and clinically useful (Chamness et al. 1982; Penney et al. 1982; Underwood et al. 1982).

We recently devised a PAP immunoperoxidase method for the demonstration of endogenous estrogen in paraffin sections of breast carcinomas. Its validity was based on experiments using the DMBA rat tumor which bound and retained intravenously injected tracer estrogen throughout the preparatory procedures for demonstration by the PAP immunoperoxidase method (Shimizu et al. 1983). The advantage of this method was the absence of previously unavoidable nonspecific staining due to the high concentrations of tracer estrogen used in other histochemical methods (Chamness et al. 1982). In the present study, the PAP immunoperoxidase method was applied to human breast carcinomas to determine the correlation with the biochemical method, the histologic types, and the clinical features.

Materials and methods

Patients: Two hundred seventy seven patients surgically treated for carcinomas of the breast during the period from June 1971 to May 1980 were studied. All but 4 patients were women, 152 were premenopausal and 121 postmenopausal at the time of mastectomy. Staging revealed 59, 136, 65 and 17 patients in stage I, II, III and IV, respectively. Detailed follow-ups for over 2 years were obtained from 87 of the 277 patients. Thirty one of these died, 25 of breast carcinomas and 6 of other causes. Of the 26 patients who relapsed, 14 did so within 2 years after remission and 12 more than 2 years after remission. The endocrine therapy included oophorectomy in 20 patients, hormone therapy in 3 patients, ovarian irradiation and hormone therapy in 2 patients, oophorectomy and hormone therapy in one patient, and orchiectomy and adrenalectomy in one male patient. The response to the endocrine therapy, according to the criteria of UICC (Hayward et al. 1977), was positive (complete or partial response of UICC) in 16 patients and negative (no change or progressive disease of UICC) in 11 patients.

Pathology and PAP immunoperoxidase technique. Mastectomy specimens from all patients were fixed in formalin and embedded in paraffin. H and E sections were studied for histological typing according to the WHO classification (WHO 1981). Additional sections cut from the same paraffin blocks were stained for endogenous estrogen with the method described previously (Shimizu et al. 1983). Briefly, the deparaffinized sections were incubated with rabbit anti 17-β-estradiol-6-bovine serum albumin (Miles Laboratories, Inc., Elkart, IN) with no pretreatment with exogenous estrogen. The incubation was followed by treatment with swine anti-rabbit immunoglobulin, PAP complex, and finally with the peroxidase reaction.

Biochemical assay of estrogen receptors. In addition to the PAP immunoperoxidase method performed on paraffin sections, 84 patients had a biochemical assay of estrogen receptors performed on fresh specimens according to the sucrose density gradient (SDG) method modified by Kato with positive values set above 10 fmol/mg cytosol protein (Kato et al. 1976).

Results

The PAP method was positive in 197 patients (71.1%) and negative in 80 patients (28.9%). Three of the 4 men studied were positive for endoge-

Table 1. Endogenous estrogen by PAP method in relation to menopause and clinical stage

	Positive	Negative	Total
Premenopause	105	47	152
Postmenopuase	89	32	121
Total	194	79	273[a]
I	43	16	59
II	98	38	136
III	43	22	65
IV	13	4	17
Total	197	80	277

[a] This study included 273 female and 4 male patients

Table 2. Endogenous estrogen in relation to the histological type

	Posite	Negative	Total (%)
1. Non-invasive			
a) Intraductal carcinoma	7	0	7 (2.5)
b) Lobular carcinoma in situ	0	0	0
2. Invasive			
a) Invasive ductal carcinoma	164	69	233 (84.1)
b) Invasive with predominant intraductal component	3	0	3 (1.1)
c) Invasive lobular carcinoma	5	1	6 (2.2)
d) Mucinous carcinoma	4	0	4 (1.4)
e) Medullary carcinoma	3	4	7 (2.5)
f) Papillary carcinoma	3	0	3 (1.1)
g) Tubular carcinoma	1	0	1 (0.4)
h) Adenoid cystic carcinoma	3	0	3 (1.1)
i) Secretory (juvenile) carcinoma			0
j) Apocrine carcinoma	2	2	4 (1.4)
k) Carcinoma with metaplasia	2	0	2 (0.7)
l) Others			0
3. Paget's disease	0	3	3 (1.1)
II c) Carcinosarcoma	0	1	1 (0.4)
Total	197	90	277

nous estrogen. Among 273 women, the positive incidence was slightly higher in postmenopausal (89 of 121 patients, 73.6%) than in premenopausal women (105 of 152 patients, 69.1%). With regard to the clinical stages, the PAP method was positive in 72.9%, 72.1%, 66.2% and 76.5% of patients, in stages I, II, III and IV, respectively (Table 1). There was no statistically significant correlation between the demonstration of endogenous estrogen by the PAP method and menopause or clinical stage.

Histological types (Table 2). The PAP method was positive in 7 out of 7 patients with non-invasive carcinomas. In the invasive carcinomas, the

Table 3. Endogenous estrogen by PAP method in relation to endocrine therapy and length of remission and survival

	Positive	Negative	Total
Endocrine therapy			
Response (+)	15	1	16
Response (−)	3	8	11
Total	18	9	27
Remission longer than 2 years			
Stage I	1/1[a]	1/1	2/2
Stage II	6/10	2/5	8/15
Stage III	2/2	0/7	2/9
Total	9/13	3/13	12/26
Length of survival			
Stage I		1 (84.0)	
Stage II	3 (20.7, 8–30)[b]	3 (20.0, 12–36)	6 (20.4, 8–36)
Stage III	2 (64.0, 56–72)	6 (15.3, 5.5–24)	8 (27.5, 5.5–72)
Stage IV	7 (26.4, 1.5–60)	3 (12.0, 4–24)	10 (22.1, 1.5–60)
Total[c]	12 (31.2, 1.5–72)	12 (15.6, 4–36)	24 (23.4, 1.5–72)

[a] Numerator and denominator respectively represent the number of patients with a remission longer than 2 years and the total number of patients who relapsed
[b] In parentheses are the mean and range of length of survival in months
[c] The only patient in stage I was excluded from the calculation

PAP method was positive in 164 out of 233 (70.4%) patients with the common invasive ductal type, and in 21 out of 22 patients with special histological types of invasive carcinoma, not including Paget's disease, medullary or apocrine carcinoma where only 5 out of 14 patients were positive. One patient with carcinosarcoma was negative for endogenous estrogen by the PAP method.

Endocrine therapy (Table 3). Among the 27 patients who underwent endocrine therapy, a positive response to the therapy was recorded in 15 of 18 patients with positive endogenous estrogen as opposed to 1 of 9 patients with negative endogenous estrogen.

Length of remission (Table 3). In 26 patients who relapsed after varied length of remission, remission lasted longer than 2 years in 9 of 13 patients with positive endogenous estrogen, as opposed to only 3 of 13 patients with negative endogenous estrogen. With regard to patients in clinical stages II and III, remission longer than 2 years was obtained by most of the patients with positive endogenous estrogen as opposed to only a few with negative endogenous estrogen.

Length of survival (Table 3). Mean survival was 31.2 months (range, 1.5–72 months) for 12 patients with positive endogenous estrogen, and 15.6 months

Table 4. Endogenous estrogen by PAP method in relation to estrogen receptor by SDG method

PAP(+), SDG(+)	54
PAP(−), SDG(−)	15
PAP(+), SDG(−)	15
PAP(−), SDG(+)	0
Total	84

(range, 4–36 months) for 12 patients with negative endogenous estrogen. In patients in stages III and IV, the average length of survival was much longer for those with positive endogenous estrogen than with negative endogenous estrogen.

SDG assay (Table 4). In the 84 patients on whom both the PAP method for endogenous estrogen and SDG assay for estrogen receptors were performed, the two results agreed in 69 patients (82.1%) of whom 54 were positive for both and 15 negative for both. The disagreement in the remaining 15 patients was all in the way of positive PAP method for endogenous estrogen and negative SDG assay for estrogen receptors.

Discussion

The present study demonstrated a good correlation between the PAP immunoperoxidase method and the biochemical assay, histological types and clinical features of patients with breast carcinomas. Patients with positive endogenous estrogen by the PAP method showed a higher rate of response to endocrine therapy, longer remission, and longer mean survival than those with negative endogenous estrogen.

Although the number of patients with the special histological types in the present study were too small for generalization, it was striking to note that the PAP method was positive in all non-invasive carcinomas and virtually all invasive carcinomas of special histological types with a few exceptions. The exceptions were medullary carcinoma, apocrine carcinoma and Paget's disease, where the PAP method was negative in the majority of cases. Similar correlations between histological types and estrogen receptors were reported by others using either biochemical or histochemical methods (Rosen et al. 1975; Antoniades et al. 1979; Fisher et al. 1980; Silfversward et al. 1980).

The PAP results agreed with the SDG assay in 82.1% (69 of 84) of patients, and the disagreement was consistent in the remaining 15 patients, all being positive in the PAP assay and negative in the SDG assay. Similarly, 5 cases negative with the dextran-coated charcoal assay were positive with the immunofluorescent or the immunoperoxidase technique, suggesting that a negative result with a biochemical assay might be less reliable (O'Connell et al. 1983).

The percentage of cases positive for endogenous estrogen (71.1%) in the present study is comparable to figures of 70.0% and 73.1% for estrogen receptors determined by biochemical methods (McGuire et al. 1975; Spaeren et al. 1973). The high rate of agreement between the 2 methods may appear

surprising since they measured different variables on different principles, that is to say, the PAP method demonstrates endogenous hormone taken up in vivo by the receptors whereas the biochemical method measures the exogenous tracer hormone taken up in vitro by the receptors. However, the two methods are not mutually exclusive. According to receptor kinetics (Jensen et al. 1973), the receptors would use some binding sites for uptake of endogenous estrogen (to be demonstrated by the PAP method) but still leave other binding sites available (for demonstration by the biochemical method).

Reviewers (Chamness et al. 1982; Penney et al. 1982; Underwood et al. 1982) have faulted the histochemical methods in general for the nonspecific staining of type II and III binders secondary to the high concentrations of tracer estrogen. The present PAP method was free of such nonspecific staining since tracer estrogen was not used. Because it is applicable to the routine paraffin sections, the method is technically much simpler and less costly that other histochemical methods. In many instances where fresh tissue is no longer available for biochemical assays, application of the PAP method to paraffin sections may be the only approach by which to predict the effect of endocrine therapy.

In conclusion, the present PAP method has shown a good correlation with the biochemical method, histological types and clinical features of patients with breast carcinomas. Although the number of patients is too small to draw a general conclusion, the method deserves further study as a potential alternative to other histochemical methods.

Acknowledgement: The authors thank Miss C. Kubota for secretarial assistance

References

Antoniades K, Spector H (1979) Correlation of estrogen receptor levels with histology and cytomorphology in human mammary cancer. Am J Clin Pathol 71:497–503

Chamness GC, McGuire WL (1982) Questions about histochemical methods for steroid receptors. Arch Pathol Lab Med 106:53–54

Fisher ER, Redmond CK, Liu H, Rockette H, Fisher B (1980) Correlation of estrogen receptor and pathologic characteristics of invasive breast cancer. Cancer 45:349–353

Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Robens RD (1977) Assessment of response to therapy in advanced breast cancer: a project of the Programme on Clinical Oncology of the International Union Against Cancer, Geneva, Switzerland. Cancer 39:1289–1294

Howanitz HJ (1981) Hormone receptors and breast cancer. Hum Pathol 12:1057–1059

Jensen EV, DeSombre ER (1973) Estrogen-receptor interaction. Estrogenic hormones effect transformation of specific receptor proteins to a biochemically functional form. Science 182:126–134

Kato J, Nomura Y, Matsumoto K, Onouchi T (1976) Radioreceptor assay of estrogen. Nippon Rinsho 34:510–517 (in Japanese)

McGuire WL, Chamness GC, Castlow ME, Richert NJ (1975) Steroids and human breast cancer. J Steroid Biochem 6:723–727

O'Connell M, Said JW (1983) Estrogen receptors in carcinoma of the breast. A comparison of the dextran-coated charcoal, immunofluorescent and immunoperoxidase technics. Am J Clin Pathol 80:1–5

Penney GC, Hawkins RA (1982) Histochemical detection of oestrogen receptors: A Progress report. Br J Cancer 45:237–246

Rosen PP, Menendez-Botet CJ, Nisselbaum JS, Urban JA, Mike V, Fracchia A, Schwartz MK (1975) Pathological review of breast lesions analyzed for estrogen receptor protein. Cancer Res 35:3187–3197

Shimizu M, Wajima O, Miura M, Katayama I (1983) PAP immunoperoxidase method demonstrating endogenous estrogen in breast carcinomas. Cancer 52:486–492

Spaeren U, Olsnes S, Brennhovd I, Efskind J, Pihl A (1973) Content of estrogen receptors in human breast cancers. Eur J Cancer 9:353–357

Underwood JC, Sher E, Reed M, Eisman JA, Martin TJ (1982) Biochemical assessment of histochemical methods for oestrogen receptor localisation. J Clin Pathol 35:401–406

World Health Organization (1981) Histological Typing of Breast Tumors. 2nd ed, International Histological Classification of Tumors, No. 2, WHO, Geneva

Accepted November 28, 1983