Effects of danazol on incidence of progesterone and oestrogen receptors in benign breast disease

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Abstract

Results from a continuing clinical trial in benign breast disease indicate that danazol may induce progesterone receptors and that this effect persists after treatment. In women given danazol the number of biopsy specimens that were positive for progesterone receptors rose from 14 out of 31 before treatment to 23 out of 30 at the end of six months' treatment and remained raised at 21 out of 31 six months later. The number of biopsy specimens that were positive for oestrogen receptors rose transiently from eight out of 31 to 12 out of 30 and then fell to six out of 31. A satisfactory clinical response was achieved in 26 of the 31 patients but was maintained in only 11 six months or more after the end of treatment. It was only in this group that a significant and long-standing increase in progesterone receptors was observed.

These findings suggest that in some women with benign breast disease who have been treated with danazol changes occur that may have long term benefit.

Introduction

Benign breast disease embraces a wide range of clinical, radiological, and pathological entities¹ and is thought to reflect the response of the breast to an abnormal hormonal milieu.^{2,4} If this were the principal aetiological factor then not only a fairly consistent hormonal abnormality but also a fairly uniform and symmetrical distribution of signs and symptoms within the breasts might be expected. This is not the case. An alternative explanation is that the principal defect lies in the target organ, which reacts abnormally to cyclic physiological changes in hormone concentrations. It is also possible that the two mechanisms could coexist.

Little information is available on the response of human breast tissue to hormonal manipulation.⁵ This is principally because in vivo studies have been hampered by the lack of a satisfactory and acceptable method for obtaining adequate serial samples.⁶ To overcome this problem we designed and developed an instrument that has enabled us to obtain sufficient material for serial receptor and histological analyses.

Danazol has been successfully used to treat benign breast disease, and the main objective of this study was to see whether it had any

effect on steroid hormone receptors in benign breast disease that could account for the favourable clinical response, which is maintained in some patients after treatment has been stopped.

Patients and methods

We are conducting a continuing open clinical trial of danazol in benign breast disease that has been approved by the district ethics committee. Patients are entered into the trial after at least two visits when explanatory notes are given and informed written consent obtained. The women in the trial are premenopausal patients over the age of 25 who present to a special benign breast disease clinic with painful or tender breasts, or both, not responding to simple measures including analgesics and severe enough to interfere with their daily activity or marital relationship, or both. Between September 1983 and March 1986, out of 403 potential patients, 39 (9.7%) met the above criteria and consented to enter the trial.

The extent and severity of breast nodularity in these patients ranged widely, and early in the study one asymptomatic patient with severe localised nodularity was also entered into the trial. Thirty one of the patients completed the study: 26 were premenopausal and five had had hysterectomies with conservation of the ovaries. Their mean ages were 36.6 years (range 25-49) and 40.8 (range 25-45), respectively. Three patients defaulted, one after the first and two after the second biopsy. They were excluded from this report owing to incomplete data. A further five patients were withdrawn because of suspected side effects of danazol.

Patients had taken no hormonal treatments or oral contraceptives for at least six weeks before the trial. Four had taken a combined oral contraceptive in the past and one was taking one when first seen; another was taking a progestogen only pill when first seen. One patient had had a 10 day course of danazol eight months previously and another a month's course of evening primrose oil two months before entering the trial.

BREAST BIOPSY

Breast biopsies were performed immediately before treatment; at six months, before danazol was tailed off; and at 12 months. Because of changes in the receptor concentration⁷ and histological appearance⁸ with the menstrual cycle biopsies were performed in the early follicular phase when this could be identified.

The most clinically affected part of the breast was chosen; subsequent biopsies were performed close to and parallel to the previous ones. Local anaesthesia was obtained with 1% lignocaine in 1/200 000 adrenaline; up to 20 ml was used depending on the size of the breast. The anaesthetic was injected at the proposed site of entry with a 23 gauge needle and the area to be biopsied was then infiltrated as a wedge by a 20 gauge spinal needle; except for the discomfort experienced during infiltration patients felt either no pain or only mild discomfort during the procedure.

Two specimens were obtained through a 5 mm stab incision with a biopsy needle designed and developed by us at The London Hospital. The instrument is based on the Tru-Cut needle but the whole procedure can be performed with one hand, leaving the other free to steady the breast, guide the needle, and compress the breast into the opened groove before firing, so that a larger specimen is obtained. A special sliding guard determines the depth at which the biopsy is started and can be adjusted to start at any depth up to 10 cm from the skin surface. Two stops vary the length of the specimen from 17 to 47 mm. Three sizes of interchangeable needles are available: small, medium, and large with outside diameters of 4.0, 5.0, and 5.5 mm with a biopsy groove cross section of 3.2×1.7, 4.0×2.5, and 4.5×2.5 mm, respectively. With a few exceptions early on in the trial when the small needle was used, all biopsy specimens were taken with the medium needle; this gave an adequate sample for histological examination and receptor analyses.

Immediately after the biopsy manual compression was applied and maintained for at least five minutes or until all oozing had stopped; a dry

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dressing was then applied. The direction and depth of the biopsy specimen was recorded for future reference. A scab formed within 48 hours, and the patients experienced only minimal discomfort during this period, rarely requiring analgesics despite their disease. Moderate bruising occurred only once, and no haematomas or other complications occurred. Samples for receptor analyses were put immediately in liquid nitrogen until assayed.

TISSUE PREPARATION

Oestrogen and progesterone receptor assays were performed by a single saturation dose method,⁹ which has been extensively validated *inter alia* by comparison with Scatchard analysis and gives an excellent correlation.¹⁰ This method was recently described in detail.¹¹ Concentrations of ≥ 5 fmol/mg protein were taken as positive.

TREATMENT REGIMEN

After biopsy treatment was started with a standard dose of danazol 100 mg twice a day for six months; after the second biopsy it was tailed off over the next three weeks. In six cases this dose was slightly altered for therapeutic reasons.

CLINICAL RESPONSE

The overall clinical response was assessed on an empirical scale of one to six (table I). Patients were classified as having had a satisfactory response to treatment and a satisfactorily maintained response after treatment at six and 12 months, respectively, if they scored no more than three for pain, tenderness, or nodularity. A score of four or more for any of these three variables after a satisfactory response at six months was classified as a relapse.

TABLE I—Clinical assessment of treatment

Score	Pain	Tenderness	Nodularity
1=Excellent	None	None	None
2=Good	Mild, occasional	Mild, occasional, not appreciable	Slight, not appreciable
3=Moderate	Occasionally troublesome, not requiring treatment	Occasionally troublesome, not requiring treatment	Moderate but response acceptable
4=Poor	Improvement slight, requiring treatment	Improvement slight, requiring treatment	Noticeable, response not acceptable
5=None	No discernible change	No discernible change	No discernible change
6=Worse	Definite deterioration	Definite deterioration	Definite deterioration

Results

Clinical response—Of the 31 patients who underwent three biopsies, 26 showed a satisfactory response at the end of six months' treatment. Six months later this response was maintained in 11 while 15 showed a relapse. Five patients failed to respond to treatment.

Progesterone receptors—Biopsy specimens were positive for progesterone receptors in 14 of the 31 patients initially, in 23 out of 30 after six months' treatment, and in 21 of the 31 at 12 months. Table II shows the results when these changes were analysed by clinical response. The increase in the proportion of biopsy specimens positive for progesterone receptors after six months' treatment was transient except in the group in which the clinical response was maintained, in which it was significantly raised at 12 months ($p=0.008$ by McNemar's test (one sided)). Table III shows the changes in activity of progesterone receptors that occurred between the initial biopsy

TABLE II—Relation between clinical response and proportion (%) of biopsy specimens positive for progesterone receptors

	Initially	At six months	At 12 months
Satisfactory clinical response maintained at 12 months	3/11 (27)	7/11 (64)	10/11 (91)
Satisfactory clinical response but relapsed by 12 months	7/15 (47)	11/14 (79)*	9/15 (60)
No clinical response	4/5 (80)	5/5 (100)	2/5 (40)

*One sample insufficient for analyses.

TABLE III—Relation between clinical response and changes in progesterone receptor activity between initial biopsy and biopsy at 12 months

12 Month biopsy:	Maintained response		Relapse		No response	
	Positive	Negative	Positive	Negative	Positive	Negative
Initial biopsy:						
Positive	3	0	6	1	1	3
Negative	7	1	3	5	1	0

and the biopsy at 12 months according to the clinical response. The trend in the group that showed no response was the opposite of that in the group in which the response was maintained for 12 months.

Oestrogen receptors—During treatment the proportion of biopsy specimens positive for oestrogen receptors increased but this proved to be transient and by 12 months there was a net decline (table IV). There were also a few changes in the activity of oestrogen receptors between the initial biopsy and the biopsy at 12 months in all three clinical groups, with 19 of the 23

TABLE IV—Relation between clinical response and proportion (%) of biopsy specimens positive for oestrogen receptors

	Initially	At six months	At 12 months
Satisfactory clinical response maintained at 12 months	3/11 (27)	5/11 (45)*	2/11 (18)
Satisfactory clinical response but relapsed by 12 months	3/15 (20)	6/14 (43)*	3/15 (20)
No clinical response	2/5 (40)	1/5 (20)	1/5 (20)

*One sample insufficient for analyses.

TABLE V—Relation between clinical response and changes in oestrogen receptor activity between initial biopsy and biopsy at 12 months

12 Month biopsy:	Maintained response		Relapse		No response	
	Positive	Negative	Positive	Negative	Positive	Negative
Initial biopsy:						
Positive	1	2	1	2	0	2
Negative	1	7	2	10	1	2

specimens that were negative for oestrogen receptors initially showing no change (table V).

Discussion

There is no consistent or convincing pattern to support the idea that the principal factor in the aetiology of benign breast disease is a hormonal abnormality. Luteal phase progesterone concentrations have been reported as low, normal, and high, and even though raised concentrations of free oestradiol have been reported, most workers have found normal oestrogen concentrations. Similarly, no clear abnormality of prolactin production has emerged. Androgen concentrations have been variously reported as low, normal, or high, and estimations of luteinising hormone, follicle stimulating

hormone, and thyroid stimulating hormone concentrations remain equivocal.¹²

Steroid receptor concentrations in the normal breast and in benign breast disease differ. Oestrogen receptors are virtually undetectable in the normal breast¹³⁻¹⁶ and are low in benign breast disease,¹⁷ the incidence and content of these receptors paralleling the intensity of hyperplasia.¹⁶ On the other hand, progesterone receptor concentration is lower in benign breast disease than in the normal breast. Concentrations greater than 10 fmol/mg protein have been found in only 12% of breasts with benign breast disease compared with 33% of normal breasts and the incidence of positive progesterone receptor concentrations in benign breast disease has been reported to be as low as 12.5%.¹⁸

Danazol, a synthetic derivative of ethisterone, has been successfully used to treat benign breast disease since the first report of its therapeutic effect in 1971.¹⁹ It is thought to act centrally, on the hypothalamus and pituitary, and peripherally on target organs, binding with steroid receptors and affecting steroidogenesis²⁰ and plasma proteins.²¹ After treatment with danazol is stopped patients with benign breast disease may show a clinical response lasting for six months or more.²² As all hormonal variables return to pretreatment concentrations within four weeks of danazol being stopped²³ this finding would be difficult to explain unless some long-standing changes had taken place. It has been suggested that the principal effect of danazol in benign breast disease could be peripheral and could take precedence over any central action.⁶ A similar local progestin-like effect rather than a systemic one may be responsible for the therapeutic effect of danazol on the endometrium.²⁴

Progesterone receptors that are independent of oestrogen can be induced by antioestrogens²⁵ and "active" serum²⁶ in breast cancer cell lines and tamoxifen in mammary cancer.^{27,28} The data presented here suggest that danazol may affect steroid receptors in benign breast disease, being associated with a rise in the proportion of biopsy specimens positive for both oestrogen and progesterone receptors after six months' treatment. Whereas the changes in oestrogen receptor concentration proved to be transient the number of samples that were positive for progesterone receptors at 12 months remained above the pretreatment number in those patients who responded to treatment. Induction of progesterone receptors could result in the breast becoming more sensitive to progestins²⁸; interestingly, induction of progesterone receptors at 12 months was significant only in the 11 patients whose clinical response was also maintained six months or more after treatment. These findings could indicate long term, if not permanent, changes in a subgroup of patients with benign breast disease that theoretically could be beneficial.

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