Eur J Cancer, Vol. 28A, No. 8/9, pp. 1404–1407, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Droloxifene, A New Anti-oestrogen in Postmenopausal Advanced Breast Cancer: Preliminary Results of a Double-blind Dose-finding Phase II Trial

Peter F. Bruning

Droloxifene (3-OH-tamoxifen citrate) is a novel anti-oestrogen with a higher affinity for oestrogen receptors (ER), a lower oestrogenic to anti-oestrogenic activity ratio and faster pharmacokinetics compared with tamoxifen. From May 1988 to April 1991, 369 postmenopausal women with metastatic or locally unresectable breast cancer of which the ER or progesterone receptor status was positive or unknown, were randomised to receive an oral dose of 20, 40 or 100 mg droloxifene once daily. 43 Brazilian, Canadian and European centres took part in this double-blind phase II trial. 60 women were ineligible for violation of entry criteria; 20 were inevaluable and 15 still await definitive response evaluation. 234 patients have been evaluated for response. The over-all objective response rate (complete plus partial) was 92/234 (39.3%): 23/74 (31.1%) for 20 mg, 33/74 (44.6%) for 40 mg and 36/86 (41.9%) for 100 mg (not significantly different within this dose range). There was no significant difference in time to tumour progression between the three doses. Toxicity was minimal at all doses. These preliminary results show that droloxifene is active against advanced breast cancer. Because of its outstanding preclinical characteristics, the drug warrants large-scale clinical investigation.
Eur J Cancer, Vol. 28A, No. 8/9, pp. 1404–1407, 1992.

INTRODUCTION

It is widely accepted that endocrine manipulation can offer the best palliation to patients with advanced breast cancer. In postmenopausal women, tamoxifen is generally the first choice of treatment; also, in premenopausal patients, tamoxifen is gaining ground as first-line hormonal therapy. Tamoxifen is active against breast cancer cell proliferation if sufficient amounts of functional oestrogen receptors are present to which it binds in competition mainly with oestradiol. Depending on the properties of the particular species, organ or tumour involved, the tamoxifen–oestrogen receptor complex can antagonise some oestrogen-responsive genes, such as pS2 and cathepsin [1], but activate other genes such as those for progesterone receptor or TGF β [2, 3]. Moreover, tamoxifen can inhibit cellular growth factor activity in the presence of oestrogen receptors, but in the total absence of oestrogens, through as yet unclear mechanisms [4]. Actually, it is 4-OH-tamoxifen, a metabolite representing only 1–3% of the total tamoxifen in circulation [5,6], which is the biologically active compound. Tamoxifen is eliminated only slowly from the human body, and both the parent compound and the main metabolites accumulate extensively [7, 8].

The intrinsic weak oestrogenic activity of tamoxifen presumably enhances breast cancer cell growth, as clinically suggested by the well-known flare-up of pain from bone metastases, and is possibly also related to the alleged increase of the incidence of thromboembolic events and endometrial carcinoma in humans [9, 10].

These properties of an otherwise very successful drug have led to the development of anti-oestrogens with a better ratio of oestrogenic to anti-oestrogenic activity and a more rapid uptake and elimination of the bioactive moiety.

Droloxifene (3-OH-tamoxifen citrate) is a drug which has been recently launched for clinical testing. The drug itself is in the bioactive form and has an affinity for the oestrogen receptor which is 10 to 60 times greater than tamoxifen affinity [11]. It is rapidly and completely absorbed from the intestinal tract, and subsequently, rapidly metabolised, mainly by methylation and glucuronidation. As a result, accumulation of droloxifene or its

Correspondence to P.F. Bruning at The Netherlands Cancer Institute (Antoni van Leeuwenhoek Huis) Plesmanlaan 121, Amsterdam 1066 CX, The Netherlands.
P.F. Bruning and the following investigators participated in the Droloxifene 002 International Study Group: R. Balas (Siegen); M. Brandtner (Wetzlar); R. Kreienberg (Mainz); G.A. Nagel, N. Marschner (Göttingen); F. Schumann, J.P. Zugelder (Bad Mergentheim); R. Kolb, E. Pilz (Vienna); G. Reiner (Vienna); F. Schmoranzer (Vienna); D. Becquart (Antwerpen); M. Beaudouin (Haine St Paul); G. Schwartsmann (Porto Allegre); R. Hegg, L.F. Aguiar (Sao Paolo); A. Malzyner, R. Caponero (Sao Paulo); F. Gomes Filho, M.L. Chueke (Rio de Janeiro); J. Cantin, R. Polsson, D. Noel (Montreal); D. Perreault (Ottawa); L. Deschênes, L. Provencher (Quebec); J. Ayoub (Quebec); K.I. Pritchard (Toronto); H. Abu Zahra (Windsor); A.H. Paterson, S. Allen (Edmonton); B. Chevallier, C. Veyret (Rouen); D. Pouillart, S. Scholl (Paris); M. Marty, M. LeBonniec (Paris); L. Mignot (Suresnes); E. Pujade-Lauraine (Paris); J. Rouesse, M. Tubiana (St. Cloud); D. Serin, F. Plat (Avignon); M. Spielmann, L. Kayitalire (Villejuif); P.F. Bruning (Amsterdam); J. Conde-Bru, A. Lluch, A. Galan (Valencia); A. Poveda, A. Ruiz (Valencia); A. Duque, M. Barros (Sevilla); E. Murillo (Sevilla); A. Moyano, C. Crespo (Madrid); J. Estapé, M. Daniels, E. Tarrago (Barcelona); L. Solé, J. Bellmunt (Barcelona); J. Lizon, F. del Moral (Alicante); L.E. Rutqvist, N. Wilking (Stockholm); A.C. Almendral, R. Ludwig, R. Roth (Basel); C. Brown (Cambridge).
Revised 21 Feb. 1992; accepted 10 Mar. 1992.

metabolites hardly occurs. Its elimination $T_{\frac{1}{2}}$, is approximately 24 h, compared with almost 1 week for tamoxifen [12]. The ratio of oestrogenic to anti-oestrogenic activity of droloxifene on rat uterine weight is lower than the tamoxifen ratio [13]. Similarly, the effect of droloxifene on the human secretion of gonadotropins or the liver production of proteins, such as sex hormone binding globulin (SHBG), was found to be small [14] compared with tamoxifen, the latter demonstrating oestrogenic activity by a significant decrease of postmenopausal gonadotropin serum levels [15] and an almost 2-fold rise of SHBG [10, 16].

In contrast with tamoxifen, droloxifene has not shown transformation of embryonic fibroblasts in the Syrian hamster or hepatic carcinoma in the rat, or ovarian or Leydig cell tumours in mice as warnings of potential carcinogenicity in humans [17]. Preliminary results have shown that droloxifene is active against metastatic breast cancer and very well tolerated.

This is a report on behalf of investigators in 11 countries, constituting the Droloxifene 002 International Study Group. It deals with the first results of a double-blind, randomised dose-finding phase II clinical trial of droloxifene in advanced postmenopausal breast cancer.

SUBJECTS AND METHODS

Eligible patients were postmenopausal women with progressive metastatic or locally advanced inoperable biopsy-proven breast cancer. The definition of postmenopausal included at least 1 year since the last menstruation or bilateral ovariectomy, or older than 50 years; patients with a history of hysterectomy with one or both ovaries left in place had to be older than 55, unless serum gonadotropins were clearly in the postmenopausal range. Patients had to have measurable or evaluable lesions according to WHO criteria [18] and should not have received previous systemic, hormonal or cytostatic antitumour treatment, except for adjuvant chemotherapy, if completed at least 1 year before entry into the study. Patients with tumours known to be positive for oestrogen or progesterone receptors (more than 10 fmole/mg cytosol protein) or unknown receptor status were eligible. Patients were ineligible if they had a performance status of four, a history of retinopathy, severe liver disease, leucocytopenia or thrombocytopenia unrelated to chemotherapy, a previous cancer other than breast cancer, except adequately treated *in situ* carcinoma of the uterine cervix or skin carcinoma. Women with central nervous system involvement, severe infections, venous thrombosis or hypercalcemia at the time of study entry were also excluded.

Study design

The study was performed as a prospective, double-blind randomised phase II trial with randomisation to an oral dose of 20, 40 or 100 mg droloxifene per capsule once daily, in blocks of 6 patients. Treatment was continued until objective tumour progression was observed.

Response evaluation

Tumour response was evaluated according to WHO criteria [18], based on two consistent observations made not less than 4 weeks apart. On entry, all patients had to be staged by chest X-ray, isotope bone scanning followed by radiograms of all suspicious lesions and by ultrasound or computerised tomography of the liver. The evolution of study target lesions had to be documented every 2 months during the first half year and every 3 months thereafter, using the same diagnostic techniques throughout the study. Complete staging was done at the start and every 6 months thereafter. Quality control of the data was assured in two ways. All case record forms were regularly checked against hospital records by clinical trial monitors of the sponsoring companies Rhône Poulenc and Klinge Pharma. Furthermore, extramural reviewing was done by regular meetings of investigators who jointly evaluated the documentation of tumour responses in all patients, including hospital records, X-rays, scans, photographs, etc.

Table 1. Classification of enrolled patients with regard to evaluability

	Dose group			
	20 mg *n* (%)	40 mg *n* (%)	100 mg *n* (%)	all doses *n* (%)
Evaluable	74 (73.3)	74 (68.5)	86 (70.5)	234 (70.7)
Ineligible	18 (17.8)	21 (19.4)	23 (18.9)	62 (18.7)
Still to be evaluated	3 (3.0)	7 (5.6)	5 (4.1)	15 (4.5)
Not evaluable	6 (5.9)	6 (5.6)	8 (6.6)	20 (6.0)
Total	101 (100.0)	108 (100.0)	122 (100.0)	331 (100.0)

Statistical methods

Response rates were compared using the χ^2 test for the comparison of proportions. Survival (time to tumour progression) curves were calculated according to the Kaplan–Meier method and compared using the log-rank test.

RESULTS

Of the 369 patients accrued, 331 were peer reviewed at response evaluation sessions. Of these, 24 were ineligible because they were primary breast cancer patients who were not operated upon for medical reasons only. 38 patients were ineligible because they were premenopausal or various other inclusion criteria were violated. 20 patients were inevaluable and 15 were too early for definitive response evaluation. The distribution over the three treatment groups is given in Table 1. Prognostic characteristics of the 234 fully evaluable cases are shown in Table 2.

The tumour responses per dose group are given in Table 3. The objective over-all response rate [complete recovery (CR) + partial recovery (PR)] was 23/74 (31.1%) for the 20 mg, 33/74 (44.6%) for the 40 mg and 36/86 (41.9%) for the 100 mg dose group. A χ^2-test showed no significant difference.

In Fig. 1, the time to tumour progression is shown per dose group with no statistically significant differences. The estimated median time to disease progression was 5.6 months in the 20 mg group, 8.3 months in the 40 mg group and 6.0 months in the 100 mg group (not significant). Although 24 patients with primary breast cancer, who were considered inoperable for medical reasons, were strictly ineligible, their responses to treatment are noteworthy. Of 6 patients treated with 20 mg of droloxifene daily, 3 had a PR; 8/11 patients on 40 mg and 7/8 women on 100 mg showed an objective response, giving an over-all objective response rate of 18/24 (75%). The median time to response in these patients was only 1 month. Fig. 2 shows the time to response for the 92 eligible responders. The over-all median time to response was 2 months. Since the study includes patients who still have stable disease, time to response has not yet been tested for possible differences between dose levels.

Table 2. Prognostic characteristics of 234 fully evaluable patients

	Dose group 20 mg (n=74)	40 mg (n=74)	100 mg (n=86)
Mean age (S.D.) y	64.6 (9.5)	65.3 (9.2)	64.3 (9.8)
	n (%)	n (%)	n (%)
Disease-free interval			
< 2 years	37 (50.0)	37 (50.0)	41 (47.7)
> 2 years	37 (50.0)	37 (50.0)	45 (52.3)
Receptor status of primary tumour			
ER + PR unknown	34 (45.9)	28 (37.8)	42 (48.8)
ER or PR positive	38 (51.4)	46 (62.2)	44 (51.2)
Prior adjuvant chemotherapy			
Yes	14 (18.9)	11 (14.9)	17 (19.8)
No	60 (81.1)	63 (85.1)	69 (80.2)
Dominant site of disease			
Soft tissue	23 (31.1)	19 (25.7)	21 (24.4)
Bone	27 (36.5)	24 (32.4)	21 (24.4)
Visceral	24 (32.4)	31 (41.9)	44 (51.2)
Including liver metastases	13 (17.6)	16 (21.6)	21 (24.4)

ER = Oestrogen receptors; PR = progesterone receptors.

Table 3. Tumour responses per dose level

Response	20 mg n (%)	40 mg n (%)	100 mg n (%)	All doses n (%)
Complete response (CR)	5 (6.8)	7 (9.5)	11 (12.8)	23 (9.8)
Partial response (PR)	18 (24.3)	26 (35.1)	25 (29.1)	69 (29.4)
No change (NC)	32 (44.6)	27 (36.5)	22 (25.6)	63 (35.0)
Progressive disease (PD)	18 (24.3)	14 (18.9)	28 (32.6)	60 (25.6)
Total	74 (100.0)	74 (100.0)	86 (100.0)	234 (100.0)
CR + PR	(31.1)	(44.6)	(41.9)	(39.9)

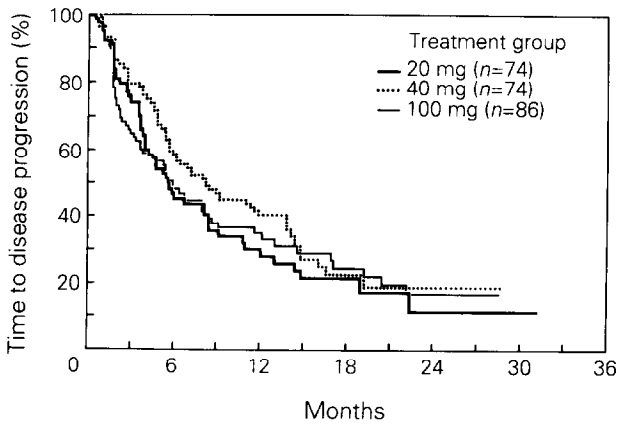

Fig. 1. Estimated progression-free survival rate per dose group (Kaplan–Meier method).

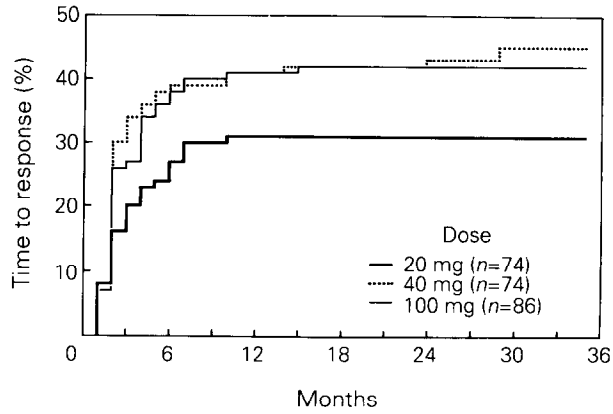

Fig. 2. Time to objective tumour response per dose group.

In general, droloxifene was extremely well tolerated, hot flushes being the complaint most frequently reported. Possible side effects could be evaluated in 331 patients; results are shown in Table 4. In 2 patients, pulmonary embolism and in 1 patient, a superficial venous thrombosis of the leg was observed. 1 patient showed erythema of the face and upper body. In another patient, a transient increase of the serum γ-glutamyltransferase levels was seen (up to 3.7 times the upper normal value), coinciding with metamizole treatment. 1 ineligible patient with hypercalcemia at the start of treatment, who was inadvertently included in the study, developed a further rise of serum calcium levels and died within a week. A decrease of bone pains within 2 weeks has been reported by several investigators.

DISCUSSION

In this dose-finding study, a 39.9% over-all objective response rate was observed with no significant difference between daily doses ranging from 20 to 100 mg. For a first-line endocrine therapy of a post-menopausal study population, in which half of the patients was known to have an oestrogen or progesterone receptor positive tumour, this response rate very well stands the comparison with established hormonal strategies. The same can be said for the duration of the time to tumour progression, which was similar in the three dose groups. Tolerance of droloxifene was rated excellent at all three dose levels.

As droloxifene itself is a bioactive compound and reaches

Table 4. Possible side-effects in 331 patients

	Dose group 20 mg (n=101)	40 mg (n=108)	100 mg (n=122)
	n (%)	n (%)	n (%)
Hot flushes	31 (30.7)	36 (33.3)	31 (25.4)
Nausea	21 (20.8)	20 (18.5)	32 (26.2)
Gastro-intestinal discomfort	16 (15.8)	12 (11.1)	17 (13.9)
Gastro-intestinal pain	9 (8.9)	6 (5.6)	12 (9.8)
Vomiting	8 (7.9)	9 (8.3)	16 (13.1)
Anorexia	10 (9.9)	11 (10.2)	18 (14.8)
Lassitude	24 (23.8)	23 (21.3)	29 (23.8)
Headache	11 (10.9)	10 (9.3)	12 (9.8)
Vaginal discharge	8 (7.9)	8 (7.4)	8 (6.6)

steady-state concentrations within days [12], it is not surprising that clinical responses have been observed within a short period of administration of the drug. This aspect of rapid droloxifene activity, which could imply an advantage over tamoxifen and other hormonal therapies, has to be further analysed.

The preclinical observations that droloxifene and its metabolites also have a short elimination half life compared with tamoxifen [12] open the possibility of using the drug for a very short period in order to block the tumour cell cycle in G_1 phase, and to subsequently recruit massive cell division with oestrogens or other factors, to finally strike with cell cycle phase-specific chemotherapeutic agents. Alternating schedules of a progestational drug with anti-oestrogen could similarly take advantage of droloxifene's rapidly versatile kinetics. The reported prolonged inhibition of droloxifene on the growth of MCF-7 cells *in vitro* when exposed to the drug for only 2 h every third day [19], not seen with tamoxifen [20], may also be exploited in the human situation to avoid the down regulation of receptors.

Preclinical data have demonstrated a lower ratio of oestrogenic to anti-oestrogenic activity of droloxifene compared with tamoxifen [19]. The present study gives no clues as to the possible superiority of droloxifene over tamoxifen in patients on this point. The recently established advantage in 10-year survival for women who were treated with adjuvant tamoxifen for at least 2 years, and the currently beginning trials of the primary prevention of breast cancer with 5 years of tamoxifen, have raised lively discussions on undesired and favourable side-effects of long-term anti-oestrogen administration. The main concern is that long-term usage of tamoxifen may cause an increase in the incidence of endometrial or other cancers [7], possibly due to the intrinsic oestrogen effect which tamoxifen has on the human endometrium. An increase in thromboembolic events could be another problem. To what extent cardiovascular mortality and morbidity from osteoporotic fractures can be reduced by tamoxifen will become clear in the next 10 years. For droloxifene, such observations will probably have to wait even longer.

Meanwhile, the proper role of droloxifene in the treatment of advanced breast cancer can now be assessed by comparative phase III clinical trials.

1. Rochefort H. Mechanism of action of high-affinity antiestrogens. *Am J Clin Oncol* (CCT) 1991, **14** (suppl. 2), S1–S4.
2. Knabbe C, Lippman ME, Kasid A, *et al.* Evidence that transforming growth factor β is a hormonally regulated negative growth factor in human breast cancer cells. *Cell* 1987, **48**, 417–428.
3. Knabbe C. Regulation of growth inhibitory polypeptides in human breast cancer. In: Voigt KD, Knabbe C, eds. *Endocrine-dependent Tumors*. New York, Raven Press, 1991, 165–177.
4. Vignon F, Bouton MM, Rochefort H. Antiestrogens inhibit the mitogenic effect of growth factors on breast cancer cells in the total absence of estrogens. *Biochem Biophys Res Commun* 1987, **146**, 1502–1508.
5. Murphy C, Fotsis T, Pantzas P, Adlercreutz H, Martin F. Analysis of tamoxifen and its metabolites in human plasma by gas chromatography-mass spectrometry (GC-MS) using selected ion monitoring (SIM). *J Steroid Biochem* 1987, **26**, 547–555.
6. Lien EA, Solheim E, Leo OA, Lundgren S, Kvinnsland S, Ueland PM. Distribution of 4-hydroxy-*N*-desmethyl-tamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. *Cancer Res* 1989, **49**, 2175–2183.
7. Adam HK, Patterson JS, Kemp JV. Studies on the metabolism and pharmacokinetics of tamoxifen in normal volunteers. *Cancer Treat Rep* 1980, **64**, 761–764.
8. Milane G, Etienne MC, Frenay M, *et al.* Optimised analysis of tamoxifen and its main metabolites in the plasma and cytosol of mammary tumours. *Br J Cancer* 1987, **55**, 509–512.
9. Fornander T, Rutqvist LE, Cedermark B, *et al.* Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. *Lancet* 1989, **i**, 117–120.
10. Jordan VC, Fritz NF, Tormey DC. Long-term adjuvant therapy with tamoxifen: effects on sex hormone binding globulin and antithrombin III. *Cancer Res* 1987, **47**, 4517–4519.
11. Roos WK, Oeze L, Löser R, Eppenberger U. Antiestrogenic action of 3-hydroxy-tamoxifen in the human breast cancer cell line MCF-7. *J Natl Cancer Inst* 1983, **71**, 55–59.
12. Grill HJ, Pollon K. Pharmacokinetics of droloxifene and its metabolites in breast cancer patients. *Am J Clin Oncol* (CCT) 1991, **14** (suppl. 2), S21–S29.
13. Löser R, Seibel K, Roos WK, Eppenberger U. *In vivo* and *in vitro* antiestrogenic action of 3-hydroxy-tamoxifen, tamoxifen and 4-hydroxy-tamoxifen. *Eur J Cancer Clin Oncol* 1985, **21**, 985–990.
14. Kvinnsland S. Droloxifene, a new antiestrogen. Hormonal influences in post-menopausal breast cancer patients. *Am J Clin Oncol* (CCT), 1991, **14** (suppl. 2), S46–S51.
15. Jordan VC, Fritz NF, Tormey DC. Endocrine effects of adjuvant chemotherapy and long-term tamoxifen administration on node positive patients with breast cancer. *Cancer Res* 1987, **47**, 624–630.
16. Bruning PF, Bonfrèr JMG, Hart AAM, *et al.* Tamoxifen, serum lipoproteins and cardiovascular risk. *Br J Cancer*, 1988, **58**, 497–499.
17. Metzler M, Schiffmann D. Structural requirements for the *in vitro* transformation of Syrian hamster embryo cells by stilbene estrogens and triphenylethylene-type antiestrogens. *Am J Clin Oncol* (CCT) 1991, **14** (suppl. 2), S30–S35.
18. Miller AB, Hoogstraten B, Staquet M, Winker A. Reporting results of cancer treatment. *Cancer* 1981, **47**, 207–214.
19. Eppenberger U, Wosikowski K, Küng W. Pharmacologic and biologic properties of droloxifene, a new antiestrogen. *Am J Clin Oncol* (CCT) 1991, **14** (suppl. 2), S5–S14.
20. Dietel M, Löser R, Röhlke P, *et al.* Effect of continuous vs intermittent application of 3 OH-tamoxifen or tamoxifen on the proliferation of the human breast cancer cell line MCF-7 M1. *Cancer Res Clin Oncol* 1989, **115**, 36–40.

Acknowledgements—This study was sponsored by Rhône Poulenc and Klinge Pharma. We thank Ms M. de Kwant for secretarial help.