High dose ketoconazole: endocrine and therapeutic effects in postmenopausal breast cancer

A.L. Harris¹, B.M.J. Cantwell¹ & M. Dowsett²

¹University of Newcastle upon Tyne, Department of Clinical Oncology, Regional Radiotherapy Centre, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE, and ²Endocrine Department, Chelsea Hospital for Women, Dovehouse Street, London, SW3 6LT, UK.

Summary  Ketoconazole, an antifungal agent, inhibits in vitro C17-20 lyase, an enzyme involved in androgen biosynthesis. Since adrenal and ovarian androgens are the main precursors of oestrogens in postmenopausal women, the endocrine and therapeutic effects of high dose ketoconazole (400 mg three times a day) were evaluated in 14 postmenopausal women with advanced breast cancer. Testosterone levels were suppressed significantly (37%, P<0.025), as was dehydroepiandrosterone sulphate, and androstenedione levels showed a similar but non-significant fall. Seventeen hydroxyprogesterone levels rose significantly, as would be expected if C17-20 lyase was inhibited. There was no suppression of cortisol or oestrone levels. There was a small suppression of oestradiol concentrations, reflecting a decrease in its precursor, testosterone. Sex hormone binding globulin levels rose, which may be due to a decrease in testosterone. All the changes are compatible with C17-20 lyase as a major site of action in vivo. No responses occurred in 12 patients treated with ketoconazole alone, but in 2 patients who were progressing on aminoglutethimide, testosterone levels were suppressed and in one patient a partial response occurred. Ketoconazole was poorly tolerated due to gastrointestinal toxicity. This study shows that C17-20 lyase is a potential target for hormone therapy, and that sequential blockade of enzymes involved in oestrogen biosynthesis should be further evaluated.

Ketoconazole is an oral antifungal agent, which was reported to produce gynaecomastia on high dosage regimens (De Felice et al., 1981). This led to investigation of its effects on testosterone production. It has been shown to inhibit testicular (Lambert et al., 1986), adrenal (Couch et al., 1987) and ovarian (Di Mattina et al., 1988) steroid biosynthesis in vitro and testicular (Pont et al., 1982a) and adrenal steroid (Pont et al., 1982b, 1984) biosynthesis in vivo. Several enzymes are inhibited, but the most sensitive is C17-20 lyase (Figure 1). Blockade of this enzyme would decrease both adrenal and testicular androgen production and ketoconazole has therefore been used to treat prostate cancer (Pont, 1987). It has been used either as a single agent or in combination with LHRH agonists (Allen et al., 1983).

Although the endocrine and therapeutic effects are well documented in men, there are no studies in women with breast cancer. In postmenopausal women, the major sources of oestrogens are adrenal and ovarian androgens (Judd et al., 1982, 1974; Grodin et al., 1973). Aromatase inhibitors that prevent this interconversion are effective therapeutically in postmenopausal (Harris et al., 1983a) and occasionally premenopausal breast cancer (Bezwoda et al., 1987; Wander et al., 1986). The latter effect has been ascribed to direct inhibition of intratumour production of oestrogens from androgens (Miller et al., 1982; Bezwoda et al., 1987), which may be a major local oestrogen source in postmenopausal women (Mehta et al., 1987). In patients failing to respond to aromatase inhibitor therapy with aminoglutethimide, rises in androgens have been reported (Santen et al., 1982).

Thus, sequential enzyme blockade to inhibit androgen production as well as inhibition of aromatase may produce greater oestrogen suppression and enhanced therapeutic effects. To evaluate this possibility, we investigated the endocrine and therapeutic effects of high dose ketoconazole in postmenopausal women with advanced breast cancer.

Patients and methods

Fourteen patients were studied. They were all postmenopausal and had progressive breast carcinoma confirmed histologically or cytologically. The majority had advanced primary local disease. Patients had stopped previous endocrine therapy, which was tamoxifen (n=7) or low dose aminoglutethimide (6) (Harris et al., 1986), a month or more previously. Two patients who were progressing on aminoglutethimide 125 mg twice daily, hydrocortisone 20 mg twice daily, had ketoconazole added to their therapy. The endocrine data for these patients was analysed separately from the others. The patient characteristics are shown in Table 1.

Response was assessed by UICC criteria (Hayward et al., 1977).

Ketoconazole was given as 200 mg three times daily (tids) with food, and, if well tolerated, the dose was increased after 1 week to 400 mg tids. Patients were seen weekly for assessment of toxicity, liver function tests and dosage modification if there were side effects.

Plasma samples were taken at each attendance for testosterone (T), 17-hydroxyprogesterone (17OHP), Δ₇ androstenedione (Δ₇A), sex hormone binding globulin (SHBG), oestrone (E), oestradiol (E₂), dehydroepiandrosterone sulphate (DHAS) and cortisol levels. They were measured by immunoassays which we have described in detail previously (Harris et al., 1983b, c).

Correspondence: A.L. Harris.
Received 20 May 1988.

---

Figure 1  Sites of action of ketoconazole. Sites at which ketoconazole can inhibit hormone synthesis are 17 hydroxylase (17OH), C17-20 lyase (17-20 lyase) and 11β hydroxylase (11OH). Aminoglutethimide inhibits aromatase (arom).
Pre- and post-treatment samples were compared by a non-parametric method, Wilcoxon ranked sums, and $P<0.05$ taken as significant.

Results

Endocrine effects of ketoconazole

Testosterone concentrations fell significantly by 37% over a 3 week period (Figure 2). Androstenedione showed a non-significant fall at week 1 (Table II). Oestrone and oestradiol both showed a fall at week 1, but only in the case of oestradiol was this significant (Figure 2). DHAS fell by week 3. In contrast, 170HP levels rose significantly at week 1 and remained elevated at week 3 (Figure 2). SHBG rose over the 3 week period (Figure 2).

No significant changes occurred in cortisol levels.

In the case of 2 patients already on therapy with AG, addition of ketoconazole produced suppression of testosterone levels (0.6 to 0.2 nM, and 1.3 to 0.3 nM).

Clinical effects of ketoconazole

No responses were seen in the 12 patients treated with ketoconazole alone. The drug, however, was poorly tolerated and 7 patients stopped therapy within 1–3 weeks because of severe nausea (5) or vomiting (2). One patient stopped because of confusion that was reversed after changing therapy. Five stopped because of progressive disease, although the drug was well tolerated.

In one patient who had shown a partial response to aminoglutethimide, addition of ketoconazole at the time of tumour progression produced a further partial response in soft tissue disease for 5 months.

The median survival from start of ketoconazole was 1 year and 3 months, median survival from first relapse or presentation with locally advanced disease was 3 years 6 months.

Discussion

The changes detected in the hormone profiles in our patients are compatible with the reported sites of action of ketoconazole, although most studies have been carried out with testicular or adrenal tissue (Nagai et al., 1987; Loose, 1983; Sikka, 1985; Kan, 1985; Kowal, 1983; Malozowski, 1985). Blood levels achieved with ketoconazole are in the range 3–20 μM (Craven et al., 1983; Brass et al., 1982) and may be expected to inhibit the following enzymes: adrenal 17 hydroxylase (K1, 0.04 μM), 17,20 desmolase (K1, 0.01 μM), 11β hydroxylase (K1, 0.01 μM) (Couch et al., 1987), ovarian 17 hydroxylase (ID50 5 μM) (Di Mattina et al., 1988). Thus inhibition of C17-20 lyase would be expected to reduce T and Δ4A levels. As a consequence of depletion of these substrates for aromatase E2 and E1, may fall. It has recently been shown that suppression of ovarian androgens by LH-RH agonists can lead to a reduction in E2 in postmenopausal women (Dowsett et al., 1988), and suppression of adrenal androgens with hydrocortisone can produce suppression of E2 and E1 (Alexieva-Figusch et al., 1987; Harris et al., 1984). However, in men treated with ketoconazole, although T fell, E2 did not (Santen et al., 1983). This may reflect differences between men and women in substrates available to aromatase.

The major precursor of Δ4, 170HP, rose significantly. It is not clear whether this is ovarian or adrenal in origin, or comes from both glands. The major adrenal androgen metabolite DHAS showed a significant fall which is probably due to adrenal blockade of C17-20 lyase.

Although 17α hydroxylase is inhibited in some studies (Couch et al., 1987), in others there is no effect (Nagai et al., 1987; Lambert et al., 1986). In our patients, 17α hydroxylase

---

Table I Patient pretreatment characteristics

|                      | Median | Mean (s.d.) | Range |
|----------------------|--------|-------------|-------|
| Age (years)          | 66     | 66 (±12)    | 47-84 |
| LMP (years)          | 20     | 15 (±9)     | 1-25  |
| DFI (months)         | 0      | 9 (±15)     | 0-60  |
| Wt. (kg)             | 65     | 61 (±12)    | 40-77 |
| Previous endocrine therapy (ET) | 11     |             |       |
| Previous chemotherapy | 4      |             |       |
| Previous radiotherapy | 8      |             |       |
| Response to previous ET | 5/11   |             |       |
| Response to subsequent ET | 3/11   |             |       |

Table II Hormone concentrations on ketoconazole

|                | Androstenedione (nM) | Oestrone (pM) | Cortisol (nM) |
|----------------|-----------------------|---------------|--------------|
|                | Week                  | Week          | Week         |
|                | 0        | 1         | 3         | 0    | 1         | 3         | 0     | 1         | 3         |
| Mean           | 1.1      | 0.6       | 1.1       | 94   | 55        | 72        | 316   | 355       | 231       |
| s.d.           | 1.1      | 0.4       | 1.2       | 115  | 37        | 38        | 93    | 105       | 99        |
| P              | —        | NS        | NS        | —    | NS        | NS        | —     | NS        | NS        |
| n              | 13       | 10        | 10        | 10   | 8         | 6         | 11    | 8         | 8         |

---

Figure 2 Endocrine effects of ketoconazole.
does not appear to be inhibited, since 17OHP levels rose, as did the levels of postmenopausal ovarian aromatase (Santer et al., 1983). Since 17α-hydroxylase and 17,20-lyase activities reside in the same enzyme, this suggests that ketoconazole interacts selectively with only one active site in C17-C20 lyase (Nakajin & Hall, 1981). Since C17-C20 lyase has been demonstrated in human breast tumours, a direct local effect may occur (Abul-Hajj et al., 1980).

The fall in oestrogens could be due to an inhibitory effect of ketoconazole on aromatase which has been reported for human placental aromatase (Ayub & Stich, 1986). However, it was not inhibitory to human ovarian aromatase (Di Mattina et al., 1988), although other imidazole compounds are potent aromatase inhibitors (Schieveck et al., 1988). Any effect on aromatase could be of additive value if combined with other classes of aromatase inhibitor.

Cortisone levels did not change significantly, although 11α-hydroxylase is inhibited in vitro (Couch et al., 1987) and in vivo (Pont et al., 1984), and urine free cortisol falls in men treated with ketoconazole (Santer et al., 1983). Thus, although it has been suggested that nausea and vomiting due to ketoconazole could be due to Addisonian crisis (White & Kendall-Taylor, 1985), this was not the case in our study. SHBG levels rose and this may be due to the reduction in testosterone levels, since androgens suppress SHBG synthesis (Anderson, 1974).

The poor tolerance to ketoconazole in this population precluded further endocrine studies. In studies with prostate cancer, there was a high discontinuation rate (Pont, 1987), although not as high as in this study. Gastric acidity is required for absorption (Van Tyle, 1984) and it may be that achlorhydria in an elderly female population led to lower absorption and higher gastrointestinal side effects. Although abnormal liver function can occur, there were no significant abnormalities in this study (McCance et al., 1987; Lake-Bakkari et al., 1987).

No responses were seen to ketoconazole alone, although the patients were not intrinsically resistant to hormone therapy, since 5 had previously responded, and 3 responded to subsequent hormone therapy. One case of male breast cancer has been described, who responded to ketoconazole (Feldman, 1986). It is likely that the poor tolerance in our study precluded adequate therapeutic assessment.

However, since one aim of the study was to assess the possible use of sequential enzyme blockade to lower intratumour oestrogen levels, ketoconazole was added to the therapy of 2 patients who had initially responded to AG and then progressed on AG. In both cases, testosterone levels fell by more than 50% and this could deplete tumours of a substrate required for intratumour oestrogen biosynthesis. One patient responded.

This study shows that inhibition of C17-C20 lyase can be achieved in postmenopausal women and produce a significant fall in androgens. Better tolerated inhibitors may produce synergistic effects with aromatase inhibitors and provide a rational target for drug development.

We would like to thank Dr M.B. Emanuel, Director of Clinical Research, Janssen Pharmaceuticals Ltd., for supplying us with ketoconazole.

References

ABUL-HAJJ, Y.J., IVerson, R. & KIANG, D.T. (1980). Metabolism of pregnenolone by human breast cancer. Evidence for 17-hydroxylase and 17,20-lyase. Steroids, 35, 817.

ALEXIEVA-FIGUSCH, J., DEJONG, F.H., LAMBERTS, S.W.J., VAN GILSE, H.A. & KLJN, I.G.M. (1987). Endocrine effects of amino-glutethimide plus hydrocortisone versus effects of high dose of hydrocortisone alone in postmenopausal metastatic breast cancer. Eur. J. Cancer Clin. Oncol., 23, 1349.

ALLEN, J.M., KERRE, D.J., WARE, H., DOBLE, A., WILLIAMS, G. & BLOOM, S.R. (1983). Combined treatment with ketoconazole and luteinizing hormone releasing hormone analogue: A novel approach to resistant progressive prostatic cancer. Br. Med. J., 287, 176.

ANDERSON, D.C. (1974). Sex hormone binding globulin. Clin. Endocrinol., 3, 69.

AYUB, M. & STITCH, S.R. (1986). Effect of ketoconazole on placental aromatase, 3-hydroxysteroid dehydrogenase-isomerase and 17β-hydroxysteroid dehydrogenase. J. Steroid Biochem., 25, 981.

BEZWODA, W.R., MANSOOR, N. & DANSEY, R. (1987). Correlation of breast tumour aromatase activity and response to aromatase inhibition with amino-glutethimide. Oncology, 44, 345.

BLAKE, R.E., RAIGURU, S., NOLAN, G.H. & AHLUWALIA, B.S. (1988). Dexemethasone suppresses sex-hormone binding globulin. Fertility & Sterility, 49, 150.

BRASS, C., GALGIANI, J.N., BLASCHKE, T.F., de FELICE, R., O’REILLY, R.A. & STEVENS, D.A. (1982). Disposition of ketoconazole, an oral antifungal, in humans. Antimicrob. Agents Chemother., 21, 151.

COUCH, R.M., MULLER, J., PERRY, Y.S. & WINTER, J.S.D. (1987). Kinetic analysis of inhibition of human adrenal steroidogenesis by ketoconazole. J. Clin. Endocrinol. Metab., 65, 551.

CRAVEN, P.C., GRAYBILL, J.R., JORGENSEN, J.H., DISMUKES, W.E. & LEVINE, H. (1985). High dose ketoconazole for treatment of fungal infections of the central nervous system. Ann. Intern. Med., 98, 160.

DE FELICE, P., JOHNSON, D.G. & GALGIANI, J.N. (1981). Gyneco-mastia with ketoconazole. Antimicrob. Agents Chemother., 19, 1073.

D’MATTINA, M., LORIAUX, D.L., MARONIAN, N., ALBERTSON, B.D. & ASHLEY, H. (1988). Ketoconazole inhibits multiple steroidogenic enzymes involved in androgen biosynthesis in the human ovary. Fertility & Sterility, 49, 62.
KOWAL, J. (1983). The effect of ketoconazole on steroidogenesis in cultured mouse adrenal cortex tumor cells. *Endocrinology, 112*, 1541.

LAKE-BAKAAR, G., SCHEUEER, P.J. & SHERLOCK, S. (1987). Hepatic reactions associated with ketoconazole in the United Kingdom. *Br. Med. J.*, 294, 419.

LAMBERT, A., MITCHELL, R. & ROBERTSON, W.R. (1986). The effect of ketoconazole on adrenal and testicular steroidogenesis in vitro. *Biochem. Pharmacol.*, 35, 3999.

LOOSE, D.S., KAN, P.B., HIRST, M.A., MARCUS, R.A. & FELDMAN, D. (1983). Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P450-dependent enzymes. *J. Clin. Invest.*, 71, 1495.

McCANCE, D.R., HADDEN, D.R., KENNEDY, L., SHERIDAN, B. & ATKINSON, A.B. (1987). Clinical experience with ketoconazole as a therapy for patients with Cushing's syndrome. *Clin. Endocrinol.*, 27, 593.

MALOZOWSKI, S., YOUNG, I., GARCIA, H., SIMONI, C., LORIAUX, D.L. & CASSORIA, F. (1985). Effects of ketoconazole on rat testicular steroidogenic enzymatic activities. *Steroids*, 46, 659.

MEHTA, R.R., VALCOURT, L., GRAVES, J., GREEN, R. & DAS GUPTA, T.K. (1987). Subcellular concentrations of estrone, estradiol, androstenedione and 17β-hydroxysteroid dehydrogenase (17β-OH-SDH) activity in malignant and non-malignant human breast tissues. *Int. J. Cancer*, 40, 305.

MILLER, W.R., HAWKINS, R.A. & FORREST, A.P.M. (1982). Significance of aromatase activity in human breast cancer. *Cancer Res.*, 42, (Suppl.) 3365.

NAGAI, K., MIYAMORI, I., TAKEDA, R., SUHARA, K. & KATAGIRI, M. (1987). Effect of ketoconazole, etomidate and other inhibitors of steroidogenesis on cytochrome P-450scI1-catalyzed reactions. *J. Steroid Biochem.*, 28, 333.

NAKAJIN, S. & HALL, P.F. (1981). Microsomal cytochrome P-450 from neonatal pig testis. *J. Biol. Chem.*, 256, 3871.

PONT, A. (1987). Long-term experience with high dose ketoconazole therapy in patients with stage D2 prostatic carcinoma. *J. Urol.*, 137, 902.

PONT, A., WILLIAMS, P.L., AZHAR, S. & 4 others (1982a). Ketoconazole blocks testosterone synthesis. *Arch. Intern. Med.*, 142, 2137.

PONT, A., WILLIAMS, P.L., LOOSE, D.S. & 4 others (1982b). Ketoconazole blocks adrenal steroid synthesis. *Ann. Intern. Med.*, 97, 370.

PONT, A., GRAYBILL, J.R., CRAVEN, P.C. & 4 others (1984). High-dose ketoconazole therapy and adrenal and testicular function in humans. *Arch. Intern. Med.*, 144, 2150.

SANTEN, R.J., WORGUL, T.J., SAMOULIK, E., BOUCHER, A.E., LIPTON, A. & HARVEY, H. (1982). Adequacy of estrogen suppression with aminoglutethimide and hydrocortisone as treatment of human breast cancer: Correlation of hormonal data with clinical responses. *Cancer Res.*, 42, (Suppl.) 3397.

SANTEN, R.J., VAN DEN BOSSCHE, H., SYMOENS, J., BRUGMANS, J. & DECOSTER, R. (1983). Site of action of low dose ketoconazole on androgen biosynthesis in men. *J. Clin. Endocrinol. Metab.*, 57, 732.

SCHIEWECK, K., BHATNAGAR, A.S. & MATTER, A. (1988). CGS 16949A, a new nonsteroidal aromatase inhibitor: effects on hormone-dependent and -independent tumours in vivo. *Cancer Res.*, 48, 834.

SIKKA, S.C., SWERDLOFF, R.S. & RAJFER, J. (1985). In vitro inhibition of testosterone biosynthesis by ketoconazole. *Endocrinology, 116*, 1920.

WANDER, H.E., BLOSSEY, H.Ch. & NAGEL, G.A. (1986). Aminoglutethimide in the treatment of premenopausal patients with metastatic breast cancer. *Eur. J. Clin. Oncol.*, 22, 1371.

WHITE, M.C. & KENDALL-TAYLOR, P. (1985). Adrenal hypofunction in patients taking ketoconazole. *Lancet*, 1, 44.