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Cytotoxicity of ketoconazole in malignant cell lines*

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Summary. The cytotoxic effects of ketoconazole, an antifungal agent known to have some activity against human prostate cancer, adrenal cancer, and male metastatic breast cancer, were evaluated using colony-growth and clonogenic assays in eight malignant cell lines. The cytotoxicity of ketoconazole showed a dose- and time-dependent pattern, with the following concentrations inhibiting 90% of the growing colonies (IC_{90}): MCF 7 (human breast cancer) 7.25 μg/ml, T 47 D (human breast cancer) 9.0 μg/ml, MiaPaCa (human pancreatic carcinoma) 10.0 μg/ml, COLO 357 (human pancreatic carcinoma), 9.5 μg/ml, HCT 8 (human colonic adenocarcinoma) 27.1 μg/ml, DU 145 (human prostatic cancer) 40.0 μg/ml, AR 42 J (rat pancreatic carcinoma) 9.0 μg/ml, and L1210 (murine leukemia) 8.6 μg/ml. Since a concentration of 10 μg/ml can be achieved in humans, the use of ketoconazole in human malignancies might be worthy of clinical evaluation.

Introduction

Ketoconazole is an orally active broad-spectrum antifungal drug [18] especially active in patients with histoplasmosis and nonmeningeal cryptococcosis [8]. At very low concentrations, it inhibits the cytochrome P-450-dependent conversion of lanosterol into ergosterol in fungi, and this is probably its mechanism of action in these organisms [23]. In mammalian cells, ketoconazole has been shown to bind directly to the cytochrome P-450 component of the monooxygenase system, inhibiting steroid hydroxylation in adrenal and gonadal tissue [21]. Gynecomastia has been reported in a few patients treated with high-dose ketoconazole [7], and further investigations have confirmed that short-term administration of ketoconazole provokes a dose-dependent, but transient, block in the production of testosterone [6, 13, 17, 19, 20] and a sharply diminished cortisol response to corticotropin [13]. This has prompted the experimental use of high-dose ketoconazole as an alternative to orchidectomy or estrogen administration in patients with prostate carcinoma [3, 22, 25]. Furthermore, there have been single case reports on the use of ketoconazole in patients with metastatic adrenal carcinoma [4] and male metastatic breast cancer [9]. To evaluate the potential role of ketoconazole in the treatment of other malignancies, we determined its in vitro cytotoxicity in eight malignant cell lines.

Materials and methods

Cell lines. The following cell lines were used: human breast carcinomas MCF 7 and T47 D, human pancreatic adenocarcinomas MiaPaCa and COLO 357, human colon adenocarcinoma HCT 8, human prostate carcinoma DU 145, rat pancreatic carcinoma Ar 42 J, and murine leukemia L1210. The L1210 cells were maintained as a stationary suspension and transferred twice weekly; the other cell lines were maintained as continuously growing monolayer cells and transferred weekly as described in detail elsewhere [5]. The doubling times under our conditions were as follows: MCF 7 cells, 28 h; T 47 D cells, 26 h; MiaPaCa cells, 21 h; COLO 357 cells, 36 h; HCT 8 cells, 12.5 h; DU 145 cells, 29 h; AR 42 J cells, 30 h; and L1210 cells, 12 h.

Drug exposure. Ketoconazole (Nizorale) was obtained from Janssen Pharmaceutic, New Jersey, as a powder. A stock solution was prepared by diluting ketoconazole to a final concentration of 1000 μg/ml in RPMI-1640 acidified with HCl to 0.1 *N*. For each cell line exposed to ketoconazole, control experiments were also performed using both untreated cells as well as cells exposed to the acidified ketoconazole diluent alone.

Clonogenic assay. Ketoconazole was added to 10 ml aliquots of L1210 cells growing logarithmically at 30000 cells/ml. Final ketoconazole concentrations ranged from 1 to 50 μg/ml. After 24 h of drug exposure (~2 doubling times), the cytotoxicity of the different concentrations was determined by cloning the cells in soft agar by a modification of techniques described previously [5]. Two milliliters of RPMI containing 15% heat-inactivated horse serum and 50 L1210 cells were transferred into 15-ml sterile plastic culture tubes to which 3 ml medium containing 10% noble agar were added. As the agar solidified, the L1210 cells remained individually suspended. Each viable cell divided

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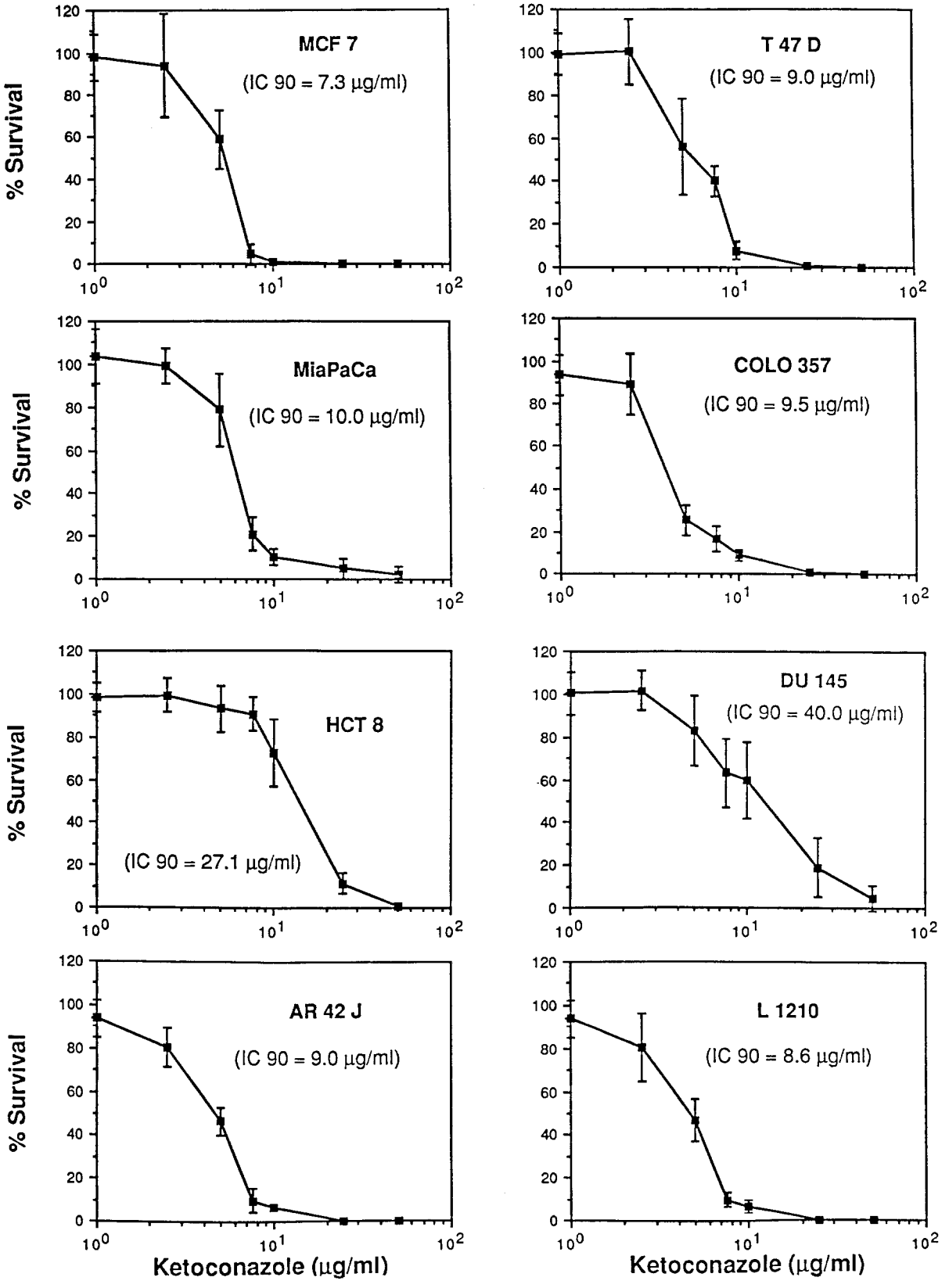

Fig. 1. Cytotoxicity of ketoconazole. Dose-response curves of 8 malignant cell lines. The percentage survival was observed in clonogenic assays and colony-growth inhibition experiments as described in Materials and methods. Each *data point* is bracketed by the standard deviation

to produce a visible colony (clone) after 10 days of incubation. The percent observed survival for each condition was calculated as [no. of colonies in each experimental condition/no. of colonies of control) X 100]. All experiments were performed in triplicate and repeated three times. The mean cloning efficiency of control cells was 81.1%.

Colony growth inhibition. A colony-growth inhibition assay of the monolayer cell lines was performed using a modification of the technique described in detail elsewhere [5]. For HCT 8 and MiaPaCa cells, 2×10^4 cells were seeded per sterile plastic 25 cm^2 culture flask with 10 ml of medium. For the other monolayer cell lines, 5×10^4 cells were seeded per flask. Twenty-four hours after seeding, cells were exposed to various concentrations of ketoconazole for a total of 72 h (~ 2 doubling times for most of the monolayer cell lines). Upon completion of exposure, the medium was aspirated off and the cells were washed twice with sterile PBS and reincubated until control cells were approximately 80% confluent (1–4 days after completion of drug exposure). The cells were stained and counted on a Biotran II counter as described in detail elsewhere [5]. The percent observed survival was calculated in the same way as described for the clonogenic assays above. Each data point represents three experiments done in triplicate.

Results

Figure 1 shows the results of the colony-growth inhibition experiments and clonogenic assays performed in the eight malignant cell lines. The colony growth of all cell lines was inhibited in a dose-dependent manner by ketoconazole. No growth inhibition of any cells treated with the acidified ketoconazole diluent alone was observed. The dose-response curves for the COLO 357, MCF 7, L1210, MiaPaCa, T 47 D, and AR 42 J cells were all very similar, the respective IC_{90}s being between 7.25 and 10.0 μg/ml. However, HCT 8 and DU 145 cells were less sensitive to ketoconazole, with IC_{90}s of >25 μg/ml. To evaluate the dependence of the growth inhibition on the length of duration of ketoconazole exposure, we did colony-growth inhibition experiments in MiaPaCa cells using different concentrations (5, 10, and 50 μg/ml) as well as different exposure times (5, 9, 24, and 72 h). Figure 2 shows that for each concentration of ketoconazole, the survival of cells decreased as the exposure time increased. Prolonged exposure to low doses of ketoconazole (5 μg/ml), however, did not substantially increase cytotoxicity in MiaPaCa cells.

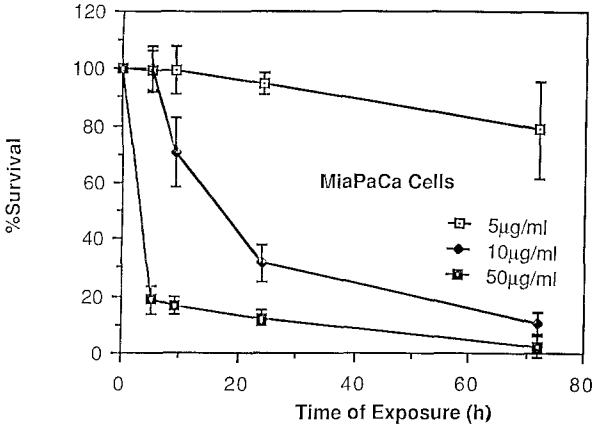

Fig. 2. Influence of exposure time on cytotoxicity at different concentrations of ketoconazole in MiaPaCa cells. Survival in percent observed in colony-growth inhibition experiments. Each *data point* is bracketed by the standard deviation

Discussion

Ketoconazole is an active agent against different malignant cell lines in vitro. Six of eight cell lines studied showed $IC_{90} \leq 10.0$ μg/ml, a concentration that can be achieved in humans in vivo. Van Tyle [24] reported peak serum levels of 3.6 μg/ml and 6.5 μg/ml after administration of single doses of 200 mg and 400 mg ketoconazole, respectively, to humans. Pont et al. [12] demonstrated mean ketoconazole concentrations of 14.5 μg/ml in five male volunteers 1 h after single doses of 600 mg. Heyns et al. [10] treated 9 patients with prostate carcinoma with high doses of ketoconazole (400 mg every 8 h) and detected serum levels of 2.0–9.1 μg/ml (means of 12 measurements recorded during 1 year of continuous treatment). Cell lines derived from the same tissue had similar IC_{90} values (human breast cancer cell lines MCF 7 and T 47 D: 7.3 and 9.0 μg/ml, respectively; human pancreatic cell lines MiaPaCa and COLO 357 10.0 and 9.5 μ/ml, respectively). Two of the eight cell lines tested (human colon carcinoma cell line HCT 8 and human prostate cancer cell line DU 145), however, were three to four times more resistant to the cytotoxic effects of ketoconazole. This insensitivity might be of clinical importance since we showed that prolonged exposure to low doses of ketoconazole in MiaPaCa cells results in only a marginal increase in cytotoxicity. Whether a more prolonged exposure to comparable concentrations of ketoconazole in humans is more cytotoxic to malignant cells cannot be deduced from these studies.

Two mechanisms of ketoconazole cytotoxicity have been proposed. The first is that ketoconazole inhibition of steroid synthesis results in the death of tumor cells dependent on specific steroids for growth in whole animals [23]. In humans, Trachtenberg [22] was the first to report antitumor effects of ketoconazole (400 mg every 8 h) in the treatment of advanced prostate cancer. Since then, other investigators have shown responses of prostate cancer [3, 25], male metastatic breast cancer [9], and metastatic adrenal carcinoma [4] to ketoconazole. Although the mechanism of action of ketoconazole in those patients remains to be elucidated, all treated patients suffered from hormone-dependent malignancies, and the clinical responses were interpreted as secondary to a decrease in androgen levels by all authors.

Heyns et al. [10], however, saw a clinical response to therapy in patients with prostate carcinoma that seemed to be better than would be expected from the measured decrease in serum testosterone levels; this suggests the possibility that ketoconazole may act on prostate cancer cells by more than one mechanism.

The second mechanism proposed is cytotoxicity resulting from ketoconazole-induced abnormal cell membrane permeability. In yeast, ketoconazole has been shown to block the conversion of lanosterol to ergosterol by inhibiting C-14-demethylation [23], thus leading to increased permeability of cell membranes [1, 23]. The cytochrome P-450-dependent C-14-demethylation of lanosterol to ergosterol in rat liver cells was similarly inhibited, although at

much higher doses. These findings were confirmed by studies on human lymphocytes in vitro [2], where ketoconazole inhibited cholesterol synthesis at doses of less than 10 μg/ml. Kraemer et al. [11] found a 27% decrease in cholesterol serum levels and a 46% increase in lanosterol serum levels in seven men with advanced prostate cancer treated with high-dose ketoconazole (3 × 400 mg/day). They also found an inhibition of cholesterol synthesis in cultured normal human fibroblasts. Ketoconazole also inhibits several other cytochrome P-450-dependent enzymes, thus blocking adrenal and testicular steroidogenesis [10, 13–16, 23, 24].

In our experiments, the growth of malignant cells was dependent on fixed extracellular steroids, including cholesterol, present in serum-enriched growth medium. This suggests that there must have been other mechanisms of action than the decrease in extracellular steroid supply whereby ketoconazole resulted in growth inhibition and cytotoxicity. The influence of ketoconazole on membrane lipid composition and permeability [1, 23], however, may be responsible for the observed cytotoxicity in the cell lines we examined, at least in part.

It is remarkable that in lymphocytes no inhibition of cell growth was seen at 10 μg/ml [2], a concentration which inhibited 90% or more of the cell growth in six of eight cell lines and at least marginally reduced the growth of the two remaining cell lines (DU145, HCT8). Selective toxicity of ketoconazole against malignant rather than normal cells might prove to be of benefit in humans. Further studies need to be performed to evaluate the exact mechanisms of action and the potential role of ketoconazole in the treatment of malignancies.

References

1. Beggs WH, Andrews FA, Sarosi GA (1981) Action of imidazole-containing antifungal drugs. Life Sci 28: 111–118
2. Buttke TM, Chapman SW (1983) Inhibition by ketoconazole of mitogen-induced DNA synthesis and cholesterol biosynthesis in lymphocytes. Antimicrob Agents Chemother 24: 478–485
3. Castillo C (1985) Ketoconazole in the treatment of advanced cancer ot the prostate: experience with 14 patients. Rev Med Chil 113: 309–311
4. Contreras P, Auristella R (1985) Regression of metastatic adrenal carcinoma during palliative ketoconazole treatment. Lancet II: 151–152
5. Damon LE, Rochlitz CF (1988) Sequence-dependent cytotoxicity between VP16 and fluoropyrimidines (in press)
6. De Coster R, Beerens D, Dom J, Willemsen G (1984) Endocrinological effects of single daily ketoconazole administration in male beagle dogs. Acta Endocrinol (Copenh) 107: 275–281
7. De Felice R, Johnson DC, Galgiani JN (1981) Gynecomastia with ketoconazole. Antimicrob Agents Chemother 19: 1073–1074
8. Dismukes WE (1983) Treatment of systemic mycosis with ketoconazole: Emphasis on toxicity and clinical response in 52 patients. Ann Intern Med 98: 13–20
9. Feldman LD (1986) Ketoconazole for male metastatic breast cancer. Ann Intern Med 104: 123–124
10. Heyns W, Drochmans A, van der Scuerren E, Verhoeven G (1985) Endocrine effects of high-dose ketoconazole therapy in advanced prostatic cancer. Acta Endocrino (Copenh) 110: 276–283
11. Kraemer FB, Pont A (1986) Inhibition of cholesterol synthesis by Ketoconazole. Am J Med 80: 616–622
12. Pont A (1982) Ketoconazole inhibits adrenal steroid synthesis. Clin Res 30: 99A
13. Pont A (1984) High-dose ketoconazole therapy and adrenal and testicular function in humans. Arch Intern Med 144: 2150–2153
14. Pont A, Goldman ES (1985) Ketoconazole-induced increase in estradiol-testosterone ratio. Arch Intern Med 145: 1429–1431
15. Pont A, Williams PL (1982) Ketoconazole blocks adrenal steroid synthesis. Ann Intern Med 97: 370–372
16. Pont A, Williams PL (1982) Ketoconazole blocks testosterone synthesis. Clin Res 30: 32A
17. Pont A, Williams PL, Azhar S, Reiter R, Bochra C, Smith ER, Stevens DA (1982) Ketoconazole blocks testosterone synthesis. Arch Intern Med 142: 2137–2140
18. Restrepo A, Stevens DS, Utz JP (1980) First international symposium on ketoconazole. Ref Infect Dis 2: 519–562
19. Santen RJ, Van den Bossche H, Symoens J, Brugmans J, De Coster R (1983) Site of action of low-dose ketoconazole on androgen biosynthesis in men. J Clin Endocrinol Metab 57: 732–736
20. Schurmeyer T, Nieschlag E (1984) Effect of ketoconazole and other imidazole fungicides on testosterone biosynthesis. Acta Endocrinol (Copenh) 105: 275–280
21. Sheets JL, Mason JI (1984) Ketoconazole: A potent inhibitor of cytochrome P-450-dependant drug metabolism in rat liver. Drug Metab Dispos 12: 603–606
22. Trachtenberg J (1984) Ketoconazole therapy in advanced prostatic cancer. J Urol 132: 61–63
23. Van den Bossche H, Willemsen G, Cools W, Cornelissen F, Lauwers W, Cutsem J (1980) In vitro and in vivo effects of the antimycotic drug ketoconazole on sterol synthesis. Antimicrob Agents Chemother 17: 922–928
24. Van Tyle JH (1984) Ketoconazole: Mechanism of action, spectrum of activity, pharmacokinetics, drug interactions, adverse reactions and therapeutic use. Pharmacotherapy 4: 343–373
25. Williams G (1986) Objective response to ketoconazole therapy in patients with relapsed progressive prostatic cancer. Br J Urol 58: 45–51

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