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Contrasting effects of fluconazole and ketoconazole on phenytoin and testosterone disposition in man

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Nine healthy male subjects received oral fluconazole 400 mg daily, ketoconazole 200 mg twice daily or no treatment for 6 days according to a randomized, cross-over design. A single 250 mg oral dose of phenytoin suspension was administered on day 5 and serum phenytoin concentrations were measured over the following 48 h. Serum testosterone concentrations were measured for 10 h after each dose of phenytoin. Ketoconazole had no significant effect on phenytoin concentrations while the mean AUC(0,48) for phenytoin was significantly higher with fluconazole (195.2 ± 47.8 μg ml^{-1} h) than control (146.3 ± 49.6 μg ml^{-1} h). At 48 h, the serum phenytoin concentration averaged 1.72 μg ml^{-1} under control conditions and 3.99 μg ml^{-1} with fluconazole (132% increase). AUC(0,10) for testosterone was 42% lower than control after ketoconazole administration ($P < 0.05$) but increased by 33% from 55.6 ± 9.4 ng ml^{-1} h (control) to 73.8 ± 12.6 ng ml^{-1} h with fluconazole. AUC(0,10) values for the testosterone precursors androstenedione and 17 α-hydroxyprogesterone were significantly higher in the fluconazole treatment phase as were concentrations of luteinizing hormone. The mechanism and clinical significance of the increase in testosterone concentration caused by fluconazole remains to be determined.

Keywords fluconazole ketoconazole phenytoin testosterone metabolism

Introduction

Fluconazole is a triazole antifungal agent which shares a number of structural and functional features with other imidazole antifungal drugs such as ketoconazole. Since the azole compounds inhibit fungal cytochrome P450 and imidazole drugs (e.g. cimetidine) are known to inhibit mammalian oxidative metabolism, there has been much interest in the potential effects of these drugs as enzyme inhibitors in man. Ketoconazole inhibits methylprednisolone clearance (Glynn *et al.*, 1986) as well as the CYP3A4 isoenzyme involved in cyclosporine metabolism (Back & Tija, 1991; First *et al.*, 1991) while having little effect on the metabolism of prednisolone, antipyrine or theophylline (Brown *et al.*, 1985; Daneshmend *et al.*, 1983; Yamashita *et al.*, 1991). In addition, ketoconazole inhibits a number of oxidative reactions involved in steroid hormone synthesis (Sonino, 1987) and significantly decreases plasma concentrations of testosterone (Pont *et al.*, 1982).

Comparative *in vitro* studies have suggested that fluconazole is significantly less potent than ketoconazole as an inhibitor of oxidative metabolism (Back & Tjia, 1991; Hanger *et al.*, 1988; Houston *et al.*, 1988). However, fluconazole lowered cyclosporine clearance by 55% in patients receiving 200 mg daily, comparable with the magnitude of interaction with ketoconazole at the same dose (Canafax *et al.*, 1991). Fluconazole also appears to be a potent inhibitor of the metabolism of phenytoin (Blum *et al.*, 1991; Lazar & Wilner, 1990).

The effect of ketoconazole on phenytoin disposition has not been reported. Furthermore, a previous study has suggested that fluconazole, in contrast to ketoconazole, may increase rather than decrease plasma testosterone concentrations (Lazar & Wilner, 1990). The purpose of this study was to compare the effects of fluconazole and ketoconazole at similar doses (400 mg daily) on serum phenytoin and testosterone concentrations in healthy male subjects.

Methods

The subjects were nine healthy non-smoking, non-obese, male volunteers between the ages of 22 and 35

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years. Each subject gave written, informed consent to participate in the study which was reviewed and approved by the Wayne State University Institutional Review Board (Detroit, MI).

Volunteers were randomly assigned to receive each of three treatments using a Latin square design with a 2 week washout period between treatments. The treatments consisted of no drug (control); ketoconazole (Nizoral; Janssen) 200 mg orally twice daily (08.00 h and 20.00 h) for 6 days; and fluconazole (Diflucan; Pfizer-Roerig) 400 mg orally once daily (08.00 h) for 6 days. On day 5 of each treatment period, subjects received 250 mg phenytoin suspension (Dilantin; Parke-Davis) orally at 08.00 h. All medications were taken on an empty stomach with approximately 4 ounces of orange juice. Venous blood samples (10 ml) were collected immediately prior to, and at 1, 2, 4, 6, 10, 24, 32 and 48 h after each dose of phenytoin. All volunteers fasted for 10 h prior to and 4 h after phenytoin administration.

Serum samples were assayed for phenytoin using fluorescence polarization immunoassay (TD_x; Abbott Laboratories). The coefficient of variation was less than 3% at 1.25 μg ml^{-1}. Testosterone concentrations were measured during the first 10 h of sample collection using a radioimmunoassay (Coat-A-Count; Diagnostic Products Corporation). Androstenedione, 17 α-hydroxyprogesterone and luteinizing hormone concentrations were also measured (control and fluconazole treatments only) by radioimmunoassay (Coat-A-Count; Diagnostic Products Corporation). The coefficients of variation for these assays were less than 10%.

The areas under the concentration *vs* time curves for phenytoin from zero to 48 h [AUC(0,48)] and for testosterone, androstenedione, 17 α-hydroxyprogesterone and luteinizing hormone from zero to 10 h [AUC(0,10)] were calculated by the linear trapezoidal rule. Phenytoin and testosterone concentrations at each time point as well as phenytoin AUC(0,48) and testosterone AUC(0,10) for each treatment phase were compared using analysis of variance (ANOVA). Tukey's *post-hoc* test was used for multiple comparisons. Androstenedione, 17 α-hydroxyprogesterone and luteinizing hormone AUC(0,10) values for the control and fluconazole treatment phases were compared using a *t*-test for repeated measures. The level of significance was set at $P < 0.05$.

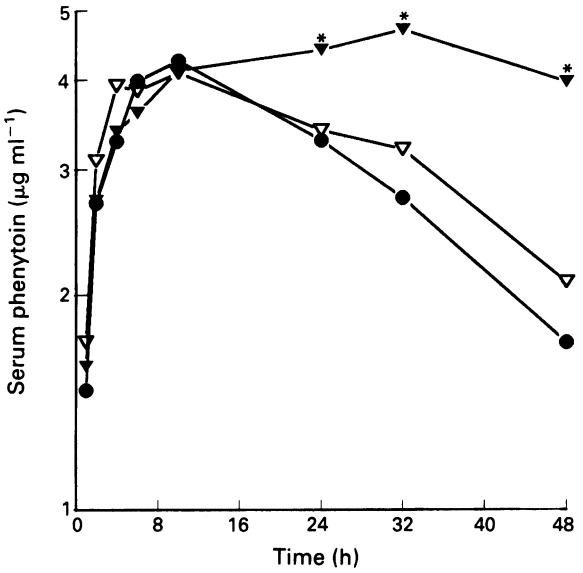

Figure 1 Mean serum phenytoin concentrations following administration of a single oral dose (250 mg) of phenytoin under control conditions (○) and with 400 mg ketoconazole (▽) or fluconazole (▼) daily for 4 days prior to and 2 days following phenytoin administration (* $-P < 0.05$ compared with both the control and ketoconazole treatments).

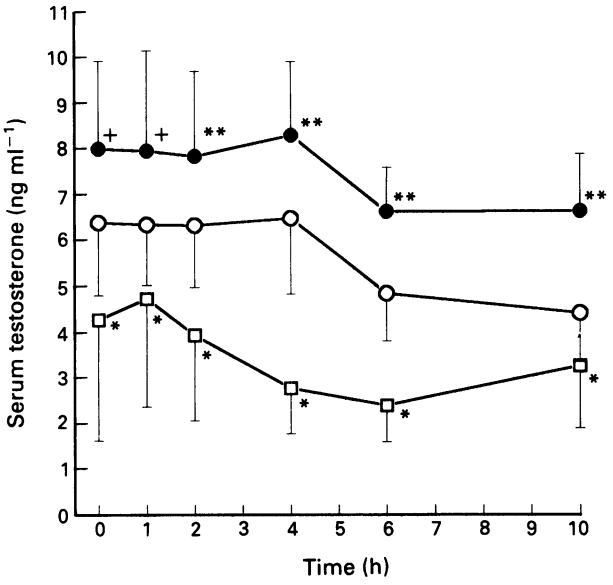

Figure 2 Mean serum testosterone concentrations (± s.d.) under baseline conditions (○) and following treatment with 400 mg ketoconazole (□) or fluconazole (●) daily for 4 days (+$P < 0.05$ *vs* ketoconazole, *$P < 0.05$ *vs* control, **$P < 0.05$ *vs* control and ketoconazole).

Results

Mean serum phenytoin concentrations for all three treatments are shown in Figure 1. Concentrations at 24, 32 and 48 h after phenytoin administration were significantly higher with fluconazole compared with control and ketoconazole treatments ($P < 0.05$). At 48 h, the phenytoin concentration averaged 1.72 μg ml^{-1} under control conditions, 2.09 μg ml^{-1} with ketoconazole and 3.99 μg ml^{-1} with fluconazole (132% increase over control). AUC(0,48) was also significantly elevated with fluconazole and averaged 146.3 ± 49.6 (108.1–184.4) (mean ± s.d. (95% confidence intervals)), 156.1 ± 49.9 (117.7–194.5) and 195.2 ± 47.8 (158.4–232.0) μg ml^{-1} h for the control, ketoconazole and fluconazole treatments.

Mean serum testosterone concentrations are shown in Figure 2. Ketoconazole administration significantly decreased testosterone concentrations at all time points when compared with control ($P < 0.05$). Fluconazole administration significantly increased the mean testosterone concentration at 2, 4, 6 and 10 h after phenytoin administration ($P < 0.05$). AUC(0,10) for testosterone averaged 55.6 ± 9.4 (48.4–62.8) ng ml^{-1} h in the control phase. Administration of ketoconazole resulted in a

42% lower ($P < 0.05$) mean testosterone AUC(0,10) (32.2 ± 13.1 (22.1–42.1) ng ml^{-1} h) while fluconazole produced a 33% increase in AUC(0,10) over control to 73.8 ± 12.6 (64.1–83.5) ng ml^{-1} h ($P < 0.05$). All nine subjects studied exhibited lower testosterone concentrations than control with ketoconazole and higher concentrations following fluconazole. AUC(0,10) was increased with fluconazole treatment (compared with control) for androstenedione (48.2 ± 14.9 (36.7–59.7) to 94.7 ± 25.8 (74.9–114.5) ng ml^{-1} h), 17 α-hydroxyprogesterone (14.8 ± 3.9 (10.1–19.5) to 33.4 ± 8.5 (26.9–39.9) ng ml^{-1} h) and luteinizing hormone (17.0 ± 7.8 (11.0–23.0) to 22.8 ± 9.9 (15.2–30.4) miu ml^{-1} h) ($P < 0.05$ for all three compounds).

Discussion

The results suggest that the effects of ketoconazole and fluconazole on serum phentyoin and testosterone concentrations are strikingly different. Administration of 400 mg/day fluconazole for 6 days produced a 33% increase in the mean phenytoin AUC(0,48). In a recent study (Blum *et al.*, 1991), fluconazole (200 mg/day for 14 days) produced a 128% increase in trough drug concentration after 4 days of phenytoin administration. This is similar to our observation of a 132% increase in the phenytoin concentration at 48 h. It should be noted that concentrations of phenytoin were relatively low in this investigation (generally less than 5 mg l^{-1}). The magnitude of this interaction is likely to be larger (and almost certainly of clinical significance) in patients since phenytoin exhibits saturable metabolism at therapeutic concentrations of 10 to 20 mg l^{-1}. The inhibition of phenytoin metabolism by fluconazole may prove to be a useful tool for studying the isoenzyme involved. It has been suggested that the hydroxylation of phenytoin and the methylhydroxylation of tolbutamide are mediated by the same isoenzyme, a member of the CYP2C gene subfamily (likely CYP2C9 or CYP2C10) (Doecke *et al.*, 1991). This is consistent with the observation that fluconazole also inhibits the elimination of tolbutamide in man (Lazar & Wilner, 1990).

Ketoconazole had no statistically significant effect on serum phenytoin concentration. This appears to confirm the selective nature of the interaction between ketoconazole and the cytochrome P450 system with inhibition primarily of CYP3A enzymes. Although a clinically significant interaction would appear to be unlikely with co-administration of ketoconazole and phenytoin, caution is still advised since the saturable metabolism of phenytoin could result in a larger interaction at therapeutic phenytoin concentrations.

Administration of 200 mg ketoconazole twice daily for 6 days decreased the testosterone AUC(0,10) by an average of 42%. This is consistent with the results of previous studies (Pont *et al.*, 1982). At the same dose of 400 mg daily, fluconazole had the opposite effect increasing testosterone concentrations in all nine subjects studied. Lazar & Wilner (1990) also noted increased testosterone concentrations after 14 days of treatment with fluconazole at doses of 200–400 mg/day but indicated that concentrations remained within the normal range (no quantitative data were presented). Testosterone is synthesized from cholesterol with the immediate precursors being 17 α-hydroxyprogesterone and androstenedione. Increased concentrations of both of these compounds as well as that of luteinizing hormone suggests that testosterone concentrations were increased with fluconazole treatment due to increased biosynthesis. A primary stimulus for luteinizing hormone secretion is the concentration of dihydrotestosterone since testosterone may undergo conversion to dihydrotestosterone by 5 α-reductase before binding to androgen receptors in the hypothalamus (Troen & Hiroyuki, 1981). Inhibition of 5 α-reductase could explain the stimulation of testosterone synthesis of fluconazole since this would result in loss of feedback inhibition of gonadotropin-releasing hormone secretion and increased luteinizing hormone production by the pituitary. This is also consistent with fluconazole altering testosterone synthesis *in vivo* while having no apparent effect *in vitro* using an isolated Leydig cell preparation (Hangar *et al.*, 1988). Unfortunately, the volume of serum collected in this study was insufficient to allow measurement of dihydrotestosterone concentrations. Further studies are needed to clarify the mechanism of increased circulating testosterone concentrations after fluconazole administration as well as the clinical significance of this observation. The identification of fluconazole as an inhibitor of 5 α-reductase could have clinical implications since testosterone and dihydrotestosterone have different physiological effects. Testosterone is involved in male sex organ development, sex drive, muscle mass increase and spermatogenesis while dihydrotestosterone is associated with scalp hair recession, increased facial and body hair as well as prostate enlargement (Metcalf *et al.*, 1989).

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