Serum protein binding of itraconazole and fluconazole in patients with diabetes mellitus

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Abstract

The protein binding of itraconazole and fluconazole in the serum of patients with insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetes mellitus was investigated*in vitro*. The unbound percentage of itraconazole in patients with IDDM and NIDDM wassignificantly higher than that in healthy volunteers. In contrast, there were no significant differences in fluconazole protein binding. A negative correlation was established between itraconazole protein binding and albumin concentration, and a positive correlation with free fatty acid concentration. The existence of a larger percentage of unbound itraconazole in diabetes patients could imply a change in drug disposition and an alteration in the effect of the drug. This should be taken into consideration in long duration treatment, especially in view of the non-linear kinetics of itraconazole.

Introduction

Azole antifungal drugs, such as itraconazole and fluconazole, are effective against a variety of superficial and systemic fungal infections. Although itraconazole and fluconazole have comparable in-vivo activity, they differ in their protein binding, which is approximately 96% for itraconazole and 14% for fluconazole, [1] and elimination routes. [2]

The main aim of antimicrobial chemotherapy is to eradicate pathogenic microorganisms, delivering an optimal amount of free (active) drug to the focus of infected tissues. Numerous factors affect the free concentration of an

antimicrobial drug at the infection site. However, the major determinant controlling the drug gradient between blood and tissue is the extent of binding of the drug to blood components, since it is widely acknowledged that only the unbound drug can pass from serum into the tissues. Changes in serum protein binding could, therefore, alter therapeutic efficacy by affecting drug distribution and clearance. [3,4]

Fungal infections are prevalent in diabetic patients, in whom treatment is often complicated by underlying renal or otherdiseases. On the other hand, the pharmacokinetics and clinical response of a number of drugs have been shown to be significantly altered by diabetes. Protein binding has been suggested as a possible cause of these changes. [5] The purpose of this paper is to study the in-vitro protein binding of two azole antifungal drugs, itraconazole and fluconazole, in serum from patients with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent (NIDDM) diabetes mellitus.

Materials and methods

Patients and healthy subjects

Serum was obtained from 22 stable IDDM patients (14 men and eight women; mean age 30 years), from 14 NIDDM patients (eight men and six women; mean age 54 years), and from 16 healthy volunteers (nine men and seven women; mean age 35 years). Patients had no other diseases. The diabetic medication taken by the NIDDM group included glibenamide, metfornine, acarbose and glipizide.Informed consent was obtained from the Ethics Committee of the Cruces Hospital.

The serum concentrations of albumin, glucose, haemoglobin A_1 (HbA_1), α_1-acid glycoprotein (AAG) and free fatty acids (FFA) were measured by standard methods in the hospital laboratory. After centrifugation of fasting blood samples from each participant, the serum was distributed in 2.5 mL aliquots and immediately frozen at -20°C.

Measurement of drug protein binding in vitro, in serum of patients and healthy volunteers

A solution of [^{3}H]itraconazole (radiochemical purity >98%, specific activity 250 µCi/mM; provided by Janssen Pharmaceutical, Beerse, Belgium) was prepared in (27:50:23) water/methanol/ acetonitrile. An aqueous solution of [^{3}H]fluconazole (radiochemical purity >98%, specific activity 500 µCi/mmol; from Pfizer, Sandwich, UK) was also prepared. Ten microlitres of each solution were added to each sample of

serum (990 µL) to achieve a final therapeutic concentration of 1 mg/L for itraconazole and 2 mg/L for fluconazole. Protein binding in serum was determined by ultrafiltration, as described by Arredondo *et al.*, [1] and the concentration of the unbound drug in the ultrafiltrate (C_U) was measured by scintillation spectrophotometry. The percentage of drug unbound was determined from the following equation:

$$\%\ unbound\ drug = (C_U / C_T) \times 100$$

where C_T is total drug concentration in serum. Additionally, the protein binding of itraconazole and fluconazole in the pooled serum of six healthy subjects and six diabetic patients was determined for a broad range of total drug concentrations in serum, ranging from 0.0625 to 2 mg/L for itraconazole and from 0.125 to 4 mg/L for fluconazole. A plot of the concentration of protein-bound drug (C_B) against the unbound drug (C_U) was used to determine saturable and non-saturable binding of the drugs and to calculate their overall affinity constant (nK_a). [6]

In-vitro protein glycosylation

A solution of [^{14}C]glucose (specific activity 50 mCi/mmol; Amersham plc, Amersham, UK) of 0.2 M was prepared in 3% methanol/water under sterile conditions. Aliquots of 2.7 mL sterile normal serum were incubated at 37°C with 0.3 mL of [^{14}C]glucose for up to 12 days. The incorporation of [^{14}C]glucose into serum protein was determined according to the technique described by Ruiz-Cabello & Erill. [7] Protein binding of itraconazole and fluconazole was studied in the same samples of serum (n= 10), before and after incubation with glucose.

Statistical analysis

The results are expressed as mean ± S.E.M. The statistical analyses included a *t*-like test to compare the unbound percentage and protein levels between healthy subjects and patients. A correlation analysis related the unbound drugs to biological data and $P<$ 0.05 was considered as the limit of statistical significance.

Results and discussion

In this study we quantified the effect of diabetes mellitus on the in-vitro serum protein binding of itraconazole and fluconazole. The results obtained are shown in theTable and indicate that,compared with binding in serum from healthy volunteers, the binding of itraconazole was decreased in sera from IDDM and NIDDM patients, while the binding of fluconazole in IDDM and NIDDM was unchanged. The concentrations of binding proteins and glycosylated haemoglobin are also given.

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Table. Biochemical data and binding characteristics of itraconazole and fluconazole in serum from patients with insulin-dependent diabetes mellitus (IDDM) or non-insulin-dependent diabetes mellitus (NIDDM), and from healthy volunteers. Data are shown as means ± S.E.M.

The alteration of itraconazole binding reported in the present study could be explained on the basis of multiple mechanismsdescribed in the literature concerning binding of other drugs in diabetes. [5] For example, the post-translational change ofalbumin due to a non-enzymatic glycosylation (associated with changes in the affinity constant of drugs) could cause interference in the serum protein binding of drugs. [7] However, in the present study the protein binding of itraconazole and fluconazole was unaffected under similar conditions (% unbound = 3.26% ± 0.006% for itraconazole in serum incubated with glucose, compared with 3.30% ± 0.006% in serum control, and 86.39% ± 0.019% for fluconazole in serum incubated with glucose, compared with 87.59% ± 0.145% in serum control). These results seem to be in accordance with the fact that the global affinity constant for the two groups of patients was not significantly different from that in healthy volunteers (Table).

It is known that FFA concentrations may be elevated in the serum of patients with either IDDM or NIDDM. [5]Additionally, in these patients a linear relationship has been observed between the serum FFA concentration and the free fraction of valproic acid [8] and diazepam. [7] In our study a positive correlation between the unbound percentage of itraconazole and the concentration of FFA was also observed in patients with IDDM (r= 0.52, P< 0.001) and NIDDM (r= 0.65, P< 0.001). This could partially explain the increase in the free percentage of itraconazole found in both groups of patients. However, because of the multiple diseases and treatments of NIDDM cases, it was not possible to find drug-free patients, and these drugs could also contribute to the decrease in the protein binding of itraconazole.

The circulating concentration of albumin and AAG seems to be in the physiological range in diabetic patients and healthyvolunteers. [5] However, the slight variation observed in the albumin concentration could be partially responsible for thedecrease in protein binding of itraconazole observed in diabetic patients (for IDDM, r= -0.47, P< 0.001; for NIDDM,r= -0.62, P< 0.001). Glucose and HbA_1 levels did not play a significant role in the serum binding of itraconazole.

The repercussions of altered serum protein binding on itraconazole kinetics and dynamics depend on its pharmacokinetic profile in humans. The volume of distribution (V_d) of this drug is very large (*c.* 700 L) and its hepatic metabolism shows non-linear kinetics in long duration treatment [2] (an increase in terminal half-life from 20 h to 30 h has been reported). Under these conditions, where the maximum elimination rate is reached, an increase in the unbound itraconazole fraction (47% in IDDM) would produce an increase of the same order in its steady-state unbound concentration. [4] Similarly, and because of the high intrinsic V_d of itraconazole, an increase in unbound fraction implies a further and proportional increasein its distribution, [4] consequently delivering more active drug to the infection site. A relationship between protein bindingand antimicrobial effect in humans has also been observed with other antimicrobial agents such as cefonicid [9] and clindamycin. [10]

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Notes

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References

1 . Arredondo, G., Martinez-Jorda, R., Calvo, R., Aguirre, C. & Suárez, E. (1994). Protein binding of itraconazole and fluconazole in patients with chronic renal failure. *International Journal of Clinical Pharmacology and Therapeutics* **32** , 361–4.[Web of Science][Medline]

2 . Grant, S. M. & Clissold, S. P. (1990). Fluconazole. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial and systemic mucoses. *Drugs* **39**, 877–916.[Web of Science][Medline]

3 . Wise, R. (1986). The clinical relevance of protein binding and tissue concentrations in antimicrobial therapy. *Clinical Pharmacokinetics* **11**, 470–82.[Web of Science][Medline]

4 . Rowland, M. & Tozer, T. N. (1995). *Clinical Pharmacokinetics. Concepts and Applications*, 3rd edn. Williams and Wilkins, London.

5 . Gwilt, P. R., Nahhas, R. R. & Tracewell, W. G. (1991). The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. *Clinical Pharmacokinetics* **20**, 477–90.[Web of Science][Medline]

6 . Wright, J. D., Boudinot, F. D. & Ujhelyi, M. R. (1996). Measurement and analysis of unbound drug concentrations.*Clinical Pharmacokinetics* **30**, 445–62.[Web of Science][Medline]

7 . Ruiz-Cabello, F. & Erill, S. (1984). Abnormal serum protein binding of acidic drugs in diabetes mellitus. *Clinical Pharmacology and Therapeutics* **36**, 691–5.[Web of Science][Medline]

8 . Gatti, G., Crema, F., Attardo-Parrinello, G., Fratino, P., Aguzzi, F. & Perucca, E. (1987). Serum protein binding of phenytoin and valproic acid in insulin-dependent diabetes mellitus. *Therapeutic Drug Monitoring* **9**, 389–91.[Web of Science][Medline]

9 . Dudley, M. N., Shyu, W. C., Nightingale, C. H. & Quintiliani, R. (1986). Effect of saturable serum protein binding on the pharmacokinetics of unbound cefonicid in humans. *Antimicrobial Agents and Chemotherapy* **30**, 565–69.[Abstract/Free Full Text]

10 . Flaherty, J. F., Gatti, G., White, J., Bubp, J., Borin, M. & Gambertoglio, J. G. (1996). Protein binding of clindamycin in sera of patients with AIDS. *Antimicrobial Agents and Chemotherapy* **40**, 1134–8.[Abstract/Free Full Text]

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