Tamoxifen metabolic patterns within a glioma patient population treated with high-dose tamoxifen

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Aims The study was designed to evaluate tamoxifen metabolic profiles in 25 patients (13 M, 12 F) suffering from recurrent high-grade cerebral astrocytomas who were treated with high oral doses of tamoxifen (120 mg/m^2 twice daily).

Methods Tamoxifen was administered for at least 8 weeks; after 4 weeks blood samples were collected 7 h post dose. Tamoxifen and metabolites were analysed by h.p.l.c.

Results Steady-state plasma concentrations (mean μM ± s.d.) were determined for tamoxifen (2.94 ± 3.44), *N*-desmethyltamoxifen (4.37 ± 2.13), *N*-desdimethyltamoxifen (1.49 ± 0.54), 4-hydroxytamoxifen (0.13 ± 0.05) and tamoxifen primary alcohol (1.07 ± 0.46). Male and female patients had comparable metabolic profiles, both qualitatively and quantitatively. The mean plasma tamoxifen concentrations were higher in dexamethasone-treated patients than untreated patients: 3.94 ± 4.35 μM (95% C.I.: 1.43–6.46) *vs* 1.67 ± 0.84 μM (95% C.I.: 1.11–2.24), with *vs* without; while phenytoin-treated patients had lower concentrations: 1.85 ± 0.87 μM (95% C.I.: 1.37–2.34) *vs* 4.58 ± 5.05 μM (95% C.I.: 0.97–8.19), with *vs* without. The differences approached but did not reach statistical significance ($P = 0.065$ and 0.078 respectively).

Conclusions There was marked interpatient variability. The observed effect of dexamethasone on tamoxifen concentrations is consistent with the involvement of CYP3A in metabolism.

Keywords: tamoxifen, metabolism, males, females, blood

Introduction

Tamoxifen is widely used in the treatment and prevention of breast cancer [1]. In addition to being antioestrogenic, tamoxifen inhibits protein kinase C, an effect which appears to be responsible for the inhibition of DNA synthesis and cell proliferation of glioma cell lines *in vitro* [2]. Clinical trials are currently underway to assess the *in vivo* efficacy of 120 mg/m^2 twice daily of tamoxifen against recurrent high-grade gliomas [3].

Tamoxifen is extensively metabolized in the liver through oxidation and conjugation [4, 5]. At 20 mg day^{-1}, steady-state plasma tamoxifen concentrations are highly variable and even higher fluctuations are observed in metabolite levels [6, 7]. This may be due to different metabolic capacities within the patient population and differences could become more marked after high doses. However, there are limited data concerning tamoxifen metabolism at high doses [8].

Most tamoxifen metabolites possess antioestrogenic actions *in vitro* [9], but their relative contribution to the overall activity or toxicity of the parent compound remains unknown. In humans, the main metabolic pathway is *N*-demethylation via cytochrome P450 (CYP) enzymes into *N*-desmethyltamoxifen (also called metabolite X), with further *N*-demethylation to *N*-desdimethyltamoxifen (or metabolite Z) and side chain deamination to the primary alcohol (or metabolite Y) [10]. Although quantitatively less important, tamoxifen also undergoes a CYP-mediated 4-hydroxylation to 4-hydroxytamoxifen (or metabolite B) [10]. The third metabolic pathway, leading to an *N*-oxide metabolite, is catalyzed by flavin-containing monooxygenases.

Glioma patients are routinely medicated with corticosteroids and antiepileptic drugs, which are known to affect hepatic metabolism. In order to describe tamoxifen metabolic profiles after high doses and to evaluate the influence of concomitant medications, we have measured steady-state plasma concentrations of tamoxifen and of four of its main metabolites in 25 patients suffering from recurrent high-grade gliomas.

Methods

Clinical protocol

The protocol was approved by the Jewish General Hospital Ethics Committee and all patients signed an informed consent prior to entry. Twenty-five patients (13 males and 12 females) with histopathologically-proven recurrent high-grade (III/IV) cerebral astrocytomas and who met the inclusion criteria were enrolled into the study [3]. Patients

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were 48 ± 11 years old and of average height (165 ± 9 cm) and weight (70 ± 12 kg; 1.78 ± 0.16 m^2). Patients were not receiving chemotherapy at the time of the study and did not suffer from any other malignancy. Concomitant medications were kept constant for a week prior to study entry and any changes during the study were recorded.

Patients did not present with renal or hepatic dysfunction, as assessed by liver enzymes and the determination of creatinine clearance. Tamoxifen was administered orally twice daily at 120 mg/m^2, for at least 8 weeks and for as long as there was evidence of stable disease or of a response to therapy. After 4 weeks of treatment, blood samples were collected into heparinized tubes, approximately 7 h post-dose.

Tamoxifen and its metabolites were analyzed by a previously described h.p.l.c. method [11]. Differences in plasma concentrations were compared with a two-way analysis of variance (ANOVA) using the analyte levels as the dependent variable and dexramethasone as grouping variables. The level of statistical significance was fixed at 0.05.

Results and discussion

Tamoxifen is a lipophilic drug with a large volume of distribution and a long elimination half-life of approximately 7 days [12, 13]. Plasma concentrations were measured after 4 weeks of daily tamoxifen treatment to ensure that steady-state had been achieved. At steady-state, tamoxifen concentrations are not expected to vary extensively within the same patient. In 19 out of 25 patients, an additional blood sample was withdrawn within two weeks to assess intrapatient variations in drug and metabolite concentrations. For tamoxifen, the second measurement averaged 104% of that at week 4. For *N*-desmethyltamoxifen, 4-hydroxytamoxifen, *N*-desdimethyltamoxifen and the primary alcohol, the second measurements averaged 104, 109, 111 and 108% of the week 4 determination, respectively. To ensure maximum uniformity of the results, only the 4 week-sample was selected for analysis.

In contrast to the intra-patient data, the concentrations of tamoxifen and its metabolites showed high inter-patient variability; for example, tamoxifen concentrations varied 60 fold. The results are presented in Figure 1 where tamoxifen concentrations are the ranking factor and the patients are presented in ascending order relative to their values. The interpatient coefficients of variation clearly exceeded those expected from the analytical method (always lower than 5%), being 117% for tamoxifen and 49, 36, 43 and 35% for *N*-desmethyltamoxifen, *N*-desdimethyltamoxifen, 4-hydroxytamoxifen and tamoxifen primary alcohol, respectively.

Apart from early single-dose pharmacokinetic studies in healthy volunteers where only the parent and *N*-desmethyltamoxifen were measured [14], this report is the first one comparing *in vivo* tamoxifen metabolism between males and females. In female patients, tamoxifen concentrations (mean ± s.d.) averaged 3.36 ± 4.71 μM compared with 2.56 ± 1.75 μM in males (mean of all patients 2.94 ± 3.44 μM), (Figure 1a). The sex of the patient did not influence the plasma concentrations (in μM) of the metabolites measured in this study: *N*-desmethyltamoxifen 4.22 ± 2.15 (female) *vs* 4.52 ± 2.19 (male), (Figure 1b); *N*-desdimethyltamoxifen 1.39 ± 0.52 (female) *vs* 1.59 ± 0.57 (male), (Figure 1c); tamoxifen primary alcohol 0.92 ± 0.39 (female) *vs* 1.21 ± 0.50 (male), (Figure 1c); 4-hydroxytamoxifen levels were 0.12 ± 0.05 (female) *vs* 0.15 ± 0.04 (male), (Figure 1e). The *in vivo* data are in agreement with *in vitro* studies using characterized human microsomes where no difference in *N*-demethylation was detected according to the gender or the age of the liver donors [15].

In all patients but one (Patient 25), *N*-desmethyltamoxifen was the major circulating metabolite, plasma concentrations being 150 to 300% that of tamoxifen. This is in agreement with previous data reported at lower doses of 20 to 80 mg daily where *N*-desmethyltamoxifen plasma concentrations were 50–200% that of tamoxifen [6, 12, 16–18]. For all patients, the plasma concentrations of 4-hydroxytamoxifen were the lowest of all four metabolites, always accounting for less than 15% of those of tamoxifen. This is also in agreement with lower dose data where plasma concentrations of 4-hydroxytamoxifen accounted for only 2–3% of parent drug concentrations [6, 16, 19, 20].

Stuart *et al.* [8] measured tamoxifen, *N*-desmethyltamoxifen and *N*-desdimethyltamoxifen levels in 10 patients following three daily doses of tamoxifen at 480 or 720 mg. Although our tamoxifen concentrations are in agreement with the values found in the previous study (3.2 to 3.5 μM), our metabolite concentrations were higher, most probably because we measured plasma levels after 4 weeks of dosing. The previous study [8] measured serum concentrations between 1 and 4 h post-dose, while absorption and distribution processes were still ongoing. Thus, these sampling times were likely to result in significantly different serum concentrations compared with those at steady-state, regardless of the metabolizing abilities of the patients.

There was no clear outlier with respect to either 4-hydroxytamoxifen, *N*-desmethyltamoxifen or *N*-desdimethyltamoxifen concentrations. However, patient 24 had much higher concentrations of *N*-desmethyltamoxifen and patient 25 had extremely high concentrations of tamoxifen, (Figure 1a). By excluding patient 25, mean tamoxifen concentrations fell to 3.33 ± 2.12 μM while interpatient variability was reduced to 66%. With the exception of desmethyltamoxifen, the metabolite concentrations for patient 25 were at the lower end of the range of the patient group as a whole (Figures 1b–e).

Out of the 25 patients, 14 were on oral dexamethasone (8–24 mg daily, mean ± s.d. 14 ± 4 mg), 15 were on oral phenytoin (200–600 mg daily, mean ± s.d. 327 ± 86 mg), while 8 were on both drugs. Dexamethasone-treated patients had the highest levels of tamoxifen. Overall, mean concentrations were higher when patients were treated with dexamethasone: 3.94 ± 4.35 μM (95% C.I.: 1.43–6.46) *vs* 1.67 ± 0.84 μM (95% C.I.: 1.11–2.24), with *vs* without dexamethasone. However, the difference did not reach statistical significance ($P = 0.065$) most probably due to low patient numbers and high inter-patient variability.

Since dexamethasone can act as both an inducer and a substrate of the CYP3A family [21], the reduction in tamoxifen metabolism could result from competitive inhibition. This *in vivo* finding is consistent with results from *in vitro* experiments using human microsomes, where tamoxifen

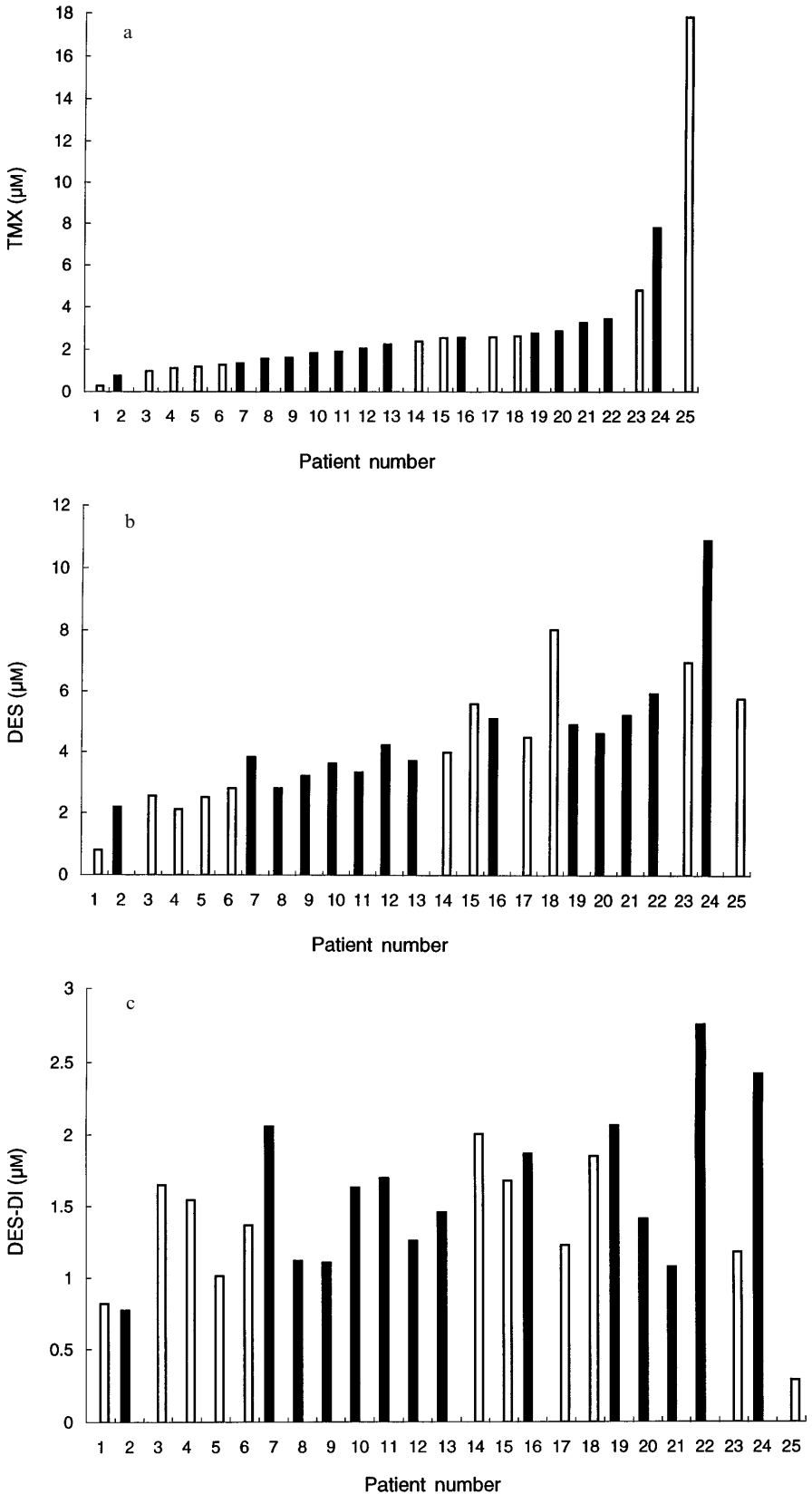

Figure 1
The plasma concentrations of tamoxifen and its major metabolites in 25 glioma patients following 4 weeks of tamoxifen at 120 mg/m^2 twice daily. In each instance, the patients are sorted in ascending order relative to their tamoxifen concentrations. Where: a = tamoxifen (TMX) concentrations; b = *N*-desmethyltamoxifen (DES) concentrations; c = *N*-desdimethyl-tamoxifen (DES-DI) concentrations; d = tamoxifen alcohol (TMX-OL) concentrations; e = 4-hydroxytamoxifen (4-OH) concentrations; and where: ■ = male patients; □ = female patients.

N-demethylation was decreased by incubation with CYP3A substrates such as cortisol or erythromycin [10].

Fifteen patients were receiving phenytoin and these patients had a trend towards lower tamoxifen concentrations than the untreated group: 1.85 ± 0.87 μM (95% C.I.: 1.37–2.34) *vs* 4.58 ± 5.05 μM (95% C.I.: 0.97–8.19), with *vs* without phenytoin. As with the dexamethasone-treated patients, the difference approached but did not reach statistical significance ($P = 0.078$) most likely due to high inter-patient variability and low patient numbers.

Unfortunately, phenytoin is a non-specific inducer with phenobarbitone-like properties and results are difficult to interpret. The agent has been shown to induce cytochromes of the 2B, 2C and 3A subfamilies [21, 24, 25]. In this study, all patients had been on phenytoin for at least 2 weeks, which is an adequate duration for the synthesis of

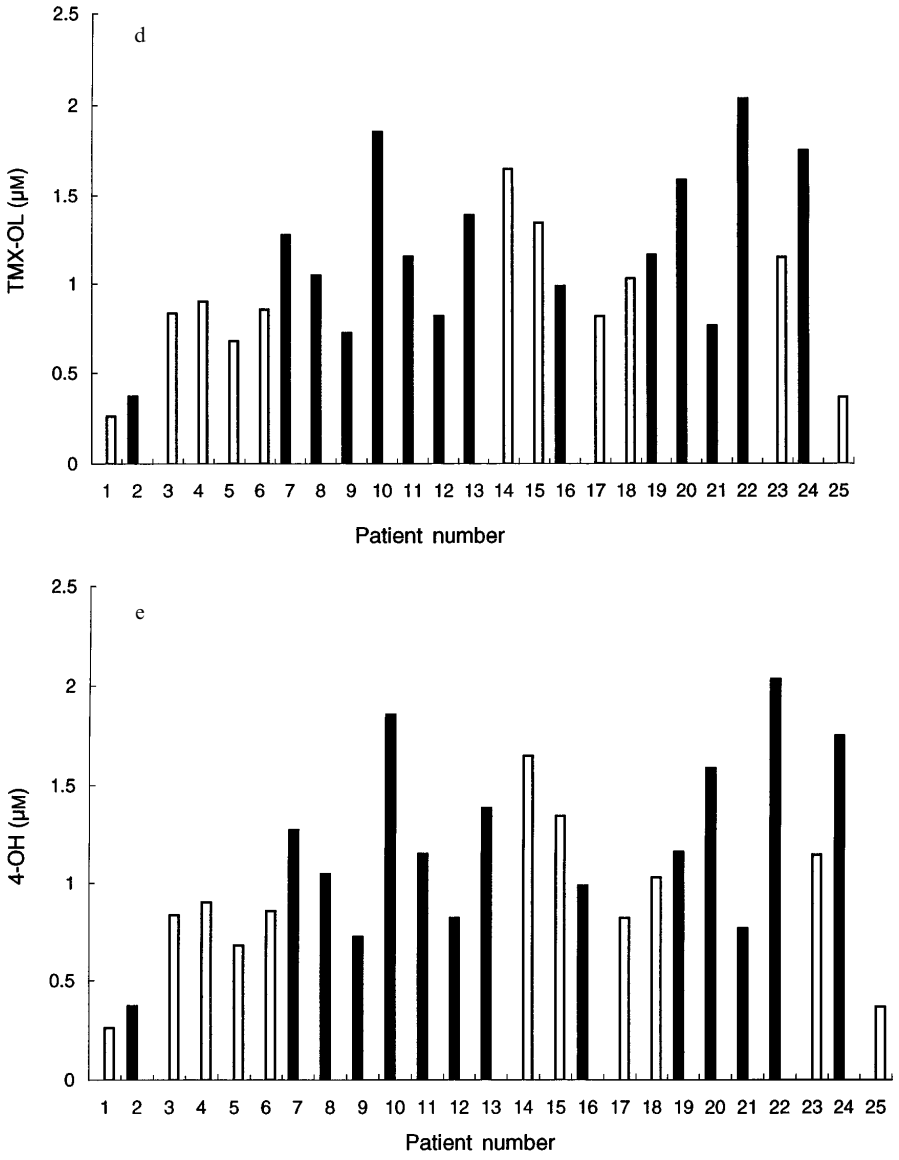

Figure 1 (*continued*)

additional cytochromes and the induction of drug metabolism.

In human microsomes, tamoxifen *N*-demethylation was found to be predominantly mediated by the CYP3A family [10, 15], whereas the isoenzymes responsible for its 4-hydroxylation have not been definitely identified and are thought to be constitutive CYPs [10]. The observed effect of dexamethasone on tamoxifen concentrations is consistent with the involvement of CYP3A in the metabolism of this agent. ANOVA analysis of tamoxifen concentrations between dexamethasone and/or phenytoin treated patients did not detect an interaction between dexamethasone and phenytoin ($P=0.200$). This may arise from the fact that the two drugs affect different CYP isoenzymes; dexamethasone selectively affecting CYP3As and the effect of phenytoin arising from induction of other cytochromes such as CYP2B and CYP2C. Indeed, in rats, tamoxifen was also demethylated by CYP1A and CYP2C isoforms in addition to CYP3A.

The results presented in this report were obtained following high doses of tamoxifen. It should be noted that the metabolism of tamoxifen might be different at lower doses, where higher affinity lower capacity enzymes are likely to play a more important role. If this is indeed the case, it may form the basis for the previously reported non-linearity in tamoxifen pharmacokinetics following high doses [8].

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