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# Evaluation of toremifene for reversal of multidrug resistance in renal cell cancer patients treated with vinblastine

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**Abstract** *Purpose*: Expression of P-glycoprotein (Pgp), which confers the multidrug resistance (MDR) phenotype, is thought to contribute to the insensitivity of renal cell cancer (RCC) to chemotherapy. The development of Pgp inhibitors for clinical application has been hampered by unacceptable toxicity at doses required to achieve adequate cellular concentration. Toremifene is able to reverse MDR and sensitise RCC to vinblastine in vitro. However, in vivo toremifene is tightly bound to serum proteins, in particular the acute phase protein $\alpha_1$-acid glycoprotein (AAG), which may limit tissue availability. In this phase I–II study we assessed the tolerability of short courses of high dose toremifene in combination with vinblastine and evaluated the key determinants of MDR reversal in vivo. *Methods*: Twenty-seven patients with metastatic RCC received escalating doses of oral toremifene for 3 days every 2 weeks in combination with vinblastine 6 mg/m$^2$ i.v. on day 3 of each cycle. The serum concentration of toremifene, its metabolites and AAG were measured and the effect of patients' serum on inhibition of Pgp in vitro was determined. *Results*: Twenty-six patients were evaluable for response. Eight patients (31%) had stable disease and 18 patients (69%) progressive disease. The mean serum concentration of toremifene at 780 mg daily for 3 days was 7.82 $\mu M$ [standard deviation (SD) 2.48, range 2.50 to 14.70], which exceeds that known to reverse MDR in vitro. The serum concentration of the major metabolite of toremifene, *N*-demethyltoremifene, which also reverses MDR, was 5.13 $\mu M$ (SD 1.78, range 1.80 to 9.00). In 60% of patients the pre-treatment AAG concentration was above that known to block the effects of toremifene in vitro. However, addition of serum from patients on toremifene to MCF-7 adr cells in vitro inhibited Pgp-mediated efflux of rhodamine 123. *Conclusions*: We have shown that short course, high-dose toremifene in combination with vinblastine is generally well tolerated and that the concentration of toremifene required to reverse MDR in vitro is achievable in vivo.

**Key words** Multidrug resistance · P-glycoprotein · Toremifene

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## Introduction

The incidence of renal cell cancer (RCC) is increasing, with an annual age-adjusted incidence of 6.3 and 3.2 per 100,000 of the male and female populations in the UK [24]. There is no effective systemic therapy for RCC, with 5-year survival less than 10% for patients with metastatic disease.

Endocrine manipulation, including tamoxifen, has been evaluated following an early report of a response rate of 30% for medroxyprogesterone [5]. Subsequent studies indicate the true response rate for hormone therapy is less than 10%. The cytokines interferon-$\alpha$ and interleukin-2 give responses of 10–30% but are associated with significant toxicity [23, 25]. In clinical trials the most active chemotherapeutic agent is considered to be vinblastine, although few studies report response rates above 10% [9, 10]. Resistance to vinblastine is thought to be mediated by the multidrug resistance (MDR) phenotype. The classical form of MDR in mammalian cells is due to increased activity of P-glycoprotein (Pgp) which acts as an efflux pump, reducing intracellular concentration of drug. Pgp is expressed by normal renal epithelial cells and contributes to their secretory capacity.

It is also commonly expressed in RCC and may limit the efficacy of cytotoxic agents, including vinblastine [8].

One approach to improving the management of RCC is to use cytotoxic drugs in combination with agents that inhibit Pgp. Several compounds including verapamil, nifedipine, chloroquine and cyclosporin reverse MDR in vitro by inhibiting Pgp [20]. In order to achieve concentrations of inhibitors sufficient to inhibit Pgp in vivo, these agents have to be given at doses that result in unacceptable toxicity. Toremifene, a triphenylethylene anti-oestrogen, is structurally similar to tamoxifen, differing only in the substitution of a chloroethyl group for the ethylgroup of tamoxifen. Tamoxifen appears to be more potent than toremifene as an MDR-reversing agent in vitro. However, tamoxifen was poorly tolerated in clinical studies at doses required to achieve effective serum concentrations [27].

The pharmacology of toremifene has been extensively studied [1]. Its metabolites are *N*-demethyltoremifene (DMT), *N*,*N*,-didemethyltoremifene (DDMT), deaminohydroxytoremifene (DAHT) and 4-hydroxytoremifene (4OHT) [15]. Cells expressing Pgp in vitro are sensitised to vinblastine by addition of toremifene in a dose-dependent manner with the most abundant metabolite, DMT, demonstrating approximately half the MDR-reversing activity of toremifene [17]. In phase I studies oral toremifene was well tolerated at doses of up to 400 mg/m$^2$/day [12, 13, 28], with gastrointestinal upset, hot flushes and vertigo being the most common adverse reactions. Over 99% of toremifene is bound to serum proteins, particularly albumin (92%) and the acute phase protein, $\alpha_1$-acid glycoprotein (AAG, 2%) [26]. In vitro addition of serum to cell culture media reduces the accumulation of toremifene in breast cancer cell lines, suggesting that much higher concentrations are required in vivo to reverse MDR [31]. AAG is frequently elevated in patients with malignant diseases, including RCC [19] and may limit the availability of toremifene to inhibit Pgp [6].

One previous study has assessed toremifene tissue distribution in patients with lung cancer. A 7-day course of toremifene was well tolerated at a dose of 480 mg/day with samples showing accumulation of toremifene in both normal lung and tumour tissues at a concentration greater than that predicted to reverse MDR [21]. Based on these experimental and clinical data, we evaluated the pharmacodynamics and tolerability of short courses of high-dose toremifene as an MDR-reversing agent in RCC, determined serum concentrations of parent drug and its active metabolites and assessed whether protein binding of toremifene limits its MDR reversing ability in vitro.

## Patients and Methods

### Patients

Twenty-seven consecutive eligible patients with histologically proven and radiologically measurable RCC were enrolled in this study. Eligibility criteria included age between 18 and 75 years, life expectancy greater than 2 months, WHO performance status 0 to 2, white blood cell count $>3.0 \times 10^9$/l, platelets $>100 \times 10^9$/l, haemoglobin $>10$ g/dl and AST and ALP less than twice the upper limit of normal (unless due to metastatic disease). Subjects gave written informed consent to take part in the study, which was conducted in accordance with the Declaration of Helsinki and approved by the Central Oxford Research Ethics Committee. Patient characteristics are summarised in Table 1.

### Treatment

Toremifene was given once daily by the oral route on days 1–3 of each 14-day cycle. Vinblastine (6 mg/m$^2$ i.v.) was administered 4 h after toremifene on day 3 of each cycle. Treatment was delayed by 1 week if either the peripheral blood leucocyte or platelet count was less than $2 \times 10^9$/l and $50 \times 10^9$/l, respectively. Patients were withdrawn from the study if a treatment delay of longer than 1 week was required. No reductions were made in vinblastine dose. The maximum tolerated dose of toremifene was determined during phase I. Nine patients received an escalating dose (cycle 1: 420 mg; cycle 2: 600 mg; cycle 3: 780 mg). Five of these patients received at least two cycles of toremifene at the 780-mg dose level. During phase II, 18 patients received 780 mg toremifene at each cycle. Treatment was continued until progressive disease was documented or until the occurrence of WHO grade 3 toxicity, up to a maximum of eight cycles.

### Toxicity/Response evaluation

Haematological indices, renal and hepatic function were assessed on day 3 of each treatment cycle, prior to vinblastine administration. Toxicity was graded according to WHO criteria except for fatigue, anorexia and vertigo, which were graded as absent, mild, moderate or severe, according to symptom diary cards kept by patients. Tumour size was measured radiologically before starting treatment and after the fourth and eighth cycles. Response was evaluated by WHO criteria. Patients withdrawn due to progressive disease after at least one cycle were considered evaluable.

**Table 1** Patients characteristics

| | |
|---|---|
| Number of patients | 27 |
| Number of patients evaluable for response | 26 |
| Median age in years (range) | 53 (30–73) |
| Male/female | 16/11 |
| Performance status (WHO scale) | |
| 0 | 8 |
| 1 | 14 |
| 2 | 5 |
| Prior therapy | |
| Nephrectomy | 15 |
| Medroxyprogesterone | 10 |
| Radiotherapy | 7 |
| Biological modifiers | 3 |
| Sites of disease | |
| Kidney or renal bed | 19 |
| Lung | 17 |
| Bone | 8 |
| Liver | 7 |
| Abdominal lymph nodes | 7 |
| Pleural effusion | 4 |
| Mediastinum | 4 |
| Adrenals | 1 |

### Pharmacokinetic assessment

*Toremifene and its metabolites*

Peripheral venous blood was taken 4 h after toremifene administration on day 3 of each cycle for measurement of serum toremifene and the metabolites DMT, DAHT and DDMT using high-performance liquid chromatography (HPLC). This time point was chosen because this is the time of peak serum concentration ($C_{max}$) for toremifene after oral administration [2]. Samples were centrifuged at 2500 $g \times 10$ min and serum aspirated and frozen at −70 °C until analysis. Toremifene and its metabolites were separated from serum using liquid-liquid extraction methodology, separated on a reversed phase HPLC column and, after post-column UV-activation, quantified by fluorescence detection as described previously [2].

### $\alpha_1$-Acid glycoprotein

Blood was taken on day 3 of each cycle for measurement of $\alpha_1$-acid glycoprotein (AAG). Serum AAG was measured on a Beckman Array Protein Analyser, which is a rate nepholometer for measuring specific proteins by an immunoprecipitation reaction. The increase in light scatter resulting from the antigen-antibody reaction was represented as a peak rate signal, which was then converted to AAG concentration units (g/l) by the analyser. The reference range for AAG in the author's laboratory is 0.33–0.88 g/l.

### Reversal of multidrug resistance in vitro

The ability of toremifene (kindly provided by the Orion Corporation, Turku, Finland) to reverse MDR was assessed using a modification of the method described by Witherspoon [30], where the fluorescent compound rhodamine 123 was used as a substrate for Pgp. MCF-7 wild-type and MCF-7adr cells (which are resistant to doxorubicin because of Pgp over-expression [3]) were grown on cover slips in 6-well plates in RPMI 1640 medium with 10% foetal calf serum in 5% carbon dioxide. When cells had reached approximately 50% confluence the medium was replaced with fresh medium containing 5 μ*M*, 10 μ*M* or 20 μ*M* of toremifene (dissolved in ethanol at a stock concentration of 50 m*M* and stored at −20 °C). Rhodamine 123 (stock solution of 1 mg/ml in ethanol stored at −20 °C) was added at a concentration of 500 ng/ml and cultures incubated at 37 °C for 2 h. Fluorescence of rhodamine 123 in individual cells was detected using confocal microscopy (excitation wavelength 488 nm by a MRC600 Fluorimeter) and results provide a semi-quantitative assessment of MDR. The presence of cells in the MCF-7adr controls was confirmed by exclusion of FITC labelled dextran (average molecular weight 50,700, stored as a stock solution of 10 mg/ml at 4 °C).

The influence of serum on the effectiveness of toremifene in the MDR assay was assessed by repeating the experiment in the presence of 50% human serum. The ability of patient sera to reverse MDR before and after toremifene was evaluated by incubating the cells with 500 ng/ml rhodamine 123 in the presence of 50% serum. There was individual variation between cells in the extent of fluorescence and data was analysed as the mean pixel intensity of all cells per field of view. During development of the assay all samples were assessed in duplicate, although subsequent analysis of patient samples was performed once only.

## Results

One hundred and sixteen treatment cycles were administered with a median of four and a range of one to eight cycles per patient. Two patients completed eight cycles. The reasons for not completing eight cycles of treatment were: progressive disease (18), toxicity (four), patients with stable disease who chose to stop treatment after four cycles (two) and death from cerebrovascular accident, considered to be unrelated to treatment, in a patient with a history of carotid artery disease (one). There were no clinical responses. Twenty-six patients were evaluable for response. Eighteen (69%) had progressive disease and eight (31%) had stable disease for 3 months (six patients), 4 months (one patient) and 12 months (one patient).

### Toxicity

Haematological toxicity is shown in Table 2. One patient experienced pyrexia associated with grade 4 neutropenia but responded to broad spectrum i.v. antibiotic treatment and was withdrawn from further study. No thrombocytopaenia was encountered and treatment delays were not required. Eleven patients developed

**Table 2** Worst toxicity (all cycles)

| | WHO grade | | | | Percentage of patients with Grade 2, 3 or 4 toxicity |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| Nausea/vomiting | 12 | 8 | 0 | 0 | 31 |
| Leucopenia | 6 | 3 | 1 | 1[a] | 16 |
| Mucositis | 6 | 1 | 0 | 0 | 4 |
| Parasthesiae | 5 | 1[a] | 0 | 0 | 4 |
| Pyrexia | 2 | 1[a] | 0 | 0 | 4 |
| Myalgia | 2 | 1[a] | 0 | 0 | 4 |
| Alopecia | 9 | 0 | 0 | 0 | 0 |
| Neutropenia | 3 | 3 | 1 | 1 | 16 |
| | Symptom diary card grade | | | | |
| | Mild (1) | Moderate (2) | Severe (3) | Percentage of patients with Grade 2 or 3 toxicity | |
| Fatigue | 6 | 14 | 1 | 58 | |
| Constipation | 7 | 5 | 0 | 19 | |
| Anorexia | 9 | 4 | 0 | 15 | |
| Vertigo | 6 | 3 | 0 | 12 | |

[a] Cause of withdrawal from study

neutropenia. Seventeen patients had anaemia, nine of whom had anaemia prior to the start of treatment. Fatigue, nausea and vomiting were the commonest non-haematological side-effects and appeared to be dose-dependent or related to chronicity of toremifene therapy. Vertigo occurred in nine out of 26 (35%) patients, of which three experienced moderate symptoms. Following dose reduction of toremifene by one dose level the symptom resolved in all cases. Three patients were withdrawn because of non-haematological adverse events: myalgia and fatigue (one patient), parasthesia (one patient). One patient was withdrawn because he developed a pyrexia considered to be vinblastine-induced because it was temporally related to vinblastine administration and was not associated with neutropenia or infection. Dose delays were necessary in two patients who were undergoing treatment for infection, although the full blood count was normal in both cases.

## Pharmacokinetics

### *Toremifene and N-demethyltoremifene*

Serum concentrations of toremifene and its metabolites at 4 h, following the third day of oral doses, are shown in Fig. 1. Mean toremifene and DMT concentrations at the highest dose level were 7.82 $\mu M$ (SD 2.48, range 2.5 to 14.7 $\mu M$) and 5.13 $\mu M$ (SD 1.78, range 1.8 to 9.0 $\mu M$), respectively. Toremifene and DMT levels were both higher during the second cycle than the first, which may be a result of variable drug absorption. This difference was significant in the case of DMT but not for toremifene ($P = 0.001$ and $P = 0.085$, respectively, unpaired Student's $t$-test) reflecting the longer half-life of DMT (14 days) compared with toremifene (5 days). There was no significant difference between serum concentrations in the second and third cycles either for toremifene or DMT ($P = 0.13$ and $P = 0.50$, respectively, unpaired Student's $t$-test). When paired data for individual patients were considered, toremifene and DMT levels were again higher after the second than the first cycle, although this was only significant for DMT ($P = 0.01$, paired $t$-test). Toremifene serum concentrations were lower after the third cycle compared with the second cycle ($P = 0.006$), while DMT remained unchanged ($P = 0.6$, paired $t$-test).

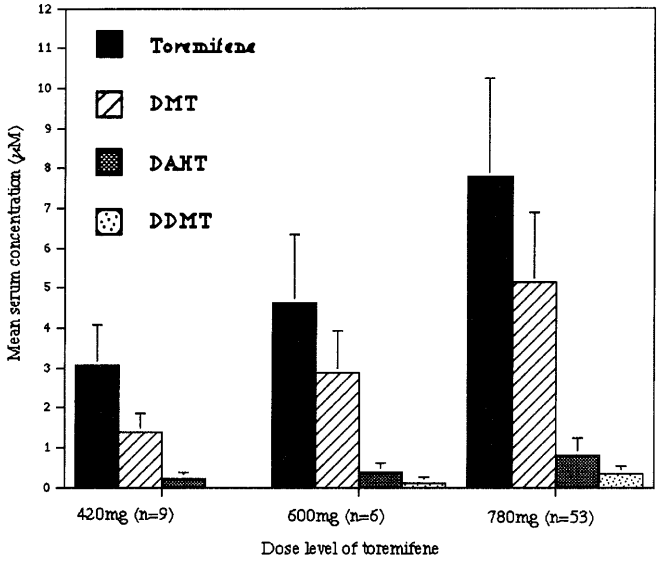


**Fig. 1** Mean serum concentration of toremifene ($\mu M$) and its metabolites at each dose level (420 mg, 600 mg and 780 mg). Samples were taken 4 h after administration of toremifene on day 3 of each cycle. *n* number of samples, *bars* standard deviation (SD)

### $\alpha_1$-Acid glycoprotein

AAG was elevated in 15 out of 19 (79%) patients studied (mean ±SD 1.64 ± 0.65 g/l AAG, range 0.67 to 2.79 g/l). Elevated AAG might be expected to correlate with poor outcome because the higher the AAG the less free toremifene is available for Pgp inhibition. However, there was no correlation of AAG levels in excess of 1.5 g/l (the concentration above which AAG modulates the effect of toremifene on MDR in vitro) with disease status ($\chi^2$, $P = 0.85$). There was a tendency for AAG levels to fall with successive treatments and for patients with stable disease to have a lower mean AAG level at each cycle than those with progressive disease. The difference in serum AAG concentration between patients with stable disease and progressive disease did not reach significance during any of the four initial cycles of treatment. When paired data for individual patients were considered, the fall in AAG between the first and second, and second and third cycles reached significance ($P = 0.0001$ and $P = 0.02$, respectively, paired $t$-test; Fig. 2).

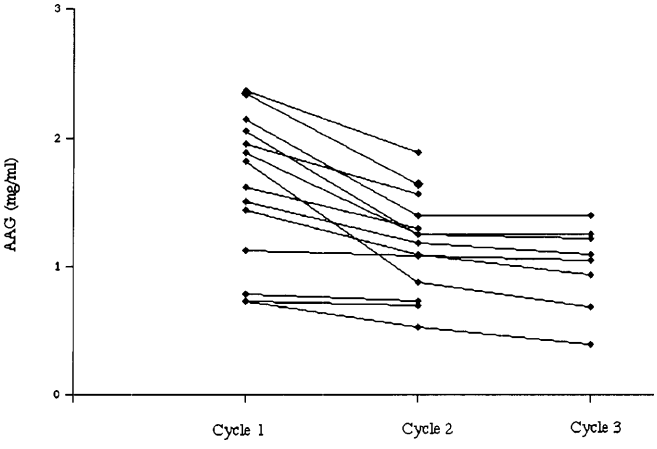


**Fig. 2** Change in AAG concentration in individual patients for cycles 1 to 2 ($n = 14$) and 2 to 3 ($n = 8$). *x-axis* cycle number, *y-axis* AAG (mg/ml). *AAG* $\alpha_1$-acid glycoprotein

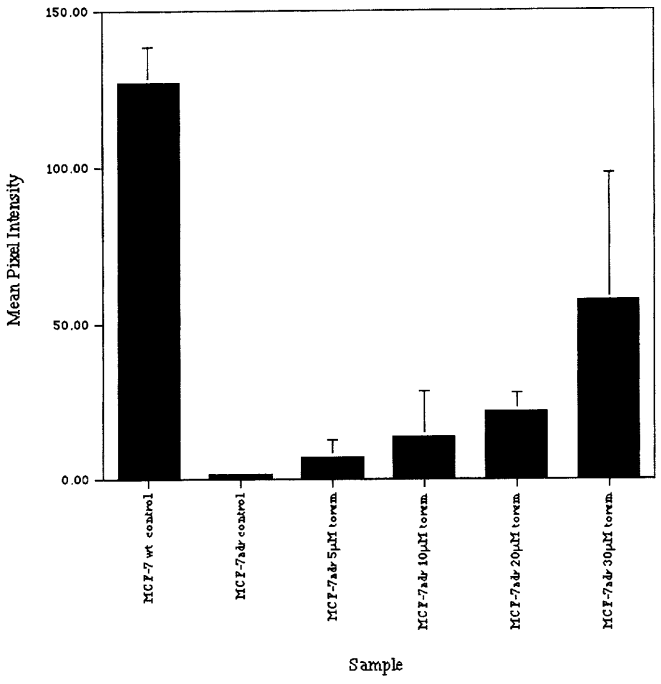


**Fig. 3** Effect of toremifene on rhodamine 123 retention by MCF-7adr cells in vitro. Rhodamine 123 is a substrate for P-glycoprotein (Pgp) and is quantified by measurement of the mean pixel intensity (y-axis) of the cells. A high value indicates that the cell retains rhodamine123 (MCF-7 wild-type), whilst a low value demonstrates that the cell actively excludes the substrate (MCF-7adr). Toremifene (*torem*) was added to cells at four different concentrations (5 μ*M*, 10 μ*M*, 20 μ*M* and 30 μ*M*) and was shown to partially reverse the effect of Pgp in the multidrug-resistant cell line MCF-7adr. *Bars* standard deviation (SD)

### Rhodamine 123 uptake

At concentrations achievable in vivo, toremifene exhibited the ability to inhibit efflux of the Pgp substrate rhodamine 123 from MCF-7adr cells in vitro in a dose-dependent fashion (Fig. 3). We were unable to demonstrate complete reversal of rhodamine 123 efflux which, at a toremifene concentration of 30 μ*M*, increased mean pixel intensity of Adriamycin-resistant cells to approximately 50% of the MCF-7 wild-type value. This may be due to the extremely high Pgp expression in MCF-7adr cells as well as the potential action of other drug resistance pathways not inhibited by toremifene. Addition of normal human serum reversed the toremifene-mediated inhibition of rhodamine 123 efflux. Serum derived from four patients treated with toremifene had a variable effect on rhodamine 123 efflux (Fig. 4). Serial rhodamine 123 uptake analysis performed in two of these patients indicated MDR reversal was maintained for up to eight cycles of toremifene.

## Discussion

Therapeutic options for metastatic RCC are limited. Although vinblastine exhibits cytotoxicity in animal models of RCC [14], response rates of more than 5% are rarely seen in clinical trials of this drug as a single agent or in combination chemotherapy. A possible explanation for the resistance of RCC to chemotherapy is the frequent finding of high expression of Pgp by these tumours. *Mdr-1* RNA was detectable in 80% of untreated RCCs, with 25% of tumours having a level of expression at least as high as in the drug-resistant cell line, KB-8-5 [11]. Toremifene was found to reverse MDR in vitro [16, 22, 29] and therefore this study was carried out to evaluate the tolerability of toremifene in vivo.

Most pharmacokinetic studies of toremifene have used a daily dosage schedule and measured steady-state serum levels. In the phase I study reported by Kohler et al. [18], the steady-state concentrations of toremifene in two patients given 400 mg/day were 3.03 μg/ml (5.07 μ*M*) and 4.37 μg/ml (7.31 μ*M*). In a study of toremifene in advanced breast cancer employing doses of 200, 300 and 400 mg/m$^2$ daily, the serum toremifene $C_{max}$ were 2.79 μg/ml (4.66 μ*M*) and 4.00 μg/ml (6.85 μ*M*) at 200 mg/m$^2$/day and 300 mg/m$^2$/day, respectively. The values for the metabolite, DMT, were 3.95 μg/ml (6.62 μ*M*) and 5.42 μg/ml (9.28 μ*M*), respectively [4]. Prolonged treatment with high-dose toremifene is poorly tolerated with gastrointestinal disturbances, vertigo, ataxia and hypercalcaemia the most common dose-limiting toxicities [4, 18]. In 7-day courses toremifene doses up to 600 mg/day are well tolerated, with serum concentrations of 4.9 μ*M* achieved. Biopsies taken from this patient group demonstrated high levels of toremifene in normal lung (175 μmol/g) and lung tumour (122.7 μmol/g) specimens [21].

In the current study, short courses of high-dose toremifene were used to test whether it was possible to achieve serum toremifene levels sufficient to reverse MDR around the time of administration of vinblastine, without inducing the unacceptable toxicity seen with prolonged toremifene administration. There was a dose-related increment in serum toremifene concentrations over the dose range used. The mean serum toremifene concentrations at the three dose levels were 3.13 μ*M*, 4.55 μ*M* and 7.82 μ*M*, respectively (Fig. 1). Toremifene was well tolerated at the top dose level of 780 mg daily for 3 days, although 35% patients experienced vertigo preventing further dose escalation. The toxicity of short-course toremifene appears to be less severe than when administered as a protracted daily dosing schedule. The combination of toremifene and vinblastine in this study had acceptable toxicity and was easily administered on an outpatient basis with vinblastine doses comparable with those used by others [9].

Only 1% of anti-oestrogens exist in the unbound state in whole blood owing to extensive protein binding, particularly to albumin. Approximately 2% are bound to the acute phase protein, AAG [26]. High serum concentrations have been shown to inhibit accumulation of toremifene in a multidrug resistant MDA-MB-A-1 breast cancer cell line [31]. The intracellular concentration of toremifene fell by 85% when drug was added in

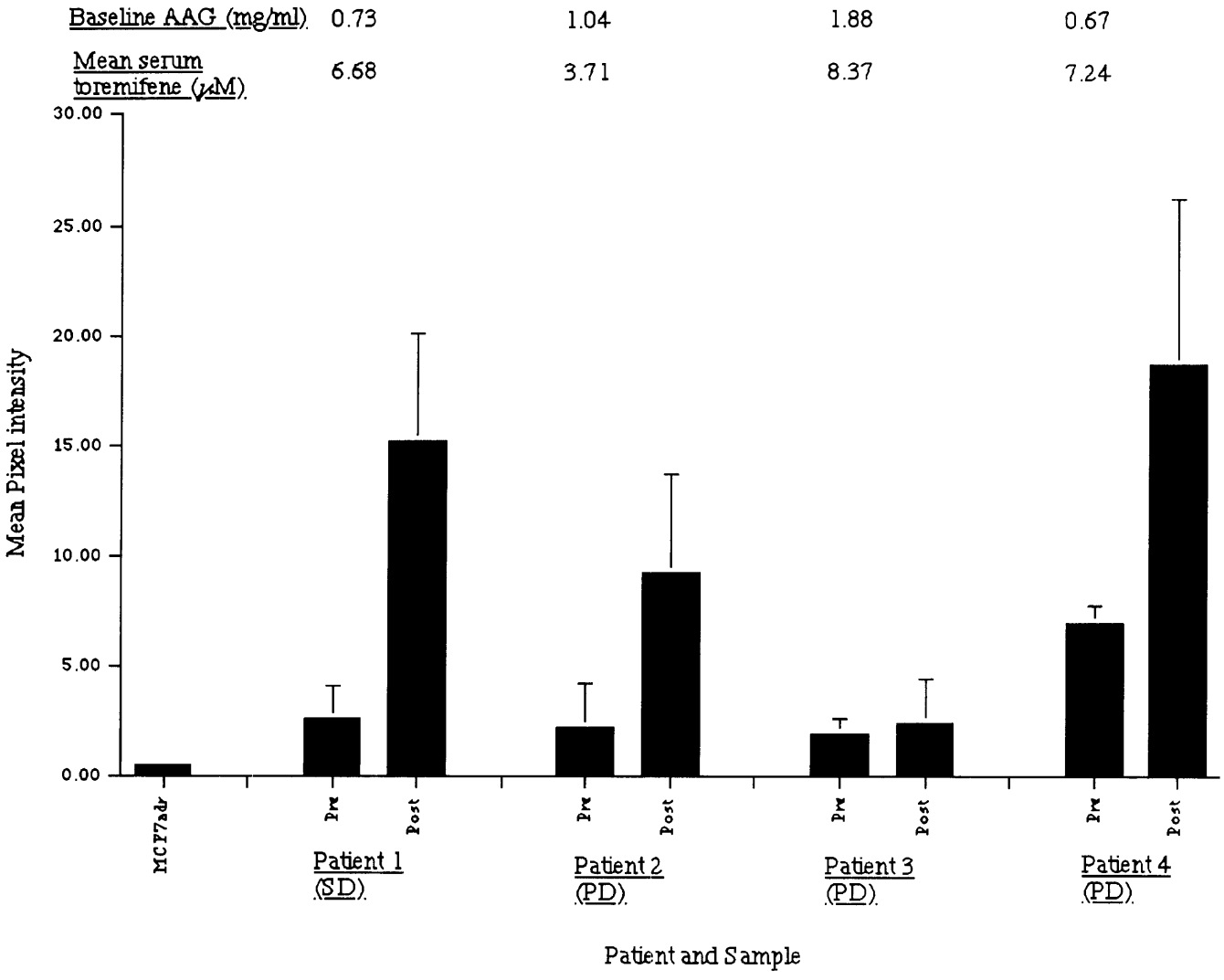


**Fig. 4** Effect of patients' serum, before and after toremifene, on rhodamine 123 retention by MCF-7adr cells in vitro. Rhodamine 123 retention is quantified by measuring the mean pixel intensity (y-*axis*). All four patients received 780 mg toremifene. The post-treatment results are presented as the mean value for all cycles of treatment administered to that patient. The mean serum toremifene concentration (μ*M*), baseline AAG (mg/ml) and patient outcome are shown. *AAG* $\alpha_1$-acid glycoprotein, *bars* standard deviation (SD)

100% serum compared with when it was added in 5% serum. In 5% serum, 2.0 μ*M* toremifene was sufficient to reverse MDR. However, in 100% serum, the effect of high protein binding was overcome by increasing the concentration of toremifene to 10 μ*M*. When AAG is added to growth medium of a multidrug resistant Chinese hamster ovary cell line (CHO-Adr$^r$) enhancement of drug sensitivity by anti-oestrogens is attenuated [6]. At an AAG concentration of 1.5 g/l the chemosensitising effect of toremifene is nearly abolished. These studies suggest that a toremifene concentration of at least 10 μ*M* is required in vivo to overcome the effects of protein binding. The mean concentration of toremifene plus DMT at the highest dose level in the present study was 13 μ*M*. It is likely that toremifene is one of the few drugs used for MDR reversal which is tolerated at doses known to be therapeutic, based on the concentrations required to reverse MDR in vitro. The serum concentrations of toremifene attained in this study were sufficient to reverse MDR in some patients as measured by rhodamine 123 efflux in vitro. Inter-patient variability in MDR reversal may, in part, have been due to differences in serum AAG (Fig. 4). The cause of the fall in AAG levels with successive cycles of treatment is not clear. It is possible that the drug combination affected tumour cytokine production and, whilst not producing clinical responses, indirectly affected acute phase proteins such as AAG.

To assess the role of protein binding of toremifene fully it is necessary to measure free toremifene concentrations. This has not been possible to date because toremifene is adherent to membranes used for separation of free and bound drug during equilibrium analysis. DeGregorio et al. investigated the chemosensitising activity of unbound plasma toremifene. Plasma from patients treated with toremifene sensitised doxorubicin-resistant MCF-7 cells, and the degree of sensitisation correlated with the dose of toremifene taken by the

patient [7]. Adequate serum levels for MDR-reversal following treatment with toremifene therefore appear to be clinically achievable and, despite poor aqueous solubility and extensive protein binding intra-vascularly, previous studies have shown that toremifene accumulates in both normal and tumour lung tissue [21]. Toremifene concentrations in renal cancer cells are not known. It is possible that *mdr-1* is so highly expressed in some renal tumours that inhibitory concentrations cannot be achieved in vivo or that alternative pathways for drug resistance exist.

An effective treatment for advanced RCC has proved elusive as the disease appears to be refractory to conventional cytotoxic chemotherapy. Further understanding of drug resistance pathways may lead to the development of new inhibitors. The response to cytotoxic drugs might be improved by the simultaneous use of two or more MDR modifiers. Combinations of MDR inhibitors with differing mechanisms of action are worthy of further clinical evaluation in order to exploit synergy and reduce toxicity.

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## References


1. Anttila M, Valavaara R, Kivinen S, Maenpaa J (1990) Pharmacokinetics of toremifene. J Steroid Biochem 36: 249
2. Anttila M, Laakso S, Nylanden P, Sotaniemi EA (1995) Pharmacokinetics of the novel antiestrogenic agent toremifene in subjects with altered liver and kidney function. Clin Pharmacol Ther 57: 628
3. Batist G, Tulpule A, Sinha BK, Katki AG, Myers CE, Cowan KH (1986) Overexpression of a novel anionic glutathione transferase in multidrug-resistant human breast cancer cells. J Biol Chem 261: 15544
4. Bishop J, Murray R, Webster L, Pitt P, Stokes K, Fennessy A, Olver I, Leber G (1992) Phase I clinical and pharmacokinetics study of high-dose toremifene in postmenopausal patients with advanced breast cancer. Cancer Chemother Pharmacol 30: 174
5. Bloom HJ (1971) Medroxyprogesterone acetate (Provera) in the treatment of metastatic renal cancer. Br J Cancer 25: 250
6. Chatterjee M, Harris AL (1990) Enhancement of Adriamycin cytotoxicity in a multidrug resistant Chinese hamster ovary (CHO) subline, CHO-Adr, by toremifene and its modulation by alpha-1-acid glycoprotein. Eur J Cancer 26: 432
7. DeGregorio MW, Ford JM, Benz CC, Wiebe VJ (1989) Toremifene: pharmacologic and pharmacokinetic basis of reversing multidrug resistance. J Clin Oncol 7: 1359
8. Fojo AT, Shen DW, Mickley LA, Pastan I, Gottesman MM (1987) Intrinsic drug resistance in human kidney cancer is associated with expression of a human multidrug resistance gene. J Clin Oncol 5: 1922
9. Fossa SD, Droz JP, Pavone-Macaluso MM, Debruyne FJ, Vermeylen K, Sylvester R (1992a) Vinblastine in metastatic renal cell carcinoma: EORTC phase II trial 30882. The EORTC Genitourinary Group. Eur J Cancer 28A: 878
10. Fossa SD, Martinelli G, Otto U, Schneider G, Wander H, Oberling F, Bauer HW, Achtnicht U, Holdener EE (1992b) Recombinant interferon-α2a with or without vinblastine in metastatic renal cell carcinoma: results of a European multicenter phase III study. Ann Oncol 3: 301
11. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazdar A, Pirker R, Green A, Crist W, Brodeur GM, et al (1989) Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 81: 116
12. Hamm JT (1997) Phase I and II studies of toremifene. Oncology 11: 19
13. Hayes DF, Van Zyl JA, Hacking A, Goedhals L, Bezwoda WR, Mailliard JA, Jones SE, Vogel CL, Berris RF, Shemano I, et al (1995) Randomized comparison of tamoxifen and two separate doses of toremifene in postmenopausal patients with metastatic breast cancer. J Clin Oncol 13: 2556
14. Hrushesky WJ, Murphy GP (1974) Evaluation of chemotherapeutic agents in a new murine renal carcinoma model. J Natl Cancer Inst 52: 1117
15. Kangas L (1990) Biochemical and pharmacological effects of toremifene metabolites. Cancer Chemother Pharmacol 27: 8
16. Kirk J, Houlbrook S, Stuart NS, Stratford IJ, Harris AL, Carmichael J (1993a) Differential modulation of doxorubicin toxicity to multidrug and intrinsically drug resistant cell lines by anti-oestrogens and their major metabolites. Br J Cancer 67: 1189
17. Kirk J, Houlbrook S, Stuart NS, Stratford IJ, Harris AL, Carmichael J (1993b) Selective reversal of vinblastine resistance in multidrug-resistant cell lines by tamoxifen, toremifene and their metabolites. Eur J Cancer 29A: 1152
18. Kohler PC, Hamm JT, Wiebe VJ, DeGregorio MW, Shemano I, Tormey DC (1990) Phase I study of the tolerance and pharmacokinetics of toremifene in patients with cancer. Breast Cancer Res Treat 16: S19
19. Kremer JM, Wilting J, Janssen LH (1988) Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacol Rev 40: 1
20. Leyland-Jones B, Dalton W, Fisher GA, Sikic BI (1993) Reversal of multidrug resistance to cancer chemotherapy. Cancer 72: 3484
21. Liippo K, Ellmen J, Vanttinen E, Anttila M (1997) Toremifene concentration and multidrug resistance in lung tumors. Cancer Chemother Pharmacol 39: 212
22. Maenpaa J, Sipila P, Kangas L, Karnani P, Gronroos M (1992) Chemosensitizing effect of an antiestrogen, toremifene, on ovarian cancer. Gynecol Oncol 46: 292
23. Medical Research Council Renal Cancer Collaborators (1999) Interferon-alpha and survival in metastatic renal carcinoma: early results of a randomised controlled trial. Lancet 353: 14
24. Parkin DM, Muir CS, Whelan SL, Gao YT, Ferlay F, Powell J (eds) (1992) Cancer incidence in five continents, vol. 6. IARC Scientific Publication No. 120. IARC, Lyon
25. Rosenberg SA, Lotze MT, Yang JC, Topalian SL, Chang AE, Schwartzentruber DJ, Aebersold P, Leitman S, Linehan WM, Seipp CA, et al (1993) Prospective randomized trial of high-dose interleukin-2 alone or in conjunction with lymphokine activated killer cells for the treatment of patients with advanced cancer. J Natl Cancer Inst 85: 622
26. Sipila H, Nanto V, Kangas L, Anttila M, Halme T (1988) Binding of toremifene to human serum proteins. Pharmacol Toxicol 63: 62
27. Stuart NS, Philip P, Harris AL, Tonkin K, Houlbrook S, Kirk J, Lien EA, Carmichael J (1992) High-dose tamoxifen as an enhancer of etoposide cytotoxicity. Clinical effects and in vitro assessment in P-glycoprotein expressing cell lines. Br J Cancer 66: 833
28. Wiebe VJ, Benz CC, Shemano I, Cadman TB, DeGregorio MW (1990) Pharmacokinetics of toremifene and its metabolites in patients with advanced breast cancer. Cancer Chemother Pharmacol 25: 247
29. Wiebe V, Koester S, Lindberg M, Emshoff V, Baker J, Wurz G, DeGregorio M (1992) Toremifene and its metabolites enhance doxorubicin accumulation in estrogen receptor negative multidrug resistant human breast cancer cells. Invest New Drugs 10: 63

30. Witherspoon SM, Emerson DL, Kerr BM, Lloyd TL, Dalton WS, Wissel PS (1996) Flow cytometric assay of modulation of P-glycoprotein function in whole blood by the multidrug resistance inhibitor GG918. Clin Cancer Res 2: 7

31. Wurz GT, Emshoff VD, DeGregorio MW, Wiebe VJ (1993) Targeting chemosensitizing doses of toremifene based on protein binding. Cancer Chemother Pharmacol 31: 412