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Absence of hepatic enzyme induction in prostate cancer patients receiving 'Casodex' (bicalutamide)

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The potential for hepatic enzyme induction by bicalutamide ('Casodex') was assessed in an open study in prostate cancer patients. A single, oral dose of antipyrine 1000 mg was given before and after 12 weeks' bicalutamide therapy [once daily 50 mg ($n=7$) or 150 mg ($n=11$)] and its pharmacokinetics and metabolism were determined. Plasma or saliva samples were taken for the measurement of antipyrine concentration. Urine samples were assayed for antipyrine and its three major metabolites. With bicalutamide 50 mg, plasma antipyrine concentrations were maximal between 2 and 4 h after administration, declined in a log-linear manner and were unaffected by bicalutamide therapy; with bicalutamide 150 mg, saliva antipyrine concentrations were maximal between 2 and 4 h, declined in a log-linear manner, and were also unaffected by bicalutamide therapy. Antipyrine half-life was 16.3% shorter after bicalutamide 50 mg ($p<0.05$); a small decrease (13.5%) in half-life after bicalutamide 150 mg was not statistically significant. A small reduction (18.6%, $p<0.05$) in the AUC_∞ for antipyrine was noted after bicalutamide 150 mg. A statistically significant reduction in antipyrine recovery was seen with the lower bicalutamide dose (23.7%, $p<0.05$). The statistically significant changes were small in absolute terms and showed no dose–response relationship. Bicalutamide does not significantly induce the hepatic enzymes responsible for antipyrine metabolism and has no obvious potential for producing clinically significant drug interactions due to enzyme induction.

Keywords: Advanced prostate cancer, antipyrine, bicalutamide, hepatic enzyme induction.

Introduction

Prostate cancer has become one of the most common malignancies in the male population worldwide and in many countries of the Western world it now represents the most frequent, newly diagnosed cancer in men. In the European Community in 1990, around 35 000 deaths were attributed to prostate cancer annually and 95 000 new cases were diagnosed per year.[1] More than 13% of all cancer deaths in the US in 1994 were due to prostate cancer and 200 000 new cases were diagnosed.[2] Between 50 and 60% of men with prostate cancer first present with metastatic disease.[3] Among the current therapeutic approaches to advanced prostate cancer is the use of antiandrogens, either as monotherapy or in combination with surgical or medical castration.[4] Bicalutamide ('Casodex'; 'Casodex' is a trademark, the property of ZENECA Pharmaceuticals Ltd) is a new potent non-steroidal antiandrogen for the treatment of advanced prostate cancer.[5]

It is common for elderly patients to suffer from more than one disease and therefore to require more than one regular medication. The concurrent use of several medications carries with it the risk of drug interactions. It has been suggested that nilutamide may directly inhibit cytochrome P_{450} activity at therapeutic doses and alter the metabolism of co-administered drugs.[6] Since bicalutamide has been shown to induce hepatic microsomal drug metabolizing enzymes in mice, rats and dogs (data on file, ZENECA Pharmaceuticals Ltd), it was important to study its potential for producing enzyme induction when given in therapeutic doses to men with advanced prostate cancer. As the profile of induction in the pre-clinical studies was similar to those produced by phenobarbitone and dexamethasone, antipyrine was considered an appropriate substrate for investigating the effects of bicalutamide on hepatic metabolism.[7] To determine whether bicalutamide changed the hepatic metabolism of antipyrine, the pharmacokinetics and metabolism of this compound were determined before and at steady state during treatment with bicalutamide. This technique is well established as a tool for the investigation of hepatic enzyme induction[8] and is further discussed below.

Materials and methods

Subjects

Twenty-two male patients with advanced prostate cancer, who were to receive treatment with bicalu-

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tamide, gave written informed consent and participated in this two-part study. In one part, 10 patients received bicalutamide 50 mg once daily and, in the second part, 12 patients received 3 × 50 mg once daily; ages ranged from 58 to 87 years (mean 73.1 years) and body weight from 48 to 98 kg (mean 74.6 kg). No patient had a residual post-micturition urine volume exceeding 20% of their normal bladder volume. No patient received concomitant treatment with phenobarbitone or any other barbiturate, or with phenytoin, carbamazepine, rifampicin, cimetidine or omeprazole during the study. The protocol called for all patients to have normal values of biochemical liver function tests (bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase) at entry, and for none to change their consumption of alcohol, caffeine or tobacco significantly during the study period. Approval for the studies was obtained from the relevant ethics committees.

Drugs

The drugs used were bicalutamide (bicalutamide 50 mg tablets for oral administration, ZENECA Pharmaceuticals Ltd, Macclesfield, UK) and antipyrine (water-soluble powder for oral administration, Fluka Chemicals, Gillingham, UK).

Study design

This was an open, non-randomized study in which patients who were already scheduled to participate in clinical trials of bicalutamide for the treatment of advanced prostate cancer agreed to have additional tests performed during 48 h periods of hospitalization. A single dose of antipyrine (1000 mg of powder dissolved in 30 ml of water) was given to patients on two separate occasions: once before and once after 12 weeks of bicalutamide therapy (either 50 or 150 mg once daily). Pharmacokinetic and other parameters of antipyrine were determined to see whether its hepatic metabolism was affected by bicalutamide (see below).

Patients receiving bicalutamide 50 mg had antipyrine assayed in plasma and urine samples. Those receiving bicalutamide 150 mg had antipyrine assayed in saliva and urine samples. Blood and saliva samples (as applicable) were collected both before antipyrine was given, and 2, 4, 6, 8, 10, 12, 24, 30 and 48 h after its administration. Patients were asked to collect and combine urine as follows: in the 50 mg group before antipyrine was given and 0–4, 4–8, 8–12, 12–24, 24–36 and 36–48 h after antipyrine administration; in the 150 mg group 1 h before antipyrine was given and 0–24 and 24–48 h after antipyrine administration.

Blood samples (10 ml) were collected in tubes containing anticoagulant. The blood was mixed thoroughly and centrifuged. The plasma fraction was transferred to plain sample tubes and frozen immediately (−20°C) until assay. Saliva samples (1.5–2.0 ml, stimulated by the patient chewing a piece of polythene) were collected in plain tubes and frozen immediately (−20°C) until assay. The total volume of urine was recorded and 10 ml aliquots were transferred from each timed sample to plain tubes and frozen (−20°C) until assay.

Plasma and saliva samples were used to determine antipyrine concentrations. Urine samples were hydrolysed and assayed for antipyrine and aglycones of its three major metabolites [4-hydroxyantipyrine (OHA), norantipyrine (NorA) and 3-hydroxymethylantipyrine (HMA)].

Analytical techniques

Plasma samples were analyzed for antipyrine using solvent extraction followed by reverse-phase HPLC with UV detection (254 nm). The internal standard added to plasma samples was 4-dimethylaminoantipyrine. Plasma samples (1 ml) were basified with 0.1 M sodium hydroxide, extracted with dichloromethane (7 ml), then rotary mixed for 10 min and centrifuged for 2 min (1000 r.p.m.) to separate the phases. An aliquot of dichloromethane (5 ml) was taken and evaporated to dryness using a stream of oxygen-free nitrogen at room temperature. The dry residue was taken up in a suitable volume of HPLC mobile phase [pH 6.5, 0.05 M phosphate buffer: acetonitrile (far UV grade) 85:15 v/v containing 1 ml/l hexylamine] and an aliquot of this was injected into a 5 μm Hypersil C18 HPLC column. Chromatographic separation was monitored using an UV detector (254 nm).

Plasma antipyrine concentration was calculated based on the peak height ratio of antipyrine to internal standard with reference to a standard calibration curve. Control samples were extracted in parallel with the test samples. Assay performance was monitored by the inclusion of quality control samples (prepared by spiking antipyrine into control human plasma) in each analytical run. The limit of detection of the assay was 0.1 μg/ml.

Saliva samples were analyzed for antipyrine by reverse-phase HPLC with UV detection by a method similar to that described for plasma and with the same limit of detection. In this method, 0.5 ml of saliva was basified with 0.5 ml of 0.1 M sodium

hydroxide and 7 ml of dichloromethane. HPLC was performed using a 5 μm Hypersil C18 column.

Urine samples were analyzed for antipyrine and the aglycones of its major metabolites, NorA, HMA and OHA, following enzyme hydrolysis. Analysis of all four compounds was by reverse-phase HPLC with UV detection. Urine samples (0.5 ml) were incubated overnight with 0.5 ml β-glucuronidase solution, type H-1 (5000 Fishman units), at pH 4.5 in acetate buffer containing 8 mg of sodium metabisulphite. Following incubation, 4-dimethylaminoantipyrine was added to the samples as an internal standard; sodium chloride 400 mg was also added to each sample which was then extracted with dichloromethane : hexane [(4 : 1) v/v, 5 ml] for 10 min. After centrifugation (1000 r.p.m., 2 min), 4 ml of the solvent was taken, blown to dryness using nitrogen, then redissolved in methanol : acetate buffer; an aliquot was injected onto a 5 μm Hypersil C18 HPLC column and eluted with pH 7.2, 0.05 M phosphate buffer : acetonitrile (far UV grade) 90 : 10 v/v containing 1 mg/l OHA. Chromatographic separation was monitored using an UV detector at a wavelength of 254 nm. The concentrations of antipyrine and its three major metabolites were determined by use of a calibrated reference curve as described for plasma (above).

Pharmacokinetic analyses

The plasma elimination half-life ($t_{½}$) was calculated by log-linear regression of the plasma antipyrine concentrations. The area under the plasma concentration–time curve up to 48 h (AUC_t) was calculated by the linear trapezoidal rule. The area under the curve to infinity (AUC_∞) was calculated using the equation:

$$AUC_\infty = \frac{AUC_t + C_t t_{1/2}}{0.693}$$

where C_t is the concentration measured at 48 h and $t_{½}$ is the half-life of the terminal elimination phase. The apparent oral clearance (Cl/F) was calculated as the ratio of the dose to AUC_∞, while the apparent volume of distribution (VD_{SS}/F) was calculated from the product of the oral clearance and the mean residence time (MRT). The MRT was calculated using the equation:

$$MRT = \frac{AUC_\infty}{AUMC_\infty}$$

where $AUMC_\infty$ is the area under the first moment curve of the antipyrine plasma concentration–time curve.

Urine concentrations were multiplied by urine volumes to give recovery of each compound in milligrams. The total recoveries for the metabolites were corrected for molecular weight and expressed as a percentage of administered dose.

Statistical analysis

Paired *t*-tests were used to compare parameters before and after bicalutamide therapy. The significance level for total clearance and half-life of antipyrine was considered to be $p < 0.05$; for secondary endpoints tested *post hoc* (AUC, volume of distribution and urinary antipyrine metabolites), the significance level was considered to be $p < 0.01$.

Results

Four out of the 22 patients did not receive the second antipyrine dose. One patient (150 mg group) was found to have had high baseline liver function test results while the remaining three (50 mg group) did not receive antipyrine for the following reasons: difficulty in obtaining blood samples; unwillingness to continue; and by decision of the investigator. Analyses are therefore based on seven patients in the 50 mg group and 11 patients in the 150 mg group.l

Mean plasma antipyrine concentrations during the 48 h following oral administration of a single antipyrine dose (1000 mg), before and after 12 weeks' treatment with bicalutamide 50 mg once daily, are shown in Figure 1(a). The pharmacokinetic parameters of antipyrine determined from these plasma concentrations are shown in Table 1. Peak plasma antipyrine concentrations occurred within 2 h of administration and were unaffected by bicalutamide 50 mg therapy. The plasma concentration of antipyrine then declined in a log-linear manner. Comparison of data before and after bicalutamide showed values of C_{max}, AUC_∞, Cl/F and VD_{SS}/F to be similar ($p > 0.05$). However, the difference in $t_{½}$ (16.3%), although statistically significant, was small and not clinically relevant; a similar change in $t_{½}$ (13.7%) after 150 mg of bicalutamide was not statistically significant and demonstrated that this effect was not dose-related.

Mean saliva antipyrine concentrations during the 48 h following oral administration of a single dose of antipyrine (1000 mg), before and after 12 weeks' treatment with bicalutamide 150 mg once daily, are shown in Figure 1(b). The pharmacokinetic parameters of antipyrine determined from these saliva

concentrations are shown in Table 1. Peak saliva antipyrine concentrations normally occurred between 2 and 4 h after administration, although in some cases the peak occurred at 6 or 12 h. The saliva concentrations are shown in Table 1. Peak saliva antipyrine concentrations normally occurred between 2 and 4 h after administration, although in some cases the peak occurred at 6 or 12 h. The saliva concentration of antipyrine then declined in a log-linear manner. Comparison of data before and after bicalutamide showed values of C_{max}, $t_{½}$, Cl/F and VD_{SS}/F to be similar ($p > 0.05$). However, the small difference in AUC_{∞} (18.6%), although statistically significant, was not clinically relevant; a similar affect in AUC_{∞} (16.7%) observed after 50 mg of bicalutamide was not statistically significant and, again, demonstrated a lack of dose-relationship.

The mean percentages of antipyrine dose recovered in urine as antipyrine and the aglycones of its metabolites before and after 12 weeks' treatment with bicalutamide are shown in Table 2. In neither dosage group were there statistically significant differences between urinary recovery of antipyrine metabolites before and after bicalutamide therapy. Although recovery of antipyrine itself was reduced to a statistically significant extent after bicalutamide treatment in the 50 mg group, the magnitude of the change was small (23.7%) and, accounting for only 3% of the dose, was clinically unimportant. There was no significant change in antipyrine recovery after bicalutamide 150 mg.

There were no adverse events considered to be serious during the 12 weeks of the present study in either bicalutamide dosage group. Tolerability of bicalutamide was typical for this patient population and consistent with that found during review of the whole bicalutamide safety database.[9]

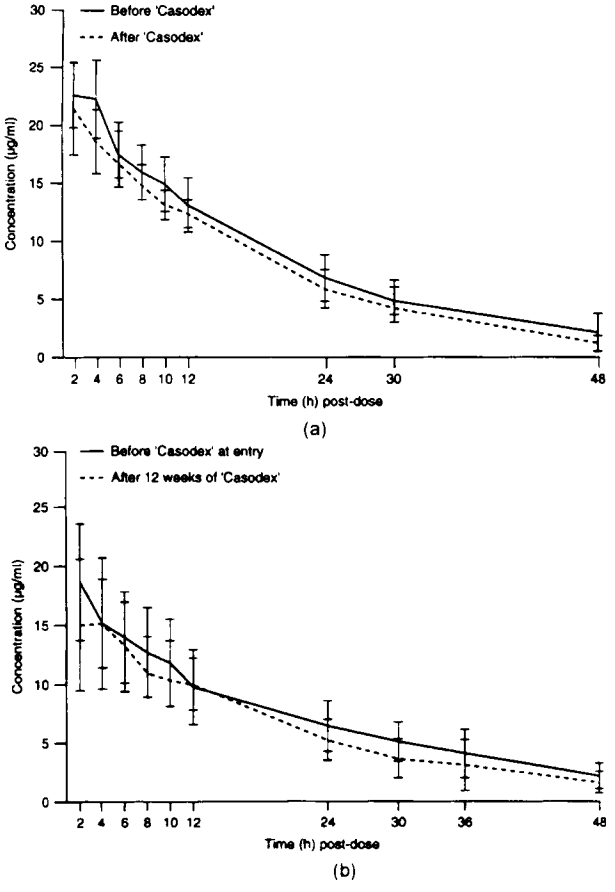

Figure 1. (a) Mean (±SD) plasma antipyrine concentrations before and after bicalutamide 50 mg once daily for 12 weeks. (b) Mean (±SD) saliva antipyrine concentrations before and after bicalutamide 150 mg once daily for 12 weeks.

Discussion

Antipyrine has become the most commonly used probe for studying the effect of drugs on oxidative metabolism; metabolite profiles provide evidence that inducers have differential effects on cytochrome P_{450} enzymes.[8] Antipyrine is cleared almost entirely by hepatic metabolism and production of the three metabolites measured in the present study involves a number of P_{450} enzymes of the mixed function oxidase family, the activity of which can be increased by various types of enzyme inducer.[10–12] For example, total antipyrine clearance is increased on average by 91% after phenytoin and by 61% after carbamazepine, and individual increases correlate well with mean plasma concentrations of the antiepileptic drug.[13]

It is therefore essential to study the metabolites of antipyrine in assessing the possible impact of a drug on its metabolism, as there may be a change in the balance of the metabolites (reflecting selective induction of certain enzymes) without a change in the overall clearance of antipyrine itself.

Previous studies of the saliva : plasma concentration ratio of antipyrine have confirmed that pharmacokinetic parameters of antipyrine can be satisfactorily established on the basis of salivary data;[14] since saliva plasma ratios are routinely below 1, this leads to somewhat higher estimates of volume of distribution and clearance than obtained from plasma antipyrine data. This observation fits well with the data provided by the present study.

In the group that received bicalutamide 150 mg once daily, the decrease in the AUC_{∞} of antipyrine, although statistically significant at the 5% level, was

Table 1. Pharmacokinetic parameters of antipyrine before and after 12 weeks of therapy with bicalutamide 50 mg (plasma samples; seven patients) and bicalutamide 150 mg once daily (saliva samples; 11 patients)

Parameter	Before		After	
	mean	SD	mean	SD
Bicalutamide 50 mg				
C_{max} (μg/ml)	22.6	2.7	21.4	4.0
$t_{½}$ (h)	13.5	5.2	11.3[a]	3.5
AUC_{∞} (μg/h/ml)	468	121	390	67
Cl/F (l/h)	2.25	0.5	2.6	0.5
VD_{SS}/F (l)	42.5	8.0	42.8	5.9
Bicalutamide 150 mg				
C_{max} (μg/ml)	19.2	4.0	16.4	5.4
$t_{½}$ (h)	16.0	4.5	13.8	3.6
AUC_{∞} (μg/h/ml)	411	118	335[a]	99
Cl/F (l/h)	2.7	0.9	3.2	0.8
VD_{SS}/F (l)	60.3	15.3	64.5	17.9

[a] $p < 0.05$.

Table 2. Mean urinary recovery (% of dose) of antipyrine and its metabolites before and after 12 weeks of therapy with bicalutamide 50 mg ($n = 7$) and bicalutamide 150 mg once daily

Parameter	Before		After	
	mean	SD	mean	SD
Bicalutamide 50 mg				
antipyrine	3.8	1.4	2.9[a]	1.2
NorA	11.5	4.2	8.3	1.1
HMA	10.1	4.1	8.9	2.6
OHA	21.1	4.8	22.3	5.4
Bicalutamide 150 mg				
antipyrine	2.3	1.1	2.4	1.3
NorA	10.9	4.1	9.1	2.5
HMA	8.6	3.0	7.7	2.4
OHA	13.2	4.3	18.3	6.1

[a] $p < 0.05$.

not considered to be clinically relevant. This parameter was used in the calculation of clearance, for which the change fell short of statistical significance. The magnitude of the change in both parameters, about 20% of the baseline value, is considerably lower than those produced by the few clinically important enzyme inducers (rifampicin, phenobarbitone, phenytoin, carbamazepine)[7,12,13,15] and unlikely to be of any clinical significance.

The changes in antipyrine pharmacokinetics observed with bicalutamide 150 mg were comparable to those noted after bicalutamide 50 mg. Thus, even a 3-fold increase in daily dose of bicalutamide (resulting in a 2.1-fold increase in steady-state plasma concentration of bicalutamide from 11.0 ± 3.4 μg/ml at 50 mg to 23.3 ± 8.3 μg/ml at 150 mg) was below the threshold required to manifest induction of hepatic mixed function oxidases in man, suggesting that bicalutamide does not have a potential for drug interactions. In over 3500 patient exposures to date, no evidence of hepatic drug interactions was seen with bicalutamide.

The impetus for the present study was the preclinical observation that bicalutamide induced hepatic microsomal drug-metabolizing enzymes in mice, rats and dogs. However, these studies were conducted using toxicological dose levels and it is not unusual to demonstrate the absence of enzyme induction in humans after animal studies had indi-

cated some enzyme-induction potential.[7] The antipyrine test has been used successfully before to demonstrate the absence of enzyme induction in humans after animal studies had suggested possible enzyme induction.[16] There is no clinical evidence to suggest that the other non-steroidal antiandrogens in clinical use can induce hepatic drug-metabolizing enzymes.

There were no serious unexpected adverse events possibly or probably related to bicalutamide in the present study and this reflects the general clinical experience to date. An overview of safety data from over 3000 patients treated with bicalutamide monotherapy in clinical trials showed that the most commonly reported adverse events were those expected with an antiandrogen (gynecomastia, breast tenderness and hot flushes); adverse events were not dose related. There was a low incidence of gastrointestinal events and only 0.3% of patients in the whole clinical trial programme had to be withdrawn because of changes in liver function; there were no clinically significant changes in mean liver function tests.[9]

Conclusions

The present study has demonstrated that bicalutamide therapy (50 or 150 mg daily) did not appreciably alter either the pharmacokinetics of antipyrine or its metabolic fate, indicating that bicalutamide has no significant effect on the hepatic enzyme systems involved in the metabolism of the probe substrate, antipyrine.

Bicalutamide, a potent non-steroidal antiandrogen, does not appear to induce hepatic enzymes significantly and is not considered to have any obvious potential to cause drug interactions related to hepatic enzyme induction.

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References

1. Müller Jensen O, Esteve J, Möller H, *et al.* Cancer in the European Community and its member states. *Eur J Cancer* 1990; **26**: 1167–256.
2. Boring CC, Squires T, Tong T, *et al.* Cancer Statistics 1994. *CA-Cancer J Clin* 1994; **44**: 7–26.
3. Schröder FH. Prostate cancer: to screen or not to screen? *Br Med J* 1993; **306**: 407–8.
4. Denis L. Prostate cancer. Primary hormonal treatment. *Cancer* 1993; **71** (3 suppl): 1050–8.
5. Soloway M, Matzkin H. Antiandrogenic agents as monotherapy in advanced prostatic carcinoma. *Cancer* 1993; **71** (3 suppl): 1083–8.
6. Harris MG, Coleman SG, Faulds D, *et al.* Nilutamide. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in prostate cancer. *Drugs Aging* 1993; **3**: 9–25.
7. Breckenridge A. Enzyme induction in man. Clinical aspects—an overview. In: Sotaniemi EA, Pelkonen RO, eds. *Enzyme induction in man.* London: Taylor and Francis, 1994.
8. Hartleb M. Drugs and the liver part II. The role of the antipyrine test in drug metabolism studies. *Biopharm Drug Dispos* 1991; **12**: 559–70.
9. Tyrrell C. Tolerability and quality of life aspects with the antiandrogen bicalutamide (ICI 176,334) as monotherapy for prostate cancer. *Eur Urol* 1995; in press.
10. Danhof M, Verbeek RMA, van Boxtel CJ, *et al.* Differential effects of enzyme induction on antipyrine metabolite formation. *Br J Clin Pharmacol* 1982; **13**: 379–86.
11. Eichelbaum M, Bertilsson L, Sawe J. Antipyrine metabolism in relation to polymorphic oxidations of sparteine and debrisoquine. *Br J Clin Pharmacol* 1983; **15**: 317–21.
12. Farrell GC, Zaluzny L. Accuracy and clinical utility of simplified tests of antipyrine metabolism. *Br J Clin Pharmacol* 1984; **18**: 559–65.
13. Shaw PN, Houston JB, Rowland M, *et al.* Antipyrine metabolite kinetics in healthy human volunteers during multiple dosing of phenytoin and carbamazepine. *Br J Clin Pharmacol* 1984; **20**: 611–18.
14. Danhof M, van Zuilen A, Boeijinga JK, *et al.* Studies of the different metabolic pathways of antipyrine in man. *Eur J Clin Pharmacol* 1982; **21**: 433–41.
15. Bachmann KA, Jauregui L. Use of single sample clearance estimates of cytochrome P_{450} substrates to characterize human hepatic CYP status *in vivo*. *Xenobiotica* 1993; **23**: 307–15.
16. Allen JG, Alderson J, Galloway DB, *et al.* The effect of midazolam (Hypnovel) administration on antipyrine pharmacokinetics in humans. *Xenobiotica* 1987; **17**: 875–9.

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