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Tenidap sodium does not alter the clearance or plasma protein binding of tolbutamide in healthy male volunteers

K. D. WILNER & M. J. GARDNER
Central Research Division, Pfizer Inc., Eastern Point Road, Groton, CT 06430, USA

1 This randomised, double-blind, parallel group study in 12 healthy young men compared the effects of tenidap sodium 120 mg day^{-1}, at steady state, with placebo on the plasma protein binding and clearance of tolbutamide.
2 Each subject received a 1000 mg intravenous infusion of tolbutamide given over 5 min on day 1 of the study, and again on day 30 following 22 days of successive tenidap or placebo administration.
3 The percentage of unbound tolbutamide in plasma was determined immediately before each infusion. Mean pharmacokinetic parameters (system plasma clearance, terminal phase rate constant, apparent volume of distribution at steady state) of tolbutamide were derived from individual tolbutamide plasma concentration-time curves generated after infusion. The within group day 30 minus day 1 differences were compared between treatment groups.
4 Tenidap was shown to have no statistically or clinically significant effects on any of the parameters assessed. These results indicate that tenidap does not induce or inhibit the P450IIC9 isozyme which metabolises tolbutamide and that tenidap does not displace tolbutamide from plasma protein binding sites.
5 Both tenidap and tolbutamide were well tolerated. No severe treatment-related adverse events were reported, no subject withdrew from the study, and there were no reports of treatment-related laboratory abnormalities, or significant variations in vital signs.

Keywords tenidap sodium tolbutamide pharmacokinetics clearance plasma protein binding

Introduction

Tenidap sodium is a cytokine modulating agent which has demonstrated clinical efficacy in the treatment of rheumatoid arthritis (RA) [1]. Tenidap reaches steady state plasma levels after 11 successive daily doses and is a low clearance, highly protein bound compound [2]. The cytokine modulatory ability of tenidap clearly differentiates it from non-steroidal anti-inflammatory drugs (NSAIDs) [3]; however tenidap also inhibits cyclo-oxygenase [4]. Some of the NSAIDs have been shown to displace drugs, such as warfarin, and methotrexate from plasma protein binding sites [5] and while most NSAIDs do not have clinically significant interactions with oral hypoglycaemic agents, phenylbutazone, azapropazone and aspirin have been found to increase the half-life of oral hypoglycaemics [5, 6].

Tolbutamide is a sulphonylurea hypoglycaemic agent used in the treatment of non-insulin dependent diabetes. It is extensively bound to plasma proteins (mainly albumin) [7], is metabolised by the P450IIC9 isozyme and is hydroxylated by an additional related enzyme, P450IIC8 [8]. Investigation of the effects of co-administration of tenidap and tolbutamide on the pharmacokinetics and plasma protein binding of tolbutamide will not only show the likelihood of a clinically significant interaction between these two agents, but may also indicate the potential for other drugs, which are also metabolised by the P450IIC9 isozyme, to interact with tenidap.

Correspondence: Dr K. D. Wilner, Central Research Division, Pfizer Inc., Eastern Point Road, Groton, CT 06430, USA

Methods

Subjects

Healthy males aged 18–45 years were eligible to take part in this study. Potential subjects were to have a full physical examination and medical history, a 12-lead ECG, and blood and urine specimens taken for routine safety tests. None was to have any evidence or history of allergic, haematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic or neurological disease, any condition that could possibly affect drug absorption, or known drug or alcohol dependence. Subjects were not permitted to take concomitant medication or donate blood or blood components during the study and they were not permitted to receive prescription or over-the-counter drug therapy and recreational drugs for at least 2 weeks, and investigational drugs for at least 4 weeks before the study began. The study was conducted in compliance with institutional review board regulations and all subjects gave written informed consent.

Protocol

This randomised, double-blind, placebo-controlled study was conducted over 30 days. An intravenous infusion of 1000 mg tolbutamide was administered over 5 min on days 1 and 30. From days 9 to 30 subjects were randomised to receive a single, daily, oral dose of either tenidap sodium 120 mg or matching placebo after an 8 h overnight fast. The second tolbutamide infusion was given 2 h after administration of tenidap or placebo. A light snack was permitted in the early morning following the overnight fast 4 h before each infusion. After each infusion subjects were required to refrain from lying down, avoid caffeinated beverages and fast for a further 4 h.

Tolbutamide plasma protein binding

Blood samples sufficient to provide at least 5 ml plasma were collected immediately before each infusion. Plasma was separated from whole blood, and stored frozen until ready for assay. Tolbutamide plasma protein binding was determined, *ex vivo*, by equilibrium dialysis using a Spectrum Equilibrium Dialyzer equipped with Plexi-glass dialysis cells. Plasma samples (1.0 ml), supplemented with 50 μg [^{3}H]-tolbutamide (1.053×10^6 d min^{-1} ml^{-1}), were dialysed against 1.0 ml sodium phosphate buffer (pH 7.4) in a water bath at 37° C for 22 h using a cellulose membrane (Spectrapor 2, 12 000–14 000 molecular weight cut-off). Tolbutamide binding to the dialysis membrane was negligible (< 0.2%). Triplicate aliquots from each chamber were pipetted into 20 ml Eclume scintillation cocktail for liquid scintillation counting. After correction for volume the ratio between the total counts in buffer and total counts in plasma was calculated as a percentage.

Tolbutamide plasma concentrations

Blood samples sufficient to give 2 ml plasma were collected on completion of each infusion, and at 5, 10 and 30 min, and 1, 2, 4, 6, 8, 12, 18, 24 and 32 h thereafter. Plasma samples were frozen at −20° C until being assayed for tolbutamide at ambient temperature using reverse-phase h.p.l.c. u.v. detection at 229 nm (dynamic range 2–200 μg ml^{-1}) [9]. The assay was validated against known concentrations of tolbutamide to show accuracy, precision and linearity of the method. The coefficient of variation was 0.03% from the standard curve. The following instrumentation was used: Walters Model 501 pump with a flow rate of 2.2 ml min^{-1}; Walters WISP 712 Autosampler with an injection volume of 15 μl; Phenomenex Bondex C18 10 μm, 3.9 × 300 mm column with a Walters RCSS C18 insert guard column; a Walters Lambda-Max 481 or equivalent detector, 0.01 AUFS; and a Hewlett-Packard Model 3390A integrator.

The pharmacokinetic parameters of tolbutamide were derived from the individual tolbutamide plasma concentration-time curves. The terminal phase rate (elimination) constant λ_z (h^{-1}), was the negative gradient calculated from least squares linear regression analysis of the plasma concentration-time curve during the log-linear elimination phase. The mean terminal half-life, $t_{1/2}$ (h) was calculated as ln2/mean λ_z. The systemic plasma clearance, CL_p (h^{-1}), was estimated as tolbutamide dose/AUC.

The apparent volume of distribution of tolbutamide at steady state, V_{ss}, was estimated as CL_p[(AUMC/AUC)-T/2], where T is the duration of the infusion. The value of AUC, the total area under the curve, was calculated as C_p/λ_z + AUC(0,t), where C_p/λ_z represents the area under the curve beyond the last measurable concentration (C_p) at time t, and AUC(0,t) represents the area under the curve up to t h after tolbutamide infusion, calculated using the linear trapezoidal method. The value, AUMC, was the area under the concentration × time versus time curve and was estimated in the same manner as AUC, with the terminal area calculated as $(C_p{*}t/\lambda_z) + (C_p/\lambda_z^2)$.

Tenidap plasma concentrations

These were determined in order to monitor compliance. Sufficient blood to provide 3 ml plasma was collected 30 min after the tolbutamide infusion on day 1, immediately before tenidap or placebo administration on days 23 and 30, and 24 h after administration on day 30. Plasma was stored at −15° C until being assayed for tenidap using reverse-phase h.p.l.c. The assay had a dynamic range of 0.5–25 μg ml^{-1}. The following instrumentation was used: a 5 μm Novapak C18, 4.6 × 200 mm column dry packed with 40 μm glass beads, preceded by an in-line guard column (3.9 × 50 mm), and maintained at 30° C; and a LDC/Milton Roy LC300 variable ultraviolet detector operated at 0.02 AUFS and a wavelength of 360 nm. The signal was acquired by a SP-4200 chart recorder in peak height mode operated at an attenuation of 8 mV, a peak width of 21 s and chart speed of 0.5 cm min^{-1}. A Zymark robot injected 50 μl aliquots at regular intervals and the mobile phase consisted of 0.025 N Tris-phosphate buffer and methanol adjusted to pH 6.3 with phosphoric acid. Single level assay calibration

standards were used, control plasma samples containing 10 μg ml^{-1} with tenidap being run after every 40 experimental samples. Intra-assay precision was determined by calculating the drug:internal standard ratio. Relative standard deviations ranged from 14% at 0.5 μg ml^{-1} to 6.6% at 150 μg ml^{-1}. Inter-assay precision was assessed by assaying control plasma samples fortified with tenidap 0.5, 5.0, and 20 μg ml^{-1} at selected intervals. This was reflected by relative standard deviation values ranging from 12.9% at 0.5 μg ml^{-1} to 6.7% at 150 μg ml^{-1}.

Statistical analyses

The effect of tenidap on the pharmacokinetics and plasma protein binding of tolbutamide was determined by comparing between groups the within group differences in means between days 1 and 30. These comparisons were made using the two-sample Student's *t*-test with 95% confidence limits. A power calculation was performed based on the results of a previous study [10]. Data from 12 subjects, six in each group, were sufficient to give statistical power. Sixteen subjects were recruited in case there were drop-outs. The data from the first 12 subjects to enter the study were to be analysed but if one of these dropped out data from a subject with the same randomisation were to be analysed.

Results

A total of 16 healthy males took part in this study, eight of whom were randomised to receive tenidap and eight to receive matching placebo. Baseline demographic characteristics of the two treatment groups were similar (Table 1). One tenidap-treated subject withdrew from the study before the second tolbutamide infusion because of a heparin lock malfunction. This withdrawal did not affect the pharmacokinetic results as only data from the first six subjects in each treatment group to complete the study were analysed.

Mean pharmacokinetic and protein binding data for tolbutamide on days 1 and 30 according to treatment group are shown in Table 2. Between treatment comparison of the day 30–day 1 changes revealed no statistically significant differences between tenidap and placebo for any pharmacokinetic parameter or for the percentage of unbound tolbutamide.

Table 1 Baseline demographic features of healthy male volunteers who received 1000 mg intravenous infusion of tolbutamide concomitantly with either tenidap (120 mg day^{-1}) or matching placebo

	Tenidap group	*Placebo group*
Number of subjects	8	8
Age (years)		
Mean	33.5	26.7
Range	26–41	19–33
Weight (kg)		
Mean	74.6	77.0
Range	69.1–84.1	66.4–85.4
Race		
White	7	5
Black	1	1
Hispanic	0	2

The mean plasma tenidap concentration, in tenidap-treated subjects was 10.4 μg ml^{-1} (range 3.1–14.6) on day 23 (before tenidap dose 15), 7.8 μg ml^{-1} (range 5.6–9.5) on day 30 (before tenidap dose 22 and tolbutamide) and 7.1 μg ml^{-1} (range 4.2–10.0) 24 h after tenidap dose 22 (after tolbutamide infusion).

Adverse events, considered by the investigator to be treatment-related, were reported in three subjects following the initial tolbutamide infusion, in three subjects receiving tenidap and in one subject receiving placebo. These adverse events included two subjects with dizziness and one with syncope in the tolbutamide group, two with dizziness and one with abdominal pain in the tenidap group, and one subject with thrombophlebitis in the placebo group. None of these adverse events was severe, or led to any subject withdrawing from the study. There were no reports of treatment-related abnormal laboratory test results, or significant changes in vital signs.

Discussion

The tolbutamide pharmacokinetic data generated by this study were consistent with those reported elsewhere [11–13]. The results demonstrated that there were no statistically significant or clinically significant differences between the tenidap and placebo groups, in the changes following 22 days continuous concomitant tenidap or placebo administration, in any of the tolbutamide parameters assessed: systemic plasma clearance, the terminal phase rate constant or the apparent volume of distribution of tolbutamide. It also appears that tenidap does not displace tolbutamide from plasma protein binding sites, in contrast with the findings of the phenytoin study where this did occur with a resultant increase of approximately 25% in unbound plasma phenytoin. This indicates that tenidap does not inhibit or induce the P450IIC9 isozyme. This finding is consistent with that of a similar study which found that tenidap did not affect the clearance of phenytoin which is also metabolised by the P450IIC9 isozyme [14].

Tenidap was well tolerated during the 22 days of continuous administration, adverse events only being of mild to moderate severity and not leading to any subject withdrawing from the study. There were no treatment-related laboratory or ECG abnormalities, or significant variations in blood pressure or pulse rate reported.

It may be concluded that this study has shown that, in normal healthy young men, tenidap 120 mg day^{-1} at steady state does not affect the pharmacokinetics or plasma protein binding of tolbutamide given as a single 1000 mg intravenous infusion. There is no evidence to suggest that tolbutamide and tenidap cannot be co-administered.

Table 2 Mean pharmacokinetic parameters of tolbutamide according to treatment group, and differences between groups in the day 30–day 1 means

	Tenidap group (n = 6)			*Placebo group* (n = 6)			*Between group difference*	*95% confidence limits*
Parameter	*Day 1*	*Day 30*	*Day 30–Day 1*	*Day 1*	*Day 30*	*Day 30–Day 1*	*Tenidap–placebo*	
CL_p ($l\ h^{-1}$)	0.74	0.84	0.10	0.79	0.84	0.05	0.06	−0.05,0.15
V_{ss} (l)	8.7	7.9	−0.8	8.4	7.8	−0.6	−0.13	−0.87,0.61
λ_z (h^{-1})	0.085	0.105	0.020	0.093	0.106	0.013	0.007	−0.001,0.015
$t_{1/2}$ (h)	8.2	6.6		7.4	6.5			
Unbound tolbutamide (%)	5.4	5.9	0.5	5.8	5.8	0.02	0.53	−0.95,2.01

All the differences between groups were not significant ($P > 0.05$).

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