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OXAZEPAM PHARMACOKINETICS IN PATIENTS WITH EPILEPSY TREATED LONG-TERM WITH PHENYTOIN ALONE OR IN COMBINATION WITH PHENOBARBITONE

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The pharmacokinetics and serum protein binding of oxazepam, a drug mainly eliminated by a single step glucuronidation reaction, were studied in nine epileptic patients treated long-term with phenytoin or phenytoin with phenobarbitone, and in nine healthy control subjects. Oxazepam elimination half-life was shorter and apparent oral clearance higher in treated patients than in age and sex matched control subjects. Serum bilirubin concentration was lower in treated patients. There was no significant correlation between serum bilirubin concentrations and oxazepam elimination. Serum α_1-acid glycoprotein concentration was higher in the treated patients than in the control group. Oxazepam was more than 93% bound to serum proteins, but the extent of binding was not significantly different between the two groups. These results show that oxazepam glucuronyl transferase activity is increased by treatment with phenytoin alone or in combination with phenobarbitone in epileptic patients.

Keywords oxazepam pharmacokinetics epilepsy enzyme induction

Introduction

Studies on the elimination of drugs and the effects of treatment with concurrent hepatic enzyme inducing agents have concentrated on drugs metabolised by the cytochrome P-450 dependent mixed function oxidase system. Less attention has been devoted to drugs undergoing metabolism by conjugation reactions, for example glucuronidation. Oxazepam and lorazepam are metabolised almost exclusively by a single step glucuronidation reaction (Greenblatt, 1981). Elimination of such drugs has not been investigated in the presence of long-term enzyme induction in man.

Phenobarbitone has been shown to increase serum concentration of α_1-acid glycoprotein (AAG) in dogs (Abramson *et al.*, 1981) and anticonvulsants have been reported to increase concentrations of AAG in man (Routledge *et al.*, 1981). These findings were associated with increased binding to plasma proteins of the basic drugs propranolol and lignocaine. The aim of this study was to determine the pharmacokinetics and serum protein binding of oxazepam in epileptic patients treated long-term with phenytoin or phenobarbitone and in control subjects.

Methods

Patient selection

Nine patients (six male, 23–60 years (mean 37); mean weight 71 kg) on long term therapy for epilepsy and nine age and sex matched healthy controls (21–60 years (mean 35); mean weight 70 kg) were selected for study. All patients with epilepsy had been on treatment with phenytoin alone or in combination with phenobarbitone for at least 3 months. Patients and controls on any other long term therapy were excluded from the study. No drugs other than the treatment for epilepsy were taken in the week before the study. All subjects had normal serum creatinine and aspartate aminotransferase concentrations. Six patients were on treatment with phenytoin alone (dose range 200–450 mg daily; mean dose 308 mg; mean plasma concentration 6 mg l^{-1} (basal sample on day of study)] and three patients were on treatment with phenytoin (dose range 200–400 mg daily; mean dose 267 mg; mean plasma concentration 7 mg l^{-1}) in combination with phenobarbitone (dose range 90–120 mg daily; mean dose 100 mg; mean plasma concentration 12 mg l^{-1}). Five of the drug-treated

patients and six controls admitted moderate alcohol consumption (≤ 10 pints/week), one control drank 10–20 pints/week, four patients and two controls were non-drinkers. Cigarette consumption was admitted by four patients (mean 20 daily) and one control (≤ 5 daily). Ethical committee approval was granted by the Area Committee and written informed consent obtained in all subjects.

Procedure and analytical methods

The volunteers attended between 08.30 and 09.00 h after an overnight fast. An indwelling cannula was inserted into a forearm vein and kept patent with a slow saline infusion. A basal blood sample was withdrawn for measurement of serum bilirubin, γ-glutamyltranspeptidase (γGT), albumin, total protein and AAG concentration and for preparation of an oxazepam standard curve. Oxazepam (15 mg) was administered orally with 50 ml water. Further 10 ml blood samples were collected into lithium heparin tubes after 20 and 40 min and at 1, 2, 3, 4, 6, 8, 12 and 24 h post-dosing. The plasma was separated immediately by centrifugation and stored at −20° C. Oxazepam is stable for at least 6 weeks under these conditions. Serum samples were collected at 1 and 2 h post-dosing for oxazepam protein binding studies.

Samples for oxazepam were analysed in duplicate by the method of Patwardhan *et al.* (1981) but sensitivity was improved to 5 ng ml^{-1} of blood using an Altex MI60 UV detector at 229 nm and connected to a Hewlett Packard 3390 integrator. The absorbance maximum of oxazepam is 230 nm and most previous studies by h.p.l.c. have used a fixed wavelength detector at 254 nm. At this wavelength sensitivity is 50% of that at 229 nm. The coefficient of variation at 20 ng ml^{-1} was 8%.

Protein binding studies were carried out by equilibrium dialysis over 4 h using M.S.E. Dianorm apparatus. Measurement of free oxazepam was carried out by h.p.l.c. because a supply of radiolabelled oxazepam with sufficiently high specific activity could not be obtained. Free oxazepam concentrations were at the lower limit of sensitivity of the assay and could not be measured in all subjects. Coefficient of variation for these measurements was 25%.

Serum α_1-acid glycoprotein concentration was measured by radial immunodiffusion using commercially prepared plates (Behring Institut). Serum bilirubin, γGT, albumin and total protein were measured by the Department of Chemical Pathology.

Oxazepam elimination half-life was calculated using linear regression analysis. Area under the plasma concentration-time curve up to the final detectable plasma concentration was obtained by the trapezoidal rule. To this was added the residual area, calculated as the final plasma concentration divided by the elimination rate constant (β), to give the total AUC. Statistical analysis was by Student's *t*-test for unpaired samples.

Results

The oxazepam kinetic parameters in the treated patients and controls are shown in Table 1. There was no significant difference between the two groups in the peak concentration (C_{max}) or time to peak concentration (t_{max}). The elimination half-life in the treated group was half that of the control group. The apparent oral clearance was almost double the value in the control group. No formulation of intravenous oxazepam was available so that the volume of distribution and total body clearance could not be calculated accurately.

Protein binding of oxazepam was measured in seven subjects in each group. The binding for oxazepam was 96.7 ± 1.8% bound in the drug-treated patients and 93.7 ± 1.9% bound in the controls, but this difference was not statistically significant at the 5% level.

The values in treated patients and controls for serum bilirubin, γGT and proteins are shown in Table 2. Serum bilirubin concentration was lower and γGT higher in the group treated with phenytoin and phenobarbitone. Serum AAG was significantly higher in the treated group. No significant difference

Table 1 Oxazepam pharmacokinetic parameters (mean value ± s.e. mean)—peak plasma concentration (C_{max}), time to reach peak (t_{max}), elimination half-life ($t_{½,z}$) area under plasma concentration time curve extrapolated to infinity (AUC) and apparent oral clearance (CL_o)—in patients treated with phenytoin alone or in combination with phenobarbitone and in age and sex matched controls.

	C_{max} ($ng\ ml^{-1}$)	t_{max} (h)	$t_{½,z}$ (h)	AUC ($ng\ ml^{-1}h$)	CL_o ($ml\ min^{-1}$)
Treated	248 ± 24	1.96 ± 0.30	3.31 ± 0.65	1030 ± 127	243 ± 30
Controls	262 ± 29	2.20 ± 0.30	6.99 ± 0.89	1864 ± 469	134 ± 34
P	NS	NS	≤ 0.01	≤ 0.05	≤ 0.05

Table 2 Serum bilirubin, γ glutamyl transpeptidase (γGT), albumin, α_1-acid glycoprotein (AAG) and total protein concentrations (mean value ± s.e. mean) in patients treated with phenytoin alone or in combination with phenobarbitone and in age and sex matched controls.

	Bilirubin ($\mu mol\ l^{-1}$)	*γGT* ($U\ l^{-1}$)	*Albumin* ($g\ l^{-1}$)	*AAG* ($mg\ l^{-1}$)	*Total protein* ($g\ l^{-1}$)
Treated	6.1 ± 1.0	149.9 ± 19.6	42.1 ± 0.9	832 ± 67	64.7 ± 1.2
Controls	10.9 ± 0.9	16.8 ± 4.8	44.4 ± 1.3	578 ± 26	67.3 ± 1.7
P	≤ 0.01	≤ 0.01	NS	≤ 0.01	NS

in albumin or total protein concentration was found between the two groups.

There was no significant correlation between serum bilirubin concentration and oxazepam half-life ($r = 0.3$) or apparent oral clearance ($r = -0.3$). Similarly no correlation was found between γGT concentration and oxazepam elimination.

Discussion

This study shows that in epileptic patients treated with phenytoin alone or in combination with phenobarbitone the oxazepam elimination half-life is reduced and the clearance increased compared with a matched control group. Oxazepam is a highly bound low clearance drug and as such its elimination depends on intrinsic hepatic enzyme activity (glucuronyl transferase) and on protein binding (Wilkinson & Shand, 1975). The most probable explanation for our findings is that enzyme activity was increased by drug treatment. Both phenytoin and phenobarbitone have previously been shown to induce bilirubin glucuronyl transferase in both animals and man (Black *et al.*, 1973).

Oxazepam and bilirubin are metabolised by glucuronyl transferase. Nevertheless the correlation between serum bilirubin concentration and oxazepam half-life and clearance was poor. This may either reflect the many other factors which determine serum bilirubin concentration, such as rate of erythrocyte destruction, or point to the involvement of different glucronyl transferases in the metabolism of oxazepam and bilirubin. Bilirubin and drug glucuronyl transferases have been shown to respond differently to inducing agents in the rat (Watkins *et al.*, 1982). Whether or not this occurs in man is not known.

There were more smokers in the drug treated group and a greater consumption of alcohol in the control group. However no significant correlation was found between these factors and oxazepam elimination. It is unlikely that these differences had an important effect on the results.

Albumin and AAG are the major drug binding proteins in serum. The drug treated group had higher AAG concentrations than the control group, confirming the findings of Routledge *et al.* (1981). Oxazepam binds to albumin (Muller and Wallert, 1973) but whether or not it binds to AAG has not been studied. The percentage protein binding of oxazepam was higher in the treated group but did not reach significance at the 5% level. The values obtained for oxazepam protein binding were in agreement with those obtained with oxazepam added to serum *in vitro* (Greenblatt *et al.*, 1980). There was no significant correlation between the percentage drug bound and AAG concentration. Investigation of oxazepam binding to individual plasma proteins requires further study using radiolabelled drug with high specific activity.

The confirmation of elevated AAG concentrations in the presence of hepatic enzyme induction also has implications for the concomitant use of basic drugs. Increased binding of such drugs will lead to an increase in systemic clearance of free drug if elimination is nonrestrictive or a reduction in systemic clearance of total drug if elimination is restrictive (Wilkinson & Shand, 1975). In practice after oral administration this is outweighed by the increased intrinsic clearance (Branch *et al.*, 1974; Alvan *et al.*, 1977). Furthermore, if plasma concentrations of such drugs are monitored in clinical practice the reduced free fraction of drug complicates interpretation of total drug concentration.

The low serum bilirubin and high γGT concentration confirm that hepatic enzyme activity in the patients was induced as described previously (Scott *et al.*, 1979; Whitfield *et al.*, 1972). This has occurred even with anticonvulsant drug concentrations below the reported therapeutic range (phenytoin 10–20 mg l^{-1}; phenobarbitone 15–30 mg l^{-1}) in seven of the nine patients. There was no clear relationship between plasma concentration of anticonvulsant and oxazepam elimination half-life or clearance.

The increased elimination of oxazepam in the presence of enzyme induction suggests that larger or more frequent dosage may be necessary to obtain an adequate therapeutic response. Studies should be undertaken to determine the effect of enzyme induction on the pharmacodynamic response to short acting benzodiazepines.

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