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Cotrimoxazole as an inhibitor of oxidative drug metabolism: effects of trimethoprim and sulphamethoxazole separately and combined on tolbutamide disposition

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The effect of separate pretreatments with cotrimoxazole, sulphamethoxazole and trimethoprim on the disposition of tolbutamide was studied in seven healthy males. Tolbutamide 500 mg intravenously was administered on four separate occasions—as a control without pretreatment and on the seventh day of separate twice daily administration of cotrimoxazole (sulphamethoxazole 800 mg plus trimethoprim 160 mg) (ST phase), sulphamethoxazole 1 g (S phase) and trimethoprim 150 mg (T phase). Tolbutamide total and unbound plasma clearance (CL) were reduced following each of the individual pretreatments compared to the control phase ($P < 0.001$). For unbound CL the reductions were 14% in the S and T phases and 25% in ST phase. Tolbutamide elimination half-life was prolonged following each pretreatment ($P < 0.001$) by 20% in the S phase, 19% in the T phase and 30% in the ST phase. Tolbutamide total steady-state volume of distribution (V_{ss}) was increased by 10% in the S and ST phases ($P < 0.01$), the increase being accounted for by an increase in tolbutamide unbound fraction. There was no change in tolbutamide unbound V_{ss} following any of the pretreatments. These results are consistent with inhibition of tolbutamide oxidation by cotrimoxazole, an additive effect of the two components sulphamethoxazole and trimethoprim. Sulphamethoxazole also reduces tolbutamide plasma protein binding.

Keywords tolbutamide/disposition cotrimoxazole sulphamethoxazole trimethoprim drug oxidation

Introduction

Available evidence suggests that the antibacterial compound cotrimoxazole (sulphamethoxazole plus trimethoprim) inhibits oxidative drug metabolism in man, as it decreases the clearance of the S(−) enantiomer of warfarin (O'Reilly, 1980) and of phenytoin (Hansen *et al.*, 1979), both of which are predominantly cleared by oxidative metabolism. This metabolic inhibitory effect of cotrimoxazole has yet to be fully characterised as it is unclear whether one or both components of the compound contribute to the effect. Previous studies have shown that some sulphonamides can inhibit oxidative drug metabolism in man (Dubach *et al.*, 1966). It has also been suggested that trimethoprim may independently inhibit oxidative drug metabolism (Hansen *et al.*, 1979). To investigate in more detail the inhibitory effect of cotrimoxazole on oxidative drug metabolism the present study in healthy volunteers has determined the effect of cotrimoxazole and its separate components on the disposition of tolbutamide, which undergoes

almost quantitative metabolism by one oxidative pathway (Thomas & Ikeda, 1966).

Methods

Subjects

The subjects were seven healthy male volunteers (ages 19–29 years, weights: 59–93 kg). Two subjects smoked up to 20 cigarettes daily and all had a modest social ingestion of alcohol. All subjects gave written consent for participation in the study, which was approved by the Clinical Investigation Committee at Flinders Medical Centre.

Protocol

For each subject the study was conducted in four phases—a nonrandomised control phase was followed by three phases each involving a 7 day pretreatment period. The separate pretreatments were cotrimoxazole as Septrim Forte (Wellcome) 1 tablet every 12 h (equivalent to sulphamethoxazole 800 mg and trimethoprim 160 mg) (ST phase), sulphamethoxazole 1 g every 12 h as two × Gantanol (Roche) 500 mg tablets (S phase), and trimethoprim 150 mg every 12 h as one half Triprim (Wellcome) 300 mg tablet (T phase). The dosing times for each pretreatment were 08.00 and 20.00 h. The order of the ST and S phases was randomised and the T phase was the fourth phase as trimethoprim tablets were not available until after these earlier phases of the study had commenced. One subject developed a rash during the sulphamethoxazole pretreatment phase and as a result did not participate in the subsequent cotrimoxazole phase; he did however complete the final phase (trimethoprim).

In each phase subjects received tolbutamide (Rastinon—Hoechst) 500 mg over 10 min by intravenous infusion into a forearm vein using an infusion pump (Braun). In the phases with pretreatments tolbutamide was administered on the seventh day of the pretreatment period and each pretreatment was continued until blood sampling had been completed on the eighth day. A washout period of at least 7 days separated each pretreatment period.

On each study day subjects attended the laboratory after having their usual breakfast. A cannula for subsequent blood sampling was then inserted into a forearm vein in the arm opposite to the intravenous infusion. The cannula was kept patent with 0.9% sodium chloride solution containing heparin 5 u/ml. Venous blood samples (10 ml into a heparinised tube) were taken before dosing, at the end of the infusion and at 5, 10, 15, 30, 45 min and 1, 2, 4, 6, 8, 24, 28 and 32 h post-infusion on each occasion. Plasma was separated and stored at −20 °C prior to assay.

Analytical methods

Tolbutamide plasma concentration was determined in each sample by high performance liquid chromatography (Nation *et al.*, 1978). Tolbutamide plasma protein binding was measured by equilibrium dialysis in the 2, 6 and 24 h samples on each occasion (Miners *et al.*, 1982). To monitor for subject safety blood glucose was measured using Dextrostix (Ames) and a reflectance meter (Ames glucometer) before and at 0.5, 1, 2, 4 and 6 h after each tolbutamide infusion.

Dispositional parameters

In each individual study, area under the tolbutamide plasma concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinite time assuming first-order kinetics. Total tolbutamide plasma clearance (CL) was then calculated as dose/AUC. Elimination half-life ($t_{½,z}$) was obtained from the slope of the terminal portion of each plasma concentration-time curve by linear least squares regression. Volume of distribution at steady state (V_{ss}) was calculated by a model-independent method (Benet & Galeazzi, 1979). The tolbutamide unbound fraction (α) in each study was calculated from the mean of the three estimates of tolubutamide protein binding. Unbound tolbutamide plasma clearance and unbound volume of distribution were calculated as CL_s/α and V_{ss}/α respectively.

Statistical analysis

For each parameter, group values are expressed as mean ± s.e. mean calculated from ANOVA. The effects of the pretreatments on the individual dispositional parameters were examined by repeated measures analysis of variance after estimation of the single missing value by least squares and appropriate reduction of the total and residual degrees of freedom (Snedecor & Cochran, 1967). Comparisons between the mean values in individual phases were performed by Newman-Keuls studentised range test. 2 × 2 factorial analysis was also employed to assess the interaction effect in the ST phase.

Results

Tolbutamide disposition parameters in each phase are summarised in Table 1. Total and unbound CL were reduced following each of the individual pretreatments compared to the control phase ($P < 0.001$). Such reductions were observed in each subject. The values for total and unbound CL respectively were similar in the S and T phases, there being a mean 11% reduction for total CL and 14% reduction for unbound CL compared to the control phase. Factorial analysis showed that the effects of S and T on both total and unbound CL were additive as seen in the ST phase in which total CL was reduced by 21% and unbound CL by 25% compared to the control phase. $t_{½,z}$ was prolonged following each pretreatment ($P < 0.001$), and was longer in the ST phase than in either the S or T phases ($P < 0.05$).

Tolbutamide unbound fraction was increased by a mean of 6% in both the S and ST phases ($P < 0.001$), factorial analysis indicating that the effect was entirely due to sulphamethoxazole. Total V_{ss} was also increased by a mean 10% in the S and ST phases ($P < 0.01$), but there was no change in unbound V_{ss} following any of the pretreatments.

Discussion

This study has shown that in healthy males pretreatment with common clinical doses of either cotrimoxazole, sulphamethoxazole or trimethoprim reduces tolbutamide total and unbound plasma clearance and prolongs elimination half-life. The effect of cotrimoxazole on tolbutamide clearance represents the additive effects of its components. In addition tolbutamide was displaced from circulating plasma protein by sulphamethoxazole both separately and in combination, accounting for the observed increase in total V_{ss}.

As the rate-limiting step for tolbutamide clearance is oxidation to hydroxytolbutamide (Thomas & Ikeda, 1966), an alteration in tolbutamide plasma clearance, particularly that of unbound drug, represents a change in its metabolic oxidation. The observed reductions in tolbutamide unbound clearance following the three different pretreatments in this study are thus consistent with partial inhibition of the oxidation of tolbutamide by cotrimoxazole and separately by each of its components, sulphamethoxazole and trimethoprim. The findings support the results of previous studies indicating that cotrimoxazole and its components inhibit the elimination of certain drugs metabolised by cytochrome P-450 (Dubach *et al.*, 1966; Hansen *et al.*, 1979; O'Reilly, 1980). Although this study has demonstrated approximately equal effects of sulphamethoxazole and trimethoprim on tolbutamide clearance, the contribution of the individual components may not be equal in a clinical situation in which cotrimoxazole is considered to be acting as an inhibitor of drug oxidation. Sulphamethoxazole and trimethoprim have different dispositional properties (Patel & Welling, 1980) and thus the active amounts of each drug in the body will not necessarily be present in predictable proportions. It should be pointed out that the metabolic inhibitory effects of sulphamethoxazole and trimethoprim may be quantitatively different for different forms of cytochrome P-450. Thus this study does not predict the magnitude of any inhibitory effect with substrates other than tolbutamide which are metabolised by different forms of cytochrome P-450.

Table 1 Tolbutamide disposition parameters (means – $n = 7$)

	Control	*ST*	*S*	*T*	*s.e. mean from ANOVA*
Total CL (ml min^{-1} kg^{-1})	0.159	0.126***	0.142**	0.141**	0.004
Unbound CL (ml min^{-1} kg^{-1})	2.13	1.60***	1.80***	1.86***	0.04
$t_{½,z}$ (h)	7.9	10.3***	9.5**	9.4**	0.2
Unbound fraction	0.075	0.079***	0.079***	0.075	0.001
Total V_{ss} (ml kg^{-1})	89	99**	97**	93	2
Unbound V_{ss} (l kg^{-1})	1.2	1.3	1.2	1.2	0.02

Pretreatments: ST—cotrimoxazole, S—sulphamethoxazole, T—trimethoprim
Significantly different from control: $^{**}P < 0.01$, $^{***}P < 0.001$.

This study was not designed to measure the effect of the interaction on the blood glucose response to tolbutamide. From the screening test for blood glucose performed during the study there was no apparent difference in response between the phases. It has previously been suggested that cotrimoxazole potentiates the hypoglycaemic effects of sulphonylureas (Mihic *et al.*, 1975), but the observed reductions in tolbutamide unbound clearance following the individual pretreatments were relatively small and thus major changes in tolbutamide response are unlikely. The situation may be different in an individual patient in whom the magnitude of the reduction in tolbutamide clearance may be sufficient to enhance the hypoglycaemic response.

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