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The effect of repeated administration of diftalone on enzyme induction

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Plasma concentrations of diftalone have been examined in normal volunteers after a single dose (500 mg) and after 500 mg doses given twice daily for one week. An increase in post dosing urinary excretion of D-glucaric acid showed a correlation with the ratio of calculated to observed areas under the plasma concentration, time curve following the final dose in the multiple dosing studies, indicating that hepatic microsomal enzymes are induced after repeated administration of the drug. Single dose studies in the presence of aluminium hydroxide and sodium bicarbonate showed that the antacids had no significant effect on the absorption of diftalone.

The non-steroidal non-acidic anti-inflammatory agent diftalone is hydrophobic and its absorption is significantly influenced by formulation factors such as micronization (Beretta, Schiatti & Tenconi, 1972), differences in the manufacture of the raw material to a finished dosage form also affect its plasma concentrations (Garnham & Stainton-Ellis, unpublished). The therapeutic activity of the drug in rheumatoid arthritis is related to its plasma concentrations (Nicolis, Buniva & others, 1974).

As the absorption of indomethacin and naproxen is affected by antacids (Segre, Sevelius & Varaday, 1974, Garnham, Raymond & others, 1975; Garnham, Kaspi & others 1977), we have examined the effect of antacids on the plasma concentration of diftalone.

Since plasma concentrations in patients have been found to be consistently lower than those found in normal volunteers (Garnham & Stainton-Ellis, unpublished) the possibility of enzyme induction has been studied by measuring serum concentrations of γ-glutamyl transpeptidase and β-glucuronidase and the 24 h excretion of D-glucaric acid, after one week of treatment with the drug at a dose of 500 mg twice a day.

MATERIALS AND METHODS

Capsules contained 250 mg of micronized drug, together with 40 mg sucrose, 6 mg polyvinylpyrrolidone, 2 mg dioctylsulphosuccinate, 10 mg sodium carboxymethylcellulose, 12 mg magnesium stearate and starch to 335 mg. Aluminium hydroxide gel suspension, contained 1·5 g in 100 cm^3 of water and sodium bicarbonate in solution, 14 g litre^{-1} of water.

Seven healthy volunteers (4 male, 3 female) 20–29 years old, 50–80 kg, participated with full knowledge of the experiment. Following an overnight fast, pre-treatment blood samples were taken, then each subject was given the drug (500 mg) on three occasions each two weeks apart together with 100 cm^3 of (i) water, (ii) sodium bicarbonate solution and (iii) aluminium hydroxide gel suspension, in randomized order. Two weeks after receiving the last of the three doses each subject took 500 mg twice a day for 15 doses.

During each experiment blood samples were taken in a heparinized tube ½, 1, 1½, 2, 3, 4, 6, 8, 12, 24, 36, 48, 74 and 84 h after the single or last dose and centrifuged immediately and stored at 0° until assay.

The assay by g.l.c. was carried out by courtesy of Professor V. James at the Department of Chemical Pathology Research, St. Mary's Hospital Medical School.

Before receiving any drug in the chronic dose experiment and two days after the last dose, each subject provided a 24 h specimen of urine for estimation of urinary D-glucaric acid excretion (Latham, 1974). Similarly, pre- and post-diftalone serum γ-glutamyl transpeptidase and β-glucuronidase values were estimated.

RESULTS

The single dose studies with water and with antacids did not show any significant differences between

¶ Correspondence.

peak plasma concentrations or between areas under the C, T curves.

Pharmacokinetic parameters for the single dose studies were obtained using the maximum point method described by Saunders & Natunen (1973).

Pre- and post-medication concentrations of γ-glutamyl transpeptidase and β-glucuronidase did not differ significantly. The values for 24 h urinary D-glucaric acid excretion did show significant change. The ratio of post- to pre-medication 24 h excretion was taken as the index of enzyme induction, R_2 in Table 2.

Table 1. *Parameters from maximum point method for single dose results with water.*

Subject	Lagtime, T_1 h	A $\mu g\ cm^{-3}$	α h^{-1}	B $\mu g\ cm^{-3}$	β h^{-1}	k_a h^{-1}
1	0·259			20·0	0·0419	1·21
2	0·395			11·0	0·0976	0·485
3	0			20·6	0·0370	0·134
4	0·720	9·06	0·146	6·71	0·0413	0·411
5	0·463			15·8	0·0811	0·261
6	0·536			8·56	0·0617	0·813
7	0·329			10·2	0·0637	1·36

DISCUSSION

The addition of antacids to the non-acidic diftalone did not affect its absortion in the same way as the absorption of indomethacin (Garnham & others, 1977) and naproxen (Segre & others, 1974) are affected.

The significant difference between D-glucaric acid before and after diftalone medication indicates that enzyme induction has occurred after multiple dosing with diftalone. This effect has been compared with the results of a kinetic analysis of the plasma concentration results which was carried out as follows.

The single dose results for diftalone with water, for each subject were interpreted by means of the maximum point method PHMKIN 3 (Saunders & Natunen, 1973). The maximum point method starts by fitting the equation

$$C = A.\exp(-\alpha.T) + B.\exp(-\beta.T) - (A + B).\exp(-k_a.T)$$

to the results. T the time is corrected for lag time. k_a is the absorption rate constant and α and β are disposition rate constants. If any one of the rate constants of the compartment model on which this equation is based becomes negative it indicates that the data do not support this model and the calculation is continued by fitting the simpler equation

$$C = B.(\exp(-\beta.T) - \exp(-k_a.T))$$

to the results.

The parameters derived from these calculations are shown in Table 1. Lag time is in hours, A and B in $\mu g\ cm^{-3}$, α, β and k_a in h^{-1}.

From these parameters a forecast was made of the area in the multidose study under the final dose C, T curve, AUC_T. By comparing the forecast value of the area up to the last data point with non zero concentration, C_z at time T_z, with that found from the experimental results, an estimate could be made of the way in which elimination after multiple doses differed from that expected as a result of the single dose studies.

T_z was used as the limit for the area because the main linear part of the log C,T plot after the final dose did not always fit the last two data points at 72 and 84 h. In two cases where positive concentrations would have been expected, zero values were found at these times.

The value of C_n, the plasma concentration at the last dose may be estimated by summing all the contributions to the plasma concentration remaining from the previous doses. If $T = 0$ at the time of the last dose and τ is the interval between doses, this summation gives geometric progressions for each exponential term in the C,T equation.

When the number of doses is large these progressions may be taken as equal to their limiting sums. The criterion for large n is that the value of $(1-\exp(-\beta.n.\tau))$ should be almost equal to 1 with the smallest value of β in the set of data. In this case the smallest value of β is 0·037, $n = 15$, $\tau = 12$ and $1 - \exp(-\beta.n.\tau) = 0{\cdot}999$ and therefore the limiting sums were used.

For monoexponential disposition,

$$C_n = B_n - D_n$$

Where $B_n = B/(1 - E_d)$; $D_n = B/(1 - E_a)$; $E_d = \exp(-\beta.\tau)$; $E_a = \exp(-k_a.\tau)$.

After the final dose estimated plasma concentrations are given by

$$C = B_n.\exp(-\beta.T) - D_n.\exp(-k_a.T)$$

for monoexponential disposition. Similar results with the addition of A and α are obtained for biexponential disposition.

By integrating these equations up to the time of the last data point, allowing for lag time if any, theoretical areas under the final dose curve, AUC_T may be assessed from the kinetic parameters derived from the single dose studies.

To give direct comparisons between the properties expected from single dose studies and the properties found after the final dose in the multidose studies, the ratio R_1 was calculated for each subject

$$R_1 = AUC_T/AUC_D$$

AUC_D is the area under the curve after the final dose, found from the data.

If this ratio is less than one, the indication is that in the multidose series the drug is accumulating more than would be expected from the single dose results, giving an area under the final curve above the predicted value, AUC_T. However, if the prolonged plasma concentrations from multiple dosing cause substantial induction of enzymes which metabolize the drug, higher values of the ratio would be expected.

A measure of enzyme induction due to multidosing is provided by the data on D-glucaric acid excretion before and after the multidosing. As an index of enzyme induction, R_2, the ratio of 24 h excretion of glucaric acid after and before the multidose studies has been used.

In Table 2 the values of the two ratios are shown in order of decreasing R_2. There is a clear correlation between R_2 and R_1 with a significance greater than 0·98.

R_1 is less than one for most of the subjects indicating that an accumulation of diftalone does occur during the multiple dosing. The correlation with R_2 shows that the effects of enzyme induction reduce this accumulation and in the cases of subjects 1 and 3 where the induction effect is most pronounced, R_1 becomes greater than one.

These results suggest that important properties of the final dose C,T curve are related to the effects of enzyme induction produced in the different subjects by the multiple dosing of diftalone for seven days.

Table 2. *Calculated properties of the final dose C,T results.*

Subject	Forecast AUC from single dose	Observed AUC_D from data	Ratio of areas R_1	Induction index, R_2
3	1084	542	2·00	25·8
1	1156	660	1·75	12·9
4	289	423	0·68	11·2
5	238	512	0·47	8·8
7	291	489	0·59	5·0
2	141	291	0·48	3·3
6	242	335	0·72	2·2

Correlation coefficient of R_1 with R_2 is $r = 0·836$, $P > 0·98$.

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