S-OXIDATION OF THIORIDAZINE TO PSYCHOACTIVE METABOLITES: AN ORAL DOSE-PROPORTIONALITY STUDY IN HEALTHY VOLUNTEERS

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SUMMARY

Thioridazine has two major active metabolites, which are formed from *S-oxidation* of its 2-methylthio group; the sulphoxide, *mesoridazine*, and the sulphone, *sulforidazine*. *Dose proportionality* of the three compounds was invesigated for the first time in 11 males after administration of three single oral doses (25, 50, and 100 mg) of thioridazine hydrochloride separated in each case by two weeks. Based on the plasma concentrations of the three analytes over 72 h following each dose, large intersubject variabilities in such parameters as AUC_0^t and C_{max} were observed for each of the three compounds. The relationships between dose and parameters such as AUC_0^t and C_{max} for each analyte were described by an equation for a straight line ($r^2 \geq 0.8$). However, the mean apparent distribution and elimination rate constants for thioridazine and mesoridazine and the mean apparent oral clearance for thioridazine decreased significantly with increasing dose, suggesting non-linearity in the elimination of thioridazine at high dose.

I. INTRODUCTION

Thioridazine (1) is a piperidine type of phenothiazine antipsychotic agent which is metabolised by various metabolic routes that include S-oxidation of the side chain and phenothiazine ring sulphur atoms /1,2/. The known major metabolites which appear in the plasma of patients under chronic treatment with the drug arise from these S-oxidations, namely, thioridazine ring sulphoxide, thioridazine side chain sulphoxide (mesoridazine, 2) and thioridazine side chain sulphone (sulforidazine, 3) /1,3/. Both mesoridazine and sulforidazine have been demonstrated to have greater potency than thioridazine in clinical /4,5/ as well as in *in vitro* studies /6/. Consequently, it is believed that mesoridazine and sulforidazine play a major role in the antipsychotic effect of thioridazine /7/, such that, in clinical pharmacokinetic studies not only the parent drug but also these two known active metabolites should be measured.

There are to date few published papers on the single dose *pharmacokinetics* of thioridazine, mesoridazine and sulforidazine /3,8-11/ and there are no reports of dose- proportionality studies on thioridazine and its major active S-oxidative metabolites. The

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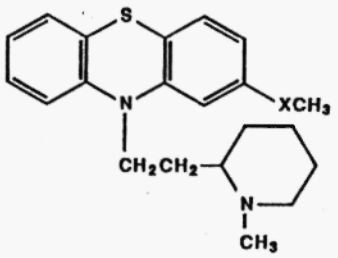

1 : X = S

2 : X = SO

3 : X = SO_2

present study was performed to investigate the effect of dose on the pharmacokinetic parameters of the psychoactive compounds thioridazine, mesoridazine and sulforidazine following administration of separate 25, 50 and 100 mg single oral doses of thioridazine to each of 11 healthy male volunteers.

II. MATERIALS AND METHODS

2.1 Subjects

Eleven healthy male volunteers (average age and weight: 21.8 ± 2.7 yr and 72.9 ± 8.5 kg, respectively) gave written consent to participate in the study. Each subject was required to pass a physical examination and to answer a questionnaire about his medical history. Standard blood and urine chemistry tests gave results within the normal ranges for healthy males in this age group.

2.2 Study design and plasma samples

The study was approved by the local committee on ethics in human experimentation. The subjects agreed to remain drug free for at least two weeks prior to the study and also abstain from alcohol 24 h prior to dosing and until 24 h after collection of the last blood sample. On three separate occasions (2 weeks apart in each case), each subject received a different dose (25, 50 and 100 mg) of

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thioridazine hydrochloride (Mellaril concentrate, Sandoz Inc., East Hanover, N.J., U.S.A) with 250 ml of water after an overnight fast. A carbonated lemon-lime beverage and a standardised meal were respectively served at 2 and 4.5 h after each dose. On each occasion blood (10 ml) was collected in evacuated glass tubes (Vacutainers®, Becton and Dickinson Co., Mississauga, Ontario, Canada) without touching the rubber stoppers at 0 (pre-dose), 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 (100 mg dose only), 24, 32, 48 and 72 h after oral administration. The plasma was immediately separated by centrifugation and stored at −20°C until analysis. The thawed plasma samples were analysed by the HPLC method (ultraviolet detector set at 254 nm; 5μm Spherisorb CN column; mobile phase −0.05 M ammonium acetate in acetonitrile, 20:80 v/v) described previously in detail /12/. The limit of quantification for thioridazine, mesoridazine and sulforidazine was 2.5 ng/ml in each case with a respective CV of 6.0, 6.5 and 11.5% using 2 ml plasma samples.

2.3 Analysis of data and statistics

The maximum plasma concentration (C_{max}) and the time to reach the maximum concentration (t_{max}) for each analyte were determined directly from the plasma concentration data. The area under the $\log_e$ plasma concentration curve up to the last available sampling time showing a detectable concentration of the analyte (AUC_0^t) was calculated by a combination of linear (ascending plasma concentrations) and log (descending plasma concentrations) trapezoidal rules /13/. The rate constant (k_a) for absorption (thioridazine) or accrual in plasma (metabolites) and the apparent distribution (α) and elimination (β) rate constants for all three analytes were calculated from the plasma concentration data for each subject by the method of residuals /14/. In general (except for a few subjects in the case of sulforidazine) it was found that the time course of analyte concentrations could be adequately described by a two compartmental model with first order absorption (or accrual) fitting the equation: $C(t) = Ae^{-kat} + Be^{-\alpha t} + Ce^{-\beta t}$, where C(t) is the plasma concentration of the analyte at any time t and A, B and C are constants. Areas under the plasma-concentration time curve extrapolated to infinity (AUC_0^∞) were calculated by adding to the corresponding AUC_0^t, the quotient of the last available plasma

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concentration in the decay phase of the curve and the appropriate β. Apparent elimination half-life ($t_{1/2}$) in plasma was calculated by using the following equation: $t_{1/2} = (\ln 2)/\beta$. Apparent oral clearance (CL_{po}) for thioridazine was calculated by dividing the weight adjusted dose by $\widehat{AUC}_0^{\infty}$.

The relationship between AUC_0^{∞}, AUC_0^t or C_{max} and dose was examined by both linear and non-linear regression analyses. Thus the data were fitted to both straight line and parabolic equations. The significance of regression coefficients and intercept terms in such equations and associated coefficients of determination (r^2) were examined by *t* tests before accepting any model. Also the appropriateness of the model was examined by determining whether the lack of fit was significant as shown by an analysis of variance (ANOVA).

Two-way ANOVA was carried out to detect the effect of the dose on the parameter means (blocked across the subjects). Student Newman-Keuls multiple comparison tests were carried out following each ANOVA to determine the significance of differences between individual treatment means. Finally, statistical power of ANOVA for AUC_0^t, AUC_0^{∞} and C_{max} was calculated by the method of Winer /15/ to determine whether the sample size was adequate to detect differences between the dose levels.

III. RESULTS AND DISCUSSION

3.1 Intersubject Variation in Plasma Concentrations

As an example for the three analytes, the mean plasma concentration-time profiles resulting from the separate oral administrations of three dose levels of thioridazine are shown for thioridazine in Figure 1. For all three analytes there were large intersubject variations in the individual plasma concentrations at each sampling time. Typically this variation was greater for each of the analytes at the low doses (25 and 50 mg) than at the high dose (100 mg). This was likely due in large part to the inclusion of a greater number of undetectable concentrations (determined as 0 ng/ml) at the two lowest doses. Also at high dose, saturation of first pass metabolism would result in less intersubject variability.

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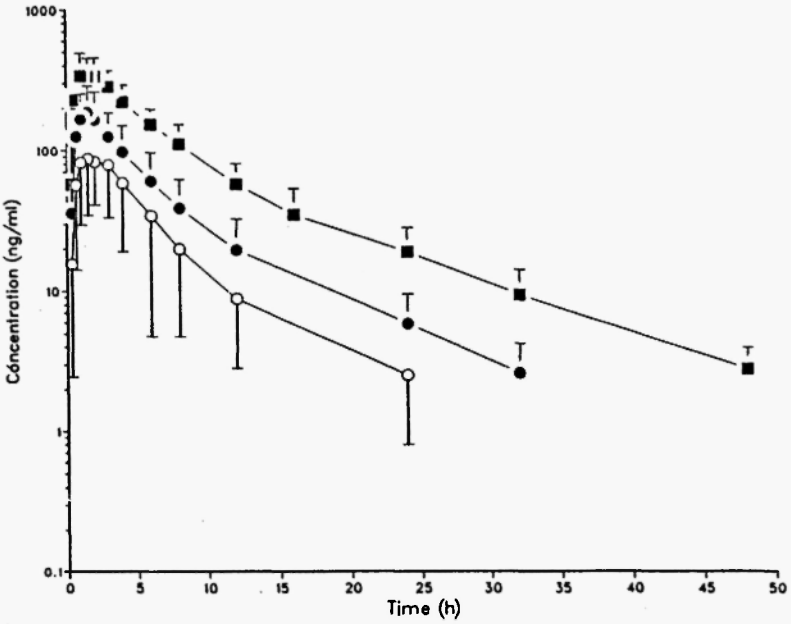

Fig. 1: Mean plasma concentration versus time profiles of thioridazine following the administration of 25 (○), 50 (●) and 100 mg (■) single oral doses of thioridazine hydrochloride to healthy subjects (n = 11).

Thioridazine was readily absorbed from the gastrointestinal tract following oral administration. In almost all subjects all three analytes were measurable for all three doses at the first sampling time (0.33 h). Also, in general, in each of the volunteers at each dose level, plasma concentrations rose faster for thioridazine than mesoridazine than sulforidazine, such that the mean t_{max} for the three analytes were in the range 1.4-1.8 h, 2.6-3.2 h and 4.7-6.4 h, respectively. After reaching the C_{max}, the plasma concentrations of thioridazine and mesoridazine declined with time in a biexponential manner in all the volunteers, whereas the decline of sulforidazine appeared to be monoexponential in a few subjects and biexponential in the others.

The mean pharmacokinetic parameters for all three analytes are given in Table 1. For each analyte there was wide intersubject variation in most determined parameters including AUC_0^t, AUC_0^∞, C_{max} and k_a. The AUC_0^t, AUC_0^∞ and C_{max} showed greater variation at the lower than the highest doses of thioridazine, as for

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TABLE 1

Mean pharmacokinetic parameters for thioridazine, mesoridazine and sulforidazine in healthy volunteers (n = 11) as determined from the plasma concentration data after separate administration of single 25, 50 and 100 mg oral doses of thioridazine hydrochloride

Dose of Thioridazine (mg)	AUC_0^t (ng-h/mL)	AUC_0^∞ (ng-h/mL)	C_{max} (ng/mL)	t_{max} (h)	k_a (h^{-1})	α (h^{-1})	β (h^{-1})	$t_{1/2}$ (h)	CL_{po} (L/kg-h)
				Parameters for Thioridazine					
25	541(51)	555(50)	111(46)	1.8[1](44)	1.70[1](55)	0.49[1](30)	0.11(27)	6.8(25)	0.83[1](51)
50	1074(54)	1084(54)	197(52)	1.4[1](29)	2.11[1](76)	0.51[1](15)	0.09[1](16)	8.2[1](18)	0.83[1](53)
100	2603(33)	2639(33)	372(37)	1.5[1](27)	2.05[1](55)	0.31(32)	0.08[1](22)	9.3[1](20)	0.59(42)
				Parameters for Mesoridazine					
25	1558(53)	1612(53)	169(48)	2.6[1](42)	1.07[1](43)	0.56(91)	0.09(22)	8.8(36)	–[2]
50	3854(39)	3964(38)	334(27)	3.3[1](33)	0.78[1](31)	0.26[1](31)	0.07[1](20)	10.4[1](20)	–[2]
100	7219(28)	7550(27)	514(34)	3.2[1](38)	0.89[1](28)	0.20[1](45)	0.06[1](12)	12.1[1](14)	–[2]
				Parameters for Sulforidazine					
25	372(43)	411(45)	32(38)	4.7[3](45)	0.74[1](41)	0.35[1,4](54)	0.09[1](44)	9.4[1](37)	–[2]
50	773(46)	901(33)	53(30)	4.9[3](55)	0.59[1](53)	0.23[1,5](78)	0.07[1](29)	10.2[1](27)	–[2]
100	1678(30)	1767(29)	90(36)	6.4[3](23)	0.54[1](67)	0.22[1,5](55)	0.07[1](19)	10.7[1](22)	–[1]

Values in the parentheses are CV%. Unless otherwise designated, for a particular analyte mean values were significantly different (ANOVA: $p < 0.05$) between each dose level.

1 Treatment means for a given analyte that are not significantly different ($p > 0.05$).

2 Not applicable.

3 ANOVA, $p = 0.043$; this significance was not detected by the multiple comparison test.

4 N = 8.

5 N = 5.

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example with thioridazine, where these parameters showed approximate variations of 5-6 fold at the 25 and 50 mg doses and 3-4 fold at the 100 mg dose. Also the interindividual variations for mesoridazine over the 25-100 mg dose range varied 2.5-7 fold for AUC_0^∞ and AUC_0^t and 3-4 fold for C_{max}, while the corresponding ranges for sulforidazine were 3-5 and 3-4 folds, respectively. It should be noted that extrapolation of AUC_0^t to infinity represented an increase of less than 10% in most volunteers for all three analytes at all three doses. Finally, regarding intersubject variations, its extent differed widely not only between doses, but also analytes. For example, in the case of k_a at the various doses, thioridazine, mesoridazine and sulforidazine showed variations of 6-11, 3-4 and 4-8 folds, respectively.

3.2 Analysis of Variance and Relationship of Pharmacokinetic Parameters for each Analyte with Dose of Thioridazine

From the analysis of both the raw and log transformed data it was found that the effect of dose on C_{max}, AUC_0^t and AUC_0^∞ was significant (two-way ANOVA; $p < 0.001$) for thioridazine, mesoridazine and sulforidazine. For each of these ANOVA the power to detect the observed differences in the mean was > 0.80, except in the case of the comparisons of 25 and 50 mg doses with respect to C_{max} for thioridazine (0.76) and AUC_0^t for sulforidazine (0.78). Also for each of the three analytes the mean value of any of these three parameters was significantly different from each other at the three dose levels of thioridazine examined (Table 1: Student Newman-Keuls multiple comparison test).

The mean t_{max} values were not significantly different between the three dose treatments (ANOVA; $P > 0.2$) for thioridazine and mesoridazine and were only of borderline significance ($p = 0.043$, not detected by the multiple comparison test) for sulforidazine. Also for each of the three analytes treatment means for k_a were not significantly different from one another (ANOVA; $p > 0.1$). These comparisons indicate that the size of dose had no effect on the rate of absorption of thioridazine from the gastro-intestinal tract.

Unlike the parameters so far discussed, comparisons of the treatment means of α and β were not similar for all three analytes. Thus whereas for thioridazine and mesoridazine, the effect of dose on both the fast and slow rate constants was significant (ANOVA, Student Newman-Keuls multiple comparison tests), in the case of

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sulforidazine, none of the treatment means of α and β were significantly different from each other. Also, whereas, in the case of mesoridazine, the mean values of both these rate constants at the 50 and 100 mg doses were significantly smaller than at the 25 mg dose, in the case of thioridazine, this was so with β but not α. Thus α for thioridazine at the 100 mg dose was significantly different (lower) from the 50 and 25 mg treatment means. In view of the above noted observations for β, it was not surprising that $t_{1/2}$ was significantly longer at the higher two doses for thioridazine and mesoridazine, but not sulforidazine.

In order to determine whether these observations just discussed are evidence for non-linearity in the disposition kinetics of thioridazine, the relationship between the AUC or C_{max} and dose was examined in detail for each of the three analytes. Except for one subject in a few cases, all the subjects for all three analytes showed an ordered response with increasing dose of thioridazine for AUC_0^t, AUC_0^∞ and C_{max}. Individual AUC_0^∞ and weight adjusted dose values were fitted separately to both linear and parabolic equations. It was found for each analyte that the values did not fit the parabolic equation in that the curvature component was not significant by a *t* test ($p \geq 0.5$). On the other hand, the relationship between individual AUC_0^∞ and corresponding weight adjusted doses was satisfactorily described by a straight line equation. Thus the respective equations of the linear regression lines for thioridazine, mesoridazine and sulforidazine were: $AUC_0^\infty = -92.9 + 1870.7$ (dose), ($r^2 = 0.61$), $AUC_0^\infty = 254 + 5075$ (dose), ($r^2 = 0.63$) and $AUC_0^\infty = 83.2 + 1161.7$ (dose), ($r^2 = 0.63$). In each of these equations the intercept was not significantly different from zero (*t* test: $p > 0.5$) but the slope and r^2 were highly significant ($p < 0.01$). Therefore, these linear relationships could be described with straight lines passing through the origin. The equations of such straight lines for thioridazine, mesoridazine and sulforidazine were respectively calculated as: $AUC_0^\infty = 1782.8$ (dose), ($r^2 = 0.86$), $AUC_0^\infty = 5316$ (dose) ($r^2 = 0.89$)and $AUC_0^\infty = 1240.5$ (dose), ($r^2 = 0.89$). Similarly, when the AUC_0^t and C_{max} values were separately regressed on the corresponding weight adjusted dose values, the regression coefficient and the coefficient of determination were only significant ($p < 0.01$) for the fits by straight lines rather than parabolas.

For all three analytes the results of the ANOVA and regression analyses indicate that the parameters AUC_0^t, AUC_0^∞ and C_{max}

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showed proportional increase with the dose of thioridazine, with no corresponding change in k_a and t_{max}. Therefore, the systemic availabilities of thioridazine and its two active S-oxidative metabolites appear to be unaffected by the size of the dose of thioridazine within the dose range studied. However, despite the observed dose-linearity in the above parameters, as previously mentioned, the mean t½ of thioridazine (6.8-9.3 h) and mesoridazine (8.8-12.1 h) increased significantly with increasing dose. In order to examine whether this observation was a reflection of reduced clearance of thioridazine at high dose(s), the effect of dose on CL_{po} of thioridazine was analysed. It was found that the mean value of CL_{po} at the 100 mg dose was significantly lower than the mean values at the 25 and 50 mg doses (ANOVA and multiple comparison tests), thereby indicating possible non-linear disposition of thioridazine at high dose(s). As previously noted, β for thioridazine was also affected by dose, however, the change in the value of β with dose does not appear to be entirely due to changes in CL_{po}. This is indicated by comparison of mean values of 25 and 50 mg doses, which show that although was significantly shorter at the higher dose, there was no change in CL_{po}. From knowledge of the concentration ratio of thioridazine in blood to plasma and CL_{po} (which is equivalent to intrinsic clearance for thioridazine) it can be shown that a decrease in total body clearance would be much lower than that in CL_{po} (18% versus 30%, respectively) and thus may not be clinically important. Nevertheless, this is the first report of a non-linear trend in the single dose pharmacokinetics at high dose of thioridazine. It is interesting to note in pilot studies involving multiple dosing that a positive correlation has been reported between the steady state serum concentrations of thioridazine and daily divided doses up to about 6 mg/kg body weight above which no such correlation was found /16,17/.

IV. CONCLUSIONS

In this study significant plasma concentrations (e.g. Cmax, Table 1) of not only thioridazine but also its active S-oxidative metabolites, mesoridazine and sulforidazine, were produced following 25, 50 and 100 mg single oral doses of thioridazine. The single oral dose pharmacokinetic parameters and interindividual variability in these

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parameters for all 3 analytes were similar to that previously reported /3, 8-11/.

For all three analytes, AUC_0^t, AUC_0^∞ and C_{max} showed proportionate increase with the dose of thioridazine and no change in k_a and t_{max}. Therefore, it appeared over the dose range studied that the systemic availability of these three active moieties may not be affected by the size of the dose of thioridazine.

Half-lives, α and β for thioridazine and mesoridazine and CL_{po} for thioridazine decreased significantly with increasing dose. Therefore, there appear to be *non-linear trends* in the elimination kinetics at high dose of thioridazine. These trends are apparent for both thioridazine and mesoridazine, but not for sulforidazine, which may indicate that different isozymes are involved in the S-oxidation of thioridazine and mesoridazine as compared to that of sulforidazine. Whether or not the observed non-linear trends in the elimination kinetics after single doses have implications in patients awaits further investigation.

V. ACKNOWLEDGEMENTS

Financial support from Sandoz Inc., East Hanover, N.J., U.S.A. is gratefully acknowledged. The authors thank Trevor Gurnsey, John Cooper and Gail Rauw for technical assistance.

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