THE INFLUENCE OF ACETYLATOR PHENOTYPE ON THE OUTCOME OF TREATMENT WITH PHENELZINE, IN A CLINICAL TRIAL

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1 It has been suggested that the rate of metabolism of phenelzine is dependent on the acetylator phenotype of the recipient and, therefore, that acetylator phenotype may be an indicator of clinical importance.

2 Acetylator phenotype was determined in a group of patients suffering from depressive, anxiety or phobic neurosis. These patients were blindly allocated to treatment with phenelzine or placebo, in addition to diazepam.

3 Ratings of clinical state were made at weekly intervals. A principle component analysis of the improvement scores on all the clinical rating scales was used to provide a slight measure of improvement for each patient. Increases in severity of undesirable symptoms or spontaneous complaints were taken to indicate side effects.

4 Assessments of whole blood MAO and 5-HT, and urinary 5-HIAA and VMA were made before treatment and at weekly intervals during the course of treatment.

5 Data from improvement scores indicate that there is a treatment effect only in the first 2 weeks and there is no significant difference between fast and slow acetylators.

6 For the dropouts, the ratio of slow to fast acetylators is not significantly different from that in the total group.

7 MAO is inhibited by phenelzine and the degree of inhibition is independent of acetylator phenotype.

8 Changes of whole blood 5-HT concentration during the course of treatment are complex and suggest that there is an interaction between treatment and acetylator phenotype. The results suggest that fast acetylation is associated with an increased metabolism of 5-HT.

9 It is concluded that acetylator phenotype should not be regarded as a prognostic indication of clinical importance and that the rate of acetylation is not directly related to the appearance or disappearance of monoamine oxidase inhibition by phenelzine.

Introduction

The similarity in structure between phenelzine and the drugs isoniazid and sulphadimidine which are known to be acetylated has led to the suggestion that phenelzine may be metabolized by acetylation. Acetylated metabolites of phenelzine have not, in fact, been identified in urine or blood of patients but other evidence has been reported which indirectly supports the acetylation theory (Evans, Davison & Pratt, 1965;

Johnstone & Marsh, 1973; Johnstone, 1976). Those authors also suggest that the patients' acetylator type, which is genetically determined, is of clinical importance in the same way as acetylator phenotype has been shown to affect the response of tuberculous patients to isoniazid (Menon, 1968). Should fast acetylators fail to respond to treatment with phenelzine as reported by Johnstone & Marsh (1973), it is clearly of great practical importance.

If acetylation is important in inactivation of phenelzine the following predictions can be made. Slow acetylators should respond clinically at a lower dosage and have more side effects than fast

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acetylators. Inactivation of phenelzine assumes a concomitant loss of its activity as a monoamine oxidase inhibition. Slow acetylators should show a more rapid and complete inhibition of monoamine oxidase and a greater and more rapid fall in 24 h urinary 5-hydroxyindoleacetic acid (5-HIAA) and vanylmandelic acid (VMA), coupled with a greater rise in blood 5-hydroxytryptamine (5-HT) than fast acetylators.

A controlled clinical trial of phenelzine in the treatment of anxiety, phobic and depressive neurosis, provided an opportunity to assess the importance of acetylator phenotype in clinical outcome. Analysis of the data from the main trial of phenelzine (Mountjoy & Roth, 1975; Mountjoy, Roth, Garside & Leitch, 1977; Marshall & Campbell, 1975) showed that phenelzine produced improvement on some anxiety scales and a greater number of side effects but, in the main, there were few significant differences between the effects of phenelzine and those of valium. For the total group of patients, neither clinical nor biochemical initial factors predicted outcome, and the improvement was unrelated to the degree of monoamine oxidase inhibition. Against such a background, significant results from this analysis, in the hypothesized direction, would lend greater substance to the acetylator status hypothesis.

Methods

Tablets containing phenelzine sulphate (15 mg active base/tablet) and placebo were supplied as identical preparations.

Clinical details

Details of the trial have been published elsewhere (Mountjoy & Roth, 1975; Marshall & Campbell, 1975; Mountjoy *et al.*, 1977). In summary, patients suffering from depressive, anxiety or phobic neurosis were randomly and blindly allocated, within diagnostic categories, to treatment with phenelzine or placebo for a period of 4 weeks. In addition, patients received diazepam in a dose determined by the consultant in charge but usually 15 mg daily. In the first fortnight the patients treated with phenelzine received 45 mg/day and in the second fortnight 75 mg/day. The supply of tablets to inpatients was supervised by the ward nurses. The compliance of outpatients with the treatment schedule was assessed by pill counts. Patients who failed to follow the dosage schedule were excluded from the trial and the data from these patients is not included in the present analysis. Ratings of clinical state were made at weekly intervals. A principle component analysis of the improvement scores on all the clinical ratings scales was used to provide a single measure of improvement for each patient. This single measure (the 1st

component) accounted for about 57% of the variance.

A side effect check list derived from Goodman & Gilman (1970) was incorporated into the systematic initial enquiry and repeated at the end of the trial or at time of drop out. Any spontaneous complaint of new symptoms was recorded and each side effect measured on a four point scale of severity. An increase in severity at the end of four weeks or a spontaneous complaint was taken to indicate side effects. Side effects were considered both in terms of mean number of side effects and of total number of patients complaining of any side effects.

Biochemistry

Patients willing to participate in a biochemical investigation provided blood and 24 h urine samples on two occasions before the start of the trial, and at weekly intervals thereafter. Some of the patients willing to have their acetylator status determined declined to provide further biochemical samples, particularly of blood.

Acetylator status was determined by the method of Schröder (1972), using sulphadimidine (10 mg/kg) as the test drug. Where the developed colour was strong, urine aliquots were diluted and the assays repeated. The phenotyping was carried out by one person and in cases of doubt confirmation was made by colleagues. Whole blood MAO activity was measured by a modification (Marshall & Campbell, 1975) of the method of Robinson, Lovenberg, Keiser & Sjoerdsma (1968). Twenty-four hour urine specimens were assayed for 5-hydroxyindoleacetic acid (5-HIAA; Contractor, 1966), vanylmandelic acid (VMA; Pisano, Crout & Abraham, 1962) and creatinine. Blood 5-HT was assayed by the method of Yuwiler, Plotkins, Geller & Ritvo (1970).

Statistics

The difference in the number of patients complaining of side effects and number of patients dropping out because of side effects were tested using Fisher's exact probability test. The number of side effects were compared by Student's *t*-test.

Clinical improvement and changes in the four biochemical measures (VMA, 5-HT, MAO, 5-HIAA) were each analysed by a two way analysis of covariance with treatment (placebo/phenelzine) as one classification and acetylation phenotype (fast/slow) as the other. The number of patients in each cell were unequal and therefore a computer programme implementing a method for unbalanced classifications (Federer, 1957) was written by one of us (IML). In each analysis the initial value of clinical severity or the initial value of biochemical measures in question was used as the covariate. The purpose of such an analysis is to allow for the disturbing effects of differing initial levels between groups of patients. By using this

procedure, group change means are adjusted for difference in initial levels giving adjusted mean changes.

Such an analysis of covariance of clinical improvement at two and four weeks was carried out. For each of the four biochemical measures at each of the four time intervals, the statistical analysis was repeated.

Results

Population characteristics

Forty-six patients treated with phenelzine and thirty-four patients treated with placebo had their acetylator status determined. Twenty-nine (36.25%) were fast acetylators and fifty-one (63.75%) were slow acetylators in the combined diagnostic groups. There

was no difference between treatment or within the diagnostic groups in the proportion of fast and slow acetylators. The distributions of fast and slow acetylators in each diagnostic treatment category is indicated in Table 1. Twenty-five fast acetylators and thirty-four slow acetylators completed 4 weeks treatment and only these patients were used in the analysis of the clinical data. Of the twenty-one clinical drop outs, four were fast and seventeen were slow. These numbers are significantly different, but not all the clinical drop outs received phenelzine. For those being treated with phenelzine, the corresponding figures for fast and slow acetylators are not significantly different. Thus the selective deficiency of rapids in the drop outs cannot be wholly attributed to the phenotype status. The numbers of patients involved in the biochemical analysis is detailed in the appropriate figures.

There was no significant difference between slow

Table 1 Acetylator phenotype by treatment showing numbers entering the trial and the numbers and reasons for withdrawal from the trial

	Placebo (n=34)				Phenelzine (n=46)			
	n	Fast (%)	n	Slow (%)	n	Fast (%)	n	Slow (%)
Neurotic depression	5	(38.46)	8	(61.54)	5	(31.25)	11	(68.75)
Neurotic anxiety states	3	(30)	7	(70)	8	(44.44)	10	(55.56)
Phobic anxiety states	4	(36.36)	7	(63.64)	4	(33.33)	8	(66.67)
Total	12	(35.29)	22	(64.71)	17	(36.76)	29	(63.04)
All dropouts	2	(28.57)	5	(71.43)	2	(14.29)	12	(85.71)
Dropouts because of side effects	0	(0)	1	(100)	1	(14.2)	6	(85.71)
Deterioration	1	(33.33)	2	(66.67)	0	(0)	3	(100)
Failure to comply with schedule	1	(33.33)	2	(66.67)	1	(25)	3	(75)

Percentages refer to the total number of patients within treatment groups.

Table 2 One-way analysis of covariance. Improvement mean scores for the first and second fortnight adjusted for the initial severity scores and *F* ratios from one-way analysis of covariance obtained from fast and slow acetylators who completed the trial

	Fast			n	Slow		
	First fortnight	Second fortnight			First fortnight	Second fortnight	n
Phenelzine	+0.21	+0.05	15		+0.45	+0.19	17
Placebo	-0.16	+0.00	10		-0.15	-0.35	17
<i>F</i> ratio	0.81	0.03			4.45	2.03	
<i>P</i>	0.38	0.86			0.04*	0.16

Figure 1 Two-way analysis of covariance for clinical improvement by treatment and acetylator phenotype adjusting for initial level of severity. Initial level of severity is represented by base line. Δ phenelzine and slow ($n = 17$); $\bullet$ phenelzine and fast ($n = 15$); $\square$ placebo and slow ($n = 17$); $\circ$ placebo and fast ($n = 10$).

and fast acetylators in respect of age, sex, duration of illness or dosage of diazepam over the trial period.

Clinical rating

Table 2 presents the results from a one-way analysis of covariance within fast and slow acetylators, for phenelzine and placebo treated patients who did not drop out. There is a significant improvement in the first fortnight, but not in the second fortnight, of treatment with phenelzine, within the group of slow acetylators.

A two-way analysis of covariance on this data (Figure 1) indicates that phenelzine causes significantly more improvement than placebo treatment after 2 weeks, but no significant difference was observed between the fast and slow acetylators. After a further 2 weeks of treatment at a higher dose there was no significant difference in improvement between treatments or between phenotypes.

Side effects

There were no statistically significant differences either between phenotypes or between treatments in the numbers of patients complaining of side effects or in the mean number of side effects for the patients who completed the trial. This applies for the total sample including patients who dropped out.

In considering the patients who did not complete the trial, seven of the eight patients who were withdrawn because of side effects were receiving phenelzine. Of these seven, one fast acetylator and two slow acetylators were withdrawn in the first fortnight and four slow acetylators were withdrawn after the dose was increased from 45 mg to 75 mg.

The ratio of slow to fast acetylators treated with phenelzine who were withdrawn because of side effects is not significantly different from that in the rest of the sample.

Biochemistry

Figures 2a, b, c and d show the mean adjusted changes for the four biochemical measures adjusted for the pretreatment values over the four week treatment period for the patients who did not drop out. In all of Figure 2, movement upwards is consistent with monoamine oxidase inhibition.

Figure 2a, illustrating the mean adjusted changes for MAO activity, shows that only a treatment effect is significant. Acetylator phenotype status appears to contribute very little to the degree of enzyme inhibition by phenelzine and treatment with placebo has very little effect on MAO activity.

The mean adjusted changes in blood 5-HT (Figure 2b) are a consequence of a significant interaction between the phenotype status and treatment. The significance of this interaction decreases as treatment progresses so that by the fourth week the interaction is no longer significant. The placebo group of patients appear to respond very slightly, irrespective of whether they are fast or slow acetylators, whereas the blood 5-HT for the fast acetylators is, in the first two weeks, decreased. It increases in the second two weeks to give the usual picture of increased 5-HT levels after monoamine oxidase inhibition. The slow acetylators, on the other hand, build up to a maximum concentration of 5-HT by the second week and by the end of treatment appear to have adapted to the monoamine oxidase inhibition by phenelzine, as indicated by the return to normal levels of the blood 5-HT values.

Figure 2 Two-way analysis of covariance for change in (a) MAO, (b) 5-HT, (c) 5-HIAA, and (d) VMA by treatment and acetylator phenotype adjusting for differences in initial values. Initial values are represented by base line. Δ phenelzine and slow ($n_{a,b,c,d} = 8, 11, 16, 16$). $\bullet$ phenelzine and fast ($n_{a,b,c,d} = 5, 9, 11, 11$). $\square$ placebo and slow ($n_{a,b,c,d} = 13, 17, 15, 15$). $\circ$ placebo and fast ($n_{a,b,c,d} = 9, 9, 10, 10$).

Time (weeks)	1	2	3	4
Treatment effect	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Acetylator phenotype effect	—	—	—	—
Interaction effect (between treatment and acetylator phenotype)	—	—	—	—

	1	2	3	4
Treatment effect	NS	NS	$P < 0.10$	$P < 0.25$
Acetylator phenotype effect	NS	$P < 0.25$	NS	NS
Interaction effect (between treatment and acetylator phenotype)	$P < 0.025$	$P < 0.05$	$P = 0.05$	NS

Time (weeks)	1	2	3	4
Treatment effect	$P < 0.10$	—	—	—
Acetylator phenotype effect	—	—	$P < 0.25$	$P < 0.005$
Interaction effect (between treatment and acetylator phenotype)	—	$P < 0.10$	—	—

	1	2	3	4
Treatment effect	$P < 0.10$	—	$P < 0.10$	—
Acetylator phenotype effect	—	$P < 0.025$	$P < 0.25$	$P < 0.10$
Interaction effect (between treatment and acetylator phenotype)	—	—	$P < 0.25$	—

In view of this interaction, these data for blood 5-HT were subjected to a further statistical analysis to determine the level of significance of the treatment or acetylator phenotype effects separately. As can be seen from Table 3a, within the placebo treated group there are no significant differences in blood 5-HT values between fast and slow acetylators whereas in the phenelzine treated group in the first 2 weeks of treatment the difference between fast and slow acetylators is significant. Within the fast acetylators (Table 3b) in the first week of treatment there is a significant difference between phenelzine treatment and placebo treatment and in the group of slow acetylators in the second and third week, the values of 5-HT are significantly different between phenelzine and placebo.

These changes in blood 5-HT concentrations are not reflected by related changes in the urinary excretion of 5-HIAA (Figure 2c). There is a strong phenotype effect in the fourth week of treatment but unexpectedly, the change in 5-HIAA excretion is an increase in fast acetylators which was present for both phenelzine and placebo treated patients. The tendency towards an increase can be seen in the third week though there is a slight suggestion of a treatment effect in the first week. Again unexpectedly the largest 5-HIAA excretion change appears to be an increase in placebo treated patients. These increases in 5-HIAA are not consistent with monoamine oxidase inhibition.

Figure 2d represents the mean adjusted changes for urinary VMA throughout the 4 weeks of treatment. The urinary VMA decreases in both groups of phenelzine treated patients which is consistent with monoamine oxidase inhibition, and it decreases more in the slow acetylators than in the fast acetylators which is the hypothesized difference in keeping with the acetylator hypothesis. However, these changes are not significant. There is a significant acetylator effect in the second week.

Discussion

The purpose of this analysis is to determine the relevance of acetylator phenotype as a prognostic indicator for treatment with phenelzine and to examine the indirect evidence that phenelzine may be acetylated. Previous indications were conflicting, in that slow acetylation had shown positive correlations with either a high number of side effects (Evans *et al.*, 1965) or a good outcome (Johnstone & Marsh, 1973).

Although the methods of determining acetylator phenotype differed, the distribution of fast and slow acetylators in our group of patients was comparable with that of Evans *et al.* (1965) and Tiitinen (1969).

The results from this study differ from those obtained in the main trial (Mountjoy *et al.*, 1977) where there were no treatment differences using the principal component of improvement at either two or four weeks. The difference between this sample of patients and the patients in the main trial was that they agreed to have their acetylator status determined. There is thus, a possibility that the response of patients for whom no acetylator phenotype could be determined, differs from the response of the patients in the present study. This hypothesis is being investigated.

In a three week trial period, Johnstone & Marsh (1973) found a significant difference in improvement between phenelzine and placebo treated patients who were slow acetylators but no such significant difference was found between patients who were fast acetylators. Within acetylator phenotypes, our findings in relation to improvement at 2 weeks parallel these observations. However, when our analyses were extended to consider the effects of acetylator phenotype and treatment by a two-way analysis of covariance, there was no suggestion of an independent phenotype effect or of an interaction between treatment and phenotype status at 2 weeks. The

Table 3 Adjusted change means at weekly intervals adjusted to account for initial values, and *F* ratios from a one-way analysis of covariance using initial and weekly 5-HT values from (a) placebo and phenelzine treated patients and (b) fast and slow acetylators

(a)	Phenelzine treated					Placebo treated				
	Week				n	Week				n
	1	2	3	4		1	2	3	4	
Fast	-0.16	-0.15	+0.03	+0.17	6	-0.05	-0.02	+0.00	-0.05	9
Slow	+0.03	+0.27	+0.20	+0.07	11	+0.05	-0.01	+0.00	+0.00	12
F ratio	6.27	5.504	3.45	0.5		1.17	1.18	3.29	2.19	
P	0.03*	0.03*	0.08	0.49		0.29	0.29	0.08	0.16	
(b)	Slow acetylators					Fast acetylators				
Phenelzine	+0.03	+0.12	+0.12	+0.02	11	-0.15	-0.14	+0.05	+0.19	6
Placebo	+0.03	+0.06	+0.01	+0.00	12	+0.02	+0.00	+0.06	-0.01	9
F ratio	0.89	5.75	13.97	1.08		8.20	1.90	0.02	1.08	
P	0.36	0.03*	0.001*	0.32		0.01*	0.19	0.89	0.32	

treatment effect, on the other hand in this analysis was significant. At 4 weeks, there were no significant effects.

Differences in drug effect indirectly due to phenotype may be more genuinely deduced from results comparing the differences between acetylator phenotypes as was done by Johnstone & Marsh (1973) and Johnstone (1976). These authors analysed two measures of improvement, namely final scores and difference between initial and final scores both of which are uncorrected for initial scores. Johnstone & Marsh (1973) found the first but not the second, and Johnstone (1976), the second but not the first, significantly different between the slow and fast acetylators. Comparing the data between acetylator phenotypes in the present study indicated no significant difference in improvement scores.

These results do not agree with those predicted on the basis of the acetylator hypothesis and cast doubt on its relevance to effective therapy with phenelzine.

The absence of a difference in side effects, again not a predicted result, may be a consequence of the virtual absence of complaints of severe side effects in our sample, or more probably due to the diagnostic difference between our patients and those in the group studied by Evans *et al.* (1965).

The capacity of phenelzine to inhibit MAO is reflected in the maximal inhibition of blood MAO in the first sample. The absence of any difference in monoamine oxidase inhibition between the phenotypes receiving phenelzine may be due to the fact that this sample was taken seven days after the beginning of treatment. Thus, any interaction between treatment and acetylator phenotypes on the inhibition of MAO could be short lasting.

The build up of amine, on the other hand, takes at least 1 week for slow acetylators and 2 weeks for fast acetylators, and significant differences between the concentration for each group of patients appears to have some dependency on acetylator phenotype. The temporal nature of the interaction effect on 5-HT concentrations lends substance to the possibility that the

contribution of acetylator phenotype is short lasting.

The actual decrease of 5-HT in the first 2 weeks in fast acetylators receiving phenelzine, suggests that there is some mechanism apparently associated with fast acetylation which either increases the metabolism or inhibits the synthesis of the amine. Phenelzine, at a dosage of 45 mg/day, does not elicit, in fast acetylators, an increase in blood 5-HT concentration consistent with its property as a MAOI. However, the change of effect of phenelzine in the third and fourth weeks, for fast acetylators, may be attributed to the increased dosage and the presumed functional increase in the concentration of phenelzine. Thus, it is evident that whatever the mechanism by which phenelzine is metabolized to prevent it acting as a MAOI in fast acetylators, it can be overloaded by administering higher doses. If higher doses are therapeutically more effective (Dunleavy & Oswald, 1973) then the importance of acetylation as a metabolic route diminishes.

Thus, the picture emerging from the biochemical data, appears, in some respects to reflect the known property of phenelzine as a MAOI. However, the biochemical responses in slow and fast acetylators are not in the directions predicted on the basis of the acetylation hypothesis and suggest that acetylation may not be the only enzyme activity which is distributed polymorphically between fast and slow acetylators (Marshall, 1976).

We have found no evidence that acetylator phenotype is a predictor of successful clinical response to treatment with phenelzine and consider that acetylator phenotype should not be regarded as a prognostic indicator of clinical importance.

The authors would like to thank the Mental Health Research Fund, and the Area Health Authority (Teaching) for financial support, William R. Warner for the supply of phenelzine and matching placebo tablets, Margaret M. Armstrong and George T. Waddell for technical assistance and the consultants who allowed their patients to take part in this study.

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(Received August 22, 1977)