The effect of minocycline on potassium leakage from red cells: a study of the genetics and relationship to vestibular adverse reactions

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SUMMARY Minocycline causes an increased leakage of potassium from erythrocytes. This effect has been quantified and found to be highly repeatable but highly variable between subjects. The variability in the population is unimodal. Random family studies show no correlation between spouses and the heritability is 0.63. The in vitro minocycline effect in 11 subjects who had experienced vestibular adverse reactions on minocycline were shown to be randomly distributed in the population.

It has been known for some time that several macrocyclic antibiotics are capable of binding to mammalian erythrocytes¹ and that some drugs cause selective leakage of ions across the red cell membrane.² In 1976 Kornguth and Kunin,³ while investigating the red cell as a possible reservoir of administered antibiotic, noted that minocycline, a highly lipophilic⁴ tetracycline antibiotic, moved freely across human red cell membranes and showed considerable binding to the membrane itself. A later study⁵ reported an in vitro leak of potassium from red cells when incubated with minocycline. This effect was dose related and not associated with increased haemoglobin leak at the minocycline concentrations used, but was nevertheless considered prehaemolytic in that, at higher concentrations, haemolysis did occur. It was not related to alteration in Na/K ATPase activity. During this investigation a subject was discovered who had a markedly augmented potassium leakage with minocycline. On testing other family members, an autosomal dominant mode of inheritance was suggested. No obvious structural or biochemical differences in the proband's red cell membranes were found.

Minocycline was introduced in 1972 with the advantages of a long half-life and high tissue penetration.⁶ Reports since 1974,^{7–12} however, have suggested a high incidence of vestibular side effects although there has been some disagreement as to the extent of the problem.^{13–15} These adverse reactions have appeared more frequently in women^{12, 16} and are probably dose related.^{17, 18} There have been no reports of permanent vestibular damage and

symptoms have usually resolved within 48 hours of cessation of minocycline. Although the administration of near-maximal non-lethal doses of minocycline to guinea pigs has been reported to cause damage to vestibular sensory cells,^{19, 20} clinical testing of auditory and vestibular function in patients receiving minocycline has revealed either no²¹ or minimal²² vestibular changes. It is likely, therefore, that minocycline produces its vestibular effects by a reversible functional change rather than by structural damage.

Menière's disease is now thought to be related to fluid movements in the semicircular canals and can be provoked by osmotically active agents.²³ It is possible, therefore, that by binding to semipermeable membranes in the inner ear, minocycline might exert its vestibulotoxic effects by causing changes in the liquid volume and ionic concentrations in the vestibular system. If this were the case, it is possible that the increased membrane permeability induced by minocycline in vitro (as measured by potassium leakage from red cells) might correlate with the occurrence of vestibular symptoms after minocycline administration in vivo.

The aims of the present research were, therefore, to investigate the genetic control of the potassium leakage effect of minocycline on erythrocytes and to see if there was any association between the vestibular adverse reaction to minocycline and a particular phenotype.

Materials and methods

Minocycline hydrochloride was obtained from Lederle Laboratories, Gosport, Hants. 2,5 p-chloro-mercuribenzenes (PCMBs), choline chloride, and

cysteine hydrochloride were obtained from Sigma Chemical Co.

Tests were carried out with blood from: (1) random healthy volunteers (age range 17 to 60); (2) 30 family units. These were two generation units comprising healthy white British subjects (age range 9 to 55); and (3) 11 subjects whose names had been obtained from the Committee on Safety of Medicines as patients who had suffered vestibular adverse effects from minocycline therapy.

The random volunteers were studied in the early part of the project with a slight overlap with the family members who were studied in the later phase of the project. For each family, the blood samples were taken on one visit to the home and taken through the laboratory procedure together. The adverse reaction subjects were studied in small groups interspersed between the family units.

TEST PROCEDURE

The method was a modification of that described by Kornguth and Kunin.⁵ A total of 20 ml of blood was taken from each subject into lithium heparin tubes. All subsequent procedures were carried out at +4°C. The blood was centrifuged at 2700 rpm for 10 minutes, the plasma and buffy coat then being discarded. The packed red cell fraction (approximately 8 ml) was washed three times in 0.01 mol/l sodium phosphate buffer, pH 7.4, containing 135 mmol/l NaCl and 5 mmol/l KCl, and was re-suspended in 8 ml of the same buffer. Thereafter, 3.6 ml of this suspension was transferred into each of four bottles (two as controls, two for minocycline addition). Into the two duplicate control bottles, 0.4 ml of buffer was added, and into the two test bottles 0.4 ml of a solution of the same buffer to which minocycline, at a concentration of 1 mg/ml, had been added, giving a final concentration in the red cell suspension of 100 µg/ml. A sample of each suspension was removed (Time T_0) for centrifugation and immediate separation of supernatant for subsequent potassium measurement. The remaining suspensions were incubated at 37°C in a shaking water bath for 3 hours. During the incubation the haematocrit of each suspension was measured. At the end of 3 hours' incubation each red cell suspension was immediately centrifuged at +4°C for 10 minutes and the supernatant taken for potassium measurement (Time T_3). Potassium assay was performed on the T_0 and T_3 supernatant samples using an IL 243 flame photometer with automatic diluter (dilution 200 : 1 with deionised distilled water).

CALCULATION OF RESULTS

The potassium efflux from the erythrocytes (K^+ efflux/l

RBC/3 hours) can be calculated by multiplying the rise in supernatant potassium concentration ($[K^+] T_3 - [K^+] T_0$) by a factor of $\frac{1 - \text{haematocrit}}{\text{haematocrit}}$ (when the haematocrit is expressed as a decimal fraction of 1). The mean potassium efflux was calculated for both the duplicate control samples and for the duplicate samples incubated with minocycline. The mean potassium efflux from the control samples was subtracted from the mean potassium efflux with minocycline to give a value of 'minocycline effect' (that is, the potassium efflux/l RBC/3 hours resulting solely from minocycline) for each subject tested.

METHOD OF ASSESSMENT OF EFFECT OF MINOCYCLINE ON POTASSIUM INFLUX INTO ERYTHROCYTES

The experiment to measure potassium influx into red cells, depleted of potassium, from a high extracellular potassium concentration with and without minocycline was modified from a technique described in detail by Garay and Meyer.²⁴ Twice the volume of blood was taken, so that after the initial K^+ depleting procedure, half the samples could be incubated with minocycline and the other half without. Unlike Garay's technique $CaCl_2$ was excluded from the incubation mixture and the K^+ and Na^+ concentrations were reversed. The composition of the incubation mixture was as follows: glucose 10 mmol/l, $MgCl_2$ 1 mmol/l, KCl 140 mmol/l, NaCl 10 mmol/l, all dissolved in 1 l of 2.5 mmol/l sodium phosphate buffer. When minocycline was added it was at a concentration of 100 µg/ml.

PROCEDURE WITH BLOOD SPECIMENS FROM ADVERSE REACTIONS SUBJECTS

While visiting subjects who had suffered vestibular effects on minocycline, it was necessary to store the blood samples for between 12 to 24 hours before testing could be carried out. Previous experiments showed that if storage was at 4°C to 8°C the results did not differ significantly from results on fresh specimens from the same subjects. Storage was therefore carried out at 4°C to 8°C in a cooled insulated container.

Results

TESTS ON UNRELATED VOLUNTEERS

The 'minocycline effect' on 86 randomly selected healthy volunteers showed wide variation between subjects (fig 1). Repeatability of results was 0.70 (fig 2). There was no significant correlation between

control and 'minocycline effect' (that is, minocycline tubes results minus control tubes results) in this population ($r = 0.028$). The control values showed considerable variability: K efflux/l RBC/3 hours ranged between 0.80 and 2.60 (mean 1.52, SD 0.36).

FIG 1 Frequency distribution histogram of minocycline effect in 96 unrelated white British subjects (mean value $2.27 \pm SD 0.45$).

FIG 2 Repeatability of test procedure. Correlation coefficient $r = 0.70 \pm 0.22$.

FIG 3 Frequency distribution histogram of minocycline effect in 133 members of 30 white British families (mean value $2.67 \pm SD 0.58$).

TESTS ON FAMILY GROUPS

Thirty families were studied. The frequency distribution histogram of 'minocycline effect' of all family members is shown in fig 3. The variability observed in the family study (coefficient of variation 21.7%) was very similar to that in the unrelated subjects (19.8%), but the mean minocycline effect in the family study was significantly higher. Analysis of the results for age and sex differences showed no significant correlation between these parameters and 'minocycline effect' (tables 1, 2).

There was a significant correlation between control and 'minocycline effects' (table 3, row 1).

Considering control values, there was a significant correlation between the parental values (table 3, row 2), and there was also a highly significant regression of mean offspring values on mid-parent values (table 3, row 4).

Considering 'minocycline effect', there was no correlation between parents (table 3, row 3, fig 4), but there was a highly significant regression of mean offspring values on mid-parent values (table 3, row 5). The last regression ($0.63 \pm SE 0.18$) is an estimate of 'heritability' $\frac{V_A}{V_P}$ (see Falconer²⁵ and fig 5).

TESTS ON MINOCYCLINE-VESTIBULAR ADVERSE REACTIONS SUBJECTS

Eleven subjects (six female and five male) who were interviewed and examined personally by BGL were judged by their histories to have experienced vestibular type side effects following minocycline administration between 1 and 6 years before interview. Minocycline had been prescribed in a dosage of either 200 mg stat and 100 mg bd, or simply 100 mg bd, for a variety of infections, mainly of the upper respiratory tract and urinary tract. None of the subjects had suffered significant vertigo before minocycline administration. A causal relationship was assumed in these 11 subjects because of the

TABLE 1 Minocycline effect in subjects of different ages

Type of subject	No of subjects	Age range (yr)	Minocycline effect	
			Mean	$\pm SE$ of mean
Parents	60	35-55	2.73	0.06
Offspring	73	9-25	2.63	0.07

TABLE 2 Minocycline effect in male and female subjects

Sex	No of subjects	Minocycline effect	
		Mean	$\pm SE$ of mean
Male	39	2.38	0.06
Female	39	2.50	0.07

TABLE 3 Relationship between control potassium efflux and minocycline effect on potassium efflux in family members

	X	Y	n	b	a	r	p
1	Control	Minocycline effect	133	0.84	1.45	0.46	<0.001
2	Father control	Mother control	30	0.46	0.90	0.42	<0.05
3	Father minocycline effect	Mother minocycline effect	30	-0.11	2.98	-0.13	>0.10
4	Mid-parent control	Mean offspring control	30	0.62	0.42	0.78	<0.001
5	Mid-parent minocycline effect	Mean offspring minocycline effect	30	0.63	0.91	0.54	<0.01

n = no of pairs of observations
 b = regression coefficient (slope)
 a = intercept (in units of y)
 r = correlation coefficient

onset of symptoms within 72 hours of starting therapy, and their cessation, usually within 24 hours, but always within 48 hours of stopping minocycline. When seen by BGL no subject had any symptoms or clinically detectable hearing impairment, loss of

balance, incoordination, or nystagmus. The severity of the vestibular symptoms which these patients had experienced varied considerably. Symptoms were assessed as mild if they caused little restriction of normal activities, moderate if normal activities were markedly restricted, and severe if the patient was virtually bed or chair bound during the episode. No statistically significant correlation was found between severity of symptoms and the in vitro minocycline effect (table 4). The overall mean of the 11 minocycline effects did not differ significantly from the mean of results from family members (table 5), and the distribution of the individual values was similar to that of the whole population tested (fig 6).

FIG 4 Correlation between minocycline effects in parents of families ($r = -0.13$).

FIG 5 Minocycline effect. Regression of mean offspring values on mid-parent values in 30 white British families.
 Regression coefficient = 'heritability' $\frac{V_A}{V_P} = 0.63 \pm SD$
 of scatter of points about the regression line of 0.32.

POTASSIUM INFLUX EXPERIMENT

When the potassium content of the red cells was reduced and the extracellular potassium increased to the extent that the passive flux of potassium was into the cell (that is, in the same direction as the

TABLE 4 Minocycline effect in patients who suffered vestibular adverse reactions of different grades of severity

Grade of severity of vestibular adverse effect	No of subjects	Minocycline effect	
		Mean	$\pm$ SE of mean
Mild	5	2.85	0.29
Moderate	4	2.55	0.28
Severe	2	2.33	0.14
Total	11	2.65	0.16

TABLE 5 Minocycline effects in patients who suffered vestibular adverse reactions compared with healthy family members

Class of subjects	No of subjects	Minocycline effect	
		Mean	$\pm$ SE of mean
Family members	133	2.67	0.05
Patients who suffered vestibular adverse reactions	11	2.65	0.16

FIG 6 Frequency distribution histogram of 229 healthy white British subjects (pooling random subjects and family members) with results of 11 patients who suffered vestibular adverse effects (■).

FIG 7 Potassium influx into erythrocytes with (●) and without (○) minocycline (mEq/l).
With minocycline $r=0.97$, slope $13.10 (\pm SE 1.05)$, intercept 7.61 .
Without minocycline $r=0.97$, slope $8.20 (\pm SE 0.59)$, intercept 11.71 .

flux produced by the active Na/K pump) then it was found that, in the presence of minocycline, the nett influx of potassium was greatly increased (fig 7). This suggests that minocycline acts directly to affect red cell membrane permeability rather than inhibiting K^+ transport (see Discussion).

Discussion

Because of the high intracellular potassium concentration in the red cell there is always a passive efflux but the steady state is maintained by an active

influx via the Na/K pump. The nett efflux of potassium from red cells observed with minocycline could, therefore, either result from inhibition of the Na/K pump mechanism or an increase in cell membrane permeability allowing more rapid leak of potassium than active transport could cope with. Inhibition of active transport seems unlikely as it has already been shown⁵ that minocycline does not reduce Na/K ATPase activity. The potassium influx experiment was designed to test this further by a different approach. By reducing the intracellular potassium to approximately 10 mmol/l and increasing extracellular potassium to 140 mmol/l, the direction of passive K^+ flux was changed from efflux to influx. The nett K^+ influx measured, therefore, would be the additive effects of both active and passive transport. If minocycline exerted its effect by inhibition of active transport, then in this experiment, addition of minocycline would have reduced the nett K^+ influx. In fact, the opposite was the case, confirming the hypothesis that minocycline acts by directly affecting membrane permeability. Because minocycline is highly lipophilic⁶ it is probable that its binding site on the cell membrane is in the lipid fraction, but this has yet to be confirmed. It is for these reasons that the inherited component of the minocycline effect is likely to be the result of variations in cell membrane lipid structure or content.

The frequency distribution of the minocycline effect is approximately normal in both random subjects and family members. One unexplained fact is that although the variability within the group of unrelated subjects is similar to that within the group which includes all family members, the mean minocycline effect of the former is significantly lower ($p<0.05$) than that of the latter. No satisfactory explanation has been found for this. There was neither any discernible change in the phenotyping procedure nor was there a change in the batch of minocycline hydrochloride used. There may, however, have been some unidentified change in the laboratory conditions between the first phase of the project in which most of the random subjects were studied and the second phase of the project during which the family members and adverse reactions patients were studied.

Repeatability²⁵ was 0.70 indicating a stability of the phenotype. There was no correlation between control and minocycline effects in random subjects. Such a correlation was, however, found in family members. This difference might be attributable to the fact that the latter were processed as individual family units.

The correlation between parental control values suggests a significant environmental influence. It is

possible that the stronger regression of mean offspring control values on mid-parent control values may indicate the existence also of a measure of genetic control on this measurement.

There was no significant correlation between parental values of 'minocycline effect' which suggests no significant environmental influence on this measurement. The regression of mean offspring 'minocycline effect' upon mid-parent 'minocycline effect' gave a regression coefficient of 0.63 indicating that 63% of the phenotypic variance is the result of the additive effects of genes.

During this study no person was encountered who possessed the phenotype described by Kornguth and Kunin⁵ characterised by an exceptionally large leakage of potassium from red cells under the influence of minocycline.

This study has revealed no correlation between a person's 'minocycline effect' and his propensity to develop vestibular symptoms after minocycline administration. The hypothesis that the vestibular effects may be the result of potassium concentration changes generally in the body, including the inner ear, is not supported by the present experiment. Minocycline may exert its vestibulotoxic effects by a mechanism totally unconnected with its capacity to bind to cell membranes and cause selective ion 'leaks', or it is possible that there are other functional changes caused by minocycline binding which do not correlate with the particular functional change measured in this study, namely potassium leakage. These possibilities will require further work.

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