Family study of antipyrine clearance

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Abstract

Antipyrine clearance was measured in 208 healthy volunteers from 78 families. After the values had been corrected for weight and sex, antipyrine clearance was observed to be significantly correlated between siblings ($r = 0.590$) and between spouses ($r = 0.320$), but not between parents and their offspring. After the clearance values had been corrected for tobacco and oral contraceptive use, there was still no significant correlation between parents and offspring.

These results are incompatible with the hypothesis that antipyrine clearance is primarily determined by genetic factors and indicate that environmental influences predominate.

Introduction

Many widely used drugs undergo metabolism by microsomal oxidation. Rates of microsomal oxidation vary widely from one individual to another,[1] however, and this is reflected by differences in plasma clearances, steady-state plasma concentrations during multiple doses, and the biological effects of drugs which are predominantly eliminated by this route.[2]

The 4-hydroxylation of debrisoquine[3] and the N-oxidation of sparteine[4] are under polymorphic genetic control, but the plasma clearance values of many drugs undergoing microsomal oxidation are continuously distributed in a population.[5] In particular, the clearance of antipyrine, which has been widely used as a "model" substrate for investigating oxidation in man, shows considerable interindividual variation and is continuously distributed in a population. Both genetic[6] and environmental[7 8] factors have been suggested as major determinants of antipyrine clearance and we therefore undertook a family study to assess the relative contributions of each.

Methods

Volunteer families were recruited from university and hospital staff and employees of local industries. Ethical approval was obtained from the ethical committee of Newcastle Area Health Authority (Teaching). Two hundred and eight individuals (106 women) from 78 families agreed to participate and fulfilled the criteria for inclusion in the study. They ranged in age from 15 to 72 years; all were healthy, none were taking any drugs apart from oral contraceptives, and all had normal haemoglobin concentrations, full blood count, serum electrolyte and urea concentrations, and liver function values (bilirubin, alkaline phosphatase, aspartate transaminase). Each subject's height and weight were measured and consumption of medicines, coffee, tea, alcohol, tobacco, and oral contraceptives was recorded. Blood was withdrawn for determining 24 genetic polymorphisms (blood group, red cell enzymes, and HLA status), and 5 ml saliva was obtained for determining secretor status. In all cases the stated family relationship was compatible with these polymorphic markers. The coffee-tea[9] index was calculated thus: cups of coffee per day + 0·6 × cups tea per day.

Antipyrine clearance was determined from six serial 5-ml saliva samples obtained 3, 5, 8, 13, 24, and 32 hours after the administration of 600 mg antipyrine (Sigma), prepared without excipient, in gelatin capsules. Preliminary studies showed the bioavailability to be 0·93 ± SE 0·02. Saliva was stored at −20°C before antipyrine was assayed (in duplicate) by gas liquid chromatography.[10] A duplicate four-point standard curve was performed with each analytical batch, together with an external quality control. Analyses were accepted only when the quality control sample fell within ±4%.

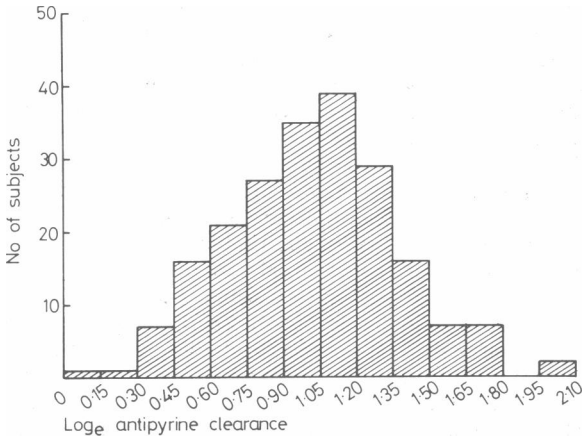
The elimination rate constant (k_{el}) was determined by least squares regression analysis of the terminal exponential decay of salivary antipyrine concentration. The mean correlation coefficient for this regression was 0·992 ± SD 0·009. The "zero-time" (Co) salivary concentration was calculated from the intercept of the regression line, and clearance (l/h) was calculated thus:

$$\text{Clearance} = \frac{\text{dose}}{\text{Co}} \times k_{el}$$

where clearance is the volume of distribution volume cleared of drug per unit time.

Results

The mean (± SD) antipyrine clearance in the population was 2·96 ± 1·07 l/h; although continuous, the distribution was skewed but was normalised by logarithmic (base e) transformation (see figure). All statistical calculations were therefore performed using the logarithm of clearance, but the mean and standard deviations have been converted back to actual measure for clarity. Log antipyrine clearance was significantly correlated with weight ($r = 0.289$; $t = 4.331$; $df = 206$; $p < 0.001$) and all clearances were therefore adjusted to the mean weight of the population (68·1 kg). Weight-corrected clearance correlated with neither height nor chronological age but was significantly higher ($t = 2.733$; $p < 0.001$) in men (2·97 ± SD 1·40 l/h) than in women (2·62 ± 1·37 l/h). All clearances were therefore also corrected for sex.



Frequency distribution of $\log_e$ antipyrine clearance in 208 subjects living on Tyneside.

Antipyrine clearance corrected for weight and sex was significantly less ($t = 2.425$; $p < 0.05$) among women who used oral contraceptives (2·40 ± 1·35 l/h) than among those who did not (2·89 ± 1·37 l/h). After correcting for oral contraceptive use we found that smoking had a striking influence on antipyrine clearance (table I). After correcting for weight, sex, oral contraceptive use, and smoking we found that

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antipyrine clearance did not correlate with either alcohol consumption or the coffee-tea index.

The correlation coefficients between family members' antipyrine clearances are shown in table II. There was no significant correlation between parents and offspring for either uncorrected antipyrine clearance, clearance corrected for the two biological variables (weight and sex), or clearance corrected also for the two environmental variables (contraceptive use and smoking). By contrast, antipyrine clearance corrected for weight and sex was significantly correlated between husband and wife and between siblings (table II).

TABLE I—*Mean (± SD) antipyrine clearance in smokers and non-smokers*

Smoking status (cigarettes/day)	No of subjects	Uncorrected antipyrine clearance (l/h)	Antipyrine clearance corrected for weight, sex, and oral contraceptive use (l/h)
0	151	2·65 ± 1·39	2·68 ± 1·36
1–9	12	3·07 ± 1·40	3·03 ± 1·38
10–19	23	2·86 ± 1·36	3·23 ± 1·3[illegible]
≥20	16	3·68 ± 1·45	3·76 ± 1·36
Pipe	6	3·36 ± 1·53	3·05 ± 1·66

Discussion

The sample we studied was broadly representative of the Tyneside population, and the blood group and other gene frequencies were not significantly different from those reported in the region.[14] The mid-parent:offspring correlations for height and weight ($r = 0{\cdot}445$ and $r = 0{\cdot}346$, $p < 0{\cdot}01$ and $p < 0{\cdot}05$ respectively) were similar to those previously reported, as were estimates of heritability derived from them. Thus, our sample possessed polymorphic and polygenic characters typical of the population from which it was drawn.

The antipyrine clearances observed in our population were similar both in size and in their frequency distribution to those reported previously.[7 11 12] The influence of sex,[13] smoking,[7 11 15] and oral contraceptives[7 16] on antipyrine clearance has also been observed in other investigations. In contrast to other studies,[11 13] we failed to detect a significant correlation between age and antipyrine clearance. The effect of age on antipyrine clearance is small,[11] however, and was probably obscured in our population because of its relatively narrow age range, which was skewed towards the younger age group. The clearance of antipyrine in our population was thus influenced by the same readily detected biological and environmental factors that have been observed by others.

The absence of significant correlations between mid-parent and offspring, father and offspring, or mother and offspring (table II) indicates that the genetic contribution to the variance in antipyrine clearance is small. Any heritability estimates derived from the regression coefficients will therefore not be significantly different from zero; their modal value is about 20%. The significant correlation of antipyrine clearance between spouses may be due either to assortative mating (the tendency of like to marry like) or to the sharing of common environmental factors. Although assortative mating has been shown in human populations for many physical characteristics,[17] this seems an unlikely explanation for our observation unless there is an association between antipyrine clearance and some other attribute relevant to the choice of spouse. We conclude that the resemblance between husband and wife is due to shared environmental factors.

The importance of environmental factors as a major determinant of the variance in antipyrine clearance is further suggested by our observations among siblings (table II). The significant sib-sib correlation after correction for weight and sex indicates either environmental or genetic influences, the latter being due to both additive and dominance effects. If heredity is indeed responsible for the magnitude of the sib-sib correlations compared with those of parent and offspring this would indicate a pronounced dominance effect. This would be unusual in a quantitative character, and, moreover, the form of the distribution of antipyrine clearance in the population (see figure) appears inconsistent with such an effect. Furthermore, the apparently higher sib-sib correlation among siblings living together ($r = 0{\cdot}764$; $n = 5$) than in those living separately ($r = 0{\cdot}356$; $n = 16$), though not significant, suggests an environmental interpretation. Thus, the absence of significant parent-offspring correlations, the significant interspouse and intersibling correlations, combined with the form of the distribution of clearances, all point to environmental, rather than genetic, factors as the primary determinants of clearance variation.

This conclusion contradicts that reached by Vesell and Page.[6] From a small twin study they concluded that 98% of the variance could be accounted for by genetic factors. In the light of our more extensive study, covering families of two generations, it seems likely that the concordance among twins observed by Vesell and Page was largely due to shared environment. Our results are not comparable with those of the pioneer study of Whittaker and Evans,[18] who used phenylbutazone as a marker drug in subjects pretreated with phenobarbitone.

Our observations do not provide much information about the nature of the environmental influences on antipyrine clearance. Although correcting for smoking and oral contraceptive use reduced the variance by 9% the remainder was unaccounted for. Dietary protein and carbohydrate,[19] indoles present in vegetables of the brassica genus,[20] insecticides,[21] and environmental pollutants[22] all affect antipyrine clearance. Therefore influences such as these are probably the major source of variation in antipyrine clearance in the population as a whole.

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TABLE II—*Correlation coefficients (r) (± SE) and regression coefficients (b) (± SE) for antipyrine clearance between family members*

Family relationship		No of subjects	Clearance (l/h)		Clearance corrected for weight and sex (l/h)		Clearance corrected for weight, sex, contraceptive use, and smoking (l/h)	
x	y		r	b	r	b	r	b
Mid-parent	Offspring	38	0·140 ± 0·159	0·191 ± 0·225	0·127 ± 0·160	0·155 ± 0·201	0·227 ± 0·154	0·344 ± 0·245
Father	Offspring	45	0·226 ± 0·141	0·213 ± 0·140	0·205 ± 0·143	0·192 ± 0·140	0·235 ± 0·141	0·234 ± 0·148
Mother	Offspring	47	0·004 ± 0·146	0·004 ± 0·148	−0·044 ± 0·146	−0·041 ± 0·141	0·079 ± 0·145	0·086 ± 0·161
Sibling	Sibling	21	0·323 ± 0·195	0·420 ± 0·283	0·590** ± 0·142	0·559 ± 0·175	0·376 ± 0·187	0·341 ± 0·193
Husband	Wife	69	0·251* ± 0·113	0·274 ± 0·129	0·320** ± 0·108	0·351 ± 0·127	0·251* ± 0·113	0·270 ± 0·127
Population mean		208	2·79		2·79		2·68	
SD			1·41		1·39		1·35	
Variance			2·00		1·92		1·83	

*p < 0·05; **p < 0·01.

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Serum retinol and the inverse relationship between serum cholesterol and cancer

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Abstract

Several human studies have shown an inverse relation between vitamin A intake (and serum concentrations of retinol and carotene) and cancer. Serum cholesterol concentrations have also been reported in inverse relation to cancer. In a study of 3102 people in Evans County, Georgia, who were followed for over 12-14 years to assess the incidence of cancer there was an inverse association between the risk of cancer and both serum retinol and serum cholesterol concentrations. The data also showed an unexpectedly strong correlation between serum retinol and total cholesterol concentrations.

The inverse relationship with cancer was stronger with serum retinol than with cholesterol, which suggested that the association with cholesterol might be secondary. This suggestion may also explain the cholesterol-cancer association reported in several other cohort studies. Further studies of the relation between serum concentrations of cholesterol, retinol, and carotene and the incidence of cancer are needed.

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Introduction

A large and rapidly expanding body of laboratory evidence shows that retinoids possess striking antineoplastic properties, inhibiting both the transformation and promotional stages of the neoplastic process.[1-3] The epidemiological data have recently been reviewed by Peto *et al.*[4] Two prospective[5 6] and several retrospective dietary studies[7-9] have shown an inverse relationship between vitamin A intake (mainly in the form of carotene) and cancer. Users of vitamin A pills showed a non-significant reduction in the risk of developing cancer.[10] Within wide bounds, blood retinol concentrations are generally little affected by varying vitamin A intake,[4] and excessive intake causes a relatively small increase in serum retinol concentrations.[11] Case-control studies of the relationship between serum retinol and serum carotene concentrations and cancer have consistently shown that serum retinol concentrations are lower in patients with cancer than in controls[12-20] and serum carotene concentrations have also been lower in those studies in which they have been measured.[12-14 18] The question whether low serum values precede the development of cancer or are a consequence of it remains open in these studies. Two prospective studies, however, one in Evans County, Georgia,[21 22] and the second in England,[23] have shown an inverse relationship between the risk of cancer and serum retinol concentrations.

Quite separately interest has grown in the inverse relationship between serum cholesterol concentrations and the incidence of cancer. Several studies have reported an inverse relationship between serum or plasma cholesterol concentrations and cancer outcomes, usually mortality.[24-28] The findings were more apparent for men than women in the three studies that included both sexes.[25 26 28] An Oslo cohort[29] and the three Chicago study cohorts,[30] however, did not show an association between cholesterol concentrations and cancer. The major methodological issue in these studies is whether the cancer produces the reduced cholesterol concentration. Rose and Shipley[31] pre-