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Geographic differences in digoxin inactivation, a metabolic activity of the human anaerobic gut flora

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SUMMARY **The inactivation of digoxin by conversion to reduced metabolites (digoxin reduction products, or DRP), a function of the anaerobic gut flora, was studied in normal volunteers from southern India and the United States. Digoxin was metabolised to DRP by 28 (13·7%) of 204 healthy south Indians in contrast to 67 (36·0%) of 186 New Yorkers ($p<1\times10^{-6}$). Only 1·0% of Indians compared with 14·0% of Americans excreted large amounts of metabolites (>40% DRP) in the urine ($p<1\times10^{-5}$). Of 104 urban Indians, 23 (22·1%) were metabolisers, in contrast with five of 100 rural villagers ($p<0·001$). Within the urban group, digoxin metabolism correlated with education, frequency of animal protein intake, and most significantly, personal income. Organisms capable of reducing digoxin *in vitro* were found with similar frequencies in stool cultures from Indians and Americans. In the cultures of some subjects, DRP production was inhibited at lower dilutions but expressed at higher dilutions. We conclude that variations in drug metabolism between population groups may result from differences in the metabolic activity of the anaerobic gut flora probably mediated by environmentally determined factors.**

It has frequently been postulated that the gut flora of people living in the tropical developing countries of the world differs from that found in inhabitants of industrialised nations, although enumeration of various species in stool cultures has often failed to reveal consistent alterations.[1-5] It is possible that differences in the metabolic actions of the bacterial flora may better distinguish between population groups.

Digoxin is inactivated in some individuals *via* saturation of its lactone ring, which converts the drug to its reduction products (or DRP).[6-8] Patients in whom DRP are formed may require increased doses of the drug.[6,7] Digoxin reduction is mediated by *Eubacterium lentum*, a constituent of the anaerobic gut flora.[8-10] The presence of this species in the colonic flora appears to be necessary, but not sufficient, for metabolism of the drug to occur, since some persons given digoxin fail to make DRP *in vivo*, even though *E lentum* capable of reducing digoxin can be isolated from cultures of their stool in high concentrations.[9-11] Digoxin metabolism *in vivo* may be used as an indicator of differences between individuals in the metabolic activity of the anaerobic gut flora.[8,9,11]

Native born residents of Bangladesh were recently found to metabolise digoxin to DRP much less frequently than Americans.[11] Correlations between digoxin metabolism and income, education, and residence in an urban area of Bangladesh during childhood suggested that environmental factors may play an important role in determining this function of the gut flora. It was speculated that living in rural areas of Bangladesh diminished the likelihood of acquiring a flora that would metabolise digoxin *in vivo*, but this was not proven, because the only Bangladeshis tested in that country were current residents of the capital city. In order to directly test the hypothesis that inhabitants of rural areas in a developing country would have a decreased tendency to inactivate digoxin, and to confirm the findings in Bangladeshis in a genetically unrelated population in a distant area of the Indian subcontinent, we studied digoxin metabolism in normal subjects living in urban

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and rural areas of southern India in comparison with Americans resident in New York City.

Methods

PATIENTS AND SUBJECTS

Two groups of asymptomatic normal adult volunteers from southern India were selected on the basis of their general good health and willingness to participate: (1) 104 Indian born medical personnel (78 men, aged 17 to 60; 71 Hindu and 33 Christian) who were employees or students of the Christian Medical College in Vellore, a town of 150000 inhabitants in Tamil Nadu, south India. All were residents of Vellore, except for 14 who resided in a rural villages and commuted to work at the Medical College. (2) 100 residents (53 men, aged 18 to 85; 98 Hindu, two Muslim) of an agricultural village (population 2300) 15 miles from Vellore who worked as farm labourers or artisans. This village is typical of the rural agricultural regions of southern India and has been the subject of an ongoing non-interventional surveillance programme of diarrhoeal disease patterns since 1964.

In addition, 186 American born medical personnel resident in New York City (NYC) (108 men, aged 21 to 48; 142 whites of European extraction, 16 blacks, 19 Caribbean Hispanics, six Chinese, three Japanese), who volunteered in response to an advertisement for various studies of digoxin metabolism[9 11 12] served as a comparison group.

Information was obtained from all subjects regarding age, sex, race, religion, occupation, previous history of diarrhoea, and antibiotic therapy in recent years. In addition, the Indian subjects were asked in a questionnaire to indicate their income, dietary protein intake, and extent of education. Information was also available for the villagers about previous episodes of illness and antibiotic therapy from the records of the morbidity surveillance. The seven subjects from Vellore who were students or laboratory trainees and therefore unsalaried were excluded from the analyses of correlations between income and other variables.

DIGOXIN ADMINISTRATION

The protocols were approved by the Research and Ethics Committee, Christian Medical College, and the Health Sciences Institutional Review Board, Columbia University. After informed consent was obtained, the subjects were given 0·25 mg digoxin from a single lot of tablets (Burroughs Wellcome, USA) of rapid dissolution rate, and a urine specimen was obtained 24 hours later. An excellent correlation between 24 hour urinary % DRP and that found in single specimens obtained at 24 hours has been shown.[11]

STOOL CULTURES

Stool specimens were obtained from randomly selected volunteers in southern India, including 28 urban and 30 rural residents. An aliquot of the stool sample was frozen at −70° within three hours of passage and transported in the frozen state by air to New York City, where after thawing, serial 10-fold dilutions in brain heart infusion medium containing 10 μg/ml digoxin were incubated under anaerobic conditions for seven days.[9] After incubation, culture supernatants were assayed for digoxin and DRP.

ASSAYS AND STATISTICAL METHODS

Digoxin and DRP in urine and culture media were measured by previously described radioimmunoassays.[13 14] Urine values are expressed as the percentage of the total excretion of digoxin and its metabolites present as DRP. Subjects with >5% urinary DRP are referred to as 'metabolisers' and with ≥40% DRP as 'heavy metabolisers'. Standard methods of statistical analysis were used.[15] In comparisons of percentages, χ^2 values were calculated using correction for continuity[15] and odds ratios were estimated taking into account the relatively small numbers of subjects.[16]

Results

DIGOXIN METABOLISM

Of the 204 subjects studied in southern India, 28 (13·7%) excreted DRP in the urine after the oral dose of digoxin and were therefore classified as metabolisers. In contrast, 67 (36·0%) of the 186 Americans tested in New York City metabolised digoxin to DRP ($\chi^2=25{\cdot}1$, $p<1\times10^{-6}$). Of the 104 subjects studied in Vellore, 23 (22·1%) were metabolisers, in contrast with five (5·0%) of the 100 volunteers resident in the rural villages ($\chi^2=11{\cdot}2$, $p<0{\cdot}001$). The percentage of metabolisers among the American subjects (36·0%) was significantly higher than that in the rural and urban Indian groups ($p<1\times10^{-7}$ and <0·02, respectively). There were only two heavy metabolisers (>40% DRP excretion), both in the urban group, among the 204 Indians (1·0%), in contrast with 26 (14·0%) of the 186 Americans ($\chi^2=22{\cdot}8$, $p<1\times10^{-5}$). Levels of DRP in the urine in the American metabolisers (Fig. 1) were significantly higher than in those from India (mean (1 SD) % DRP excretion, 34·8 (17·4)% *versus* 20·3 (11·4)%, $p<0{\cdot}001$). The extent of digoxin metabolism (Fig. 1) appeared to be greater among the urban metabolisers (mean % DRP, 22·0 (11·6)%) than in those from the village (12·5 (6·1)%) but the difference was not significant (0·10> p>0·05).

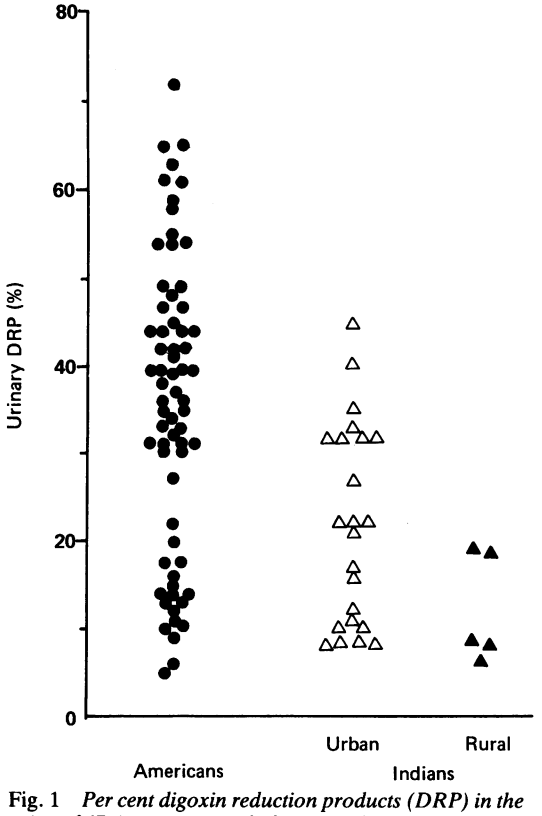

Fig. 1 *Per cent digoxin reduction products (DRP) in the urine of 67 American metabolisers resident in New York City, 23 urban Indian metabolisers living in Vellore and five Indian metabolisers resident in rural villages 24 hours after an oral 0·25 mg dose of digoxin.*

DIET AND SOCIOECONOMIC FACTORS IN RURAL AND URBAN SOUTHERN INDIANS

The mean monthly income of the urban subjects, 1076 (733) Rupees (Rs) was much higher than that of the villagers, 177 (126) Rs ($p<0·0001$). There were also marked differences between the two groups in education beyond secondary school, frequency of ingestion of dietary items containing animal protein (eggs, fish, or meat) and the number of strict lacto-vegetarians in the urban group (Table 1).

Table 1 *Differences in income, education, and animal protein intake between urban and rural Indian subjects*

	Urban		*Rural*		
	n/total	*%*	*n/total*	*%*	*p<*
Income ≥200 Rs/month	90/104	86·5	29/100	29·0	1×10^{-7}
Education beyond secondary school	63/104	60·6	1/100	1·0	1×10^{-7}
Intake of animal protein other than milk at least once/week	82/104	78·8	40/100	40·0	1×10^{-6}
Strict lactovegetarians	8/104	7·7	23/100	23·0	0·005

Table 2 *Income, education and animal protein intake as predictors of digoxin metabolism in residents of Vellore*

	Number of metabolisers/total (%)	*Odds radio**	*p<*
Income			
≥1000 Rs	18/44 (40·9)	6·0	0·001
<1000 Rs	5/52 (9·6)		
Education			
Postgraduate	15/36 (41·7)	5·1	0·002
Less than postgraduate	8/68 (11·8)		
Animal protein intake other than milk			
≥6 days/week	16/38 (42·1)	5·8	0·001
<6 days/week	7/66 (10·6)		
Vegatarian status			
Strict lactovegetarian	1/8 (12·5)	0·7	NS
Other	22/96 (22·9)		

*Likelihood of being a digoxin metaboliser associated with higher *v* lower income, greater *v* lesser education, or differences in animal protein intake. NS: not significant.

The five rural metabolisers did not significantly differ in diet, income and education from the rural nonmetabolisers, but the number of metabolisers in the villages was so small as to preclude meaningful statistical analysis. Among the urban subjects the likelihood of being a metaboliser was significantly greater in those with higher income, postgraduate education after college, or a reported intake of foods containing animal protein other than milk at least six days a week (Table 2; odds ratios greater than 5·0 in each instance). Strict lactovegetarianism was not predictive of metabolic status (Table 2). Significant quantitative correlations were also present in the urban subjects between % urinary DRP and income, education and dietary animal protein intake (Table 3). After a multiple linear regression analysis, however, the only variable that retained a significant correlation with % DRP was income ($r=0·37$, $p<0·002$).

Table 3 *Correlations between % urinary DRP and income, diet, and animal protein intake in 104 subjects from Vellore*

	Correlation with % DRP	
Variable	*r*	*p*
Simple linear regression analysis		
Income	0·39	<0·001
Education	0·23	<0·05
Animal protein intake	0·24	<0·05
Multiple linear regression analysis		
Income	0·37	<0·002
Education	0·01	NS
Animal protein intake	0·08	NS

When only those subsets of urban volunteers with greater income, education or animal protein intake were considered (Table 2), the prevalence of metabolisers (40·9, 41·7, and 42·1%, respectively) did not differ from that in the New Yorkers (36·0%; $p>0·05$ for each comparison). In the urban Indian subjects, there was no relationship apparent between digoxin metabolism and birthplace in a rural or urban setting. Those urban volunteers who were born in rural areas who were metabolisers had lived in Vellore for longer periods (mean 19·0 (10·1) years, n=14) than those who were not (mean 6·2 (6·8) years, n=39, t=5·28, $p<0·0001$). Among the rural born subjects the metabolisers also differed significantly from the non-metabolisers in personal income and protein intake (data not shown). No correlations were present in either the urban or rural subjects between metabolic status and age, sex, religion, previous antibiotic use, or history of diarrhoea in the past.

STOOL CULTURES

In the cultures from the urban subjects, DRP-producing organisms were detected in the stool of all the five metabolisers and 18 of the 23 non-metabolisers tested (Table 4). The stool culture from the single metaboliser studied from the village produced DRP as did eight of the cultures from the 29 rural non-metabolisers. The urban:rural difference in the percentage of cultures from non-metabolisers containing DRP-producing organisms (78·2 *versus* 27·6%) was highly significant ($p<0·001$). The concentration of DRP-producing organisms in the stool cultures of the metabolisers did not differ significantly from those of the non-metabolisers and did not vary with place of residence.

Three stool cultures from urban and three from rural non-metabolisers showed an 'inhibitor' pattern – that is, little or no DRP was formed in the lowest one or two dilutions of stool but was clearly present in higher dilutions. Repeated cultures of frozen stool specimens from each of these six subjects consistently demonstrated the inhibitor pattern.

Table 4 *Formation of digoxin reduction products by stool cultures from urban and rural Indian subjects*

	Subjects cultured (n)	*Subjects with stool cultures producing DRP* (n)	(%)
Urban subjects	28		
Metabolisers	5	5	100
Non-metabolisers	23	18	78·2*
Rural subjects	30		
Metabolisers	1	1	100
Non-metabolisers	29	8	27·6*
All subjects	58		
Metabolisers	6	6	100
Non-metabolisers	52	26	50

DRP: digoxin reduction products. *$p<0·005$.

COMPARISON OF STOOL CULTURE FINDINGS IN INDIANS AND AMERICANS

The culture results in the 58 southern Indians were compared with those of a previously published study of 72 New Yorkers.[4] Of the 58 Indians tested, DRP-producing organisms were recovered from all of six metabolisers and 26 of 52 non-metabolisers (Table 4); thus of 32 people whose gut flora was colonised by DRP-forming bacteria, only six (18·8%) metabolised digoxin *in vivo*. In contrast, of the 72 Americans, such organisms were recovered from all of 25 metabolisers and 22 of 47 non-metabolisers. Therefore, of the 47 Americans whose gut flora was colonised by the organisms, 25 (53·2%) were metabolisers, which was a significantly higher percentage than that in the Indians ($p<0·005$). These differences are sum-

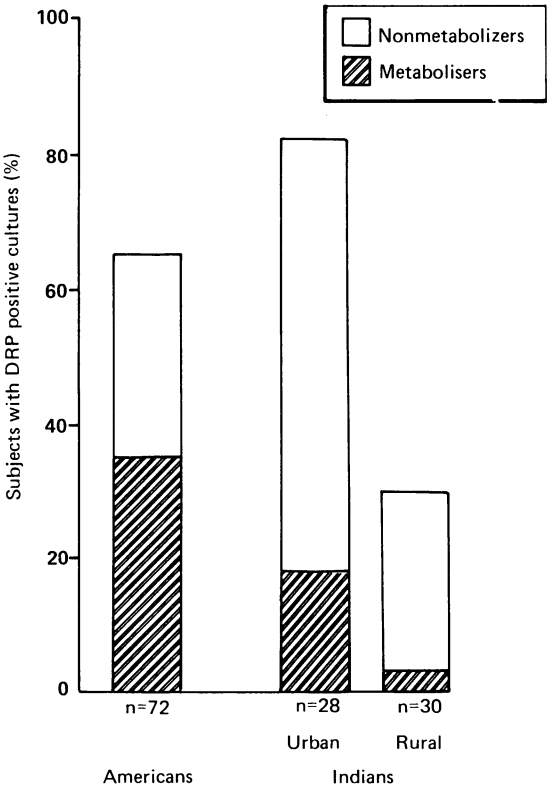

Fig. 2 *Per cent of Americans, urban Indians and rural Indians with stool cultures positive for DRP-forming organisms. The black areas indicate the percent of subjects who also excreted DRP in the urine after an oral dose of digoxin and were therefore classified as metabolisers. DRP: digoxin reduction products; n: number of subjects.*

marised in Figure 2. On the other hand, the percentage of all Indians studied from whom DRP-forming bacteria were recovered (32 of 58, or 55·2%) was not significantly lower than that in the Americans (47 of 72, or 65·3%).

Discussion

The prevalence of digoxin reducing organisms in the stool of indigenous southern Indians and residents of New York City was similar although the proportion of subjects in whom the organisms actually metabolised digoxin *in vivo* was significantly less in southern India. The overall percentage of metabolisers in the 204 southern Indians (13·7%) was quite similar to that found in a recent study[11] in Bangladeshis in Dhaka (13·9%) and in marked contrast with the prevalence of 35–36% that has been repeatedly found in American subjects.[9 11 17] In individuals who were metabolisers, the extent of digoxin metabolism was also less in the Indians (Fig. 1) and in the Bangladeshis[11] than in Americans.

This study shows that variations between ethnic groups in drug disposition, which have usually been attributed to genetic diversity in the actions of hepatic enzymes, may also be the result of differences in the metabolic activities of the gut flora. Although patients receiving digoxin were not investigated, it is likely that resistance to the drug caused by reductive inactivation[6 7] would be encountered less frequently in southern Indians, particularly in rural areas.

Environmental factors were found to strongly correlate with the ability to metabolise digoxin. Significant associations were noted between DRP in the urine and personal income and education in both southern India and Dhaka, as well as with dietary protein intake (which was not assessed in the Bangladesh study) in India. In both the developing countries it was possible to identify a subset of subjects with favourable socioeconomic indices in whom the prevalence of DRP excretion was similar to that in residents of New York City. This finding strongly suggests that the overall differences are related to environmental rather than genetic determinants. Previous investigators have suggested that environmental factors may influence other metabolic functions of the human anaerobic microflora, including methane production[18] and bile salt transformations.[19] The difference in DRP production between urban and rural southern Indians is consistent with the observation in Bangladesh that the strongest variable that influenced digoxin metabolism was a history of childhood residence in an urban rather than a rural area,[11] as the characteristics of the anaerobic gut flora may well be established during the first decade of life.[11 18 20 21] The proportion of digoxin metabolisers in the Indian village population (five of 100) is the lowest found in any group of adult humans studied.[7 9 11 13 17 22]

Digoxin was not metabolised *in vivo* by the majority of southern Indian subjects whose stool cultures contained organisms that were able to metabolise digoxin *in vitro*, as was also found in Bangladeshis.[11] This suggests that constituents of the colonic flora may be inhibited in their metabolic activities in the *milieu* of the lumen of the bowel. Such inhibition also appears to occur in a minority of New York City residents (Fig. 2). This suppression of DRP formation was also evident *in vitro* in stool samples from several southern Indians. Little or no DRP was produced in the first one or two dilutions after incubation, while it was formed in abundance in higher dilutions, an observation we have also made in a minority of American subjects. The suppression of DRP formation *in vitro* despite the presence of high concentrations of DRP-forming organisms suggests an inhibitory effect which is no longer seen in the higher dilutions. This could be related to the availability of substrates or cofactors generated or consumed by the metabolic activities of the other bacteria, which in turn influence the reactions promoted by *Eubacterium lentum*. Arginine, for example, which stimulates the growth of *E lentum*, progressively inhibits DRP generation when added in increasing concentrations to human faecal emulsions or pure cultures of the organism.[9]

In addition to metabolic inhibition, failure of *E lentum* to colonise the gut flora appeared to contribute to the low prevalence of digoxin reduction in the rural subjects, since DRP-forming organisms were recovered from their stool cultures significantly less frequently than in the urban residents. An earlier study in children also showed a quantitative difference in the normal gut flora between residents of this village and of Vellore, in that the anaerobe, *Sarcina ventriculi*, was found much more frequently in rural subjects.[23] The factors such as low income, lesser education, decreased frequency of animal protein intake and residence in villages, which were associated with low DRP production and low prevalence of DRP-producing bacteria, are likely to be surrogates for as yet poorly understood variables that affect gastrointestinal luminal colonisation and the metabolism of the flora. The effect of differences in diet on the human gut flora remains to be clearly established.[2-5] Environmental sanitation was much lower in the village (no latrines or protected water supply) compared with that of the urban hospital employees, many of whom drink chlorinated or boiled water. A high prevalence of gastrointestinal pathogens has been recorded in the village studied[24] and repeated infection with such organisms might alter the normal

composition of the colonic flora and its metabolic activities. Changes in transit time[25] might also be important.

Our observations extend the work of other investigators indicating variation between populations in metabolic functions of the gut flora, as reflected in the faecal concentrations of bacterial enzymes, microbially derived mutagens and metabolites of cholesterol and bile acids.[26-28] The findings reported here and in the recent study in Bangladesh[11] show striking geographic differences in the metabolic activity of the human anaerobic gut flora, as indicated by the *in vivo* inactivation of digoxin by a single species of bacteria, *Eubacterium lentum*. The presence of DRP-forming bacteria in the microflora of many southern Indians in whom digoxin was not inactivated *in vivo* emphasises the importance of assessing the metabolic activity of enteric bacteria, rather than their numbers and species. The factors controlling that activity, which appear to be strongly influenced by environmental variables, and which may be related to susceptibility to intestinal infection and neoplasia, remain to be determined.

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