Evaluation of dihydropyrimidine dehydrogenase activity in South-west Asian, Kenyan and Ghanaian populations

Janine M. Morsman,¹ Julieann Sludden,¹ Margaret-Mary Ameyaw,^{1,2} Jessie Githang'a,³ Anne Indalo,³ David Ofori-Adjei² & Howard L. McLeod¹

¹Department of Medicine & Therapeutics, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK, ²Centre for Tropical Clinical Pharmacology, University of Ghana, Accra, Ghana and ³University of Nairobi, Kenyatta National Hospital, Nairobi, Kenya

Aims Dihydropyrimidine dehydrogenase (DPD) reduces endogenous pyrimidines and therapeutic analogues such as the anticancer agent 5-fluorouracil (5FU). Among Caucasian populations DPD activity is highly variable and subject to polymorphic regulation. To evaluate interethnic influence, DPD activity was assessed in South-west Asian, Kenyan and Ghanaian populations.

Methods DPD activity was determined in peripheral mononuclear cells using [¹⁴C]-5-fluorouracil and h.p.l.c. analysis.

Results A high degree of variation in DPD activity was observed within each population (range CV = 34–48%). Median DPD activity also varied between these populations. South-west Asian and Kenyan subjects exhibited almost identical median values (192 and 193.5 pmol min⁻¹ mg⁻¹, respectively), which were similar to Caucasians (median 215 pmol min⁻¹ mg⁻¹). A significantly lower median DPD activity (119 pmol min⁻¹ mg⁻¹) was observed in the Ghanaian population.

Conclusions The similarity in DPD activity between Caucasian, Kenyan and South-west Asian populations suggests that the incidence of 5FU-related toxicity may be comparable in these groups. The pharmacokinetic implications of lower activity amongst Ghanaians needs to be evaluated.

Keywords: 5-fluorouracil, dihydropyrimidine dehydrogenase, drug metabolism, ethnic variability

Introduction

Interethnic variation in drug-metabolizing capacity is a well-known phenomenon that is most readily evident amongst polymorphic enzymes [1, 2]. For example, the poor metabolizer phenotype for CYP2D6 occurs in 5–10% of Caucasians, while in Chinese and Japanese the frequency is less than 1% [1]. Conversely, 18–23% of Chinese and Japanese are poor metabolizers of (S)-mephenytoin (CYP2C19-mediated) compared with 2–5% of a Caucasian population [1]. Subjects heterozygous for thiopurine methyltransferase (TPMT) mutant alleles appear to be less common in South-west Asians (2%) and

more common in Ghanaians (15%) compared with Caucasians (10%) or Kenyans (10.2%) [3–5]. This has therapeutic relevance as TPMT S-methylates 6-mercaptopurine and the immunosuppressive azathioprine [6]. As such, rheumatoid arthritis patients with mutant TPMT alleles are at a higher risk of developing severe side-effects in response to azathioprine treatment [7].

Dihydropyrimidine dehydrogenase (DPD; E.C. 1.3.1.2) reduces uracil and thymine and is rate-limiting in the biosynthesis of β -alanine, a putative neurotransmitter [8]. DPD is also involved in the degradation of the pyrimidine analogue 5-fluorouracil (5FU), an anticancer agent commonly used in the treatment of solid tumours such as head and neck, breast and colon. Liver exhibits the highest DPD activity, and peripheral blood mononuclear cells are used as a surrogate tissue in clinical studies [9]. Deficiencies in DPD activity reduce the clearance of 5FU which results in severe adverse reactions including neutropenia, gastrointestinal toxicity and neurotoxicity

Correspondence: Dr Howard L. McLeod, Department of Medicine & Therapeutics, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, United Kingdom. Tel.: 441224 552730; Fax: 441224 273066; E-mail: h.l.mcleod@aberndeen.ac.uk.

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[10–12]. Analysis of DPD has so far focused on Caucasian populations [13–16], and it is not yet known whether there are differences among ethnic groups. In this report, DPD activity was evaluated in Caucasian, South-west Asian, Kenyan and Ghanaian populations.

Methods

Subjects were unrelated blood donors of South-west Asian (Indian, Pakistani, Sri Lankan), Kenyan, Ghanaian and British Caucasian origin, as detailed previously [3, 5]. Following Ethics Committee approval, informed written consent was obtained from all individuals and DPD activity measured in peripheral mononuclear cells according to a method described previously [17]. Mononuclear cells were purified from 20 ml heparinized blood by Ficoll-hypaque density centrifugation and stored at -20°C for at least 15 days in order to avoid the fluctuation in activity evident before this timepoint [17]. Cytosol was subsequently prepared and protein content estimated using the Bio-Rad protein assay [18]. Cytosolic incubations were carried out in 35 mM sodium phosphate buffer pH 7.4 containing 2.5 mM magnesium chloride, 10 mM 2-mercaptoethanol, 125 μM NADPH and 20 μM [^{14}C]-5-fluorouracil (Amersham, UK). Metabolite production was quantified by h.p.l.c. analysis with radio-detection. DPD activity was expressed in pmol metabolite formed $\text{min}^{-1} \text{mg}^{-1}$ protein. The coefficient of variation for the assay was 8%. The influence of ethnic group on DPD activity was assessed using the Kruskal–Wallis test, and gender differences (within an ethnic group or for all subjects combined) analysed by the Mann–Whitney test. The difference in DPD activity between ethnic groups was also evaluated using the Mann–Whitney test.

Results

DPD activity was measured in 97 South-west Asians and ranged from 28 to 367 $\text{pmol min}^{-1} \text{mg}^{-1}$, with a median value of 192 $\text{pmol min}^{-1} \text{mg}^{-1}$ (Figure 1; Table 1). Variability within the Indian ($n=43$) and Pakistani ($n=49$) subgroups was similar, with values of 43–367 (median 194) and 28–324 $\text{pmol min}^{-1} \text{mg}^{-1}$ (median 189), respectively. For the Kenyan population ($n=78$), DPD activity varied 7.7-fold (62–480 $\text{pmol min}^{-1} \text{mg}^{-1}$) and the median activity was 193.5 $\text{pmol min}^{-1} \text{mg}^{-1}$ (Figure 1). Ghanaian DPD activity was also highly variable (41–482 $\text{pmol min}^{-1} \text{mg}^{-1}$), although the median activity was significantly lower at 119 $\text{pmol min}^{-1} \text{mg}^{-1}$ ($n=80$, $P<0.001$, Figure 1). In a British Caucasian group DPD activity ranged from 70 to 334 $\text{pmol min}^{-1} \text{mg}^{-1}$ ($n=41$) with a median value of 215 $\text{pmol min}^{-1} \text{mg}^{-1}$ (Figure 1). A significant difference in median DPD activity was detected between

Figure 1 Influence of ethnicity on mononuclear cell DPD activity. Horizontal line is the median value and open circles represent values for outliers which are $>95\%$ ile.

Ghanaian and Caucasian (difference 96 $\text{pmol min}^{-1} \text{mg}^{-1}$, 95% confidence interval 62–118), Kenyan (75 $\text{pmol min}^{-1} \text{mg}^{-1}$, 95% CI 58–93), and South-west Asian (73 $\text{pmol min}^{-1} \text{mg}^{-1}$, 95% CI 55–90) subjects (all $P<0.001$). The coefficient of variation of DPD activity was similar for the four populations; 34% in South-west Asians, 35% in British Caucasians, 37% in Kenyans and 48% in Ghanaians.

The influence of gender was also assessed in each population. For the majority of individuals, median DPD activity was higher in males than females; 13% for South-west Asians, 30% for Kenyans and 45% for Ghanaians (Figure 2). The British Caucasian group however, had a 44% higher median DPD activity in females compared with males (Figure 2). Overall, there was no significant difference in DPD activity between males and females when all subjects were combined ($P=0.26$).

Discussion

5FU has been used clinically for more than 40 years in the treatment of solid tumours across diverse populations, but

Table 1 Mononuclear DPD activity in world populations.

Individuals	n	DPD activity range ($\text{pmol min}^{-1} \text{mg}^{-1}$)	Median DPD activity ($\text{pmol min}^{-1} \text{mg}^{-1}$)
Caucasian UK	41	70–334	215
South-west Asian	97	28–367	192
Kenyan	78	62–480	194
Ghanaian	80	41–482	119
Korean [ref. 19]	114	60–800	280 (mean)
Caucasian French [ref. 14]	124	65–559	227 (mean)

Figure 2 Influence of gender (open bars male; closed bars female) on mononuclear cell DPD activity. Horizontal line is the median value and open circles represent values for outliers which are >95%ile.

has primarily been evaluated in the context of Caucasian subjects. Likewise, the enzyme that inactivates 5FU, DPD, has only been studied extensively in Caucasians, and has demonstrated widely variable activity. In this study, a wide range of activity was observed across the ethnic groups. The largest range was seen in South-west Asian subjects (13-fold), followed by Ghanaians (12-fold), Kenyans (8-fold) and the British Caucasians (5-fold). Median DPD activity was similar between the South-west Asian, Kenyan and British Caucasian populations (Table 1), and was analogous to that of Scottish colorectal cancer patients ($210 \text{ pmol min}^{-1} \text{ mg}^{-1}$) [15] and French cancer patients ($211 \text{ pmol min}^{-1} \text{ mg}^{-1}$) [14]. These findings are consistent with other populations studied so far, in that ranges of activity appear fairly constant, while median activities tend to differ. African Americans and Caucasian Americans were previously shown to have similar activities (overall range $24\text{--}354 \text{ pmol min}^{-1} \text{ mg}^{-1}$; mean $189 \text{ pmol min}^{-1} \text{ mg}^{-1}$) [13]. Although the authors stated that no significant difference was observed between these populations, specific data on ethnicity and DPD activity was not provided. In contrast, a Korean population was found to have higher activity in terms of both range and mean DPD activity (Table 2) [19].

DPD activity for a Ghanaian population was lower with a median value of $119 \text{ pmol min}^{-1} \text{ mg}^{-1}$. As DPD is responsible for inactivating over 80% of administered 5FU, low DPD activity may predispose an individual to severe toxicity [10]. In addition, a linear relationship exists between DPD activity and 5FU systemic clearance in cancer patients [20]. The lower median activity of the Ghanaian group may imply different pharmacokinetics from the Caucasians, Kenyans and South-west Asians, and a lower clearance of 5FU could be expected. This suggests

that standard doses of 5FU would result in a greater incidence of significant toxicity in Ghanaian subjects. However, pharmacokinetic studies have not been carried out in different ethnic groups, so the DPD-clearance relationship needs to be ascertained in non-Caucasian subjects. The lower activity of the Ghanaians is difficult to explain, but may be related to external influences such as diet. Rat hepatic DPD activity was found to be lower in those fed a low protein diet [21]. Likewise, a deficiency in a micronutrient (vitamin B2) also lowered DPD activity, and these findings may extrapolate to humans [22].

A high degree of variation in DPD activity was observed within each ethnic group. The CVs for the South-west Asian, Kenyan and British Caucasian populations were 34%, 37% and 35%, respectively. These values are similar to those obtained for British (37%) [17] and North American Caucasian populations (34%) [13] and are also comparable with those seen in cancer patients, both French (37%) [14] and Scottish (37%) [15]. The degree of variability was higher though for the Ghanaian subjects (CV 48%). Overall, there is no obvious link between variability in DPD activity and ethnicity. Consequently, it may be valid to assume that studies in one population provide a general predictor of other groups' behaviour.

The influence of gender on DPD activity is unclear. Higher DPD activity was evident in male subjects of the South-west Asian, Kenyan and Ghanaian populations which is in agreement with the British and French populations examined previously [14, 17]. In contrast, the British Caucasians in the present study had higher DPD activities in the female subjects. Likewise, mononuclear cell and liver DPD activities have also been found to be higher in females than males in North American populations [13, 23]. With relatively small differences in activity between gender (13–45%), it would seem unlikely to have any significant clinical impact.

To date there have been no reports detailing the frequency of low DPD activity within other ethnic groups. This study has demonstrated similarities in DPD activity between Kenyan, South-west Asian and Caucasian groups. As such, it could be anticipated that the findings of further studies in Caucasian populations alone may well be applicable to other ethnic groups.

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