Link between Dihydropyrimidine Dehydrogenase Activity in Peripheral Blood Mononuclear Cells and Liver

M. Chazal, M. C. Etienne, N. Renée, A. Bourgeon, H. Richelme, and G. Milano

OncoPharmacology Department, Centre Antoine Lacassagne, 33 avenue de Valombrase, 06050 Nice, Cedex 1 [M. C. E., N. R., G. M.], and Department of Surgery, Hopital Pasteur, Nice, Cedex [M. C., A. B., H. R.], France

ABSTRACT

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme of 5-fluorouracil (FU) catabolism, which occurs mainly in the liver. Several cases of severe FU-related toxicity have been reported in patients exhibiting a marked DPD deficiency measured in peripheral blood mononuclear cells (PBMCs). In addition, it has been shown that PBMC-DPD activity correlates to systemic FU clearance. The purpose of the present study was to closely evaluate the link between DPD activity measured in PBMCs and in liver samples obtained from the same patients.

This prospective study was conducted on 27 patients (18 men and 9 women) who underwent laparotomy for various pathologies. Liver biopsies were performed in normal liver and immediately stored in liquid nitrogen. Biological liver function tests were within normal values for all patients. Concomitantly to the liver biopsy, a blood sample was taken and PBMCs were immediately isolated and stored at -80°C. 

Lever-DPD and PBMC-DPD activities were measured by a radioenzymatic assay using [14C]-FU as substrate (sensitivity limit, 5 pmol/min/mg protein; inter assay reproducibility, 10%). Liver-DPD (mean, 178; median, 186; range, 54–290 pmol/min/mg protein) and PBMC-DPD (mean, 196; median, 205; range, 80–275 pmol/min/mg protein) exhibited the same pattern of distribution. Neither liver-DPD nor PBMC-DPD was significantly different between men and women. A significant linear correlation was demonstrated between liver- and PBMC-DPD activity (r = 0.56, P = 0.002). Interestingly, the patient who exhibited the lowest PBMC-DPD activity (80 pmol/min/mg protein, at risk value for developing FU-related side effects) also had very low liver-DPD activity (98 pmol/min/mg protein). In conclusion, in patients with normal liver function, DPD activity measured in PBMCs reflects DPD activity expressed in the liver. The demonstrated link between liver- and PBMC-DPD activity reinforces the interest in DPD investigation in PBMCs for selecting, before FU-containing chemotherapy, patients at risk of developing severe toxicities due to impairment of FU clearance.

INTRODUCTION

In eukaryotic cells, the first step in the catabolism of pyrimidine bases, thymine and uracil, is a hydrogenation by the reverse enzyme DPD (1). Naguib et al. have measured DPD activity in several normal and neoplastic human tissues (2) and found the highest DPD activities in PBMCs and liver in comparison to those found in the pancreas, lung, and intestinal mucosa.

Tuchman et al. (3) were the first to describe a patient with a severe FU toxicity associated with a disorder of pyrimidine metabolism (3). Based on another case report, Diasio et al. (4) measured DPD activity in PBMCs and demonstrated a profound deficiency of this enzymatic activity in a patient having developed a marked toxicity to FU. Since then, further similar syndromes were reported by the same group (5) and others (6), including ourselves (7).

To evaluate the incidence of complete or partial DPD deficiency, two prospective studies have been conducted so far. Both Lu et al. (8) and ourselves (9) made the observation of a Gaussian distribution for PBMC-DPD with marked differences between subjects, as shown by a 10-fold range variability (65–559 pmol/min/mg protein; Ref. 9). It has also been shown that PBMC-DPD activity was significantly correlated to FU clearance (10, 11). However, a study on a large group of FU-treated patients revealed that the correlation between PBMC-DPD and FU clearance, although highly significant, was relatively weak (9), leading to question whether PBMC-DPD was a reliable indicator of liver DPD which plays the central role for clearing FU from the organism (12). Interestingly, Lu et al. (8) reported low DPD activity in the liver for two patients exhibiting a DPD deficiency at the PBMC level. The aim of the present study was thus to prospectively evaluate the link between DPD activity measured in PBMCs and in liver samples obtained from the same subject. These investigations were performed in 27 patients requiring a laparotomy.

PATIENTS AND METHODS

Patients. This prospective study was performed on 27 patients [18 males and 9 females; mean age, 61.5 (range, 34–81) years] who underwent laparotomy for various pathologies including cancer (see details in Table 1). All patients had a liver biopsy either because it is systemically scheduled before surgery or due to a doubt concerning diagnosis during laparotomy. For all studied patients, there was no histological evidence of any liver abnormality. In addition, the preoperative biological check-up including bilirubin and hepatic enzymes were in all

Received 11/9/95; accepted 11/20/95.

1 To whom requests for reprints should be addressed. Phone: (33) 92 03 15 53; Fax: (33) 93 81 71 31.

2 The abbreviations used are: DPD, dihydropyrimidine dehydrogenase or dihydropyrimidine reductase (EC 1.3.1.2); PBMC, peripheral blood mononuclear cell; FU, 5-fluorouracil; FUDR, dihydropyrimidinase; FUPA, α-fluoro-β-ureidopropionic acid; FBAL, α-fluoro-β-alanine.
cases within the range of normality. In all cases, biopsies were performed in normal liver. A portion of the biopsy was transmitted for analysis by a pathologist, and the other portion was immediately stored in liquid nitrogen for DPD determination. Concomitantly, a blood sample was collected (20 ml on lithium heparinate) for PBMC-DPD measurement. PBMCs were immediately isolated using Histopaque (Sigma) and washed twice with PBS. RBCs were hypotonically lysed. PBMCs (25,000 cells/μl in 35 mmol/liter sodium phosphate buffer containing 10% glycerol) were then stored at −80°C until assayed.

Chemicals. FUH2, FUPA, and FBAL were kindly provided by Roche Laboratories (Neuilly, France). 14C-FU labeled at position 6 (55 Ci/mol) was obtained from Amersham (Buckinghamshire, United Kingdom). All other chemicals were obtained from Sigma Chemical Co. (Sigma Diagnostics, St. Louis, MO) and were of the highest purity available.

DPD Determination. The day of the assay, PBMCs were freeze thawed three times, sonicated on ice, and centrifuged for 30 min at 28,000 × g (+2°C). Frozen biopsies [mean weight, 233 (range, 46–659) mg] were homogenized on an ice bed using a Polytron (Polylabo, Paris, France) in 1 ml buffer containing 10 mmol/liter Tris-HCl, 1 mmol/liter edathamil, 0.5 mmol/liter dithiothreitol, and 10 mmol/liter sodium molybdate (pH 7.4). After a 10-min centrifugation (800 × g at +2°C), the supernatant was collected and centrifuged for 30 min at 28,000 × g (+2°C). For both liver biopsies and PBMCs, the resulting cytosolic fractions were immediately assayed for DPD activity. Cytosolic proteins were quantified according to the Bradford assay (Bio-Rad SA, Munich, Germany) using BSA as standard.

DPD activity was measured according to the method described by Harris et al. (10). The assay consisted of incubating 50 μl cytosol with 14C-FU (20 μmol/liter final), β-nicotinamide adenine dinucleotide phosphate (250 μmol/liter final), and magnesium chloride (2.5 mmol/liter final). Total volume was 125 μl (in 35 mmol/liter sodium phosphate buffer, pH 7.5, containing sodium azide). The duration of incubation was 30 min at +37°C. The reaction was stopped by addition of 125 μl ice-cold ethanol followed by 30-min storage at −20°C. The samples were centrifuged (5 min, 400 × g) to remove proteins, and the supernatant was analyzed for the presence of 14C-FUH2, 14C-FBAL, and 14C-FUPA using a high-pressure liquid chromatographic method. Detection was performed using a radioactive flow monitor (LD 506; Berthold, Wildbad, Germany). DPD activity was calculated by taking into account the sum of FUH2, FBAL, and FUPA peaks. DPD activity was expressed as pmol 14C-FU catalyzed per min and per mg protein. Each sample was assayed in duplicate. The sensitivity limit was 5 pmol/min/mg protein. The stability of DPD activity during storage evaluated by the interassay reproducibility (pooled cytosol) gave a coefficient of variation of 10.3% on average.

Statistics. Both liver-DPD and PBMC-DPD distributions appeared to fit a Gaussian distribution, therefore, parametric tests were performed. Statistics were done on Statgraphics Plus software (Uniware, Paris, France).

RESULTS

Patient characteristics and DPD measurements are given in Table 1. Both liver-DPD and PBMC-DPD exhibited a wide intersubject variability. DPD activity ranged between 54 and 290 pmol/min/mg protein in liver biopsies (mean, 178; median, 186; SD, 49) and between 80 and 275 pmol/min/mg protein in PBMCs (mean, 196; median, 205; SD, 46). Although evaluated on a limited sample of 27 subjects, DPD activity expressed in liver and PBMCs seemed to fit a Gaussian distribution in both cases, as shown by mean and median values. The liver-DPD: PBMC-DPD ratio ranged between 0.48 and 1.44, with a mean value at 0.93 (median, 0.91). Comparison between liver-DPD and PBMC-DPD activity shows that DPD activity was significantly higher in PBMCs as compared to liver (Student’s paired test, P = 0.046). However, this difference was weak (18 pmol/min/mg protein on average). Comparison of DPD activity between men and women did not reveal any significant difference (Student’s test, P = 0.18 for liver-DPD and 0.45 for PBMC-DPD), although mean DPD activities were higher in men (187 and 201 pmol/min/mg protein in liver and PBMCs, respectively) as compared to women (160 and 186 pmol/min/mg protein in liver and PBMCs, respectively). Neither liver-DPD nor PBMC-DPD activity were influenced by the age of patients (linear regression, P = 0.71 for liver-DPD and 0.62 for PBMC-DPD). A significant linear correlation was demonstrated between liver-DPD activity and PBMC-DPD activity (liver-DPD = 0.6 × PBMC-DPD + 59, r = 0.56, P = 0.002, Fig. 1). One patient of the 27 investigated (i.e., 3.7% of the whole study group) exhibited a PBMC-DPD activity at 80 pmol/min/mg protein, i.e., below the threshold value linked to an elevated risk of severe FU toxicity due to DPD deficiency (<100 pmol/min/mg protein; Ref. 13). Interestingly, for this patient DPD activity was also very low in the liver biopsy (98 pmol/min/mg protein). This DPD-deficient patient was a woman.

DISCUSSION

Recent data from the literature (3–8), including ours (9), have suggested that DPD activity measured in PBMCs is, when markedly diminished, strongly related to the risk of developing severe and even lethal FU-related toxicity due to FU systemic overexposure induced by a depressed drug clearance. However, the site for FU catabolism is the liver and PBMCs is used as a surrogate since PBMCs are much more accessible than a liver biopsy. It was thus justified to examine the relationship between DPD activity expressed in PBMCs and liver biopsies. Moreover, due to a circadian variability previously demonstrated for DPD activity (10), it was necessary that both PBMCs and liver samples from the same patients were obtained at the same time during the day. The present study group was relatively small (27 patients) due to the weight of the present investigations planned on healthy liver samples obtained during laparotomy in different clinical situations. Nevertheless, one can consider this group as representative of a cancer patient population as concerns the age [mean, 61.5 (range, 34–81) years]. In addition, the mean PBMC-DPD activity presently found (196 pmol/min/mg protein) is very close to that previously reported by Lu et al. (189 pmol/min/mg protein; Ref. 8) on a large set of 123 healthy volunteers and also very close to that we previously reported in a group of 185 cancer patients (222 pmol/min/mg protein; Ref. 9).

The first new information herein provided is that the pattern of DPD activity expressed in the liver is very similar to that
Table 1  Patients and DPD activities measured in PBMCs and liver

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Clinical status</th>
<th>DPD activity (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>57</td>
<td>Exploratory laparotomy</td>
<td>225</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>47</td>
<td>Colectomy</td>
<td>235</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>53</td>
<td>Exploratory laparotomy</td>
<td>205</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>55</td>
<td>Postgraft laparotomy</td>
<td>220</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>61</td>
<td>Liver metastases</td>
<td>205</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>81</td>
<td>Cholecystitis</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>70</td>
<td>Cancer (liver)</td>
<td>240</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>71</td>
<td>Gallbladder lithiasis</td>
<td>163</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>70</td>
<td>Polyposis + gallbladder lithiasis</td>
<td>178</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>65</td>
<td>Cancer (pancreas)</td>
<td>225</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>50</td>
<td>Pancreas cyst</td>
<td>204</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>54</td>
<td>Cancer (stomach)</td>
<td>196</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>57</td>
<td>Cancer (stomach)</td>
<td>227</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>76</td>
<td>Cancer (colon)</td>
<td>223</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>68</td>
<td>Cancer (colon)</td>
<td>176</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>52</td>
<td>Cancer (rectum)</td>
<td>236</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>34</td>
<td>Cancer (Hodkin)</td>
<td>120</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>60</td>
<td>Cancer (rectum)</td>
<td>275</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>72</td>
<td>Cancer (rectum + liver metastases)</td>
<td>151</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>70</td>
<td>Cancer (rectum)</td>
<td>134</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>61</td>
<td>Cancer (stomach)</td>
<td>185</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>66</td>
<td>Waldenstrom</td>
<td>225</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>69</td>
<td>Cancer (stomach)</td>
<td>234</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>72</td>
<td>Cancer (colon + liver metastases)</td>
<td>231</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>62</td>
<td>Cholecystitis</td>
<td>161</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>55</td>
<td>Pancreas cyst</td>
<td>219</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>53</td>
<td>Gallbladder lithiasis</td>
<td>112</td>
</tr>
</tbody>
</table>

Men (n = 18)
Mean | 201 | 187 | 0.94 |
Median | 205 | 191 | 0.97 |
SD | 42 | 52 | 0.26 |
Women (n = 9)
Mean | 186 | 160 | 0.89 |
Median | 219 | 165 | 0.85 |
SD | 54 | 39 | 0.19 |
All (n = 27)
Mean | 196 | 178 | 0.93 |
Median | 205 | 186 | 0.91 |
SD | 46 | 49 | 0.24 |

F, female; M, male.

found in PBMCs. Moreover, a significant linear correlation was demonstrated between DPD activity measured in PBMCs and DPD activity measured in liver. This means that globally, DPD determination in PBMCs reflects DPD activity expressed in the main site of FU catabolism, i.e., the liver. However, a closer examination of this relationship points to a $r^2$ value at 0.31, which is relatively modest. This weak correlation could be explained by differences in the regulation of DPD expression involving the known circadian variability for DPD (10), which could be different between the two cell types investigated. Considering that DPD is the rate-limiting enzyme of FU catabolism which occurs mainly in the liver (12), one could anticipate that PBMC-DPD is not a strong and reliable indicator of FU clearance. Yet, we recently reported on a large set of 90 chemotherapy cycles for FU-treated patients a positive and statistically significant but weak correlation between pretreatment PBMC-DPD activity and FU clearance ($r^2 = 0.09, P = 0.002$; Ref. 9). As a practical consequence, PBMC-DPD itself cannot be recommended as a faithful prediction of FU clearance and for pretreatment individual FU dose tailoring, which could be theoretically feasible since maximum tolerated exposure for FU treatment has been clinically established (15–17).

It must be emphasized that the frequency of depressed PBMC-DPD activity (below 100 pmol/min/mg protein) presently found (1 patient of 27, i.e., 2.7%) is similar to that clinically validated from population studies (8, 9, 14), i.e., 2–3%. Of interest is the fact that the woman with the lowest PBMC-DPD activity also expressed a low DPD activity in liver. Also, Lu et al. (8) made the observation that a decreased liver DPD activity was present in two cancer patients exhibiting a DPD deficiency in PBMCs. This parallel deficiency in DPD activity observed both in PBMCs and liver could reflect a common regulation mechanism of DPD expression mediated by hormones or other systemic factors. Also, a possibility for explaining the origin of this parallel DPD deficiency could lie in a DPD gene mutation. Consistent with this hypothesis is the fact that familial syndromes for DPD deficiency have been described (4) and a point mutation in the DPD open reading frame has been recently reported (18).
It seems important to point out that a marked discrepancy between hepatic- and PBMC-DPD activity has been recently observed in our institute for a 43-year-old white woman with multifocal hepatic metastases and liver function test abnormalities (19). This woman experienced a dramatic prolonged overexposure to FU in blood and cerebrospinal fluid despite treatment cessation and died from FU-related neurotoxicity. DPD activity in PBMCs was in the normal range. In contrast, DPD activity measured in a liver biopsy taken from a healthy part of the liver was strongly depressed, explaining the very low FU clearance. Moreover, we have previously measured liver-DPD activity in three additional patients with liver abnormalities as attested by the pathological analysis: two cases of cirrhosis and one case of cirrhosis complicated by icterus. In these three cases, PBMC-DPD activity was in the normal range (303, 245, and 179 pmol/min/mg protein, respectively), whereas it was depressed in the parallel liver biopsy taken from the healthy part of the liver (184, 157, and 101 pmol/min/mg protein, respectively). Taken together these data point out that DPD deficiency can be accurately predicted by PBMC investigation in patients with normal liver function, but in the case of marked liver abnormalities, caution must be observed for the interpretation and the predictability of a normal PBMC-DPD finding. The cost:benefit ratio of prospective identification of FU-treated patients with DPD deficiency remains to be evaluated. The recent development of polyclonal antibodies against human DPD protein (20) could be a valuable analytical tool for such DPD deficiency screening.

REFERENCES


Link between dihydropyrimidine dehydrogenase activity in peripheral blood mononuclear cells and liver.

M Chazal, M C Etienne, N Renée, et al.


Updated version

Access the most recent version of this article at:

http://clincancerres.aacrjournals.org/content/2/3/507

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/2/3/507. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.