Cancer Chemother Pharmacol (2000) 45: 477–482 

ORIGINAL ARTICLE

**Rosine Guimbaud · Sylvie Guichard · Caroline Dusseau
Viviane Bertrand · Thomas Aparicio · Isabelle Lochon
Etienne Chatelut · Daniel Couturier · Roland Bugat
Stanislas Chaussade · Pierre Canal**

# Dihydropyrimidine dehydrogenase activity in normal, inflammatory and tumour tissues of colon and liver in humans

Received: 22 November 1999 / Accepted: 25 January 2000

**Abstract** *Background/Purpose*: Dihydropyridmidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the catabolism of 5-fluorouracil (5FU). Although this catabolism is likely to occur in the liver in humans, there may be a local inactivation in tumours, modifying the efficacy of 5FU. The aim of this study was to examine the DPD activity in normal, inflammatory and malignant tissues from both the colon and the liver to assess the modifications of DPD activity in the process of tumourigenesis. *Methods*: DPD activity was evaluated in 107 patients, corresponding to 194 samples (70 colorectal tumour and normal colon, nine metastases secondary to a colon cancer, ten inflammatory colon, 20 samples of normal liver, seven from primary liver cancer, and eight from inflammatory liver). DPD activity was determined using an enzymatic reaction followed by analysis of 5FU and its catabolite dihydro-5FU by high-performance liquid chromatograph. Results were expressed as pmol of 5FU catabolized/min · mg protein. *Results*: DPD was highly variable in tumour and normal tissues, both from colon and liver. In colon, the correlation between DPD activity in tumour and normal mucosa was weak, even if it was statistically significant due to the higher number of samples. In inflammatory colon tissue (ulcerative colitis or Crohn's disease), DPD activity was significantly higher than in normal tissue ($P = 0.006$). In liver metastases from colon cancer, DPD activity was not significantly different from that observed in primary colon tumour ($P = 0.32$). In liver, DPD activity was significantly lower in primary liver tumour than in uninvolved liver specimens ($P = 0.001$). In inflammatory liver tissue (hepatitis), DPD activity ranged between normal and tumour tissues, and did not differ significantly either from normal tissue or primary liver cancer. *Conclusions*: DPD activity was modified in colon and in liver during a pathological process and the dysregulation of DPD increased from a benign to a malignant tissue.

**Key words** Dihydropyrimidine dehydrogenase · Colon carcinoma · Liver cancer

R. Guimbaud · S. Guichard · C. Dusseau · I. Lochon
R. Bugat · E. Chatelut · P. Canal (✉)
Groupe de Pharmacologie Clinique et Expérimentale,
Institut Claudius Regaud, 20–24 rue de Pont Saint-Pierre,
31052 Toulouse Cedex, France
e-mail: canal@icr.fnclcc.fr
Tel.: +33-5-61-424222; Fax: +33-5-61-424177

R. Guimbaud · V. Bertrand · T. Aparicio · D. Couturier
S. Chaussade
Service d'Hépato-Gastroentérologie, Hôpital Cochin, Paris, France

E. Chatelut · R. Bugat
Université Paul Sabatier, Toulouse, France

## Introduction

Dihydropyrimidine dehydrogenase (DPD, EC 1.3.1.2) is the initial and rate-limiting enzyme in the three-step metabolic pathway leading to the catabolism of the pyrimidine bases uracil and thymine [14]. This is the only metabolic pathway in the biosynthesis of $\beta$-alanine in mammals. DPD is also the key enzyme that degrades the structurally related pyrimidine antimetabolite 5-fluorouracil (5FU), a common anticancer drug used in the treatment of colorectal, breast, head, neck and ovary cancers.

Since 70–80% of the administered 5FU is degraded in vivo by DPD to fluorinated $\beta$-alanine, the level of DPD activity is a major determinant in the toxicity of 5FU [6]. Variability in 5FU pharmacokinetics was due to the variability in DPD activity determined in peripheral mononuclear cells (PMNC) [10]. DPD activity was found in most tissues, with the highest content in liver and PMNC [15, 19]. A high degree of variation in PMNC activity was observed in the general population (up to 20-fold range) [7, 11, 19, 20]. DPD activity has also been measured in surgical specimens from head and neck and colorectal cancer patients before administration of 5FU chemotherapy [8, 22]. A large variability in DPD activity was observed in both localizations and a

relationship was demonstrated between DPD tumour-normal ratio and response to a 5FU therapy in patients with head and neck cancers. Moreover, relatively high DPD activity has been reported in hepatocellular carcinoma [17].

The apparent relation of causality between the variability of DPD activity and the variability of 5FU pharmacology made attractive the development of DPD inhibitors to eliminate the variations due to DPD. Different new drugs (UFT, S1 and eniluracil) are being developed in this field [1, 12]. Thus, a deeper knowledge of DPD activity in tumours and in normal tissue is recommended. In the present study, we examined DPD activity in normal, inflammatory and malignant tissues from both the colon and the liver in order to assess the modifications of DPD activity in the process of tumourigenesis.

## Material and methods

### Patients and chemicals

DPD activity was assessed in normal and pathological (neoplastic and inflammatory) tissues from liver and colon in a total of 107 patients, corresponding to 194 samples. Informed consent was obtained from each patient before surgery. [6–$^{14}$C] 5FU was purchased from Moravek, Brea, Calif., USA.

### Colon samples

The population comprised 70 patients with colon carcinoma. Tumour colon tissues and their normal counterparts were obtained during surgical procedure (resection of colon tumour) for most of the patients ($n = 68$). After resection, portions of viable pathological and adjacent normal tissues ($> 5$ cm from tumour) were removed by an experimental gastrointestinal pathologist. For the two last patients, colon biopsies were obtained during diagnosis colonoscopy. The local stage of the colon tumour was classified according to Duke's classification as follows: stage A = 13 patients, B = 21 patients and C = 36 patients. Moreover, nine patients with hepatic metastases from primary colon cancer were investigated. For each of them, both metastatic and normal liver tissues were obtained. In two cases, these metastases were synchronous with a primary tumour.

Evaluation of DPD was also conducted in ten patients suffering from an inflammatory bowel disease [ulcerative colitis (UC) five patients, Crohn's disease (CD) five patients]. Samples of inflammatory colon tissue were obtained by colonoscopy in macroscopically inflammatory area. In each case, the intensity of the inflammation (moderate to severe) was assessed according to specific indexes (Best's index for CD and Truelove's index for UC).

### Liver samples

DPD activity was determined in seven patients with primary tumours of the liver. The primary tumour was a hepatocarcinoma in four cases and a cholangiocarcinoma in three cases. Biopsies of tumour were performed during surgical procedures for tumour resection. Moreover, inflammatory liver tissue was obtained in eight patients with chronic inflammatory disease: active viral hepatitis B or C ($n = 6$ patients) and alcoholic hepatitis with cirrhosis ($n = 2$ patients). Samples were obtained by transparietal (Thrue cut needle) or surgical biopsy.

Normal liver samples were obtained in 20 patients (nine with metastatic disease from colon cancer and 11 other patients without any liver disease). The median age of the overall population was 51 years (range 18–93). Immediately after biopsy, samples of tissue were frozen in liquid nitrogen and were stored at −80 °C until analysis of enzyme activity.

### Dihydropyrimidine dehydrogenase activity

Tumour and normal tissues were homogenized in 35 m*M* sodium phosphate buffer pH 7.5. The cellular suspension was ultracentrifuged at 20,000 *g* for 30 min at 4 °C. Cytosolic protein concentration was determined with the Bradford method [2]. DPD activity was immediately measured according to the method described by Harris et al. [16]. The assay consisted in incubating 50 µl of cytosolic proteins with [$^{14}$C]5FU (20 µ*M* final), NADPH (250 µ*M* final) and $MgCl_2$ (2.5 m*M* final) in a final volume of 125 µl in 35 m*M* sodium phosphate buffer pH 7.5. The duration of the incubation was 30 min at 37 °C. The reaction was stopped by the addition of 125 µl ice-cold ethanol. The samples were centrifuged (400 *g*, 5 min) to remove proteins and the supernatant was analysed for the presence of [$^{14}$C]5FUH$_2$ using a high-performance liquid chromatography method. [$^{14}$C]5FU and [$^{14}$C]5FUH$_2$ were separated on a Prontosil column (5 µm, 250 × 4 mm), eluted at 0.8 ml/min with potassium phosphate 1.5 m*M* and tetrabutylammonium hydrogenosulfate 5 m*M*, pH 8.0. Fractions corresponding to [$^{14}$C]5FU and [$^{14}$C]5FUH$_2$ were collected and radioactivity was counted in a tri-carb Packard scintillation counter. DPD activity was expressed as pmol of [$^{14}$C]FU catabolized per min and per mg of protein. Each sample was assayed in duplicate and DPD activity was measured in two independent experiments. During each series of experiments, a control consisting of the determination of DPD activity in WiDR cells was performed. The series was validated only if the value of WiDR activity varied by less than 20% of the target values. The sensitivity of the method was 2 pmol/min · mg protein. The linearity with the protein concentration (in the range 0.1–0.4 mg/ml) and with incubation time (between 5 min and 60 min) were verified.

### Statistical analysis

Statistical tests were performed in two different ways (i.e. parametric or non parametric analysis) according to the size of compared groups. The analysis on paired samples of colon tumour and adjacent normal mucosa was performed with parametric tests. The normality of the two distributions and of the difference between the two distributions was verified by a Kolmogorov-Smirnov test. The homogeneity of the variances was verified using an *F* test ($P = 2.5\%$). The comparison of means between tumour and normal tissue was performed with a paired Student's *t*-test. The correlation between tumour and normal DPD activity was evaluated with a test of linearity. The significance level was 5% in all tests. For comparison of means in subgroups among either colon or liver tissues, the large difference of patient samples between the different subgroups necessitated the use of distribution-free tests (Kruskall Wallis and Mann-Whitney tests).

## Results

### Dihydropyrimidine dehydrogenase activity in colon tissue

DPD activity was detectable in all colon specimens. It was highly variable in normal tissue (%CV = 50%), in tumour (%CV = 67%; Fig. 1A) and in liver metastases from colon cancer (%CV = 77%). The mean activities were 41.9 pmol/min · mg protein and 40.3 pmol/min · mg protein in tumour and normal tissues, respectively,

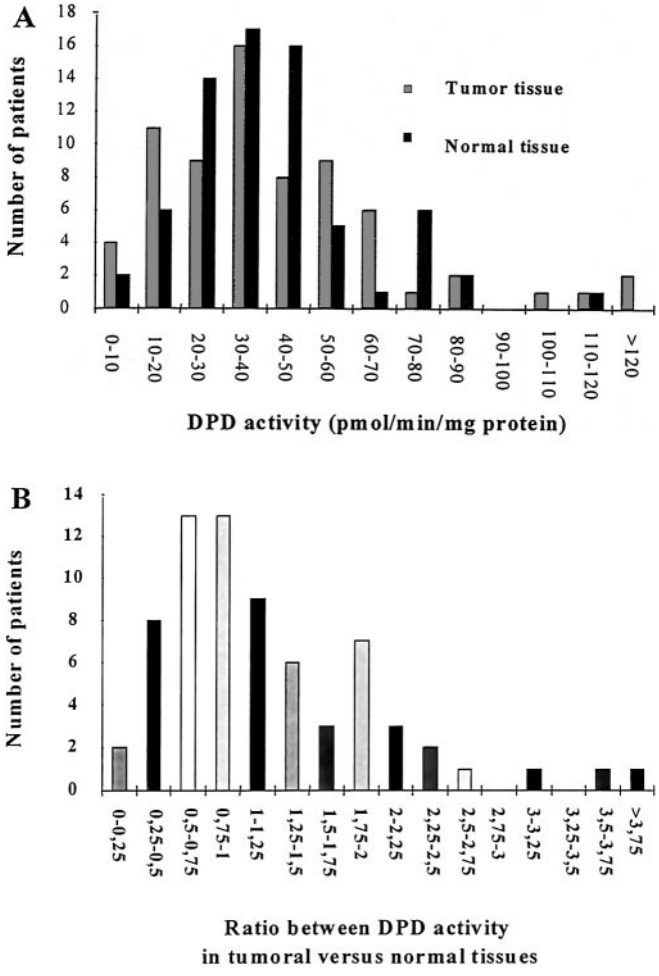


**Fig. 1** Dihydropyrimidine dehydrogenase (*DPD*) activity (pmol/min · mg protein) in colon carcinoma and normal colon tissue in the population of 70 patients. **A** Distribution of DPD activity among the population. **B** Distribution of the tumour-normal DPD activity ratio among the population

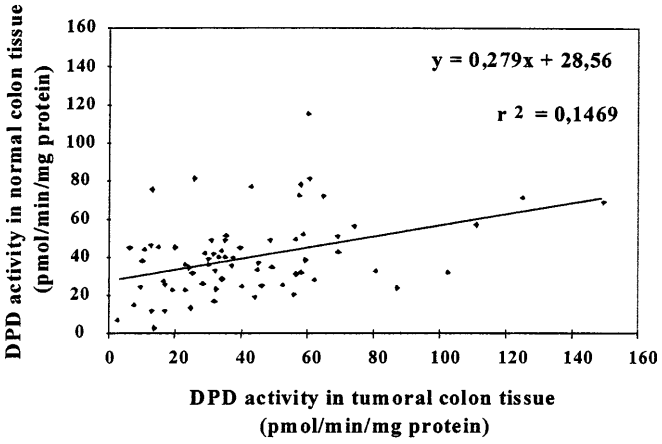


**Fig. 2** Correlation between dihydropyrimidine dehydrogenase (*DPD*) activity (pmol/min · mg protein) in colon tumour and adjacent normal tissue ($n = 70$)

demonstrating no significant difference ($P = 0.61$, ddl = 69; Table 1). The median tumour-normal ratio of DPD activity was 0.97 (range 0.14–4.82) and 37 out of 70 patients exhibited a ratio ranging from 0.6 to 1.4 (Fig. 1B). The correlation between DPD activities in tumour and normal mucosa (Fig. 2) was very weak, although statistically significant ($r^2 = 0.147$, $P = 0.001$, ddl = 68). No relationship existed between tumour DPD activity or tumour-normal ratio and sex, age, tumour Duke's stage or differentiation. In inflammatory colon tissue, i.e. UC or CD, the DPD activity was significantly higher than that observed in normal tissue ($P = 0.006$; Fig. 3). In liver metastases from colon cancer, DPD activity was not significantly different from that observed in primary colon tumour ($P = 0.32$; Fig. 3).

## Dihydropyrimidine dehydrogenase activity in liver tissues

DPD activity was evaluated in 20 normal liver, eight inflammatory liver and seven primary liver cancer specimens. The variability was high in inflammatory liver (%CV = 71%) and in liver tumours (%CV = 77%), but lower in normal liver (%CV = 34%). The DPD activity was significantly lower ($P = 0.001$) in primary liver tumour than in uninvolved liver specimens (Table 1; Fig. 4). In inflammatory liver tissue (hepatitis, cirrhosis), DPD activity ranged between normal and tumour tissues. However, this activity did not differ significantly either from normal tissue or primary liver cancer.

**Table 1** Characteristics of the population and dihydropyrimidine dehydrogenase (*DPD*) activity (pmol/min/mg protein) in the different tissues studied. *SD* standard deviation

| | Number of specimens | Mean age (range) | Sex (male/female) | DPD activity [mean ± SD (range)] |
|---|---|---|---|---|
| Normal colon tissue | 70 | 70 (38–93) | 41/29 | 40.3 ± 20.4 (2.9–115.5) |
| Inflammatory colon tissue | 10 | 38 (18–68) | 9/5 | 67.5 ± 16.7 (25.6–84.9) |
| Colon carcinoma | 70 | 70 (38–93) | 41/29 | 41.9 ± 27.9 (2.9–149.5) |
| Liver metastases | 9 | 60 (48–76) | 4/5 | 59.8 ± 46.4 (3.96–144.4) |
| Normal liver tissue | 20 | 60 (39–83) | 8/12 | 167.6 ± 57.6 (67.8–283.2) |
| Inflammatory liver tissue | 8 | 53 (32–60) | 7/1 | 119.5 ± 84.5 (2.01–242.2) |
| Primary liver cancer | 7 | 57 (48–72) | 5/2 | 50.5 ± 58.8 (2.5–171.8) |

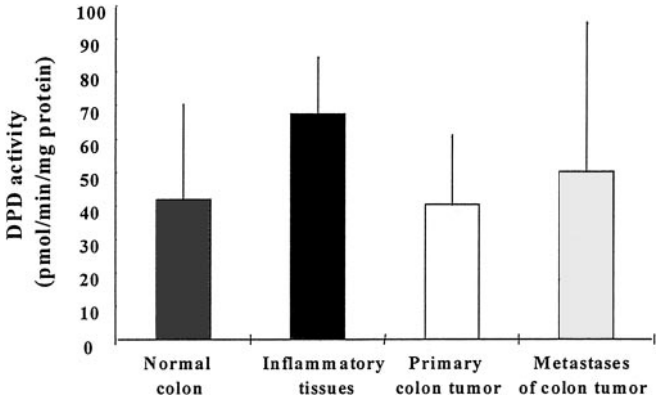


**Fig. 3** Comparison of dihydropyrimidine dehydrogenase (*DPD*) activity (pmol/min · mg protein) in normal ($n = 70$), inflammatory ($n = 10$), tumour colon tissue ($n = 70$) and liver metastases from a colon carcinoma ($n = 9$)

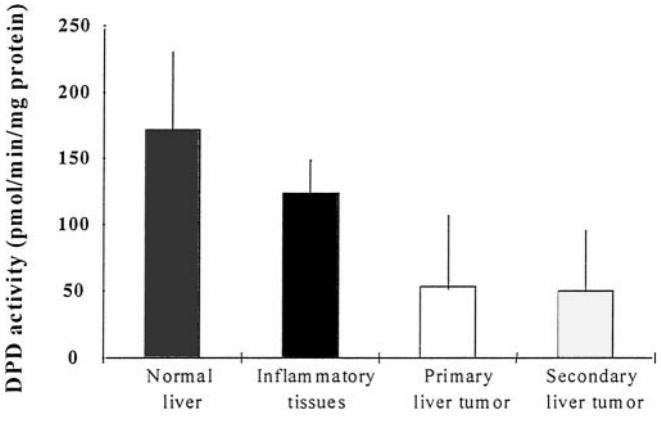


**Fig. 4** Comparison of dihydropyrimidine dehydrogenase (*DPD*) activity (pmol/min · mg protein) in normal ($n = 20$), inflammatory liver tissue ($n = 8$), and primary liver tumour ($n = 7$) and liver metastases from a colon carcinoma ($n = 9$)

## Discussion

Characterization of DPD in colon and liver diseases is of clinical interest because it is the initial and rate-limiting enzyme in the metabolic pathway of 5FU, the main chemotherapeutic agent used to treat colorectal cancer. Moreover, several studies demonstrated that DPD was responsible for most of the variability noted after administration of 5FU and contributed to 5FU clinical toxicity and tumour response [1, 4]. Our study is the first one to describe DPD activity in both colon and liver in normal, pathological but non-malignant and malignant tissues. Our goal was to find out whether a malignant process could contribute to change the DPD activity. DPD activity was found in most tissues, with the highest content in liver and peripheral blood mononuclear cells (PBMC) [5, 20, 21]. In our population, DPD was highly variable in tumour and normal tissues. The degree of variation was similar in tumour and normal tissue, both from colon and liver. Our results are in good agreement with those published by McLeod et al. [22] and Uetake et al. [25]. Fernandez-Salguero et al. [9] observed a significant increase in 5FU toxicity in patients with a DPD level in PBMC two-fold lower than the mean population. Thus a two-fold difference in activity could be related to a clinical effect, and the coefficient of variation on both tumour and normal mucosa in our population could be relevant, both in terms of toxicity and efficacy. Although a statistically significant correlation was observed between the DPD activity in normal and tumour tissues, its clinical relevance is questionable due to the large dispersion of paired values.

We evaluated the ratio of DPD activity between tumour and normal colon to assess both the degrees of efficacy and toxicity of 5FU. The tumour-normal ratio of DPD activity for colorectal tumours had a median value of 0.97, which was consistent with the data reported in head and neck tumours [8]. However, it differed from that reported by McLeod et al. [22] showing a ratio of tumour to normal colon of 0.76. Nine of 70 patients (13%) had a ratio greater than 2 in our series, which was higher than what reported by McLeod et al. [22] (5%). The significance of DPD activity in relation to 5FU responsiveness was demonstrated by considering the ratio of tumour DPD versus non-tumour DPD activity in patients with head and neck tumours: complete response exhibited a significantly lower ratio than partial or non-responding patients [8].

In inflammatory diseases of the colon (CD and UC), DPD activity was significantly higher than in normal or tumour tissues ($67.5 \pm 16.7$ versus $41.9 \pm 27.9$ and $40.3 \pm 20.4$ pmol/min · mg protein, respectively). Although we did not perform a histological analysis of the samples, the infiltration of the colon tissue by lymphocytes and inflammatory-related cells is a major feature of these diseases. Several reports showed that DPD activity in PBMC was important [5, 19]. The DPD activity in PBMC of healthy donors ranged from 50 to 660 pmol/min · mg protein, which is higher than in normal colon. So, the presence of lymphocytes associated with the inflammation could explain the high DPD level observed in these tissue specimens.

As reported in many studies, DPD activity in normal liver was the highest tissue activity [3, 23]. Our data are consistent with those published by Chazal et al. [3], obtained in 27 patients and showing a mean DPD activity of 178 pmol/min · mg protein ($168 \pm 58$ in our series). These results disagreed with those published by Jiang et al. [17], who showed, in a large series of 50 liver specimens in Chinese cancer patients with hepatocellular carcinoma, that DPD activity was approximately two-fold higher than in our experience: 450 pmol/min · mg protein (range 190–850). The DPD activity was lower in both tumour and normal colon than in normal liver, suggesting that the liver is the major detoxification site for 5FU.

Our data showed that the DPD activity in hepatocellular carcinoma was significantly lower than in uninvolved liver specimens, which is consistent with

previous studies in animals [24], as well as in humans [17]. However, our results disagreed with the latter report, which showed very high levels of DPD activity in hepatocellular carcinoma (340 ± 30 pmol/min/mg protein) compared with our series (50.5 ± 58.8 pmol/min · mg protein). This discrepancy could be explained either by the difference in the studied population (Asian versus Caucasian subjects) or by a methodological difference. DPD activity in primary liver cancer was comparable to the activity in primary colon cancer as found in other reports on colon [22] or head and neck cancer [8]. The low DPD activity observed both in liver metastases from colon cancer and from hepatocellular carcinoma is consistent with the report of Johnston et al. [18]. These authors showed that DPD mRNA expression, DPD activity and DPD protein content were lower in liver metastases from colon cancer than in normal liver. They suggested that the regulation of DPD activity in liver metastasis was not dependent on the liver environment but rather due to a hepatocyte-specific transcriptional control [18]. A similar process could take place in hepatocellular carcinoma.

In viral and alcoholic hepatitis, the level of DPD activity was between that in normal liver and in primary liver tumours. A disease-specific differential expression of xenobiotic-metabolizing cytochrome P450 (CYP) was demonstrated in patients with liver disease. The pattern of expressed CYP was different between normal liver, hepatocellular carcinoma and cirrhosis, and this expression seemed to be regulated both at the pre- and post-translational levels [13]. Given the potential value of DPD as a determinant of both tumour responsiveness and toxicity associated with 5FU [5], recent attempts have focused on modulating the activity of 5FU through inhibition of DPD. Several agents are undergoing clinical testing including UFT, eniluracil and S-1 [1, 12].

**Acknowledgements** This study has been supported by a grant from The Ferring Laboratory.

## References


1. Allegra CJ (1999) Dihydropyrimidine dehydrogenase activity: prognostic partner of 5-fluorouracil? [editorial]. Clin Cancer Res 5: 1947
2. Bradford MM (1976) A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248
3. Chazal M, Etienne MC, Renee N, Bourgeon A, Richelme H, Milano G (1996) Link between dihydropyrimidine dehydrogenase activity in peripheral blood mononuclear cells and liver. Clin Cancer Res 2: 507
4. Diasio RB, Johnson MR (1999) Dihydropyrimidine dehydrogenase: its role in 5-fluorouracil clinical toxicity and tumor resistance [editorial]. Clin Cancer Res 5: 2672
5. Diasio RB, Lu Z (1994) Dihydropyrimidine dehydrogenase activity and fluorouracil chemotherapy [editorial; comment]. J Clin Oncol 12: 2239
6. Diasio RB, Beavers TL, Carpenter JT (1988) Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. J Clin Invest 81: 47
7. Etienne MC, Lagrange JL, Dassonville O, Fleming R, Thyss A, Renee N, Schneider M, Demard F, Milano G (1994) Population study of dihydropyrimidine dehydrogenase in cancer patients. J Clin Oncol 12: 2248
8. Etienne MC, Cheradame S, Fischel JL, Formento P, Dassonville O, Renee N, Schneider M, Thyss A, Demard F, Milano G (1995) Response to fluorouracil therapy in cancer patients: the role of tumoral dihydropyrimidine dehydrogenase activity. J Clin Oncol 13: 1663
9. Fernandez-Salguero P, Gonzalez FJ, Etienne MC, Milano G, Kimura S (1995) Correlation between catalytic activity and protein content for the polymorphically expressed dihydropyrimidine dehydrogenase in human lymphocytes. Biochem Pharmacol 50: 1015
10. Fleming RA, Milano G, Thyss A, Etienne MC, Renee N, Schneider M, Demard F (1992) Correlation between dihydropyrimidine dehydrogenase activity in peripheral mononuclear cells and systemic clearance of fluorouracil in cancer patients. Cancer Res 52: 2899
11. Fleming GF, Vokes EE, Buse JB, Mick R, Dushay J, Levitan D, Dolan ME (1996) Peripheral blood mononuclear cell dihydropyrimidine dehydrogenase activity in volunteers with and without diabetes mellitus. Cancer Chemother Pharmacol 37: 569
12. Fukushima M, Shimamoto Y, Kato T, Uchida J, Yonekura R, Ohshimo H, Shirasaka T (1998) Anticancer activity and toxicity of S-1, an oral combination of tegafur and two biochemical modulators, compared with continuous i.v. infusion of 5-fluorouracil. Anticancer Drugs 9: 817
13. George J, Liddle C, Murray M, Byth K, Farrell GC (1995) Pre-translational regulation of cytochrome P450 genes is responsible for disease-specific changes of individual P450 enzymes among patients with cirrhosis. Biochem Pharmacol 49: 873
14. Gonzalez FJ, Fernandez-Salguero P (1995) Diagnostic analysis, clinical importance and molecular basis of dihydropyrimidine dehydrogenase deficiency. Trends Pharmacol Sci 16: 325
15. Grem JL, Yee LK, Venzon DJ, Takimoto CH, Allegra CJ (1997) Inter- and intraindividual variation in dihydropyrimidine dehydrogenase activity in peripheral blood mononuclear cells. Cancer Chemother Pharmacol 40: 117
16. Harris BE, Carpenter JT, Diasio RB (1991) Severe 5-fluorouracil toxicity secondary to dihydropyrimidine dehydrogenase deficiency. A potentially more common pharmacogenetic syndrome. Cancer 68: 499
17. Jiang W, Lu Z, He Y, Diasio RB (1997) Dihydropyrimidine dehydrogenase activity in hepatocellular carcinoma: implication in 5-fluorouracil-based chemotherapy. Clin Cancer Res 3: 395
18. Johnston SJ, Ridge SA, Cassidy J, McLeod HL (1999) Regulation of dihydropyrimidine dehydrogenase in colorectal cancer. Clin Cancer Res 5: 2566
19. Lu Z, Zhang R, Diasio RB (1993) Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients, and clinical implication in 5-fluorouracil chemotherapy. Cancer Res 53: 5433
20. Lu Z, Zhang R, Diasio RB (1995) Population characteristics of hepatic dihydropyrimidine dehydrogenase activity, a key metabolic enzyme in 5-fluorouracil chemotherapy. Clin Pharmacol Ther 58: 512
21. Lu Z, Zhang R, Carpenter JT, Diasio RB (1998) Decreased dihydropyrimidine dehydrogenase activity in a population of patients with breast cancer: implication for 5-fluorouracil-based chemotherapy. Clin Cancer Res 4: 325
22. McLeod HL, Sludden J, Murray GI, Keenan RA, Davidson AI, Park K, Koruth M, Cassidy J (1998) Characterization of dihydropyrimidine dehydrogenase in human colorectal tumours. Br J Cancer 77: 461
23. Naguib FN, el Kouni MH, Cha S (1985) Enzymes of uracil catabolism in normal and neoplastic human tissues. Cancer Res 45: 5405

24. Queener SF, Morris HP, Weber G (1971) Dihydrouracil dehydrogenase activity in normal, differentiating and regenerating liver and in hepatomas. Cancer Res 31: 1004

25. Uetake H, Ichikawa W, Takechi T, Fukushima M, Nihei Z, Sugihara K (1999) Relationship between intratumoral dihydropyrimidine dehydrogenase activity and gene expression in human colorectal cancer. Clin Cancer Res 5: 2836