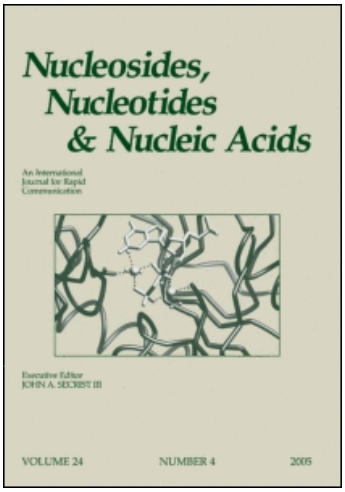

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Activity of Pyrimidine Degradation Enzymes in Normal Tissues

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ACTIVITY OF PYRIMIDINE DEGRADATION ENZYMES IN NORMAL TISSUES

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□ *In this study, we measured the activity of dihydropyrimidine dehydrogenase (DPD), dihydropyrimidinase (DHP) and β-ureidopropionase (β-UP), using radiolabeled substrates, in 16 different tissues obtained at autopsy from a single patient. The activity of DPD could be detected in all tissues examined, with the highest activity being present in spleen and liver. Surprisingly, the highest activity of DHP was present in kidney followed by that of liver. Furthermore, a low DHP activity could also be detected in 8 other tissues. The highest activity of β-UP was detected in liver and kidney. However, low UP activities were also present in 8 other tissues. Our results demonstrated that the entire pyrimidine catabolic pathway was predominantly confined to the liver and kidney. However, significant residual activities of DPD, DHP and β-UP were also present in a variety of other tissues, especially in bronchus.*

Keywords Pyrimidine degradation; Dihydropyrimidine dehydrogenase; Dihydropyrimidinase; ß-ureidopropionase

INTRODUCTION

In man, the pyrimidine bases uracil and thymine are degraded via a three-step pathway. Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme, catalyzing the reduction of thymine and uracil to 5,6-dihydrothymine and 5,6-dihydrouracil, respectively. The second step consists of a hydrolytic ring opening of the dihydropyrimidines which is

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catalysed by dihydropyrimidinase (DHP). Finally, the resulting N-carbamyl-*β*-aminoisobutyric acid and N-carbamyl-*β*-alanine are converted in the third step to *β*-aminoisobutyric acid and *β*-alanine, ammonia, and CO_2 by *β*-ureidopropionase (ß-UP). It is generally believed that the liver is the major organ for pyrimidine catabolism to occur. However, conflicting reports exist as to the expression of the enzymes from the pyrimidine degradation pathway in extrahepatic tissues.[1–4] In this study, we measured the activity of DPD, DHP, and ß-UP, using radiolabeled substrates, in 16 different tissues obtained at autopsy from a single patient.

MATERIALS AND METHODS

Patient

The patient was a man 50 years of age, who died from a glioma. Brain tissues were obtained 4–5 hours post mortem and the other tissue samples were obtained 5–6.5 hours post mortem. There was no indication for liver damage or failure. Informed consent was obtained from the family of the patient.

Enzyme Analysis

The DPD and DHP activity was determined using [4-^{14}C]-thymine and [2-^{14}C]-dihydrouracil, respectively, followed by separation of the substrates from the radiolabeled products by reversed-phase HPLC with on-line detection of the radioactivity.[5,6] The activity of ß-UP was determined using [^{14}C]-N-carbamyl-ß-alanine as a substrate and quantification of the reaction product $^{14}CO_2$ by liquid scintillation counting.[7]

RESULTS

The activity of DPD could be detected in all tissues examined, with the highest activity being present in spleen and liver (Table 1). The highest activity of DHP was present in kidney followed by that of liver. In contrast, the highest activity of ß-UP was detected in liver followed by that of kidney. Furthermore, a low DHP and ß-UP activity could be detected in 8 other tissues as well. Apart from testis, all tissues expressing ß-UP also expressed DHP. In cerebellum, only the activity of DPD and that of DHP could be detected but not that of ß-UP.

DISCUSSION

Previously, the tissue-specific expression of DPD has been investigated using tissues from different individuals.[1,2] A large variation in

TABLE 1 Activity of Pyrimidine Degradation Enzymes in Human Tissues

Tissue	DPD activity (nmol/mg/h)	DHP activity (nmol/mg/h)	ß-UP activity (nmol/mg/h)
Bronchus	0.66	0.27	1.1
Adrenal gland	1.4	0.06	0.06
Duodenum	1.8	0.11	0.38
Liver	2.3	12	36
Lung	1.1	0.10	0.30
Spleen	4.1	0.013	0.013
Kidney	0.67	17	4.4
Pancreas	1.3	0.05	0.14
Prostate	1.0	0.04	0.04
Thyroid gland	0.13	n.d. (<0.01)	n.d. (<0.004)
Muscle	0.21	n.d. (<0.02)	n.d. (<0.006)
Testis	1.8	n.d. (<0.01)	0.17
Cerebellum	0.18	0.1	n.d. (<0.009)
Cortex	0.21	n.d. (<0.03)	n.d. (<0.008)
Gray matter	0.24	n.d. (<0.02)	n.d. (<0.005)
White matter	0.10	n.d. (<0.03)	n.d. (<0.01)

n.d., not detectable

the DPD activity was observed, not only between different types of tissue but also within the same type of tissue obtained from different individuals.[1,2] To exclude the inter-individual variation, we have investigated the activity of the three enzymes of the pyrimidine degradation pathway in 16 different tissues obtained at autopsy from a single patient.

Our results demonstrated that the entire pyrimidine catabolic pathway was predominantly confined to the liver and kidney. Nevertheless, the activity of DPD could be detected in all tissues investigated. Furthermore, the DPD activity in spleen was even higher than that of liver, which is in apparent contrast with the results obtained by Ho and coworkers.[1] In their study, using tissues from different individuals, the DPD activity was on average 10-fold higher in liver when compared to that observed in other tissues.[1] Until now, the activity of DHP and ß-UP has not been measured in human tissues. Northern analysis suggested that human DHP and ß-UP are only expressed in liver and kidney.[3,4] However, using highly sensitive radiochemical assays, significant residual activities of DHP and ß-UP could be detected in a variety of other tissues, especially in bronchus. In cerebellum, only the activity of DPD and DHP could be detected indicating that adult human brain cells are not able to synthesise *ß*-alanine via the catabolism of uracil. In this respect, it should be noted that partial ß-UP cDNAs have been detected in human EST libraries of infant brain.[8]

REFERENCES

1. Ho, D.H.; Townsend, T.; Luna, M.A.; Bodey, G.P. Distribution and inhibition of dihydrouracil dehydrogenase activities in human tissues using 5-fluorouracil as a substrate. *Anticancer Res.* **1986**, 6, 781–784.
2. Naguib, F.N.M.; el Kouni, M.H.; Cha, S. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res.* **1985**, 45, 5405–5412.
3. Hamajima, N.; Matsuda, K.; Sakata, S.; Tamaki, N.; Sasaki, M.; Nonaka, M. A novel gene family defined by human dihydropyrimidinase and three related proteins with differential tissue distribution. *Gene* **1996**, 180, 157–163.
4. Sakamoto, T.; Fujimoto-Sakata, S.; Matsuda, K.; Horikawa, Y.; Tamaki, N. Expression and properties of human liver ß-ureidopropionase. *J. Nutr. Sci. Vitaminol.* **2001**, 47, 132–138.
5. Van Kuilenburg, A.B.P.; Van Lenthe, H.; Tromp, A.; Veltman, P.C.J.; van Gennip, A.H. Pitfalls in the diagnosis of patients with a partial dihydropyrimidine dehydrogenase deficiency. *Clin. Chem.* **2000**, 46, 9–17.
6. Van Kuilenburg, A.B.P.; Van Lenthe, H.; Van Gennip, A.H. Radiochemical assay for determination of dihydropyrimidinase activity using reversed-phase high-performance liquid chromatography. *J. Chrom. B* **1999**, 729, 307–314.
7. Van Kuilenburg, A.B.P.; Van Lenthe, H.; Van Gennip, A.H. A Radiochemical assay for ß-ureidopropionase using radiolabeled N-carbamyl-ß-alanine obtained via hydrolysis of [2–^{14}C]5,6-dihydrouracil. *Anal. Biochem.* **1999**, 272, 250–253.
8. Vreken, P.; van Kuilenburg, A.B.P.; Hamajima, N.; Meinsma, R.; van Lenthe, H.; Göhlich-Ratmann, G.; Assmann, B.E.; Wevers, R.A.; van Gennip, A. cDNA cloning, genomic structure and chromosomal localisation of the human BUP-1 gene encoding ß-ureidopropionase. *Biochim. Biophys. Acta* **1999**, 1447, 251–257.