Ned Tijdschr Klin Chem Labgeneesk 2006; 31: 215-216

Activity of pyrimidine degradation enzymes in normal tissues

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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, catalysing 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 catalysed by dihydropyrimidinase (DHP). Finally, the resulting N-carbamyl-*ß*-aminoisobutyric acid and N-carbamyl-*ß*-alanine are converted in the third step to *ß*-aminoisobutyric acid and *ß*-alanine, ammonia and CO_2 by *ß*-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

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.

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Discussion

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

Table 1. Activity of pyrimidine degradation enzymes in human tissues; n.d. means not detectable

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)

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 synthesize *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).

Acknowledgements

We thank dr. C. van Noesel and prof. dr. D. Troost of the department of Pathology of the Academic Medical Center for providing the tissue samples.

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Ned Tijdschr Klin Chem Labgeneesk 2006; 31: 216-218

Analysis of pyrimidine synthesis de novo intermediates in urine and dried urine filter-paper strips with HPLC-electrospray tandem mass spectrometry

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Pyrimidine nucleotides are essential for a vast number of biological processes such as the synthesis of RNA, DNA, phospholipids, glycogen and the sialylation and glycosylation of proteins. Pyrimidines can be synthesized de novo in mammalian cells through a multistep process and via the salvage of the pyrimidine nucleosides uridine and cytidine. Proliferating cells usually require a functional pyrimidine de novo pathway to sustain their increased demand for nucleotides. Therefore, a number of inhibitors have been developed against enzymes of the pyrimidine de novo pathway, such as dihydroorotate dehydrogenase, which show potent antiproliferating effects in tumor cells or proliferating T-lymphocytes. Pathological conditions, such as a deficiency of UMP synthase or a urea-cycle defect can result in altered excretion of metabolites of the pyrimidine de novo pathway (1, 2).

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Patients with a deficiency of UMP synthetase excrete excessive amounts of orotate in their urine. In contrast, patients suffering from a urea cycle disorder can, in addition to orotate, also excrete strongly elevated levels of orotidine, uridine and uracil (2). We have developed a fast and sensitive method, using HPLC-tandem mass spectrometry, allowing the detection of all pyrimidine de novo metabolites within a single analytical run of 14 min, using urine or urine-soaked filter paper strips (3).

Materials and Methods

Urine samples were centrifuged and 10 µl of the IS and 2 µl of 25% (v/v) HCOOH was added to 100 µl of clear urine. After centrifugation, 50 µl of this urine was injected into the HPLC-MS/MS system.

Urine (200 µl) was added to filter paper strips, dried at room temperature and 20 µl of the IS was deposited on the strip. After drying, the strip was extracted with 1.5 ml of 75% (v/v) methanol, dried at 40 °C and subsequently dissolved in 200 µl of 50 mM HCOOH (pH 2.6) and sonificated for 5 min. After centrifugation, 50 µl of the clear extract was injected into the HPLC-MS-MS system.