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HUMAN TISSUES DEGRADE URIDINE MUCH LESS THAN THYMIDINE. POSSIBLE CONSEQUENCE FOR 5-FLUOROURACIL THERAPY

B. LIERMANN, E. MATTHES and P. LANGEN
Central Institute of Molecular Biology, Department of Cell Kinetics, 1115 Berlin-Buch G.D.R.

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Abstract—In view of clinical trials to improve FUra chemotherapy of cancer by combined application with Urd and dThd, we investigated the capacity of human tissues to split these nucleosides.

All human normal and neoplastic tissues gave a uridine-splitting activity which can be inhibited by β-L-pTdR and behaves in this respect as uridine-deoxyuridine phosphorylase (EC 2.4.2.3). dThd splitting, however, which is 2–9-fold higher than that of Urd, is insensitive towards β-L-pTdR, confirming earlier results that it is due to thymidine phosphorylase (EC 2.4.2.4).

On the other hand, tissues, e.g. spleen of rats and mice, in which dThd and Urd are split by uridine-deoxyuridine phosphorylase, degrade 2–5-fold more Urd than dThd. Thus, free pyrimidine base competing with FUra for degradation and thus prolonging the life time of the drug in the body, will be formed mainly from dThd in the human body but more so from Urd in the rat or mouse.

Introduced more than 20 years ago, FUra has been one of the most widely applied cancerostatic agents. In recent time some efforts have been made to improve the chemotherapeutic effect of FUra by combination of the drug with natural metabolites, as dThd [e.g. 1]; or by use of the depot form 5′-deoxy-5-fluorouridine, which depends on activation by a uridine-splitting enzyme activity [2–4]. The complex metabolism of FUra and its interaction with metabolic products formed from dThd or Urd are shown in Fig. 1. The final products of FUra anabolism, FdUMP or FUTP are responsible for the cytostatic effect of the drug, brought about in two different ways, i.e. inhibition of thymidylate synthetase and thereby of DNA replication, and impairment of RNA function. Both modes of action are operative in cells, depending in their relative importance on drug concentration and cell type [e.g. 5–8]. dThd bypasses the thymidylate synthetase blockade and should thus prevent the effect on DNA synthesis. Combination of FUra with dThd has been studied in animals and patients and improvement of cancerostatic [1, 5, 7, 9–11] effect as well as increased toxicity [12–14] have been reported. Urd will largely prevent the FUra effect on RNA by competing with the formation of FUTP and with its incorporation into RNA. This should render FUra into an S-phase specific agent with the corresponding advantages and disadvantages. Furthermore, since FUrd is more toxic than either FUra or FdUrd [12, 15], it could be assumed that interference with the conversions of the ribose derivatives of FUra will lessen toxicity. The combination with Urd to our knowledge has been tried in mice only, again with the result of increased efficiency [1, 16]. So it was shown recently that mice can be rescued from the lethal toxicity of FUra by infusion of Urd but not of dThd or dUrd [17].

As follows from the scheme, dThd and Urd undergo still another metabolic conversion of decisive importance for FUra activity: by degradation of these nucleosides, thymine or uracil are formed competing with the degradation of FUra and thereby prolonging its half life time and increasing its efficiency [18, 19]. In this way the long known "paradoxical" fact is explained that dThd which should lower or prevent the FUra effect (and *in vitro*, where dThd splitting is low, does so) actually increases its effect *in vivo* [20].

It follows that for the application of FUra itself,

Abbreviations: Urd, uridine; dUrd, 2′-deoxyuridine; dThd, thymidine; FUra, 5-fluorouracil; β-L-pTdR,1-(β-L-2-deoxyribopyranosyl) thymine; β-D-pTdG, 1-(β-D-2-deoxy-glucopyranosyl)thymine; CML, chronic myelogenous leukemia.

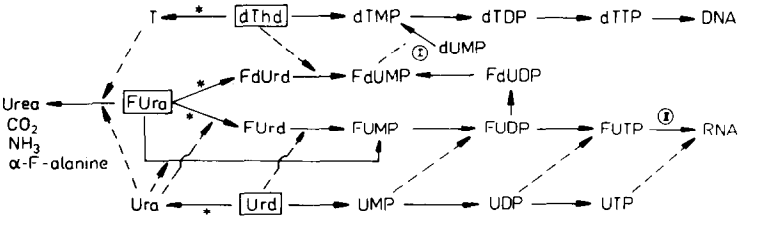

Fig. 1. Pathways of FU, dThd and Urd metabolism. Dotted arrows point to sites of interference by inhibition or competition: Reactions of pyrimidine nucleoside phosphorylases. I, II modes of FUra action.

its depot form 5'-deoxy-5-fluorouridine and the combination of FUra with dThd and Urd the role of pyrimidine nucleoside phosphorylases is of crucial importance; they contribute to the activation of FUra, they liberate the drug from the depot form mentioned and, by splitting dThd and Urd, largely determine the outcome of a combination with these nucleosides.

The tissues of the most frequently used laboratory animals (rats and mice, but also cats and dogs) contain two different pyrimidine nucleoside phosphorylases, either thymidine phosphorylase (EC 2.4.2.4) being strongly specific to deoxyribonucleosides [21–23], or uridine-deoxyuridine-phosphorylase (EC 2.4.2.3) which catalyzes the cleavage of Urd, dUrd and dThd [24] and can be inhibited by pyranosyl nucleosides [25–30]. For human tissues the situation is not clear. To our knowledge, dThd is split solely by thymidine phosphorylase [25–27, 31]; but little information is available concerning Urd catabolism. Krenitsky *et al.* [32] described a uridine-cleaving enzymatic activity in human liver and fibrosarcoma extracts which was unseparable from uric acid ribonucleoside phosphorylase. Gallo and Perry [33] reported on the cleavage of Urd by crude leucocyte extracts from healthy donors and patients with CML. Woodman *et al.* [34] have described a uridine-splitting activity in HeLa cells which could be inhibited by the pyranoid nucleoside β-D-pTdG. In contrast to the results of Gallo and Perry, however, these authors did not find any uridine-splitting activity in partially purified preparations of human leucocytes. It seems that the uridine-splitting activity in leucocytes is very unstable and was possibly destroyed by the purification steps used by Woodman. Recently, Piper and Fox [8] demonstrated the presence of uridine phosphorylase in a human B cell line. In a search for differences between normal and neoplastic tissues, Pauly *et al.* [35] found a significantly higher thymidine phosphorylase in the plasma of cancer patients than in plasma of control persons, but in both groups the amount of Urd catabolized was negligible.

We therefore studied whether and to what extent human normal and neoplastic tissues (surgical samples, bone marrow, leukemic blood) are able to split Urd and how this is related to their dThd splitting capacity. The results were compared with those obtained with rats and mice, the main objects of experimental studies on FUra-therapy modulation.

MATERIALS AND METHODS

Substance. Urd was purchased from Serva, Heidelberg; dThd was obtained from Sigma. β-L-pTdR was synthesized in our department. (Methyl-3H)-thymidine (sp. act. 24 Ci/mmole) was obtained from the Nuclear Research Institute, Rossendorf and (6-3H)-uridine was purchased from UVVVR, Prague.

Tissues. Histologically verified tumor material and normal tissues of man were obtained from the department of histology of the Robert Roessle Klinik, Berlin-Buch.

Enzyme isolation. The tissues were washed twice in ice-cold 0.9% NaCl solution, homogenized in 3 vol of 5 mM potassium phosphate buffer (pH 7.0), containing 5 mM β-mercaptoethanol and 1 mM EDTA. After centrifugation at 16,000 *g* for 30 min at 4°, the supernatant was taken as enzyme source.

Pyrimidine nucleoside phosphorylase assay. The arsenolytic cleavage of dThd or Urd was assayed as follows: The incubation mixture (1 ml contained 48 mM arsenate–80 mM Tris–HCl buffer (pH 7, 4); 1mM unlabelled nucleoside (final concentration), in inhibition studies 1mM β-L-pTdR; 1 μCi of the appropriate radioactive nucleosides (which were demonstrated by paper chromatography to be more than 97% pure); 5–7 mg protein (only in 2 samples of mammary carcinoma the content was 2 mg). The assay mixture was incubated at 37° for 5–40 min. The reaction is linear for 30 min, for Urd splitting in some cases (as mammary carcinoma) for 60 min. Small deviations from linearity did not alter the conclusions from the results. The reaction was stopped by boiling for 3 min, and the precipitates were removed by centrifugation. The supernatant fractions were analyzed for tritiated bases (products) and nucleosides (substrates) by paper chromatography, using ethyl acetate : water : formic acid (12 : 7 : 1; by vol.).

The radioactivity of the u.v.-absorbing spots was estimated in a liquid scintillation counter.

RESULTS

The data presented in Table 1 show that a phosphorolytic splitting of Urd occurs in crude extracts of normal (spleen, liver bone marrow) and malignant (mammary carcinoma, small intestine carcinoma, leucocytes of a patient with CML) human tissues.

This cleavage is strongly inhibited by β-L-pTdR. Therefore it is not due to thymidine phosphorylase which is not inhibited by this compound. Urd cleavage in the investigated tissues is between 9 and 33% at 1mM substrate concentration during 30 min incubation.

The high substrate concentration was used in correspondence to the one achieved in clinical studies of the modulation of the cancerostatic action of FUra by dThd [1]. The data in the right column, the ratio of dThd: Urd cleavage, show that in each case thymidine phosphorylase activity is much higher (2–9-fold) than uridine-splitting activity.

For comparison of phosphorylase activities in human and animal tissues, Table 2 shows the cleavage of Urd and dThd by extracts from spleen and liver of man, rat and mouse. Resulting from the exceptionally high thymidine phosphorylase activity in human spleen and the lacking activity of this enzyme in the spleen of rat and mouse [see also 27], the ratio of dThd : Urd cleavage shows great differences: man 2.9; rat 0.2 and mouse 0.4. The values for the spleen of these laboratory animals agree well with those for their tumors described in the literature, e.g. Novikoff hepatoma 0.1 and Ehrlich ascites carcinoma 0.2 [34].

For liver, however, the situation is different. The data in Table 2 show that here for rats and mice—as in man—splitting of dThd is not inhibited by β-L-pTdR and, therefore, is due to thymidine phosphorylase. Accordingly, the ratio of dThd : Urd cleavage is > 1. These data confirm that liver—in

Table 1. Pyrimidine nucleoside phosphorylase activity in human tissues

Tissue	Uridine-splitting activity Urd	% inhibition by β-L-pTdR§	Thymidine phosphorylase dThd	% inhibition by β-L-pTdR	Ratio of dThd : Urd cleavage
Spleen*	24 ± 8.0	92	71 ± 10.4	3	2,9
Liver†	13 (9; 16)	99	43 (42; 48; 50;)	2	3,3
Bone marrow‡	13	92	80	2	6,1
Small intestine‡ carcinoma	15	87	n.e.‖	n.e.	–
Blood (CML)‡	33	99	75	0	2,2
Mammary* carcinoma	9 ± 6,0	89	80 ± 4,2	4	8,9

Details of assay are described in Methods. Substrate and inhibitor concentrations were 1 mM. Incubation time was 30 min at 37°. Values for nucleoside phosphorylase activity correspond to percent cleavage of substrate.

* Mean value and standard deviation of 7 (spleen) or 6 (mammary carcinoma) samples.

† Mean value, in brackets single values.

‡ Single sample.

§ Values from 2 to 3 experiments.

‖ n.e., not estimated.

contrast to the other organs of rats and mice with uridine-deoxyuridine phosphorylase activity such as kidney [27], lung (unpublished results) and transplantable tumors [25–27]—contains thymidine phosphorylase [21–23, 26, 34]. As shown by Niedzwicki *et al.* [36], mouse liver also contains an uridine phosphorylase with a low dThd degrading capacity. Thus, our data for mouse liver confirms their results.

DISCUSSION

In this study we present evidence that crude extracts of all investigated normal and neoplastic tissues (liver, spleen, mammary carcinoma, small intestine carcinoma, leucocytes of a patient with CML) contain a uridine-splitting activity. Therefore, the capacity for catabolizing Urd seems to be a general property of human tissues.

The fact that only occasional and partly controversial findings have been published on this matter [8, 32–35] may be explained by the relatively low uridine-splitting activity compared with that of thymidine phosphorylase. Based on our early observation that uridine-deoxyuridine phosphorylase of animal tissues (mouse, cat, rat) can be selectively inhibited by pyranoid thymine nucleosides, e.g. β-L-pTdR [25], we now show that the uridine-splitting activity in human tissues is also inhibited by this compound. In contrast, in human tissue homogenates, degradation of dThd is almost totally due to thymidine phosphorylase as shown by its insensitivity towards pyranoid nucleosides. The same is true for dUrd (not shown). The situation in human tissues differs from that in mice and rats. In the latter, the cleavage of all three natural substrates (Urd, dUrd, dThd) can be inhibited in most tissues (except liver) and all tumors by pyranoid nucleosides [25–29], indicating that a uridine-deoxyuridine phosphorylase is involved. In all these tissues, the splitting of Urd is much higher than that of dThd.

As the opposite is true for human tissues, this should have consequences for clinical trials on the combination of FUra with dThd or Urd. Since in humans much less uracil is formed from Urd than thymine from dThd, the base-dependent increase in toxicity should be considerably lower for the combination of FUra with Urd than the one observed after its combination with dThd.

Our results demonstrate that experiments with FUra, which are depending in a special way on the

Table 2. Phosphorolytic splitting of Urd and dThd in spleen and liver of man, rat and mouse

Tissue	Source	Urd	% inhibition by β-L-pTdR	dThd	% inhibition by β-L-pTdR	ratio of dThd : Urd cleavage
Spleen	Man	24	92	70	3	2,9
	Rat	50	94	9	99	0,2
	Mouse	16	81	7	57	0,4
Liver	Man	12	99	43	2	3,5
	Rat	19	95	31	7	1,6
	Mouse	14	93	33	6	2,4

Details of assay are described in Methods. Substrate and inhibitor concentrations were 1 mM. Incubation time was 30 min at 37°. Values for nucleoside phosphorylase activity correspond to percent cleavage of substrate. Livers or spleens of 4 or 5 animals were pooled.

pyrimidine nucleoside phosphorylases, carried out on mice and rats, might not properly reflect the situation in human beings.

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