Urol Res (1985) 13:219–221

Urological Research

Short Report

Pseudouridine and Uridine in Normal Kidney and Kidney Cancer Tissues

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Accepted: November 15, 1984

Summary. The tissue concentrations of a modified nucleoside, pseudouridine, and a normal nucleoside, uridine, were measured with high-performance liquid chromatography. Human kidneys were obtained from five patients with renal cell carcinoma and divided into a noncancerous part and a cancerous part. The pseudouridine concentration in the cancerous part of the kidneys ranged between <2–2.8 nmoles/g and in the noncancerous part 4.3–19.4 nmoles/g (mean 10,9 nmoles/g). The uridine concentration in the cancerous and noncancerous parts of the kidney ranged between 19.6–179.1 nmoles/g (mean 110.7 nmoles/g) and 117.5–235.6 nmoles/g (mean 191.5 nmoles/g), respectively. The pseudouridine concentration appeared to be approximately seven times higher in the noncancerous part as compared to the cancerous part of the kidney. In the case of uridine, the difference was less pronounced.

Key words: Pseudouridine, Uridine, Renal cell carcinoma, High-performance liquid chromatography.

Introduction

The urinary excretion of modified nucleosides has been found to be elevated in a variety of neoplastic diseases (for a review see [3]). Such modified nucleosides occur primarily in transfer RNA (tRNA). Following transcription, some of the bases of the tRNA chains are modified by specific enzymes [1]. Later, when the tRNA breaks down, these nucleosides are liberated. Free modified nucleosides do not appear to be catabolized or reutilized by cells and are therefore rapidly excreted [13]. The dominant modified nucleoside is pseudouridine (Ψ).

Ψ has been found to be elevated in both urine and blood in several neoplastic diseases [11, 12]. We have previously shown that Ψ elevations is associated with carcinoma of the kidney, bladder, ovary and uterus [6]. It is therefore of interest to analyse the tissue concentrations in renal cell carcinoma and in the non-cancerous part of the same kidney, utilizing a new high-performance liquid chromatography (HPLC) procedure. The normal nucleoside, uridine, was measured simultaneously.

Material and Methods

Clinical Material

Five kidneys were removed surgically for renal cell carcinoma. One kidney was removed because of tumor of the ureter. The cancerous and the noncancerous portions of the kidneys were frozen separately immediately after removeal and stored at −40 °C until analysis.

Tissue Extraction

250 to 500 mg of tissue was disrupted mechanically in 2.0 ml of 6% trichloracetic acid [5]. The suspension was centrifuged at 2,500 g for 30 min, 4 °C. The supernatant was mixed with an equivalent volume of a 1:2 solution of tri-n-octylamine and 1,1,2 trichlorotrifluoroethane. The sample was centrifuged at 1,000 g for 5 min at 4 °C, and the top layer collected. The nucleosides were recovered from the extract by the boronate gel method developed by Davis et al. [4]. 0.1 ml of 2.5 M ammonium acetate was added to every ml of sample. The samples were loaded onto columns and washed with 8 ml of 0.25 M ammonium acetate. The nucleosides were eluted in 12 ml of 0.1 M formic acid and lyophilized.

Reagents

Nucleoside standard samples of analytical grade were purchased from Sigma (St. Louis, MO, USA).

Chromatographic Procedure

The lyophilized sample was dissolved in 550 µl of H_2O and a 50 µl aliquot was analysed on a 5 µm NOVA-PAC C18 column (8 x 100

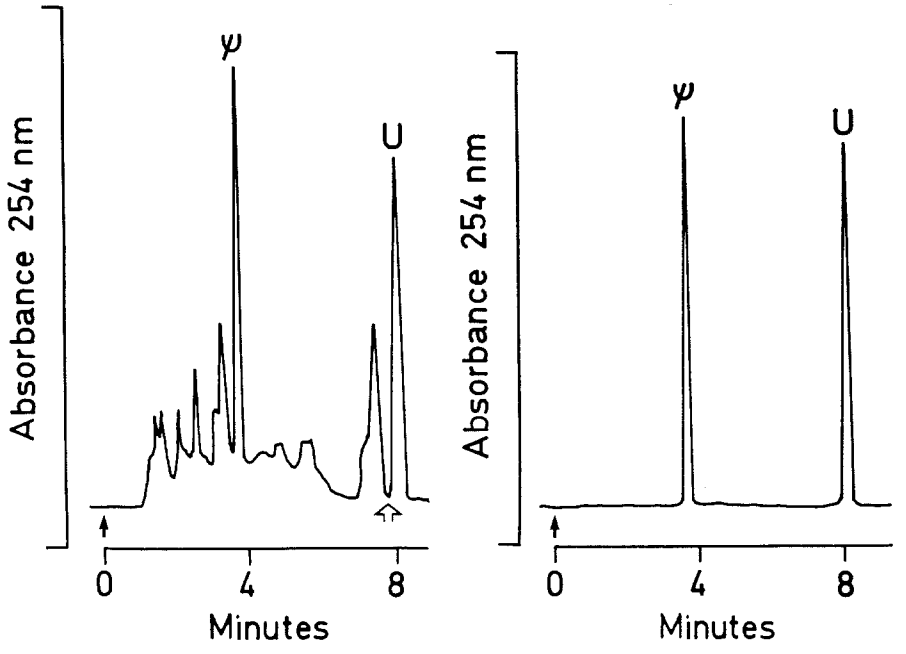

Fig. 1A, B. Reversed-phase HPLC separation of nucleosides in noncancerous portion of neoplastic kidney (Case 5) (A); sample corresponding to 25 mg of tissue. Detector sensitivity = 0.02 changed to 0.2 at mark ↶. B Reversed-phase HPLC separation of pseudouridine and uridine standard samples, 500 pmol of each nucleoside. Detector sensitivity = 0.02. Other conditions as in Material and Methods

Table 1. Concentrations of pdeusouridine and uridine in noncancerous and cancerous portions of kidneys

Case No.	Pseudouridine[a] (nmol/g)		Uridine[a] (nmol/g)	
	Noncancerous part	Cancerous part	Noncancerous part	Cancerous part
1	6.6	<2	204.8	159.8
2	4.3	<2	117.5	69.5
3	5.4	<2	206.2	19.6
4	18.8	2.5	193.4	179.1
5	19.4	2.8	235.6	125.4
6[b]	7.6	–	53.0	–

[a] Each value is the average to two different samples of the same material

[b] Normal kidney removed because of tumor of the ureter

mm, Waters Associates, Milford, Mass., USA). The mobile phase consisted of 10 mM acetate buffer, pH 4.7, flow rate 2.0 ml/min, ambient temperature.

Identification of the eluted UV-absorbing compounds was done in three ways:

1) by comparing retention times of standard nucleosides with those of the unknown peaks;

2) by comparing the absorbance ratios (A280/A254) of standard nucleosides with those of the unknown peaks;

3) by chromatography of the sample together with standard nucleosides.

Results

Tissue Sample Analysis

The HPLC separation of pseudouridine and uridine present in the extracts from the noncancerous part of a neoplastic kidney is shown in Fig. 1A. For comparison, a standard chromatogram of pseudouridine and uridine is included in Fig. 1B. Table 1 shows the concentrations of pseudouridine and uridine in noncancerous and cancerous tissues of six kidneys. The range of pseudouridine in the noncancerous portions was 4.3–19.4 nmol/g (mean 10.9 nmol/g). The values in cancerous tissues ranged from <2 to 2.8 nmol/g.

The amount of uridine in noncancerous portions of the kidneys ranged from 117.5 to 235.6 nmol/g (mean 191.5 nmol/g), and in the cancerous part from 19.6 to 179.1 nmol/g (mean 110.7 nmol/g).

Nucleoside Recovery

Recovery by the present method was checked by adding known amounts of Ψ and was found to range between 80% and 95%.

Some authors have noted that xanthine and hypoxanthine interfere in HPLC measurement of uridine [5, 7]. In our assay, however, these substances were completely separated from uridine and did not interfere.

Discussion

We have measured Ψ and uridine in neoplastic and noncancerous portions of tumor-bearing human kidneys.

The results indicate that the noncancerous portion of a neoplastic kidney contains approximately seven times more Ψ than the cancerous portion. The Ψ concentrations in the noncancerous part of the neoplastic kidneys were similar to the Ψ concentration found in a single normal kidney. The Ψ concentrations in kidney appeared to be of the same order of magnitude as the blood concentration found by others [12].

Tumor tissues are reported to exhibit an elevated tRNA turnover rate compared to normal counterparts [2]. The post-transcriptional modification enzyme activities (tRNA methylases) appear to be elevated in cancerous tissues [8].

Infussion of uridine is known to counteract antitumor and toxic effects of certain cancer chemotherapeutic drugs [14]. Examples are 5-fluoroudine, 5-fluorouracil and other pyrimidine antimetabolites. It should therefore be important to know the intracellular concentrations of uridine.

The noncancerous portions of neoplastic kidneys contained 1–10 times more uridine than the cancerous parts. The uridine concentrations of the tumor kidneys were somewhat higher than those in the single normal kidney. The kidney uridine concentrations measured here were more than ten times higher than the blood concentrations found by Karle et al. [5]. This is interesting in the light of experiments by Moyer and coworkers [9], who infused radioisotope labelled uridine i.v. in rats and found a highly specific uptake in lung, spleen and kidneys.

It is not known why the noncancerous portion of a neoplastic kidney contains more Ψ and uridine than the cancerous part. Dedifferentiated tissue may have lower amounts of several intracellular compounds. Two reports have described decreased levels of Ψ in tRNA in cancer tissues compared to the normal counterparts [8, 10]. Our results are in accordance with those reports.

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