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Urinary Modified Nucleosides as Tumor Markers in Cancer of the Urinary Organs or Female Genital Tract

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Summary. Using a sensitive and specific method involving high-performance liquid chromatography, urinary levels of four modified nucleosides – pseudouridine (Ψ), 1-methylinosine (m^1I), 1-methyladenosine (m^1A), and 1-methylguanosine (m^1G) – were investigated before and after treatment in 31 patients with cancer of the urinary organs or the female genital tract. Before treatment m^1I was the most frequently elevated nucleoside (77%). Pretreatment urinary levels of Ψ, m^1I, and m^1A in patients with stage 2–4 cancer of the female genital tract were significantly elevated compared to human healthy volunteers ($p < 0.005$). Compared with the other nucleosides, Ψ appeared to correlate more closely with the clinical outcome (progression or regression) of patients with cancer of the female genital tract. In the case of patients with cancer of the urinary organs, m^1I followed the clinical outcome better than the other nucleosides measured. Therefore Ψ and m^1I seem to be useful for monitoring genito-urinary cancers.

Key words: Urinary modified nucleoside, Cancer of the urinary organs, Cancer of the female genital tract, High-performance liquid chromatography.

Introduction

Transfer RNA (tRNA) contains the four major nucleosides as well as a variety of post-transcriptionally modified nucleosides. Catabolism of tRNA breaks the chain down into its constituents. The major nucleosides are then broken down further or reused in the cell through the salvage pathway. The modified nucleosides are handled in a different way. They appear to be excreted from the body unchanged [9]. Measurement of modified nucleosides in the urine may therefore be a way to estimate the tRNA turnover rate in the body [2, 9]. Neoplastic diseases may be associated with increased tRNA turnover rate [2] but more research is needed on this point. It is, however, an established fact that many cancer patients have an elevated level of modified nucleosides in the urine (for review see [3], It has therefore been suggested that modified nucleosides may be useful as tumor markers.

A few cases of patients with cancer of the urinary organs or female genital tract have been reported to have elevated modified nucleosides [10, 13, 15]. We have therefore measured four different modified nucleosides in the urine of patients suffering from cancer of the cervix, uterus, ovary, kidney or bladder. The pre- and post-treatment values have been compared with the extent of tumor disease and with the short-term clinical course.

Materials and Methods

Normal Controls

Urines from 21 normal healthy adults, 16 females and 5 males, were used as controls.

Clinical Materials

Eighteen patients with cancer of the cervix, uterus or ovary, five patients with bladder cancer and eight patients with renal cell carcinoma were selected for analysis of modified nucleoside levels in urine. Urine samples were obtained aseptically from the patients at initial diagnosis and after treatment, and stored at −20 °C until analysis. The bladder and kidney tumors were staged according to the TNM system, the gynecological tumors according to the FIGO classification (Annual Report Gynecological Cancer, Vol. 18).

Urine Sample Preparation

The nucleosides were recovered from the urine samples by using the boronate gel method originally developed by Davis et al. [5], and modified by Rasmuson and coworkers [11].

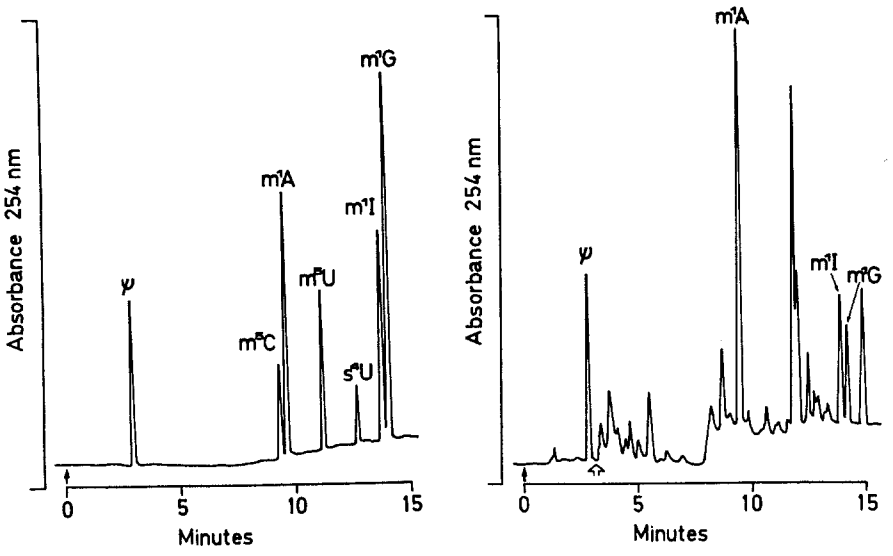

Fig. 1A, B. Reversed-phase HPLC separation of a mixture of seven modified nucleoside standard samples (A), 250 pmol of each nucleoside. Detector sensitivity = 0.02, other conditions as in Material and Methods. B Reversed-phase HPLC separation of nucleosides in normal control urine. 100 µl, equivalent to 10 µl of urine. Detector sensitivity = 0.2, changed to 0.01 at mark↑. Other conditions as in Material and Methods

Table 1. Concentrations of modified nucleosides in urine of human healthy volunteers

Nucleoside	[(mean ± 2 SD) nmoles/µmoles urinary creatinine]				
	Speer 1979	Gehrke 1979	Colonna 1980	Borek 1983	Koshida 1984
Pseudouridine (ψ)		22.6 ± 4.32	17.77 ± 6.36	22.4 ± 4.20 26.7 ± 9.0	19.3 ± 5.38
1-Methyladenosine (m^1A)	1.77 ± 0.58		4.32 ± 1.58	1.77 ± 0.58 1.76 ± 0.96	1.63 ± 0.50
1-Methylguanosine (m^1G)			0.96 ± 0.44	1.06 1.07	0.59 ± 0.20
1-Methylinosine (m^1I)			1.04 ± 0.42	1.15 ± 0.54 1.18 ± 0.78	1.12 ± 0.34

Reagents

Nucleoside standard samples of analytical grade were purchased from Sigma (St. Louis, MO, USA). Methanol of analytical grade was obtained from Riedel-de Haen, Hannover, FRG. Acetonitrile was of chromatographic grade (Merck, Darmstadt, FRG). Urinary carcinoembryonic antigen (U-CEA) was measured by a double antibody radioimmunoassay [17].

Chromatographic Apparatus

The nucleoside analysis was performed on a high performance liquid chromatograph (Waters Assoc., Milford, Mass., USA), consisting of one Model 6000A pump and one Model 510 pump, a Model U6K manual injector, a Model 440 dual wavelength (254, 280 nm) UV absorbance detector and an automated gradient controller (Model 680).

Chromatographic Procedure

Analytical 5 µm NOVA-PAK C_{18} (8 x 100 mm) columns from Waters Assoc., Milford, Mass., USA, were used. The movile phase consisted of 10 mM acetate-buffer, pH 4.7, flow rate 2.5 ml/min, ambient temperature. After the first 4 min under isocratic conditions, from 4 min to 10 min a linear gradient from 0% to 4% acetonitrile was used. Urinary excretion levels of pseudouridine (Ψ), 1-methyladenosine (m^1A), 1-methylinosine (m^1I) and 1-methylguanosine (m^1G) were quantitated within 15 min (Fig. 1). The excretion level of each nucleoside was expressed relative to the urine creatinine concentration.

Results

Modified Nucleosides in the Urine of Human Healthy Adults

Four modified nucleosides (Ψ, m^1A, m^1I, m^1G) were measured in the urine of 21 human healthy volunteers. The mean values and standard deviations (SD) are listed in Table 1 and are compared to values found by other groups.

Modified Urinary Nucleosides of Patients with cancer of the Female Genital Tract

The four modified urinary nucleosides were measured before and after treatment of 18 patients suffering from cancer of the female genital tract. The results are shown in Fig. 2 and

Table 2. The relationship between increased urinary levels of modified nucleosides (over mean + 2 SD) and clinical diagnosis

	Cancer of the cervix, uterus or ovary			Bladder cancer	Renal cell carcinoma	Total
	stage I	II–IV	all stages			
ψ	0/5	6/13	6/18	3/5	4/8	13/31 (42%)
m^1I	2/5	12/13	14/18	4/5	6/8	24/31 (77%)
m^1A	1/5	7/13	8/18	1/5	3/8	12/31 (39%)
m^1G	0/5	5/13	5/18	0/5	2/8	7/31 (23%)
at least one nucleoside elevated	2/5	12/13	14/18	4/5	6/8	24/31 (77%)

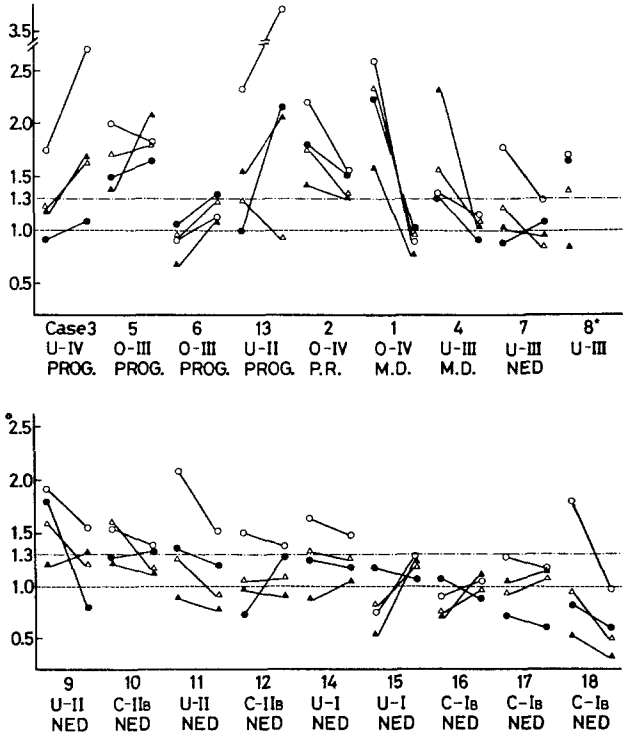

Fig. 2. The urinary levels of four modified nuecleosides in 18 patients with cancer of the uterus and ovary.

$$\text{Y-axis} = \frac{\text{Concentration of urinary modified nucleosides}}{\text{Average of urinary concentration of modified nucleosides}}$$

$$\frac{\text{(nmoles)/}\mu\text{moles creatinine in urine}}{\text{of 21 healthy adults (nmoles)/}\mu\text{moles creatinine in urine}}$$

●–● Ψ, ○–○ m^1I, △–△ m^1A, ▲–▲ m^1G levels of urinary modified nucleosides before and after treatment. – – · – – = $\frac{\text{Average + 2SD}}{\text{Average}}$ of urinary concentration of modified nucleosides in 21 healthy adults (nmoles)/μmoles creatinine in urine. The exact values were found to be 1.28 for Ψ, 1.30 for m^1I, 1.31 for m^1A and 1.33 for m^1G. *PROG,* progressive disease; *P.R.*, partial response; *M.D.*, minimal disease; *NED,* no evidence of disease; *U,* uterine cancer; *O,* ovarian cancer; *C,* cervical cancer; *I–IV:* stage of disease. ☆ died due to complication other than cancer

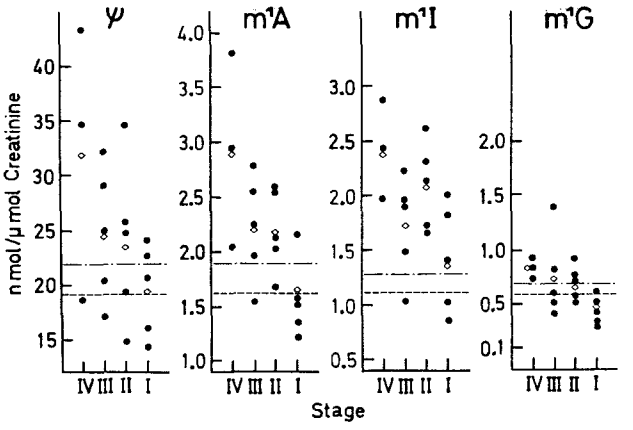

Fig. 3. Concentration of urinary modified nucleosides of 18 patients with cancer of the female genital tract and clinical stage. • Concentration of urinary modified nucleoside, ◇ Average concentration of urinary modified nucleoside in each group; – – – Average concentration of each modified nucleoside in the urine of 21 healthy adults; – · – Average concentration + SD of each modified nucleoside in the urine of 21 healthy adults; I–IV Clinical stage

Table 2. Patients no. 3, 5, 6 and 13 all had progressive disease in spite of treatment. As indicated in Fig. 2, 3–4 measured nucleosides were increased in these four patients. The exceptions were m^1I and m^1A, each of which decreased in one of the four patients. Thirteen patients had regression of diesase and were divided into two sub-groups according to tumor classification. In four patients with clinical stages III–IV (nos. 1, 2, 4, 7) all measured nucleosides showed a decrease after treatment in all patients, except for one nucleoside in case 7. Out of nine patients with stages I–II [9–12, 14–18], who had no evidence of disease after treatment, six showed declining levels of the majority of the modified nucleosides measured (three or all four nucleosides were declining). In the remaining three, who were all stage I disease with minimal distribution, the values did not exceed the normal range.

The measured urinary levels of the four modified nucleosides at the time of diagnosis were related to the patients' clinical stages (Fig. 3). m^1I was elevated above the means + 2 SD in 78% of the cases, which is more often than the other three nucleosides. The pretreatment levels of Ψ, m^1A, m^1I in patients with stage II–IV disease were all significantly increased as compared to the levels in human healthy voluneers ($p < 0.005$, Student's t-test).

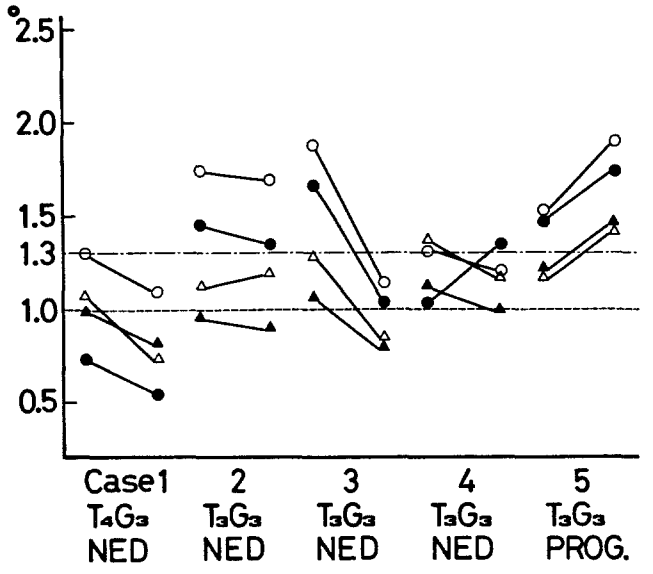

Fig 4 The urinary levels of four modified nucleosides in 5 patients with bladder cancer. Signs and symbols as in Fig. 2

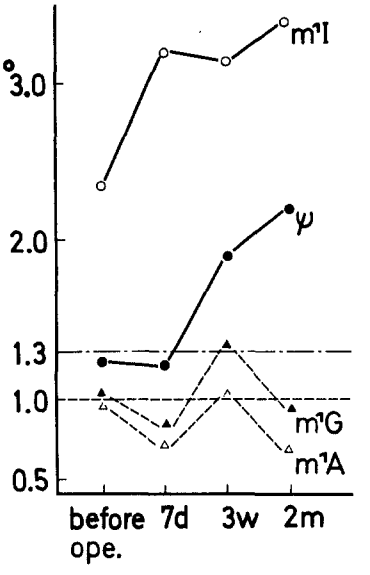

Fig. 6. A follow-up study of the urinary levels of four modified nucleosides in one progressive case of renal cell carcinoma (case 2). Signs and symbols as in Fig. 2

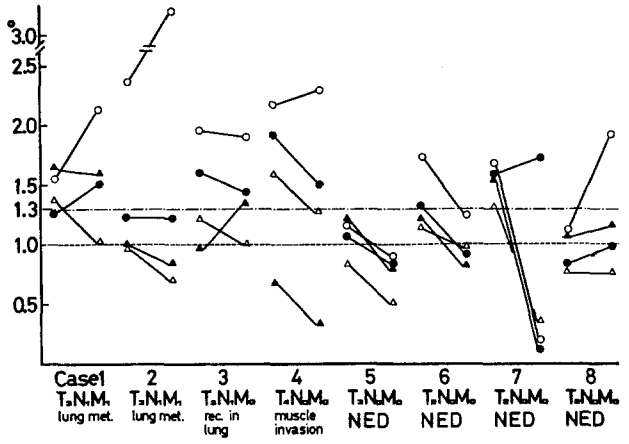

Fig. 5. The urinary levels of four modified nucleosides in 8 patients with renal cell carcinoma. Signs and symbols as in Fig. 2

Urinary Modified Nucleosides of Patients with Bladder Cancer

Five patients with bladder cancer who had an initially high level of U-CEA and a marked decrease following therapy, were selected. Modified nucleosides were measured before and after treatment (Fig. 4). Four patients had clinical regression of their cancer following radiation therapy and one had progression. Elevated pretreatment levels of m^1I were found in four out of five patients and for Ψ in three out of five. The urinary levels of two of the nucleosides (m^1I, m^1G) reacted in accordance with the clinical outcome in all five cases and the other two did so in four of the five. Interestingly, recurrence of tumor was detected in case no. 5 seven months after evaluation of nucleoside levels. All four nucleosides were then increased in spite of a normalized U-CEA level.

Urinary Modified Nucleosides of Patients With Renal Cell Carinoma

Figure 5 shows the results from eight patients with renal cell carcinoma who underwent nephrectomy. A post-operative evaluation was done 7–10 days after operation except in case 3, which was investigated 4 months later when recurrent tumor appeared in the lung. In six out of eight patients, elevated excretion of one or more nucleosides was observed before operation. Two patients (cases 1, 2) who had metastasis to the lung at the time of the operation, continued to excrete elevated levels of m^1I.

A follow-up study of case 2 whose disease was progressive, showed a good correlation of levels of Ψ and m^1I with clinical status (Fig. 6). In case 3, elevated levels of Ψ, m^1I and m^1G were detected in post-operative investigation at the time of tumor recurrence in the lung.

Case 4 had tumor invasion to the psoas muscle and also continuously elevated excretion of m^1I and Ψ.

Observed Relationships

The relationship between clinical outcome and increase or decrease of urinary modified nucleosides is shown in Table 3. Urinary Ψ and m^1I appeared to be more closely associated with clinical outcome than the other two. Progressive cancer of the uterus or ovary was found to be associated with increases of Ψ in each of the four cases. Successful treatment was followed by declining levels of Ψ in 10 out of 13 cases and for m^1I in 11 out of 13.

The kidney and bladder cancers appeared to be best monitored by m^1I. m^1I was elevated (above the mean +

Table 3. The relationship between urinary modified nucleosides before-after treatment and clinical outcome

Diagnosis (no.)	ψ		m^1I		m^1A		m^1G		Total	
	increase	decrease	increase	decrease	increase	decrease	increase	decrease	increase	decrease
Gynecological[a] ca										
progression (4)	4	0	3	1	3	1	4	0	14	2
Part. resp. (3)	0	3	0	3	0	3	0	3	0	12
NED (10)	3	7	2[b]	8	4[c]	6	5[d]	5	14[e]	26
Bladder ca										
progression (1)	1	0	1	0	1	0	1	0	4	0
NED (4)	1	3	0	4	1	3	0	4	2	14
Renal cell ca[f]										
progression (1)	0	1	1	0	0	1	0	1	1	3
NED (4)	2	2	1	3	0	4	1	3	4	12

[a] One patient died
[b] Both cases occurred in stage I disease and the levels were within the normal range
[c] Three out of four cases occurred in stage I disease and the levels were within the normal range
[d] Four out of five cases occurred in stage I disease and the levels were within the normal range
[e] Nine out of fourteen cases occurred in stage I disease and the levels were within the normal range
[f] Three cases with metastases were omitted

2 SD) in 10 out of 13 cases before treatment (Table 2) and responded with increasing levels in both cases of progressive disease. Successful treatment lowered the level of m^1I in 7 out of 8 cases (Table 3).

Discussion

A significant elevation of nucleosides was defined as a value higher than the average plus two standard deviations determined in urines of a control group. The urine from 31 patients suffering from cancer of the urinary organs or female genital tract was analysed before and after treatment. The results indicated that m^1I and Ψ were elevated before treatment more frequently than the other two nucleosides. The same two nucleosides were also elevated in 4 and 5 out of 6 progressive cases after treatment.

Some associations were found between clinical outcome (progression or regression) and an increased or decreased level of modified nucleosides in the urine. Ψ seems to be the most appropriate nucleoside to monitor female genital cancer, while m^1I appears to be better for bladder and kidney cancer. It may therefore be of value to assay both Ψ and m^1I when monitoring these diseases.

The methylated bases in tRNA are synthesized by post-transcriptional modification of the RNA. Methyl groups are transferred from S-adenosyl-methionine to the appropriate base by highly specific enzymes. These enzymes have been found to be species specific [14]. The halflife of total tRNA in the liver has been estimated to be about 72 h [2]. The turnover rate of tRNA has been found to be elevated in tumor tissues compared to their normal counterparts [2]. Furthermore, tRNA methylase activity has been found to be elevated in a variety of tumors (for review see [1]). Experiments with radioactively labelled substances have shown that pseudouridine lacks any signifikant catabolism in vivo [6, 16]. These factors taken together may therefore explain the observation that many tumor patients exhibit elevated levels of modified nucleosides in the urine.

The elevation of modified nucleosides in the urine of cancer patients before treatment is only slight [7, 11, 13 and this paper]. The values are generally only just above the average plus two standard deviations of a control group. This is in contrast to fetal antigens like CEA, where marked elevation may occur [12]. Modified nucleosides have much shorter half-lifes in the body than proteins (hours as opposed to days; [4, 8]). A faster response to therapy and recurrence of disease is therefore feasible. Modified nucleosides may therefore be of value for a rapid assessment of disease course in monitoring genito-urinary cancers.

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