Pergamon

European Journal of Cancer, Vol. 34, No. 9, pp. 1460–1462, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(98)00079-3

Short Communication

Urinary Levels of Cyclic Guanosine Monophosphate (cGMP) in Patients with Cancer of the Uterine Cervix: a Valuable Prognostic Factor of Clinical Outcome?

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Changes in urinary cyclic nucleotide levels have been reported in patients with various types of cancers. The present study was conducted to relate changes in urinary levels of cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) to the clinical outcome of 11 patients treated for cancer of the uterine cervix. Urine was sampled for 24 h before and 3 months after primary treatment. The levels of cGMP increased in all the patients ($n = 5$) who relapsed within the observation period of 39 months. 4 of these patients showed an increased cGMP/cAMP ratio. In the patients without relapse ($n = 6$), the cGMP levels decreased, whereas the cGMP/cAMP ratios were unchanged. No marked changes in the levels of cAMP were observed for either of the groups. The measurement of urinary cGMP levels seems to be a valuable tool in the follow-up of patients with cancer of the uterine cervix. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: tumour marker, gynaecological cancer, cGMP, cAMP
Eur J Cancer, Vol. 34, No. 9, pp. 1460–1462, 1998

INTRODUCTION

CLINICAL STUDIES have reported that patients with gynaecological cancers have elevated urinary cyclic guanosine monophosphate (cGMP) levels in advanced disease, and that levels are normalised after successful treatment [1–3]. In order to mimic the *in vivo* situation, we established an *in vitro* model with four human cell lines (C4-I, C33A, ME-180, Si-Ha) derived from cancers of the uterine cervix [4]. During growth, levels of cGMP increased, whereas cyclic adenosine monophosphate (cAMP) levels fell and a cell density-dependent increase in ratio between extracellular levels of cGMP and cAMP was observed. In contrast, the ratios between extracellular cGMP and cAMP decreased in the non-transformed human fibroblasts (Wi-38) with increasing cell densities [5]. With this background, the urinary levels of cGMP and cAMP in patients with carcinomas of the uterine cervix were determined before and 3–4 months after completed primary treatment, and compared with the clinical outcome.

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Received 6 Oct. 1997; revised 2 Feb. 1998; accepted 4 Mar. 1998.

MATERIALS AND METHODS

Subjects

11 patients with diagnosed cancer of the uterine cervix were included when admitted to hospital for primary treatment. Stage, age, menstrual status, therapy, performance status and the clinical outcome are given in Table 1. Body weight and serum creatinine were obtained from the case records. Informed consent was given by all participants. The maximal follow-up time was 39 months. All patients were included in a control programme of follow-up by an experienced gynaecologist at the local hospital.

Urine sampling

The samples were obtained prior to treatment and 3–4 months thereafter. Urine was sampled for 24 h, frozen immediately, and stored at − 22°C until analysis.

Analysis of cyclic nucleotides

The urines were thawed, mixed with trichloroacetic acid to achieve a final concentration of 0.5% (v/v) and then neutralised with $CaCO_3$. The concentrations of cAMP were determined in duplicate by radioimmunoassay (RIA) using antiserum, raised and tested in our own laboratory,

Table 1. *Patient characteristics: clinical stage, grade, age (years), menstrual status and performance status (a.m. Karnofsky) at the time of primary diagnosis and admission to hospital for therapy, treatment and clinical outcome*

Subject	Stage	Grade	Age	Menstrual status	Performance status	Treatment	Relapse
1	Ib	2	37	Normal	100	Surgery a.m. Wertheim	No
2	IIa	2	37	Normal	100	RT (Gy): 40 + 20	Yes
3	IIb	2	29	Normal	100	RT (Gy): 50 + 22.8	No
4	IIb	3	33	Normal	90	RT (Gy): 46 + 22	No
5	IIb	2	42	Normal	90	RT (Gy): 46 + 24	Yes
6	IIb	2	50	Climaceric	100	RT (Gy): 50 + 22.8	No
7	IIb	2	68	Postmenopausal	90	RT (Gy): 50 + 22.8	No
8	IIb	2	74	Postmenopausal	90	RT (Gy): 45 + 20	Yes
9	IIb	2	75	Postmenopausal	100	RT (Gy): 46 + 22	Yes
10	IIIb	1	68	Postmenopausal	90	RT (Gy): 46 + 0	No
11	IIIb	2	69	Postmenopausal	90	RT (Gy): 60 + 14.8	Yes

RT, radiation therapy with fractionated doses (brachytherapy). a.m. Karnofsky: radical hysterectomy and removal of pelvic lymph nodes. a.m. Wertheim, radical hysterectomy and removal of pelvic lymph nodes.

[^{3}H]-cAMP (Amersham International, Amersham, U.K.) and cAMP (Sigma Chemical Co., St. Louis, Missouri, U.S.A.). The concentrations of cGMP were determined in duplicate by RIA with cGMP antiserum, [^{3}H]-cGMP and cGMP (Amersham International).

Data presentation

Since cyclic nucleotides are excreted in the urine and quantitatively glomerular filtration seems to prevail over tubular secretion [6], the urinary concentration was related to creatinine clearance (Cl_{creat}) to account for any changes in renal function due to disease or treatment.

RESULTS

For all the patients participating in the study, at least two measurements of urinary samples were performed, with each subject being its own control. The data from the patients are shown in Tables 2 and 3. For the patients with relapse, $cGMP/Cl_{creat}$ increased in all patients (Table 2), in contrast to the patients without disease recurrence (Table 3). The corresponding values for $cAMP/Cl_{creat}$ showed no consistent changes. The concentration ratios between urine levels of cGMP and cAMP showed an increase in 4 of 5 patients with relapse (Table 2) and a small, but inconsistent reduction was observed in the patients without recurrent disease (Table 3).

Table 2. *Urinary excretion of cyclic nucleotides in patients with relapse. The data are given as the ratio between pmol cyclic nucleotide/ml urine and creatinine clearance (ml plasma/min)*

	$cGMP/Cl_{creat}$		$cAMP/Cl_{creat}$		cGMP/cAMP	
Subject	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
2	1.4	1.6	1.8	2.2	0.76	0.73
5*	2.0	2.9	4.4	4.7	0.45	0.62
8	3.4	5.7	2.8	3.6	0.70	1.25
9	4.4	4.8	4.2	3.4	1.04	1.40
11	1.9	2.6	2.8	2.8	0.68	0.96
Mean	2.6	3.5	3.2	3.3	0.70	1.00
SD	1.2	1.7	1.1	0.9	0.20	0.30

cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; Cl_{creat}, creatinine clearance; SD, standard deviation. *A third sample was taken 6 months after primary treatment. The measured values were GMP/Cl_{creat}: 4.9, $cAMP/Cl_{creat}$: 5.3, cGMP/cAMP: 0.94.

Table 3. *Urinary excretion of cyclic nucleotides in patients without relapse. The data are given as the ratio between pmol cyclic nucleotide/ml urine and creatinine clearance (ml plasma/min)*

	$cGMP/Cl_{creat}$		$cAMP/Cl_{creat}$		cGMP/cAMP	
Subject	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
1	2.1	1.9	3.1	2.4	0.66	0.80
3	3.0	1.8	1.7	2.6	1.75	0.71
4	1.0	0.7	0.9	1.0	1.10	0.75
6	1.6	1.5	3.7	2.4	0.44	0.62
7	3.3	2.7	3.3	3.6	0.99	0.75
10	3.9	2.9	2.8	3.6	1.38	0.81
Mean	2.4	1.9	2.8	2.6	0.9	0.7
SD	1.2	0.9	1.1	1.1	0.4	0.1

cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; Cl_{creat}, creatinine clearance; SD, standard deviation.

DISCUSSION

During the last few decades, several authors have studied variations in cyclic nucleotide levels in plasma and urine as prognostic factors for the outcome of various malignancies [7–11]. Some reports on gynaecological cancers do exist [1–3, 12]. In the present study, 5 patients with cancers of the uterine cervix had a recurrence within 12 months after primary diagnosis and for all the subjects, urinary cGMP levels increased. In contrast to this, in the other 6 patients with no recurrent disease after an observation period of more than 3 years, the cGMP levels fell markedly. Our finding is in agreement with a previous clinical study which found raised urinary cGMP levels in 93% of subjects with carcinoma of the uterine cervix, but in only 30% of patients with intraepithelial neoplasia [1].

In an early study, elevation of urinary cGMP excretion was observed in patients with various epithelial cancers, but not in patients with mesenchymal tumours [7]. Elevated cGMP levels were also found in patients with cancers of the lung, breast, colon and with Hodgkin's disease [8, 10]. In patients with cancers of the gastrointestinal tract and ovaries, elevation of cGMP levels was observed in the subjects where tumour reduction was impossible, whereas the levels were unchanged in patients who had their tumours completely removed [9]. In another study, no change in cGMP excretion was found in patients with cancers of the colon/rectum, stomach, breast, bladder and prostate, and elevation was only found in patients with cancer of the lung and oesophagus [13]. Furthermore, compared with healthy individuals, urinary excretion of cGMP was significantly greater in patients with lymphoid tumours (both sexes) and in male patients with myeloid tumours or malignant melanoma, but normal in patients with breast carcinoma or colonic adenocarcinoma [14]. Another study of 10 different types of cancer also showed normalisation of cGMP levels in patients who attained complete remission [11]. The discrepancies in the above-cited studies may be explained by the fact that a great interindividual variability exists in excretion of cGMP. Only the studies where the patients were their own controls [11], including the present one, seem to be consistent.

The mechanism beyond the elevated cGMP levels in urine is unclear, but a previous study indicated that elevation was not a result of increased plasma clearance, but rather increased rate of appearance in plasma [10]. It seems unlikely that a small tumour mass of malignant cells is responsible for such an increase. Another possibility is that the growing cells excrete paracrine and endocrine factors that may enhance the cellular cGMP export from normal cells.

No marked changes were found in cAMP levels in either of the two groups in the present study. This is in agreement with most studies where no difference in cAMP excretion between cancers and controls has been found [8, 10, 11]. However, decreased cAMP excretion in human premalignant and malignant disease has also been reported [7, 12].

Since cGMP levels seem to be elevated and cAMP levels unchanged or reduced, the concentration ratio between cGMP and cAMP has been proposed as a tumour marker for certain types of malignant diseases. In former *in vitro* studies, we found a cell density-dependent increase in the ratios between extracellular cGMP and cAMP levels of transformed human cell lines derived from the uterine cervix [4, 5], mimicking a growing tumour *in vivo*. In the present study, these observations were extended to a clinical situation. The data showed that 4 of the 5 patients with relapse had an increased urinary cGMP/cAMP ratio, whereas the cGMP/cAMP ratios were unaltered in patients without recurrent disease. Another study revealed a high correlation between the concentration ratios cGMP/cAMP at or above 0.2 and findings of abnormal cytopathology [12]. After surgery, the ratios normalised. However, in contrast to most other studies, the cAMP levels were significantly lower in the cases, whereas the mean level of cGMP showed no consistent elevation.

Cyclic nucleotides are not organ specific markers. In addition, they show considerable interindividual variations in excretion. However, the elevations of urinary cGMP in several types of human cancers are well documented and an increased level of cGMP within an individual appears to be a sensitive marker of recurrent disease. Standardised measurement of the urinary excretion of cGMP in patients with cancers of the uterine cervix seems to be an additional useful tool to monitor the disease. Based on the present results, a more comprehensive study has been initiated in order to correlate cGMP excretion and other tumour markers to clinical outcome after treatment of gynaecological cancers.

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Acknowledgements—The study was supported with grants from the Norwegian Cancer Society. Thanks are due to the nursing staff at the Department of Gynaecological Oncology, University Hospital of Tromsø, for the sampling of urine specimens.