Europ. J. Cancer Vol. 15, pp. 211–1218
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0014-2964/79/1001-1211 $02.00/0

Urinary Cyclic AMP and Bone Histology in Walker Carcinosarcoma: Evidence of Parathyroid Hormone-like Activity*

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Abstract—*Plasma and urinary cyclic AMP were determined in* 8 *control rats and* 8 *rats with Walker carcinosarcoma* 256 *both at the sixth and the eighth day after transplantation. In addition, serum parathyroid hormone,* 25-OH-*vitamin D and a* 12 *channel chemistry profile were determined. Histology of the bone was carried out and evaluated.*

Six days after transplantation only a slight degree of hypercalcemia existed, however, hypophosphatemia was marked, so that a "parathyroid hormone-like activity" might have been responsible for these alterations. In contrast to hyperparathyroidism in man, serum chloride was not increased at this time.

The animals exhibited severe hypercalcemia 8 days *after transplantation and died within* 10 *to* 11 days. *Calcinosis of kidneys and the heart was observed.*

Urinary cAMP markedly increased in the animals and was statistically significant above normal as early as 3 days *after transplantation.*

Bone histology showed marked osteoclastic activity. Because the "osteoclast activating factor" and prostaglandins of the E-group according to the literature don't increase urinary cAMP excretion, a "parathyroid hormone-like substance", which is able to stimulate renal adenylate cyclase, might be responsible for the changes observed in Walker carcinosarcoma in the rat.

INTRODUCTION

THE SYNDROME of hypercalcemia is known to occur in several malignant tumours of non-endocrine tissues in man. In 1956, Plimpton and Gellhorn described hypercalcemia in bronchial and renal carcinoma, and these cases resembled hyperparathyroidism in respect to high serum calcium and low serum phosphorus [1]. Wilson and colleagues reported on animals bearing tumors which led to hypercalcemia [2]. Additional observations were published by Rice *et al.* [3, 4].

Accepted 23 February 1979.
*Supported by a grant from the Deutsche Forschungsgemeinschaft (Schm 400/1 and 400/3).
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An increase in serum calcium during growth of Walker carcinosarcoma 256 in the rat was described by Hilgard in 1970 [5]. Further investigations of this group were reported to detect the cause of hypercalcemia in this tumor [6, 7].

Hypercalcemia in malignant disease may have several causes. In the majority of patients there is spread of tumor to bone, and the hypercalcemia is caused by local osteolytic action of the metastases. In these cases generally phosphorus is not lowered because of liberation from bone. In other cases of hypercalcemia, for example in multiple myeloma, an "osteoclast activating factor" is formed [8]; these cases also are generally not lowered in phosphorus. Recently, Seyberth *et al.* have described increased prostaglandin E-M excretion, the main urinary metabolite of prostaglandins of the E-group [9]. These

patients exhibited serum phosphorus levels lower than the mean of a control group. Interestingly, urinary cyclic AMP (cAMP) was not increased in patients with high prostaglandin E-M excretion [10]. It is known that cAMP responds very sensitively to parathyroid hormone (PTH) [11]. After injection of PTH in man, within minutes an increase of urinary cAMP occurs. Urinary cAMP may serve as an indicator of PTH-like activity.

If a substance is secreted by the tumor which behaves like PTH, i.e., is able to activate adenylate cyclase in renal cortex, this PTH-like activity will cause an increase in urinary cAMP. This PTH-like activity may not be detected by several antisera used for the PTH radioimmunoassay, because of slight differences in the structure. In some cases of tumor-hypercalcemia an increase of serum PTH measured by radioimmunoassay was reported [12, 13]. A comparison of immunoreactivity of circulating PTH-like material in ectopic hyperparathyroidism (=tumor hypercalcemia with low serum phosphorus) exhibited a significant difference of the slope of the dilution curves which indicated a lack of immunological identity ([13], for review [14]).

The purpose of this study is to investigate the mechanism of hypercalcemia in Walker carcinosarcoma, especially the role of cyclic AMP.

MATERIALS AND METHODS

Female Sprague-Dawley rats of about 200 g served as experimental animals (Süddeutsche Zuchtanstalt in Tuttlingen). The animals lived in specific pathogen free area and were fed with Altromin standard diet (Altromin, Hagen/Lippe) and water *ad libitum*. The tumor groups got 0.1 ml (=80,000 Walker ascites carcinosarcoma cells, passage 945) s.c. The animals were divided into the experimental groups by random numbers. Urine was collected daily from the first to the tenth day after transplantation in a vial containing 0.2 ml of sodium azide (2 M). Blood was sampled at day 6, 8 and 10 after transplantation of the tumor by aortic puncture. The animals were prepared for histopathological examination; the tissues were stained with hematoxylin-eosin, Masson-Goldner and impregnation. The data were examined for significant differences by so called "simultaneous test procedure".

In addition, a comparison of the median was determined by Kruskal-Wallis test [15]. Finally, according to Dunn [16], pairs of groups were compared and a probability of 0.05 was chosen as limit of significant differences.

Clinical chemistry results were obtained by 12-channel serum profile on ISMA 12/60 (Technicon Corporation, N.Y., Tarrytown, U.S.A.). Sodium, potassium, chloride, calcium, phosphorus, iron, uric acid, urea, creatinine, glucose, cholesterin and triglycerides). In addition, alkaline phosphatases and gamma-glutamyl-transpeptidase were determined (Eppendorf-Enzymstrasse 5020). PTH was determined according to Bouillon with antiserum A VI 2 [17], and urinary cAMP was determined according to Tovey [18]. 25-OH-vitamin D was measured by competitive protein binding assay according to Belsey [19].

RESULTS

Weight of the tumor and of the animals

The tumor weight increased from about 2 g at day 6 to 15 g at day 8 and 24 g at day 10. From day 8 to 10, the animals lose about 40 g of weight. At day 6, the tumor was very small and the animals had lost no weight compared to the control group.

The clinical results are shown in Table 1.

As is shown in the table, a slight degree of hypercalcemia is present at day 6 and the inspection of the original data shows that there exists nearly no overlap between serum calcium concentration between controls and hypercalcemic animals. At the 6th day, serum phosphorus is markedly lower in the tumor animals than in the controls and serum chloride tends to drop too. At this time, kidney function is normal (serum creatinine in both groups 0.6 mg/dl). Serum sodium concentration tends to drop too. As in most tumors, serum iron drops. A comparison of alkaline phosphatase (AP) and gamma-glutamyl-transpeptidase (GGT) in serum of control animals and Walker-tumor bearing animals is shown in Table 1.

Statistically significant differences ($P<0.05$) were observed at day 6 between control animals and tumor bearing rats in serum phosphorus and triglycerides. Serum calcium at day 6 is elevated, however, 95% confidence are not achieved ($0.05<P<0.10$).

25-hydroxy-vitamin D (25-OH-D)

25-O-H-D in Walker rats was lower than

Table 1. Parathyroid hormone (parathyroin), 25-OH-vitamin D (25-OH-D) and clinical chemistry results of rat serum and cyclic AMP in rat plasma

No.	Parameter	Unit	Controls		Day 6		Day 8	
			Mean	Range	Mean	Range	Mean	Range
1	Parathyrin	pmole/l	33	27–41	31	26–34		
2	25-OH-D	nmole/l	151	75–250	112	47–245	—	—
3	Cyclic AMP	nmole/l	66	50–88	77	48–126	—	—
4	Calcium	mEq/l	5.3	4.7–5.6	5.9	5.4–7.3	8.6	7.6–9.5
5	Phosphorus	mg/dl	7.3	6.9–7.6	4.9	4.0–5.7	5.4	3.8–6.1
6	Chloride	mEq/l	103	102–105	102	100–106	88	86–92
7	Creatinine	mg/dl	0.6	0.6–0.8	0.6	0.6–0.6	1.5	0.9–1.7
8	Urea	mg/dl	41	39–44	38	32–46	158	75–240
9	Uric acid	mg/dl	1.8	1.5–2.4	2.4	2.2–2.6	2.7	2.4–3.0
10	Sodium	mEq/l	144	143–145	139	138–140	136	134–138
11	Potassium	mEq/l	5.1	4.2–7.1	4.9	4.1–5.5	4.3	4.1–4.7
12	Iron	μg/dl	457	290–520	214	140–310	90	40–126
13	Glucose	mg/dl	161	115–190	149	140–170	142	120–170
14	Cholesterol	mg/dl	61	50–68	66	54–80	38	30–50
15	Triglycerides	mg/dl	95	80–110	193	120–270	251	190–365
16	Alkaline phosphatase	U/l	187	144–215	227	175–482	—	
17	γ-Glutamyl-transpeptidase	U/l	1.85	1.0–2.3	1.93	1.1–2.9	–	—

Comparison of control rats ($n=8$) and Walker carcinosarcoma 256 s.c. bearing rats at day 6 ($n=8$) and day 8 ($n=8$) after transplantation.

in control animals: a mean of 112 and a standard deviation of 58 compared to a mean of 151 and standard deviation of 59 in controls ($P<0.10$).

Serum PTH

This was determined in control animals ($n=8$) and at day 6 after transplantation of the tumor ($n=8$). In tumor animals PTH was slightly lower, the difference, however, was statistically not significant. Day 6 was chosen for PTH, 25-OH-D and cAMP determination, because at this time kidney function was normal.

Urinary cAMP

Urinary cAMP was determined from days 3 to 8 in Walker animals and at day 6 in control animals. The result is shown in Fig. 1.

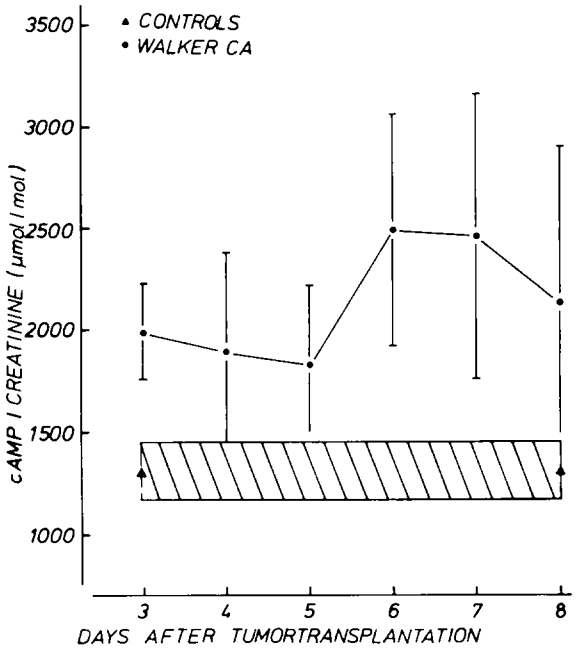

Fig. 1. Urinary cAMP related to urinary creatinine in control animals (measurement at day 6) *and in animals with Walker carcinosarcoma* 256. *In tumor bearing animals, urinary cAMP was determined from days* 3 *to* 8.

Figure 1 demonstrated the major difference in urinary cAMP excretion at day 6. The difference in the tumor bearing animals compared to the control animals is statistically different as early as day three (it is assumed that the control animals would not change very much in urinary cAMP excretion).

The plasma levels did not differ significantly as is shown in Table 1. The levels are higher than in man (20 nmole/l) and an influence of stress (handling of the animals) might account for these concentrations (about 70 nmole/l).

Histopathology

In tumor-bearing animals 6 days after transplantation thyroid and parthyroid were not different to controls. Kidneys in Walker animals differed markedly from controls. Severe nephrocalcinosis with involvement of the tubular system without glomerular alterations was observed.

Bone. The spine and long bones contained no metastases of the tumor. Femur and tibia were observed in detail for osteolytic activities, a typical lesion is shown in Fig. 2.

DISCUSSION

Clinical chemistry

The rat is an animal with high serum phosphorus levels. Because serum phosphorus is controlled by PTH and other different factors, the decrease observed might be caused by PTH-like activity, or by volume expansion or a prostaglandin. It was shown in the classical, prostaglandin producing tumor (VX-2 carcinoma of the rabbit), that in these animals serum phosphorus drops too [2]. The decrease in serum chloride is not suggestive for PTH-like substances, because this hormone, at least in man, produces hyperchloremia. Sodium drops like chloride and this may result from natriuresis produced by hypercalcemia. Because the enzyme GGT remained normal, the increase in alkaline phosphatase is more likely to result from activated bone cells than from liver damage.

Serum PTH

The production of "PTH-like activity" is not ruled out by our results, because of limited crossreactivity of rat PTH to the antibody (A VI 2) used, which detects mainly human and bovine and rabbit PTH (unpublished results).

Minne *et al.* [20] detected large quantities of immunoreactive PTH in extracts of Walker tumors.

25-OH-*vitamin D*

There is a decrease (statistically not significant, $P<0.10$) compared to controls. Whether there exists enhanced turnover [21] and an increase of 1,25-$(OH)_2$-vitamin D, the most active of the known vitamin-D metabolites, has to be determined in the future. However,

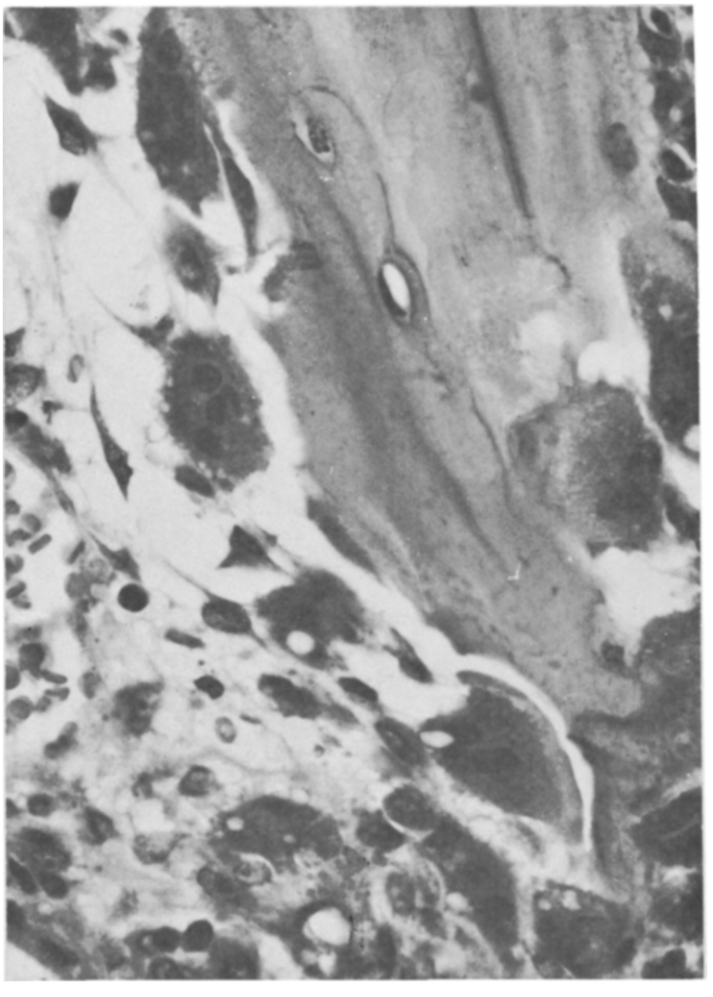

Fig. 2. The metaphyseal area of the femur is shown. Multinucleated giant cells (osteoclasts) are found with numerous vacuoles. The osteoclasts were actively resorbing bone. Howships lacunae are present.

this seems to be not very likely because vitamin-D intoxication generally lowers urinary cAMP.

Prostaglandins or other factors

Seyberth *et al.* [10] observed that patients with tumor hypercalcemia and elevated prostaglandin E-M excretion had low-normal cyclic AMP excretion. Therefore, our results in Walker animals (elevated urinary cAMP) argue against one of the known prostaglandin E-M precursors.

Because plasma levels of cyclic AMP were not significantly higher in tumor animals, the increase of urinary cAMP is caused by nephrogenous cAMP. Of several hormones (glucagon, vasopressin, calcitonin, PTH), only the latter is able to increase nephrogenous cAMP production [22]. Our results indicate that a PTH-like activity is most likely responsible for the syndrome produced by Walker carcinosarcoma.

Acknowledgements—We thank Dr. Zentgraf for statistical evaluation and Mrs. Petra Förster for skillful technical assistance.

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