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Electrolyte and Glucose Metabolism in VX-2 Carcinoma of the Rabbit*

HEINRICH SCHMIDT-GAYK,†‡ HEINZ LÖHRKE,§ BRIGITTE HESSE,§ ALFRED FISCHKAL§ and KLAUS GOERTTLER§

†*Clinical Laboratory, Department of Surgery, University of Heidelberg and* §*Institute of Experimental Pathology, German Cancer Research Center, Heidelberg, F.R.G.*

Abstract—*Biochemical and other parameters in VX-2 carcinoma in rabbits were evaluated. VX-2 carcinoma not only produced hypercalcemia but also hypophosphatemia and* 25-OH-*vitamin D deficiency. An increased turnover of* 25-OH-*vitamin D seems likely. Serum parathyroid hormone and urinary cyclic AMP did not increase. Hypokalemia occurred in association with hypophosphatemia and lowered blood glucose within* 1 week *after tumor transplantation. At the end of the experiment glucose and insulin were both below the control range. It is concluded that VX-2 carcinoma in rabbits yields much more complex biochemical alterations than reported before on calcium metabolism.*

INTRODUCTION

MALIGNANT tumors of non-endocrine origin are able to produce polypeptide hormones and to manifest different paraneoplastic endocrine syndromes. Besides excess adrenocorticotropic hormone (ACTH), gonadotropin and other hormones presenting with specific syndromes depending on the secreted substance, the hypercalcemic syndrome is of clinical interest [1]. The paraneoplastic hypercalcemia syndrome caused by tumors of non-endocrine tissue-origin is known to be frequent in the case of some human malignancies and obligatory for some animal transplantable tumors. The syndrome is marked by a combination of an obligatory high serum calcium level and either a low or a nearly normal serum phosphorus level.

Both in human neoplasia and in hypercalcemia associated with experimental animal tumors different mechanisms are described to produce the syndrome (for review see [2, 3]). Besides the metastatic bone involvement, the hypercalcemic syndrome may by caused by (a) the ectopic production of parathyroid hormone (PTH) or PTH-like substances (experimental tumors [4–6]; human tumors [7–11]); (b) the excessive liberation of prostaglandins (PGs) [12–17]; and (c) a not exactly defined osteoclast-activating factor (OAF), supposed to permit the bony penetration of myelomas and other lymphoproliferative diseases [18, 19].

Animal tumors are commonly used as models for the aforementioned etiopathogenetic mechanisms. The Walker carcinosarcoma 256 is accompanied by hypercalcemia [6] and severe nephrocalcinosis due to PTH or PTH-like substances, and the rabbit carcinoma VX-2 is designated as an example for a tumor secretion of PG-E_2 [12, 20]. Other tumors producing the hypercalcemia syndrome are the $HSDM_1$ (which is also supposed to be accompanied by an excessive liberation of PGs [21]) and, not so often employed, the Leydig-cell tumor of the Fischer rat (probably producing an osteolytic sterol [22]).

In the case of the VX-2 carcinoma the results of both gas chromatography/mass spectrometry (GC/MS) and radioimmunoassay (RIA) were in good agreement and the role of PG-E_2 as a tumor-dependent bone-resorbing substance was confirmed [20, 23]. The mode of action of PG-E_2 and of the derived metabolites on bone was confirmed using bone organ cultures [13, 24]. Subsequently, the PG-mediated bone resorption leads to pertubations in the calcium homeostasis, which is reflected by changes of the PTH-secretion. Little is known on the role of vitamin D-metabolites in tumor hypercalcemia.

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‡To whom requests for reprints should be addressed at: Clinical Laboratory, Surgical University Hospital, Im Neuenheimer Feld 110, 6900 Heidelberg, F.R.G.

The purpose of our study was an assessment including complex biochemical and pathological findings occurring after transplantation of VX-2 carcinoma. Special interest is drawn on the endocrine alterations corresponding to the supposed tumor-associated secretion of PGs.

MATERIALS AND METHODS

Animals

Female Yellow-Silver rabbits of 2.5 kg were used (E. Stark, GmbH, Gelnhausen, F.R.G.). The rabbits were kept in standardized conditions and fed with Altromin standard diet and water *ad libitum*.

Tumor

The tumor was obtained from Hoechst (Frankfurt, F.R.G.) by the generosity of Prof. Dr. Gehricke. After the generation of a tumor suspension, avoiding the use of necrotic parts of the tumor tissue, 0.2 ml of the suspension was injected in the right thigh of each animal.

Sampling

Once a week, starting 1 week before tumor transplantation, the rabbits were kept in metabolic cages to collect urine under refrigerated conditions. Likewise, blood was taken by heart puncture at the same frequency and before housing in the cages was performed.

Morphology

If one of the 12 animals was considered to be moribund it was killed by use of diethylether. A complete necropsy was carried out. Representative samples of tissues from various organs including the tumor on the right thigh were processed for routine light microscopic evaluation. Sections of tissues were cut at 5 μm and stained with hematoxylin-eosin.

Clinical chemistry

Clinical chemistry results were obtained by a 12-channel serum profile on SMA 12/60 (Technicon Corp., Tarrytown, NY, U.S.A.): sodium, potassium, chloride, calcium, phosphorus, albumin, protein (total), uric acid, urea, creatinine, glucose and total bilirubin.

Furthermore, an hepatic profile with gamma-glutamyl-transpeptidase (γ-GT), glutamic-pyruvic-transaminase (GPT, ALT), glutamic-oxaloacetic-transaminase (GOT, AST), alkaline phosphatases and total bilirubin was established as well as a 'Coulter counter' red and white blood cell count, determination of hematocrit and mean cell volume of erythrocytes. Also, a urine profile (with calcium, phosphorus, potassium, sodium, creatinine) was obtained (urine measured at two dilutions on SMA 12/60), and urinary calcium was determined by atomic absorption and urinary sodium and potassium by flame photometry ('KliNa', Beckman Instruments, Fullerton, CA, U.S.A.).

Other biochemical parameters

Parathyroid hormone was determined according to Bouillon *et al.* [25] with antiserum guinea pig VII and A VI 2, and urinary and plasma cAMP was determined according to Tovey *et al.* [26]. 25-OH-Vitamin D was measured by competitive binding assay according to Belsey *et al.* [27]. Insulin was determined by RIA according to Yalow [28].

Statistics

The data were examined for significant differences by the so-called 'simultaneous test procedure'. In addition, a comparison of the median was determined by the Kruskal-Wallis test [29]. Finally, pairs of groups were compared and a probability of 0.05 was chosen as the limit of significant differences, according to Dunn [30].

RESULTS

Pathology of the tumor-bearing rabbits

Four animals were found moribund and killed 4 weeks after transplantation, 1 rabbit was killed in terminal state after 5 weeks, 4 more after 6 weeks and the last 3 were killed after 7 weeks. The dissection of the animals as well as the later histological examination confirmed the appearance of lung metastases besides the *per continuitatem* infiltration of the pelvis. Other parenchymatic organ metastases were rare and infrequent. Also, no metastatic spread involving bone was seen. In particular, the right thighs neighbouring the implantation site, although sometimes surrounded completely by tumor tissue, were never infiltrated and destructed locally or in a metastatic mode. Moreover, rarification of osseous structures was not demonstrable and also osteoclast activation was not prominent by the microscopic evaluation of the bony specimen. A large increase in the number of osteoclasts did not occur. Final tumor mass of the animals exceeded 4 cm in diameter.

The performed clinical chemistry at first sight revealed no direct evidence of hypercalcemia. By use of a correction method commonly applied to calculate the relevant ultrafiltrable calcium (= ionised and complexed calcium), we have corrected the measured total calcium serum levels for the serum albumin levels [31]. The used calculation resulted in a hypercalcemia of a slight degree (Table 1, Fig. 1). A significant difference of the serum calcium levels was observed during

Table 1. 25-OH-*Vitamin D, insulin and clinical chemistry in serum*

No.	Parameter	Unit	Weeks after tumor transplantation: Controls Mean ± S.D.	1st Mean ± S.D.	2nd Mean ± S.D.	3rd Mean ± S.D.	4th Mean ± S.D.	5th Mean ± S.D.	6th Mean ± S.D.	7th Mean ± S.D.
1	25-OH-Vitamin D	nmol/l	225 ±94	220 ±70	173 ±55	168 ±90	105 ±36	63* ±26	52* ±17	30* ±22
2	Insulin	μU/ml	16.35 ±4.99	—	—	26.95* ±6.47	—	10.12 ±4.34	—	—
3	Calcium	mEq/l	6.71 ±0.34	6.16 ±0.19	5.91 ±0.28	5.79 ±0.23	6.09 ±0.27	6.22 ±0.51	6.82 ±0.62	6.32 ±2.51
4	Phosphorus	mg/dl	5.53 ±0.38	3.57* ±0.25	3.66* ±0.57	3.52+ ±0.20	3.98 ±0.26	4.30 ±0.62	3.93 ±0.54	2.90* ±1.04
5	Potassium	mEq/l	5.52 ±1.08	3.48* ±0.11	3.78* ±1.56	3.09* ±0.24	2.61* ±0.13	2.81* ±0.36	2.47* ±0.24	3.27 ±0.80
6	Glucose	mg/dl	219.7 ±29.6	152.3* ±9.1	153.3* ±17.4	160.3* ±14.5	155.1* ±7.9	143.5* ±12.7	127.9* ±8.4	134.3* ±30.3
7	Total protein	g/dl	6.3 ±0.27	6.08 ±0.33	6.06 ±0.10	6.26 ±0.25	6.27 ±0.22	6.07 ±0.08	5.99 ±0.23	4.43* ±1.93
8	Albumin	g/dl	4.32 ±0.2	3.82 ±0.2	3.91 ±0.27	3.60* ±0.3	3.53* ±0.22	3.41* ±0.29	3.05* ±0.2	2.20* ±1.55
9	Calcium (Alb. Corr.)	mEq/g g/dl/Alb.	6.16 ±0.33	6.47 ±0.44	6.12 ±0.6	6.59 ±0.72	7.10* ±0.55	7.29* ±0.54	7.25* ±0.54	6.65 ±2.47

*Significant differences between the values of the control group and every weekly sample group (according to Dunn [30], $P \leqslant 0.05$). The experiment started with 12 rabbits; 4 animals were killed 4 weeks post-transplantation, 1 after 5 weeks and 4 more after 6 weeks.

Table 2. *Urinary cyclic AMP and urinary clinical chemistry (all urinary values are corrected to urinary creatinine)*

No.	Parameter	Unit	Weeks after tumor transplantation: Controls Mean ± S.D.	1st Mean ± S.D.	2nd Mean ± S.D.	3rd Mean ± S.D.	4th Mean ± S.D.	5th Mean ± S.D.	6th Mean ± S.D.
1	cAMP	μmol/mol	376.0 ± 35	438.0 ± 46	386.0 ± 49	359.0 ± 34	381.0 ± 51	350.0 ± 67	394.0 ± 31
2	Calcium	mEq/mol	0.242 ±0.086	0.259 ±0.108	0.183 ±0.007	0.170 ±0.055	0.246 ±0.11	0.243 ±0.152	0.36* ±0.11
3	Potassium	mEq/mol	2.61 ±1.64	2.95 ±1.57	2.72 ±2.92	1.86 ±0.89	2.32 ±1.82	1.04 ±1.12	0.77* ±0.71
4	Sodium	mEq/mol	72 ±17.2	138.6 ±32.3	67.8 ±20.3	34.3* ±7.1	49.8 ±8.1	52.1 ±19.2	46.8 ±11.1
5	Phosphorus	mg/mol	0 0.108 ±0.02	0.138 ±0.087	0.137 ±0.023	0.107 ±0.024	0.146 ±0.026	0.246* ±0.111	0.279* ±0.022
6	Creatinine	mmol/l	156.5 ±32,9	136.5 ±35.7	211.2 ±43.4	249.2* ±22.9	177.5 ±26.6	170.3 ±34.9	218.3* ±18.6

*Significant differences between the values of the control group and every weekly sample group (according to Dunn [30], $P \leqslant 0.05$). The experiment started with 12 rabbits; 4 animals were killed 4 weeks post-transplantation, 1 after 5 weeks and 4 more after 6 weeks.

weeks 4, 5 and 6 (Table 1). The albumin-corrected calcium levels varied for the groups of tumour-bearing animals of weeks 4–6 post-transplantation between 7.1 and 7.25 (mean values) in relation to a mean of 6.16 mEq/l for the control animals. Of stronger significance and starting earlier in the malignant disease, the serum phosphorus level decreased (Table 1, Fig. 2). The third parameter disclosing a perturbation of calcium metabolism is the constant decrease of the 25-OH-vitamin D (25-OH-D) serum levels to the significantly lower mean values of weeks 5–7 (Table 1, Fig. 3). In parallel, the excretion rate increased in the case of phosphorus to the 2-fold amount on weeks 5 and 6 (Table 2). In addition, the calcium excretion increased significantly ($P < 0.05$) in the 6th post-transplantation week group. The parathyroid hormone (PTH) levels were measured by use of both an N- and a C-terminal-directed radioimmunoassay for PTH (antisera guinea pig VII and A VI 2). It was known from antiserum A VI 2 that it cross-reacted to rabbit PTH: elevated PTH levels were measured in induced secondary hyperparathyroidism. The determined PTH values were, however, mainly in the undetectable range. An indirect indicator of the PTH serum levels is the amount of the excreted urinary cAMP [32]. The cAMP excretion of the measured urine samples remained in the same range as the control values (Table 2) throughout the entire investigation, suggesting that the non-measurable PTH levels behaved in the same way.

The plasma levels of sodium, chloride, urea and creatinine remained essentially unchanged during the investigation.

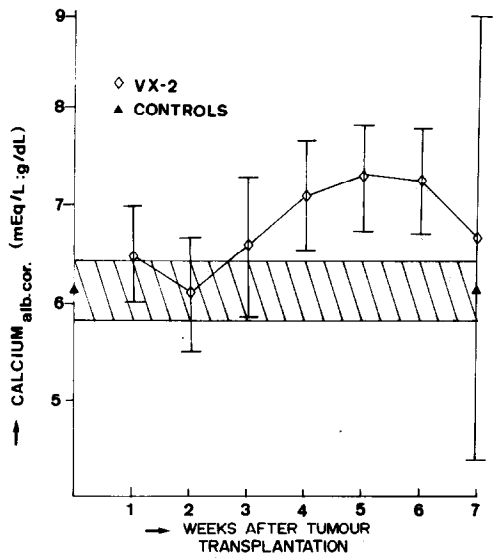

Fig. 1. Serum calcium corrected for serum albumin in controls (shaded area) and tumor-bearing rabbits. Mean ± 1 standard deviation is shown.

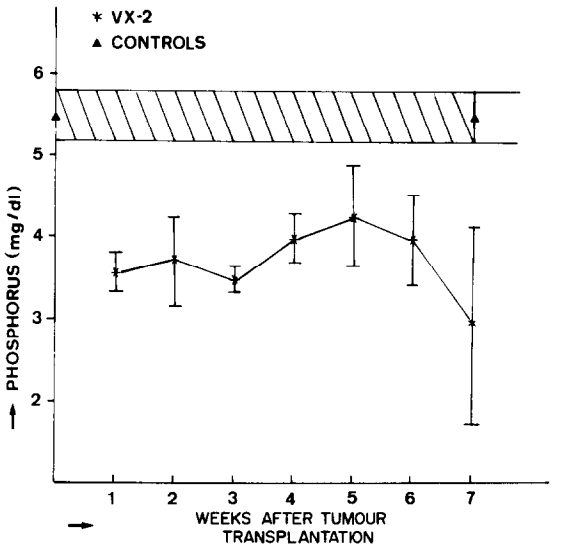

Fig. 2. Serum phosphorus levels in controls and tumor-bearing rabbits.

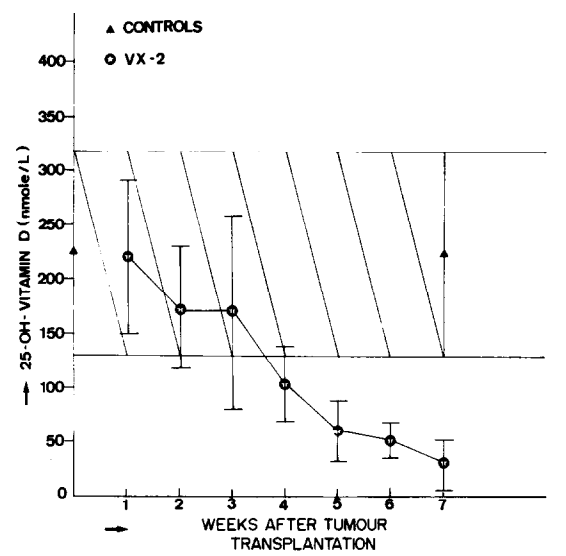

Fig. 3. Serum levels of 25-OH-vitamin D in controls and tumor-bearing rabbits.

The hypoglycemic state

The serum glucose levels decreased constantly from the beginning to about 60% of the control mean (7th week; Table 1, Fig. 4). A high value of 219 mg/dl for the control group and a low value of 127 mg/dl for the 6-week group of the tumor-bearing animals were demonstrable. Moreover, the potassium serum levels were paralleling the decrease of glucose (Table 1, Fig. 5). The urinary excretion of potassium remained in the range of the control mean value until the 3rd week of the investigation and rose significantly in the case of the last group, consisting of 7 animals. The determination of insulin [(a) control group: blood samples of 9 rabbits; (b) 3 weeks post-transplant: blood sample of 9 animals; (c) 5 weeks post-

transplant: consisting of 6 blood samples] exhibited an increase (cf. control group to 3rd week group) and later a decrease (significant differences for the levels of the 3rd week group in relation to the 5th week group) in insulin secretion (Table 1, Fig. 6).

Other parameters

The albumin concentration and, less significantly, the serum total protein levels decreased constantly (Table 1). Other parameters like the hepatic profile (γ-GT, alkaline phosphatases, GPT, GOT and bilirubin) and the Coulter counter (hematocrit and leucocyte and erythrocyte counts) showed no changes of relevance besides a slight anemia (statistically not significant) in the group of rabbits of the 6th and 7th weeks.

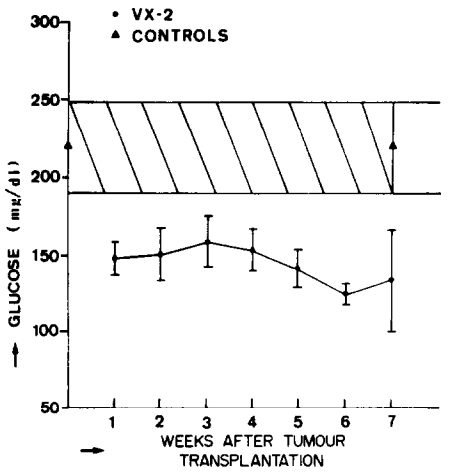

Fig. 4. Serum glucose levels in controls and tumor-bearing rabbits.

DISCUSSION

Pathology of the tumor-bearing rabbits

Our rabbits demonstrated lung metastases as has been reported for VX-2 carcinoma [33, 34]. In addition, no metastases to skeletal tissues were observed, in accordance with the literature [33, 34]. In contrast to these authors, however, the effect of the tumor on bone was less prominent.

Biochemical and clinical parameters with respect to hypercalcemia

The minor effect of our tumor on bone histology is in accordance with the occurrence of only mild hypercalcemia in this series. Differences in tumor biology were also noted by Young *et al.* [33]: from 45 tumor-bearing rabbits only 20 developed overt hypercalcemia. Wolfe *et al.* [35] observed different degrees of hypercalcemia, depending on the source of the tumor.

In contrast to the tumor of Young *et al.* [33], our animals immediately developed hypophosphatemia and hypokalemia. Phosphorus remained normal in their hypercalcemic animals and potassium increased in their normocalcemic and hypercalcemic VX-2 rabbits. A large variation in serum calcium levels was also noted by Wilson *et al.* [36, 37], and their rabbits developed hypophosphatemia like our animals.

To our knowledge no reports have appeared on vitamin D-metabolites in VX-2 carcinoma. The constant decrease of this metabolite suggests an increased turnover to other metabolites such as 1,25-$(OH)_2$-vitamin D or 24,25-$(OH)_2$-vitamin D. Hypophosphatemia could stimulate an increased production of 1,25$(OH)_2$-vitamin D formation [38]. An elevated level of 1,25-$(OH)_2$-vitamin D could stimulate bone resorption and elevate the serum calcium level, thereby suppressing para-

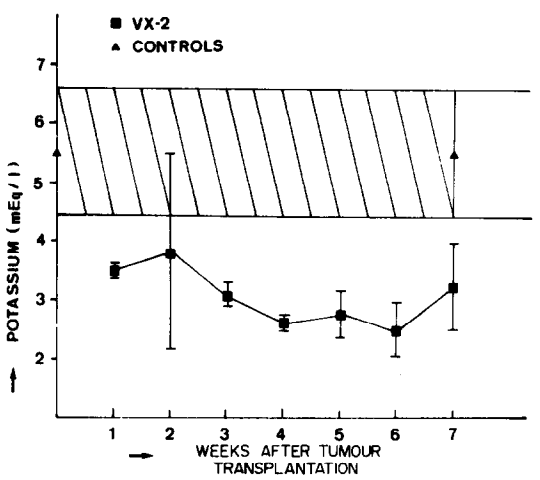

Fig. 5. Serum potassium levels in controls and tumor-bearing rabbits.

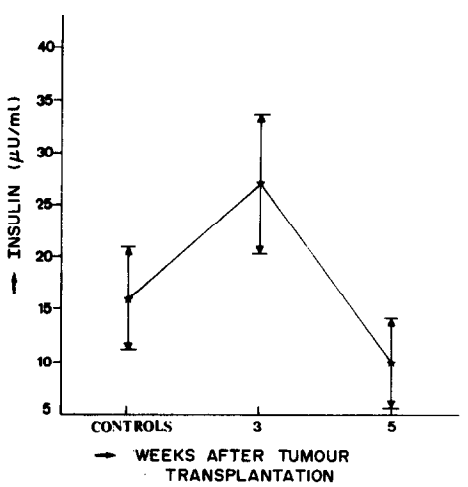

Fig. 6. Serum insulin levels in controls and tumor-bearing rabbits.

thyrin secretion. Our data on serum parathyrin (low levels) are compatible with this mechanism. Urinary cyclic AMP remained fairly constant, indicating that no increase in parathyrin secretion occurred. More detailed studies of vitamin D metabolites in VX-2 carcinoma are necessary to elucidate the cause of 25-OH-vitamin D consumption.

At this time the cause of the hypokalemia in our rabbits is unknown and is in contrast to the report of Young *et al.* [33], who observed increased potassium levels in all VX-tumor-bearing rabbits. Hypokalemia may also occur as a consequence of prostaglandin excess, as in Bartter's syndrome [39]. Hypokalemia did not result from nephrogenous loss: urinary potassium did not increase. Hypokalemia developed within 1 week after tumor transplantation, as did hypoglycemia. As a cause of the latter, hyperinsulinism was excluded: serum insulin levels were lower at the end of the experiment. The assay, of course, measured circulating insulin; this has been demonstrated previously in rabbits before and after glucose injection. Whether the potassium followed the glucose from extracellular to intracellular fluid, as in the treatment of diabetes, remains unknown. The tumor mass may have resulted in increased glucose consumption at the end of the experiment, as has been suggested by Kahn [40]. Semi-starvation could certainly have produced some of the changes seen, e.g. low serum levels of potassium, glucose and insulin.

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