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Relative Role of Bone and Kidney in the Hypercalcaemia Associated with the Rat Walker Carcinosarcoma 256

J. Hurtado, P. Esbrit and A. Rapado

The rat Walker carcinosarcoma 256 is an animal model for humoral hypercalcaemia of malignancy (HHM). In this model, the relative contribution of bone and kidney in the hypercalcaemia of tumour-bearing rats was investigated. Daily administration of pamidronate, a bone resorption inhibitor, for 2 days prevented the increased fasting Ca^{2+} excretion observed in the hypercalcaemic rats, although serum Ca^{2+} remained high. However, the high serum Ca^{2+} normalised after the acute injection of ethiofos, an inhibitor of renal Ca^{2+} reabsorption, which was associated with a marked increase of Ca^{2+} excretion. Changes in Ca^{2+} were accompanied by similar changes in Mg^{2+}. The results indicate that altered renal Ca^{2+} handling has a key role in the hypercalcaemia associated with this HHM model.
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INTRODUCTION

THE WALKER carcinosarcoma 256 implanted in the rat is one of the few known models for humoral hypercalcaemia of malignancy (HHM) [1]. Both increased bone resorption and stimulation of renal calcium (Ca^{2+}) reabsorption appear to contribute to the hypercalcaemia associated with this tumour [2–4]. A factor(s) which interacts with adenylate cyclase-coupled parathormone (PTH) receptors in bone cells has been partly purified from Walker tumour cells [5]. In addition, we have isolated from this tumour a chromatographic fraction displaying PTH-like and growth factor activities for renal cells (ref. 6 and unpublished). However, the relation between these activities and either the increased bone resorption or the stimulated tubular Ca^{2+} reabsorption is unclear [7].

The present study was done to evaluate the relative contribution of bone and kidney in the pathogenesis of the hypercalcaemia associated with this tumour. We studied the efficacy of pamidronate, a known inhibitor of bone resorption [8], and of ethiofos, a compound that inhibits renal Ca^{2+} reabsorption [9] in the treatment of hypercalcaemia in Walker tumour bearing rats.

MATERIALS AND METHODS

The Walker tumour 256, supplied by Prof. A.J.S. Davies (Institute of Cancer Research, London), was continuously transplanted into female Wistar rats (200 g) [3]. On day 8 after tumour implantation, the animals were put into restrictive cages. 3 days later, one group received pamidronate 21 μmol/kg (a gift from Ciba-Geigy, Basel) or saline daily for 2 days subcutaneously. This dose inhibits bone resorption in the rat [8]. On days 10 and 13, rat urine and plasma were collected after a 24 h fast [3].

Ethiofos, supplied by Dr M. Attie and Dr J.P. Bonjour (National Cancer Institute, Bethesda and University Hospital of Geneva, respectively) was dissolved in saline and adjusted to pH 7.0 with 1 mol/l sodium bicarbonate. On day 14 after tumour implantation, 5 ml 20% mannitol solution was administered intraperitoneably to promote an adequate urine flow. Urine was collected over 2 h, and blood was then taken by cardiac puncture under light ether anaesthesia. Then, ethiofos 0.7 mmol/kg or its solvent was given subcutaneously. This dose is adequate in the acute control of the hypercalcaemia associated with the Leydig cell tumour in the rat [10]. Blood and urine were again collected 2 h later. In some animals, ethiofos was administered after pamidronate treatment. Blood and urine were taken before and 2 h after ethiofos injection. On days 12 and 13 after tumour implantation, all the animals were given 10 ml saline intraperitoneally. This amount was sufficient to maintain normal sodium (Na^+) excretion [3].

Ca^{2+} and magnesium (Mg^{2+}) were measured by atomic absorption spectrometry. Inorganic phosphate (Pi) and creatinine were measured by colorimetry [11, 12]. Urinary Ca^{2+}, Mg^{2+} and Pi excretions were expressed as a molar ratio relative to urine creatinine.

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The results are means (S.E.). The significance of differences between groups was evaluated by one-way analysis of variance and Scheffé's test, or Student's t test where appropriate.

RESULTS

At the time of study, tumour-bearing rats were hypercalcaemic and hypercalciuric. Treatment with pamidronate for 2 days significantly decreased Ca^{2+} excretion. At the same time, the drug prevented the further increase in serum Ca^{2+} (Fig. 1). In the control group, serum and urine Ca^{2+} increased with tumour growth. No significant changes in Mg^{2+} and Pi in serum or in Mg^{2+} excretion were detected compared with the controls (not shown). However, a significant decrease in urinary Pi excretion, from 6.8 (1.8) to 4.6 (0.5) mol/mol ($P < 0.01$), was induced by pamidronate.

Ethiofos induced a significant reduction of serum Ca^{2+} within 2 h of injection. Moreover, this fall of serum Ca^{2+} was associated with increased Ca^{2+} excretion, indicating an enhanced tubular Ca^{2+} reabsorption in tumour-bearing rats (Fig. 2). These changes in Ca^{2+} were not accompanied by significant changes of serum or urine Mg^{2+}, although a tendency towards increased Mg^{2+} excretion was observed (urinary Mg^{2+}/creatinine ratio 0.93 [0.32] vs. 3.61 [1.06], before and after treatment, respectively). Serum Ca^{2+} and Mg^{+} as well as urinary excretion of both cations remained unchanged in rats receiving solvent alone.

To further assess the effects of these drugs on the treatment of the hypercalcaemia associated with this tumour, some tumour-bearing rats treated with pamidronate were then given ethiofos.

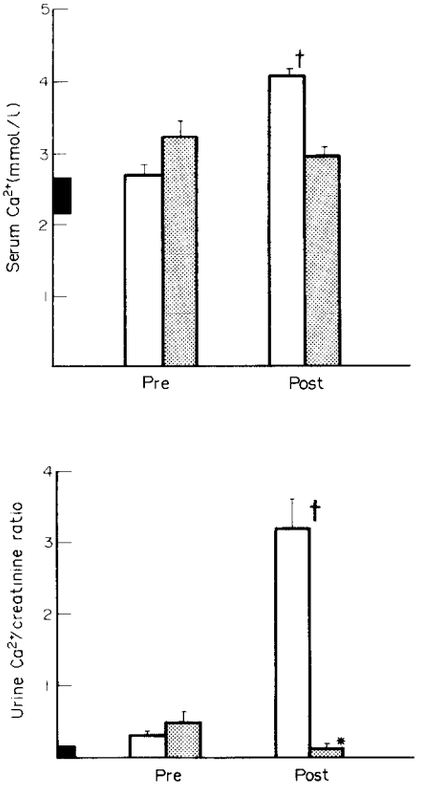

Fig. 1. Effect of pamidronate (21 μmol/kg) on serum and urinary Ca^{2+} in rats implanted with Walker carcinosarcoma 256. Ca^{2+} was assayed before (pre) and after (post) treatment (▨) or vehicle (□). *$P < 0.05$ and †0.01 compared with corresponding pretreatment value. Means (S.E.), $n \geq 5$. ■ = normal range.

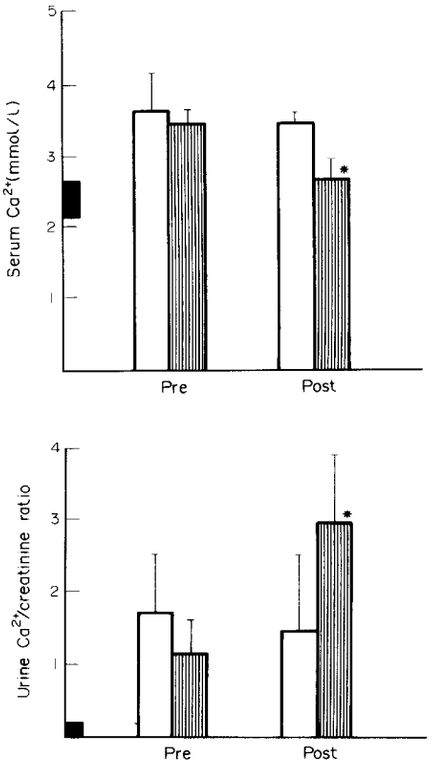

Fig. 2. Effect of ethiofos (0.7 mmol/kg) on serum and urinary Ca^{2+} in rats implanted with Walker 256. ▨ = ethiofos and □ = solvent.

As shown in Fig. 3, the combined effects of both drugs induced a fall in serum Ca^{2+}, which normalised at the expense of an acute increase of Ca^{2+} excretion produced by ethiofos. These changes in Ca^{2+} were accompanied by normalisation of serum Mg^{2+}, which was increased above normal values after pamidronate treatment, associated with a dramatic rise of Mg^{2+} excretion (Fig. 3).

Serum and urine Ca^{2+} in the control group increased in accord with the pattern of tumour growth. Serum and urine Mg^{2+} remained unchanged in rats receiving the solvent alone.

All animals in this study had a normal renal function, estimated by creatinine clearance (not shown).

DISCUSSION

The present study shows that a dose of pamidronate, known to block bone resorption, given to Walker tumour-bearing rats normalised the elevated Ca^{2+} excretion (an index of bone resorption) in these animals. However, the drug was unable to lower the high serum Ca^{2+} in tumour-bearing animals. These results suggest that stimulation of tubular Ca^{2+} reabsorption has a significant role in the hypercalcaemia associated with the Walker tumour, in agreement with our previous report [3]. These findings are consistent with results obtained by others who tested the efficacy of various bisphosphonates to prevent the hypercalcaemia associated with this tumour model [4, 13]. Our results are also in agreement with the incomplete Ca^{2+} lowering response to inhibitors of bone resorption found in cancer patients with hypercalcaemia and an increased tubular Ca^{2+} reabsorption [14].

The observed decrease of Pi excretion after pamidronate treatment is consistent with previous findings, and it appears to be related to bone resorption inhibition [15].

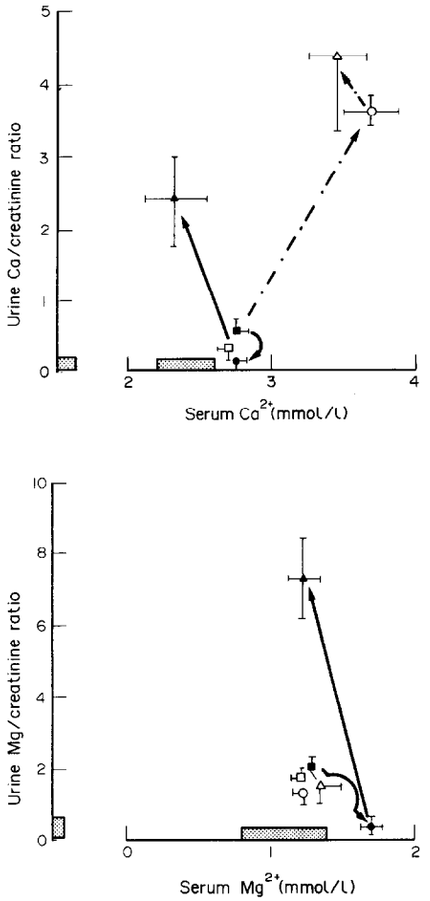

Fig. 3. Urine Ca^{2+} or Mg^{2+} excretion in relation to serum Ca^{2+} or Mg^{2+} after acute administration of ethiofos in Walker tumour-bearing rats pretreated with pamidronate for 2 days. Solid arrows indicate changes in renal handling of corresponding cation before (solid squares) and after treatment with pamidronate (solid circles) followed by ethiofos injection (solid triangles) in the treated group of rats. Open symbols represent corresponding values, whose changes are indicated by broken arrows, obtained in control group of animals injected with vehicle. ▨ = normal range.

In contrast to that observed with pamidronate treatment, the acute administration of ethiofos, an inhibitor of tubular Ca^{2+} reabsorption, induced a significant decrease of serum Ca^{2+} accompanied by elevated Ca^{2+} excretion. Furthermore, ethiofos given to tumour-bearing rats pretreated with pamidronate rapidly normalised serum Ca^{2+}, and was associated with a dramatic increase of Ca^{2+} excretion. The latter appears to be independent of Na^{+} changes, since all animals were similarly repleted with saline. These results are similar to those found in another rat model for HHM and those obtained in hypercalcaemic rats infused with an aminoterminal fragment of a PTH-related protein isolated from HHM-associated tumours [10, 16].

These data strongly suggest that the effects of ethiofos are likely to be due to an inhibition of tubular Ca^{2+} reabsorption in Walker tumour bearing rats. Nevertheless, other known extrarenal effects of this drug might contribute to the hypocalcaemic effect. Thus, ethiofos inhibits PTH secretion [9, 17]. Although in the experiments throughout this study we used intact rats, the degree of hypercalcaemia should have allowed only a minimal PTH secretion, if any. On the other hand, a possible inhibitory effect of ethiofos on bone resorption seems to require a longer exposure to the drug [18]. Moreover, such an effect should have been reflected in a decrease instead of an increase of Ca^{2+} excretion, as observed after ethiofos administration. Therefore, these effects of ethiofos are unlikely to play a significant role in the control of hypercalcaemia induced in the Walker tumour-bearing rats. However, the present data cannot rule out a possible inhibitory effect of this drug on secretion of a factor(s) that would alter renal Ca^{2+} handling. This factor is unlikely to be a PTH-related protein, since inhibition of its secretion would have been associated with a decrease of Pi excretion, which was not observed in ethiofos treated rats (not shown).

The effect of ethiofos on Ca^{2+} excretion was accompanied by a similar effect on Mg^{2+} excretion. This suggests an inhibitory effect of this drug on tubular Mg^{2+} reabsorption, as previously reported [19], and it is consistent with the current concept that both cations share a common transport mechanism in the renal tubule [20].

On the other hand, some of the pamidronate-treated rats (Fig. 3) showed an increase of serum Mg^{2+} with a decrease of Mg^{2+} excretion, as previously reported [21]. These animals displayed less hypercalcaemia (−0.5 mmol/l) compared with the other group of rats also treated with pamidronate (Fig. 1), which would result in a lower renal Ca^{2+} load. Therefore, the correlation between the fractional excretion of Ca^{2+} and Mg^{2+} manifested more clearly, resulting in an increase of serum Mg^{2+} [21].

Our study shows that besides an increased bone resorption, an altered renal Ca^{2+} handling appears to have a key role in the hypercalcaemia associated with the Walker tumour 256 in the rat. Therefore, this tumour should be added to the growing list of malignancies where changes in tubular Ca^{2+} reabsorption have been reported [14, 22]. These results also emphasise the need for a therapeutic approach based on an inhibition of renal Ca^{2+} reabsorption, such as treatment with ethiofos, for the normalisation of the hypercalcaemia associated with these tumours.

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Induction of Natural Killer Activity by Xanthenone Analogues of Flavone Acetic Acid: Relation with Antitumour Activity

Lai-Ming Ching, Wayne R. Joseph, Li Zhuang, Graham J. Atwell, Gordon W. Rewcastle, William A. Denny and Bruce C. Baguley

Flavone-8-acetic acid (FAA) induces haemorrhagic necrosis and tumour regression in experimental tumours and induces natural killer (NK) activity. Xanthenone-4-acetic acid (XAA) forms the basis of a series of analogues of FAA which vary in antitumour potency. FAA, XAA and 15 XAA derivatives were tested for their ability to induce either NK activity in mouse spleens or haemorrhagic necrosis in mouse colon 38 tumours. Some derivatives were active in both assays (one at a dose 8-fold lower than that of FAA). When both assays were quantitated, a significant correlation ($r = 0.85$; $P < 0.001$) was found. NK assays could be useful in screening compounds such as FAA and XAA analogues which appear to mediate their antitumour activity by biological response modification. Since tumour necrosis may not be mediated directly by NK cells, FAA and active XAA derivatives may exert pleiotropic effects that include NK induction and tumour necrosis by acting on host cells to release cytokines.
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INTRODUCTION

FLAVONE-8-ACETIC ACID (FAA) is a synthetic flavonoid with high activity against several tumours in mice [1, 2] but low clinical antitumour activity [3]. The mechanism of action of FAA appears to be indirect and different from that of conventional agents [4]. FAA acts as a biological response modifier, inducing natural killer (NK) activity in mouse spleen [5] and other organs [6], as well as in human peripheral blood [7]. It induces cytokine synthesis in the mouse [8–10], and it is likely that FAA activates pleiotropic host cell mechanisms, which include tumour cell lysis, cytokine production and NK induction. To determine whether the induction of NK activity and of tumour necrosis are part of the same pleiotropic response, it would be useful to correlate the two effects with structurally similar FAA analogues differing from highly active to inactive. Such a series is provided by derivatives of xanthenone-4-acetic acid (XAA, Fig. 1), a drug containing a fused tricyclic pharmacophore which has similar effects to those of FAA against the murine colon 38 tumour [11]. Our studies on XAA derivatives have identified 5-methyl-XAA, a drug with similar activity to FAA at approximately one-eighth of the dose, and other XAA derivatives which vary from

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