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Research Article

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Normalization of Hypercalcemia Associated with a Decrease in Renal Calcium Reabsorption in Leydig Cell Tumor-Bearing Rats Treated with WR-2721

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Abstract

The Rice-500 Leydig cell tumor (LCT) in Fischer rats is a model of humoral hypercalcemia of malignancy (HHM). In this model, the elevation of plasma calcium (Ca) does not result merely from an increased bone resorption, but also from an enhanced tubular Ca reabsorption (TRCa).

We investigated the hypocalcemic response to WR-2721 [S-2,2-(3-aminopropylamino)-, ethylphosphorothioic acid] in LCT-bearing Fischer rats. WR-2721 is a potent inhibitor of normal and aberrant parathyroid hormone (PTH) secretion. Moreover, it exerts a PTH-independent inhibitory effect on TRCa.

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Introduction

The Rice-500 Leydig cell tumor (LCT)[1] in Fischer rats is considered a model of humoral hypercalcemia of malignancy. It is associated with hypercalcemia, hypophosphatemia, and elevated urinary cyclic (c) AMP excretion (1–4). Increased osteoclastic bone resorption, inhibition of bone formation (5), and, more recently, stimulation of the tubular calcium (Ca) reabsorption (6) have been described in this model. It has been suggested that so far unidentified molecules such as parathyroid hormone (PTH)-like or transforming growth factors released by the tumoral cells might be responsible for the alterations in Ca metabolism (7–10).

In LCT-bearing rats the administration of some powerful inhibitors of bone resorption, such as diphosphonates (11), entailed a complete correction of the hypercalciuria, a response indicating that the antiosteolytic therapy was successful. Nevertheless, the diphosphonate treatment did not normalize the plasma Ca level (11). This failure could be related to the inefficacy of diphosphonates for counteracting the stimulated tubular Ca reabsorption that is present in LCT-bearing animals (6). It thus appears to be of interest to test whether a drug displaying an inhibitory activity on the tubular Ca reabsorption could influence the calcemia of LCT-bearing rats.

The radioprotective agent WR-2721 [S-2,2-(3-aminopropylamino)-, ethylphosphorothioic acid] inhibits normal and abnormal PTH secretion in human beings (12–14) and experimental animals (15–17). In addition, this drug also promotes a PTH-independent reduction in the tubular Ca reabsorption, which can be observed without any consistent change in the renal handling of sodium (16). We report on the very effective and rapid hypocalcemic action of WR-2721 in LCT-bearing animals; calcemia was normalized while the renal Ca reabsorption was markedly reduced.

Methods

Animal preparation. 180–200-g male Fischer rats (F344) obtained from IFFA Credo (L'Arbresle, France) were pair-fed during a 14-d period elapsing between the tumor implantation and the last day of the experiment. The diet was a standard rat chow (Kliba, Kligenthalmuhle AG, Kaiseraugst, Switzerland). The evening before the experiment food was provided for the last time.

Leydig cell tumor. A dose of 0.25 ml of a 1:1 Leydig tumor cell suspension in RPMI medium (kindly provided by Dr. A. C. Santora II, National Institute of Arthritis, Digestive Diseases and Kidney, Bethesda, MD) was injected into each flank under light ether anesthesia. The tumor was palpable at day 7 and grew rapidly thereafter. The experiments were carried out on day 13 and 15 after tumor implantation.

Drug preparation. WR-2721 [H2N-(CH2)3-NH-(CH2)2-S-PO3H2] was kindly provided by the National Cancer Institute (Bethesda, MD). Before each injection one dose was dissolved in 0.2 ml Hepes-containing solution and the pH adjusted to 7.4 by Tris-base. At the dose delivered the drug did not induce any toxic side effects.

Effect of WR-2721 on calcemia 13 d after tumor implantation. In the morning of day 13 after tumor implantation conscious animals were placed into restrictive cages and blood was sampled from a tail vein. After a first blood collection the drug or its solvent was injected subcutaneously at a dose of 0.7 mmol/kg body wt. A second blood sample was collected 2 h after drug administration.

Determination of the renal handling of Ca by clearance studies. On day 15 after tumor implantation animals were placed into restrictive cages and a tail vein catheter was inserted into a tail vein to administer a continuous intravenous infusion of a 0.15-M NaCl solution containing 50 μCi/100 ml of methoxy-[^{3}H]inulin at a rate of 5 ml/h throughout the

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1. *Abbreviations used in this paper:* GFR, glomerular filtration rate; LCT, Leydig cell tumor; PTH, parathyroid hormone.

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experiment. After an equilibration period of 30 to 60 min, 0.8 ml of a 5% mannitol solution was injected intravenously in all animals in order to promote a sufficient urine flow rate. During the following 120 min urine was collected and a blood sample was taken at the end of this period. Then, 0.7 mmol/kg of WR-2721 or its solvent was injected subcutaneously. Rats that received WR-2721 on day 13 were reinjected with the drug, whereas control animals were again given the solvent as on day 13. Then, a second urine collection of 120 min was started, at the end of which a second blood sample was taken.

Analytical methods. Plasma and urine Ca were measured by atomic absorption spectrometry. Urinary inorganic phosphate (Pi) was determined by colorimetry, urinary cAMP by a protein binding assay (18), and the activity of [^{3}H]inulin in a scintillation spectrometer.

Statistical analysis. All results are expressed as mean±standard error of means (SEM) unless otherwise specified. The significance of differences was evaluated by a two-sided unpaired Student's *t* test.

Results

Effect of WR-2721 on plasma Ca in LCT-bearing rats. As shown in Fig. 1, LCT-bearing rats displayed a marked hypercalcemia with a mean value of 3.24±0.05 mmol/liter (n = 10) on day 13 as compared with 2.71±0.02 mmol/liter (n = 10) before tumor implantation. WR-2721 was able to normalize the plasma Ca level within 2 h after its administration (2.72±0.09 mmol/liter), while the solvent injection had no influence on hypercalcemia (3.33±0.05 mmol/liter). Without any further treatment plasma Ca increased in both groups of rats during the following 48 h (Fig. 1). However, the WR-2721 pretreated group remained significantly less hypercalcemic (3.24±0.12 mmol/liter) than the control group (3.62±0.10 mmol/liter) on day 15 ($P < 0.05$). A second injection of WR-2721 normalized plasma Ca (2.66±0.23 mmol/liter) as rapidly as on day 13 (Fig. 1).

Effects of WR-2721 on renal Ca handling in LCT-bearing rats 15 days after tumor implantation. Renal clearance studies were conducted on day 15 before and 2 h after the administration of WR-2721. As illustrated in Fig. 2 the drug provoked a marked alteration in the renal handling of Ca. Indeed, the decrease in plasma Ca was accompanied by an elevation in urine Ca excretion indicating an inhibition of tubular Ca reabsorption. In control animals the moderate fall in calcemia was accompanied by a slight augmentation in urinary Ca excretion (Fig. 2) that may be related to the continuous saline infusion. In both groups urinary sodium excretion tended to increase during the experiments. Although the mean natriuresis of the WR-2721-treated rats was slightly greater 2 h after injection as compared with that of the solvent-injected animals (4.79±0.88 vs. 3.21±0.93 μmol/ml GFR), there was an important overlapping of the individual values recorded in the two groups. In WR-2721-treated rats no significant alteration in the clearance of inulin, urinary Pi, or cAMP excretion was observed as compared with the values monitored in the solvent-injected counterparts (Table I).

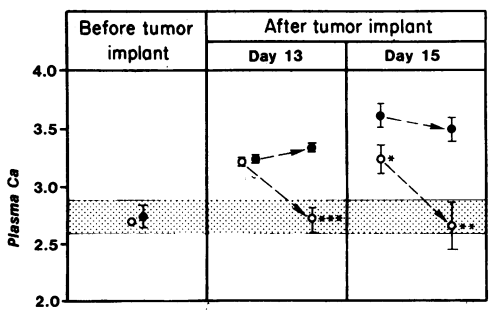

Figure 1. Effect of WR-2721 on plasma Ca (millimoles per liter) in LCT-bearing rats. 13 and 15 d after tumor implantation, the animals received one dose (0.7 mmol/kg s.c.) of WR-2721 (o) or its solvent (•). Calcemia was determined 2 h after the injection (→). Each dot represents the mean±SEM of five rats. *$P < 0.05$, **$P < 0.01$ as compared with controls. The shaded area corresponds to the mean±2 SD of the calcemia recorded in all animals (n = 10) before tumor implantation.

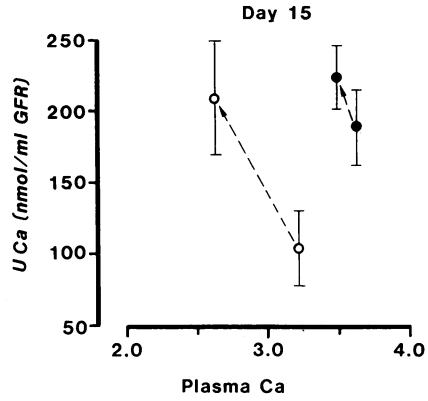

Figure 2. Urinary Ca excretion in relation to plasma Ca (in millimoles per liter) after one injection of WR-2721 in LCT-bearing rats. 15 d after tumor implantation renal clearance experiments were performed to determine urinary functions and Ca excretion before and after an injection (0.7 mmol/kg) of WR-2721 (o) or its solvent (•). 2 h later (→) plasma Ca and urinary Ca excretion were determined. Each dot represents the mean±SEM of five rats.

Discussion

In this study the organic thiophosphate WR-2721 appears to be a very effective drug for rapidly lowering plasma Ca in malignant hypercalcemia provoked by the LCT in Fischer rats. We observed a normalization of hypercalcemia within 2 h after treatment and 2 d later, there was still a significant difference in the plasma Ca level between the treated and nontreated animals. Simultaneous to the decrement in plasma Ca, we observed an important rise in urinary Ca excretion signifying a reduction of tubular Ca

Table I. Renal Parameters before and 2 h after the Injection of 0.7 mmol/kg of WR-2721 in LCT-bearing Rats

	Before injection		2 h after injection	
	Solvent	WR-2721	Solvent	WR-2721
GFR (ml/min)	1.30±0.22	1.42±0.14	1.00±0.12	1.34±0.23
UNa (μmol/ml GFR)	2.04±0.66	1.74±0.56	3.21±0.93	4.79±0.88
UPi (nmol/ml GFR)	541±103	491±129	563±80	445±71
UcAMP (pmol/ml GFR)	94±11	99±11	82±7	87±8

Values are means±SEM of five rats per group. GFR, glomerular filtration rate; UNa, urinary excretion of sodium; UPi, urinary excretion of inorganic phosphate; UcAMP, urinary excretion of cyclic AMP.

reabsorption. This finding strongly suggests that the renal effect may play a major role in the normalization of plasma Ca in this experimental model.

The nature of this tubular action of WR-2721 remains very puzzling. From the results one could not completely rule out that it might be related, at least in part, to an inhibition of renal sodium reabsorption. As previously reported (16), however, WR-2721 can promote a conspicuous decrease in the tubular Ca reabsorption without consistently altering the renal handling of sodium. In this respect, its mode of renal action sharply differs from that of natriuretic agents, such as furosemide. Furthermore, the fall in Ca reabsorption is independent of the inhibitory effect of the drug on PTH secretion since it is also present in thyroparathyroidectomized animals (16). With regard to the consequences of this renal action, previous observations did not suggest that the tubular effect of WR-2721 could have an important impact on the level of plasma Ca in animals deprived of parathyroid glands. However, as tubular Ca reabsorption is already reduced in thyroparathyroidectomized rats, any further inhibition might not markedly affect the plasma Ca concentration. In contrast, in situations with enhanced renal Ca reabsorption, as induced by implantation of LCT in rats, an inhibition of this process may be expected to have a much more important repercussion on calcemia.

In LCT-bearing rats the rise in plasma Ca is associated with stimulation of both bone resorption (2–5) and tubular Ca reabsorption (6). A substantial contribution of the renal component to hypercalcemia could be indirectly inferred from studies showing that diphosphonates given in doses blocking bone resorption were unable to normalize completely the plasma Ca level of LCT-bearing rats (11). The present study brings direct support in favor of a contributing role of the kidney, since inhibition of tubular Ca reabsorption by WR-2721 was accompanied by a dramatic reduction in the plasma Ca level.

That other mechanisms could still have contributed to the WR-2721-induced hypocalcemic response cannot be excluded from the foregoing experiments. Firstly, WR-2721 is a very potent inhibitor of PTH secretion (12–16). Since we used intact rats, this property of the drug could play a contributing role in the hypocalcemic response. At plasma Ca of about 3.4 mmol/liter, however, PTH secretion should already be fully inhibited. Several studies found undetectable PTH serum levels in LCT-bearing rats (4, 9, 19). Secondly, Attie et al. (14) reported an additional inhibitory effect of WR-2721 on bone resorption. The reduced osteoclast activity observed in vitro occurred only after 4 d of incubation with WR-2721. Reduction of bone resorption could thus contribute to the fall of plasma Ca in LCT model at a later phase and perhaps explain the slight but significant difference in plasma Ca observed 48 h after WR-2721 injection (Fig. 1). An immediate and major contribution of a reduced Ca release from bone appears, however, unlikely because it should be accompanied by a fall in urinary Ca excretion. Finally, WR-2721 can inhibit the aberrant PTH secretion of parathyroid cancer cells (15, 16). One might speculate, therefore, that this agent could exert an inhibitory influence on malignant cells that produce PTH-like factors as demonstrated for the Leydig tumor (20, 21). However, neither the urinary cyclic AMP excretion nor the phosphaturia were altered by WR-2721 injection. We therefore have no evidence in favor of this hypothesis.

Hypercalcemia of malignancy in human patients is very often associated with a marked increase in osteolysis (20, 22). However in some patients a stimulation of tubular Ca reabsorption has also been documented (23, 24). In those individuals, like in the LCT rat model (11), the hypocalcemic response to diphosphonate therapy appears to be relatively modest as compared to that recorded in subjects in whom osteolysis represents the prevailing hypercalcemic process (24). Therefore, in hypercalcemic patients with enhanced tubular Ca reabsorption a drug such as WR-2721 that is able to inhibit this process could be of therapeutic interest.

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