Renal osteodystrophy in rats with reduced renal mass

A. Moscovici¹, J. Bernheim², M. M. Popovtzer¹ and D. Rubinger¹

¹Nephrology and Hypertension Services, Hadassah University Hospital, Jerusalem, and ²Department of Nephrology, Meir Medical Center, Kfar Saba and Sackler Faculty of Medicine, Tel Aviv University, Israel

Abstract. The rat remnant kidney, a popular model of experimental renal failure, has also been used to assess the histological bone changes associated with reduction in renal mass. The suitability of this model has been challenged, especially with regard to the standardization of animals from different age groups and various degrees of renal failure. The present study was undertaken: (1) to better evaluate the histomorphometric changes associated with reduction in renal mass; (2) to assess these changes over a longer follow-up period as compared to those of matched intact animals; (3) to define the response to pharmacological doses of calcitriol.

Male rats were evaluated 4 and 8 weeks after induction of renal failure (5/6 nephrectomy) and compared with age-matched control rats with intact kidneys. Plasma biochemistry, creatinine clearance and parathyroid hormone (PTH) were obtained from normal rats and from the rats with chronic renal insufficiency. Histomorphometric studies were performed in all animals on the right tibial bone.

Induction of renal failure of 4 weeks’ duration (short-term study, age 16 weeks) was associated with increased PTH and bone resorption, but suppressed bone formation. At long-term follow-up (rats aged 20 weeks), the suppression in bone formation was even more prominent. These changes were assessed considering normal bone histomorphometry during the process of growing.

Calcitriol administration was associated with a significant suppression of bone resorption and a spectacular increase in all osteoid parameters. The bone formation rate, however, remained in the same low range as that in untreated animals.

This model of renal osteodystrophy is similar to human secondary hyperparathyroidism with regard to the enhanced bone resorption, but differs by the marked decrease in bone formation. There was a remarkable similarity between the response of rats with renal failure and that of patients with severe secondary hyperparathyroidism to large doses of calcitriol, leading in both to adynamic bone disease. Keeping in mind the species differences, the bone changes developing in the uraemic rats may be useful in understanding certain aspects of human renal osteodystrophy.

Key words: calcitriol; renal failure; renal osteodystrophy; secondary hyperparathyroidism

Introduction

For many years, it has been considered as essential ‘to develop animal models for human diseases, with disease onset mechanisms as those of humans, to study the causes, ... therapeutic and preventive methods as well as to develop new drugs’ [1]. This plea applies to the spectrum of metabolic bone diseases, including various forms of renal osteodystrophy. Renal osteodystrophy, the term used to describe the skeletal complications related to renal diseases, is a multifactorial disorder, and is classified as osteitis fibrosa cystica, osteomalacia, mixed type and adynamic bone disease [2–4].

Historically, osteitis fibrosa due to secondary hyperparathyroidism is the predominant bone lesion in chronic renal failure. Even though a variety of independent factors have been identified in the development of the hyperparathyroid state in uraemia, considerable controversy still exists regarding the sequence of the events and the early histological changes associated with this condition [3,4]. A reproducible animal model presents an attractive way to investigate the occurrence and evolution of secondary hyperparathyroidism and of the bone morphological alterations in incipient renal insufficiency. Such a model will have the advantages of providing a homogeneous study population with regard to the aetiology of the renal disease, age, nutrition and the degree of impairment of renal function. By contrast, similar criteria are much more difficult to fulfil in the context of even a well-controlled clinical study.

The rat remnant kidney is one of the most used models to assess pathophysiological aspects of chronic renal failure. For the last 15 years it has also been used to assess the histological bone changes in uraemia. The main histological findings which were described
Rat model of renal osteodystrophy

in rats with reduced renal mass include increase of total eroded surface, osteoclast number and surface covered by osteoclasts [5–7]. The increased bone resorption which paralleled elevated parathyroid hormone (PTH) is considered to be suggestive of secondary hyperparathyroidism. These changes could be partly prevented using inhibitors of PTH secretion [8]. Severe secondary hyperparathyroidism was also found in rats with experimental glomerulonephritis [9] and hereditary polycystic disease [10], in mice and dogs with impaired renal function due to subtotal nephrectomy [11,12], and in elderly rats with age-related nephrosclerosis [13].

When animals with reduced renal mass were fed a low calcium and/or a low phosphorus diet, an osteomalacic type of bone disease was observed. The osteomalacic changes were enhanced by aluminium, fluoride, and calcitriol administration [14–18]. While aluminium loading in animals with intact parathyroid is generally associated with excessive osteosclerosis, its presence in parathyroidectomized rats led to impaired osteoid formation, decreased osteoblastic surface and decreased bone formation resembling the human aplastic (adynamic) bone disease [19,20].

Despite its widespread use, the suitability of the rat remnant kidney model to evaluate bone histomorphometric variations in renal disease has been challenged, especially with regard to the difficulties of standardization of animals with different degrees of renal failure [5].

The present study was undertaken:

(1) to better evaluate the static and dynamic histomorphometric changes associated with chronic reduction in renal mass in rats;
(2) to assess the evolution of these changes over a longer follow-up period as compared to those of rats with intact renal mass, age and weight matched;
(3) to define the histomorphometric changes following the administration of pharmacological doses of calcitriol at short-term and long-term follow-up.

Subjects and methods

In all our studies we used male adult Sabra rats of the Hebrew University strain with a body weight of 180–200 g. The rats were fed standard pellet chow with a calcium and phosphorus content of 0.78% and 0.51% of 100 g dry weight, respectively, and drank tap water ad libitum.

Chronic renal failure was induced by 5/6 nephrectomy in two stages: first, 2/3 reduction of the mass of the right kidney by surgical ablation of the upper and lower poles, and 1 week later total left nephrectomy.

The experiments were performed at two time intervals: 4 weeks after 5/6 nephrectomy (short-term study) and 8 weeks after 5/6 nephrectomy (long-term study).

Data obtained from short- and long-term studies of rats with chronic renal insufficiency were compared with control rats with intact kidneys matched for weight and age and with similar rats with 5/6 nephrectomy, treated with calcitriol 54 ng/24 h, administered subcutaneously for 1 and 3 weeks in short- and long-term studies, respectively.

Prior to sacrifice, the animals were kept in metabolic cages for 7 days. A 24 h urine collection was obtained in the last day of the study.

The biochemical measurements, performed with an automated analyser, included determination of plasma and urine creatinine, calcium and phosphate. PTH was determined with a commercial radioimmunounassay kit (Incarta Corp., Stillwater, MN, USA) using rat-specific antibodies directed to the mid-portion of rat PTH.

Histomorphometric studies were performed on the right tibial bones [21]. Tetracycline (Ledermycin, Lederle, Germany), 25 mg/kg, was injected intraperitoneally twice, 5 days and 1 day prior to sacrifice.

The tibial bones were removed, further fixed in Carson–Milloning’s solution, and embedded undecalcified in historesin kit after dehydration in alcohol.

Sections of 5 and 10 μm were cut in antero-posterior planes. To identify mineralized bone, osteoid, osteoblasts and osteoclasts, the 5 μm sections were stained by the von Kossa method.

The following parameters were calculated according to Parfitt et al. [22]:

(1) Osteoblast surface (Ob.S/BS), the fraction of trabecular surfaces covered by osteoblasts.
(2) Osteoid surface (OS/BS), the fraction of trabecular surface covered by osteoid.
(3) Osteoid volume (OV/BS), the fraction of trabecular bone volume occupied by osteoid.
(4) Osteosclerotic surface (Oc.S/BS), the fraction of trabecular surface covered by osteoclasts.
(5) Osteoclast number (OC/NTA), the number of osteoclasts per mm² of cancellous bone.
(6) Osteoid thickness (O.Th) calculated as the ratio of osteoid volume to osteoid surface x 100.
(7) Eroded surface (ES/BS), the fraction of trabecular surface covered by lacunae (including ‘active’ lacunae with osteoclasts and lacunae in reversal phase, i.e. the period in which the resorption lacunae are filled with inactive-looking mononuclear cells).
(8) Double-labelled tetracycline surface, the fraction of trabecular surface covered by double-labelled tetracycline.
(9) Mineralized surface, given by the total extent of double labels plus half the extent of single labels.
(10) Bone formation rate (BFR/BS) at tissue level, obtained by multiplying the mineralized surface by mineral oppositional rate.
(11) Mineral appositional rate (MAR), obtained by measuring the mean distance between tetracycline labels and dividing by the number of days between doses of tetracycline.

The results were expressed as mean ± SE. Comparisons between two groups were performed with the Wilcoxon rank sum test. Data of more than two groups were compared using non-parametric one-way analysis of variation (the Kruskal–Wallis test) and the SPSS statistical package.

Results

Plasma and urine biochemical data and PTH from intact control rats and rats with chronic renal failure of 4 weeks’ duration are listed in Table 1.

Table 2 depicts the histomorphometric data of intact rats and those of rats with reduced renal mass of 4 weeks’ duration. Induction of renal failure was associ-
Table 1. Plasma biochemistry, creatinine clearance, fractional excretion of phosphate (CP/CCr) and parathyroid hormone (PTH) level in intact control rats and in rats with chronic renal failure (CRF) of 4 weeks' duration (short-term).

<table>
<thead>
<tr>
<th></th>
<th>Intact (n=10)</th>
<th>CRF (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (µmol/l)</td>
<td>53.0±0.05</td>
<td>73.4±4.4*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phosphate (mEq/l)</td>
<td>2.1±0.07</td>
<td>2.3±0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Calcium (mEq/l)</td>
<td>4.5±0.2</td>
<td>4.7±0.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>114±9.1</td>
<td>278±21*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CCr (µl/min)</td>
<td>1042±174</td>
<td>608±43*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CP/CCr</td>
<td>0.22±0.01</td>
<td>0.33±0.03**</td>
<td>&lt;0.0001</td>
</tr>
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</table>

*P < 0.001, **P < 0.005 vs intact.

Table 2. Bone resorbing and bone forming parameters in intact control rats and in rats with short-term chronic renal failure (CRF).

<table>
<thead>
<tr>
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<th>Intact (n=10)</th>
<th>CRF (n=10)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>ES/BS (%)</td>
<td>15±3.8</td>
<td>31±3.1</td>
<td>&lt;0.0045</td>
</tr>
<tr>
<td>Oc.S/BS (%)</td>
<td>0.93±0.4</td>
<td>9.5±1.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oc.N/TA (1/mm)</td>
<td>0.28±0.13</td>
<td>19±0.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OS/BS (%)</td>
<td>17.6±3.1</td>
<td>58±1.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OV/BV (%)</td>
<td>3.76±0.65</td>
<td>1.28±0.45</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BFR/BS (mm/mm/year)</td>
<td>1.27±0.14</td>
<td>0.58±0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MAR (mm/year)</td>
<td>0.19±0.01</td>
<td>0.16±0.01</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

For abbreviations, see 'Subjects and Methods'.

The effect of growing on bone histomorphometry in normal male rats is shown in Figure 1. Figure 1 compares histomorphometric data from normal rats aged 16 and 20 weeks. The effect of growing in rats with chronic renal failure as well as the effect of 1,25(OH)2D3 is depicted in Figures 2 and 3. In normal rats, there was a remarkable increase in bone turnover with growing (Figure 1). It consisted of a 5-fold increase in the bone formation rate, a 3-fold increase in the osteoclast number, and an increased osteoclast surface. There was also elevation in the osteoid (OS/BS) and the osteoblast (Ob.S/BS) surfaces. Likewise, in rats aged 20 weeks there was a modest but significant decrease in PTH compared to animals aged 16 weeks (114±9.1 and 72±3.3 pg/ml, P<0.01).

By contrast, it is noteworthy that in rats of the same age (20 weeks, Figures 2 and 3) but with chronic renal failure, the increase in bone resorptive parameters (ES/BS, Oc.B/B, Oc.N/TA) was less prominent than that seen in rats aged 16 weeks (Figure 2). The bone forming parameters (OV/BV, OS/BS, Ob.S/BS, BFR) were significantly suppressed compared to those seen in rats with normal renal function. The degree of suppression of bone forming parameters in rats with chronic renal failure was similar at 16 and 20 weeks (short-term and long-term follow-up, respectively).

The effects of calcitriol challenge in both groups of rats with chronic renal failure are also shown in Figures 2 and 3. The duration of the calcitriol treatment was 1 week in the short-term and 3 weeks in the long-term group. Calcitriol administration was associated with a significant suppression of bone resorptive parameters already after 1 week of therapy. After 3 weeks of calcitriol administration, ES/BS, Oc.S/Bc and Oc.N/TA reached values lower than those of the controlled age group animals with normal renal function.

The administration of calcitriol was associated with a remarkable increase in all osteoid parameters after 1 week, but even more spectacular after 3 weeks of therapy. It is noteworthy that after 3 weeks of calcitriol treatment 85% of the bone perimeter was covered with
osteoid (OS/BS) of which 61% was osteoblastic osteoid (Ob.S/BS).

Despite the suppression of bone resorption and the increase in osteoid parameters seen after calcitriol, there was no change in the bone formation rate (BFR) which remained in the same low range as that in untreated animals.

Figure 4 combines representative histological sections from a tibia of a normal rat, a bone section from a rat with chronic renal failure, and sections from tibiae of rats with chronic renal insufficiency treated with calcitriol for 1 and 3 weeks.

Calcitriol administration was associated with a significant increase in plasma Ca (6.14 ± 0.14 vs 4.7 ± 0.11 mEq/l in control after 1 week, $P < 0.001$, and 6.95 ± 0.11 vs 4.92 ± 0.08 mEq/l in control after 3 weeks, $P < 0.001$). There were no significant changes in plasma phosphate or creatinine. PTH was 178 mg/ml in the untreated animals with chronic renal failure and decreased to 125 ± 6 pg/ml ($P < 0.05$) after calcitriol administration.

**Discussion**

For interpretation of bone histomorphometric data of rats with reduced renal mass, several points have to be kept in mind: (1) bone histology—in contrast to humans, rats exhibit a different bone structure; (2) the principal biological mechanisms responsible for bone gains and losses during normal growth and adult life, however, are the same in humans and in rats [23]. Furthermore, secondary osteons, as in humans, can be found in rat bones after skeletal injuries and metabolic changes [24].

Is the rat remnant kidney model suitable to elucidate the mechanism of human bone disease of renal origin? The classic and the most common histological form of renal osteodystrophy in patients with end-stage renal disease is osteitis fibrosa in correlation with increased PTH [25,26]. It is present mainly in severe renal failure, but can be found also in moderate renal insufficiency [26,27]. It is characterized by rapid bone turnover, therefore featuring both increased number and activity of osteoclasts and increased bone formation, as reflected by increased amounts of osteoid, osteoblastic activity and non-lamellar bone. A hallmark of osteitis fibrosa is marrow fibrosis with fibrous tissue occupying the peritabecular spaces [25–27].

Two additional distinct forms of bone disease in patients with chronic renal diseases are osteomalacia and adynamic or aplastic bone. Osteomalacia is characterized by low rates of bone turnover and mineralization defect with accumulation of unmineralized osteoid. The most common cause of osteomalacia is aluminium intoxication, causing defective mineralization, increasing matrix synthesis and long-term inhibition of osteoblast differentiation [28].

The pathogenesis of adynamic bone disease is still poorly understood. It is characterized by very low bone turnover but with no obvious abnormalities in the static parameters of bone morphometry [25,26,29]. This form of bone pathology has been variously attributed to suppression of parathyroid function by high
Fig. 4. (A) Tibia of a rat with normal renal function (age 16 weeks). (B) Tibia of a rat with normal renal function (age 20 weeks). There is a marked increase in osteoid and active osteoblast surfaces (see arrow). (C) Tibia of a rat with chronic renal failure (age 16 weeks). Note the marked increase in the eroded surfaces and in osteoclast numbers (see arrow). (D) Tibia of a rat with chronic renal failure treated with calcitriol for 1 week. Note the decreased eroded surface and the moderate increase in surfaces covered by osteoid and active osteoblasts (arrow). (E) Tibia of a rat with chronic renal insufficiency treated with calcitriol for 3 weeks. There is an additional increase in the osteoid and in active osteoblast surface (arrow) as compared with (D).

calcium concentration in the dialysate and/or by excessive concentrations of circulating calcitriol such as those that follow intermittent high dose intravenous administration of this derivative [30–32].

In rats with chronic renal failure, the most striking histological feature was enhanced active bone resorption. Other remarkable findings include: (1) reduced osteoid surface and volume and (2) reduced bone
formation rate. Since at the time when 5/6 nephrectomy was performed, the rats were still in the process of growing, we compared the animals with chronic renal insufficiency with age-matched normal controls. Thus, taking into consideration the effects of growing on normal histology, the increased osteoclastic bone resorption and the suppressed bone formation seen at short term were still seen at long-term follow-up. These observations suggest abnormal bone remodelling in rats with chronic renal failure with dissociation between forming and resorbing parameters. In this regard, the renal osteodystrophy in our animal model resembles the picture seen in patients with chronic renal insufficiency by the presence of enhanced resorbing activity, but it differs from the human type of secondary hyperparathyroidism because of the suppressed bone formation. The reason for the uncoupling of bone resorbing and forming parameters in our chronic renal failure model is not apparent.

The putative mechanisms by which bone resorption and formation are coupled include mechanical signals generated by bone weakened by resorption, the action of growth factors released by bone matrix during resorption, and cellular interaction between osteoblasts and osteoclasts, also mediated by lymphokine growth factors or matrix components [4,33].

The uncoupling between bone resorptive and formation in our model could be indicative of impaired osteoblast function reflected as both diminished matrix formation and decreased mineralization of matrix. The pathogenesis of osteoblastic failure remains unknown. However, uraemic toxins and systematically or locally produced cytokines could interfere with osteoblast function [4].

Recent observations by Chow et al. [34] shed new light on the sequence of events in bone remodelling in rat. Opposite to the adult human, in which the coupling between formation and resorption involves a specific sequence of events in which bone resorption is followed at the same site by bone formation, in rats 16 weeks to 5 months bone formation does not necessarily occur at a previously resorbing site. The above animals were in the process of growing. By contrast, in rats 2 years old, in which bone had ceased to grow in length, there was a greater site specificity of coupling between resorption and formation, as in adult humans.

Thus, the apparent decrease in bone formation seen in the rats with chronic renal failure aged 16 weeks may reflect not necessarily an osteoblastic failure, but a lack of site-specific coupling of resorption formation. It is worthwhile mentioning that BV/TV (total bone volume) in rats aged 16 weeks with chronic renal failure was significantly increased compared to that of the control group (25.95 ± 0.85 and 14.38 ± 1.9, P < 0.001, data not shown in tables), while in rats aged 20 weeks BV/TV was similar in animals with renal insufficiency and with normal kidney function (16.56 ± 4.5 and 16.9 ± 2.9%, NS). These values, which hardly match other histomorphometric data, especially BFR, might be interpreted as an indication of the uneven distribution between areas of increased bone resorption and bone formation in growing rats. Chow et al. suggest that in growing rats mechanical signals rather than local release of cytokines couple bone resorption and formation.

This hypothesis, however, cannot provide the sole explanation for the consistent finding of decreased BFR, at both 16 and 20 weeks, in the rats with chronic renal failure, and mechanisms of osteoblastic failure have to be taken into consideration.

Calcitriol challenge to the rats with renal insufficiency brought a total suppression of all resorbing parameters to less than those observed in untreated animals. The administration of calcitriol to animals with renal failure was associated with a remarkable increase in all osteoid parameters (OV/BV, OS/BS, Ob.S/BS). There was no change in the BFR, which remained low. It is noteworthy that in the long-term chronic renal failure rats treated with calcitriol, 85% of bone perimeter was covered with osteoid, of which 60% was osteoblastic osteoid. Thus, the administration of calcitriol transformed the mainly resorptive bone lesion with reduced matrix that characterizes the chronic renal failure in rats into a pure form of osteomalacia. It appears that in this animal model, treatment with calcitriol was associated with an improved osteoblastic function only with regard to organic bone matrix formation but without improvement in matrix mineralization. In this respect it is noteworthy that in calcitriol-treated rats, the mineralization lag time was 2-fold greater than in untreated intact animals (data not shown), underscoring the defect in bone mineralization in the calcitriol-treated group leading to osteomalacia.

In certain aspects our findings in calcitriol-treated animals with long-term chronic renal failure are reminiscent of the reported observations in patients with secondary hyperparathyroidism treated with high doses of calcitriol. In these patients calcitriol administration was associated not only with decreased bone resorption, but also with suppressed bone formation, leading in certain cases to adynamic bone disease [32,35,36]. In summary, our animal model of chronic renal disease is similar to the clinical equivalence of hyperparathyroid bone disease with regard to the significant enhancement of bone resorption. In contrast with the human disease, where high turnover and increased bone formation rate are usual features, this animal model features a marked decrease in bone formation. It appears that the uraemic state modified the bone response to PTH. While the osteoclast-stimulating effect of PTH remains intact, the bone-forming effect is suppressed. The peculiar absence of marrow fibrosis in the uraemic rats is also noteworthy.

There is a remarkable similarity between the response of the rats with renal failure to pharmacological doses of calcitriol and that of patients with severe secondary hyperparathyroidism receiving large doses, especially intravenous pulses of the metabolite. In both categories the striking feature was suppressed bone formation rate, suggestive of adynamic bone disease. These observations may provide a warning against
overzealous use of high doses of active vitamin D metabolites in clinical settings. Thus, keeping in mind the species differences, the bone changes developing in the remnant kidney model can be useful in understanding certain aspects of human renal osteodystrophy.

References