Kidney International, Vol. 63, Supplement 85 (2003), pp. S125–S128

Bone remodeling after renal transplantation

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Bone remodeling after renal transplantation. Several studies have indicated that bone alterations after transplantation are heterogeneous. Short-term studies after transplantation have shown that many patients exhibit a pattern consistent with adynamic bone disease. In contrast, patients with long-term renal transplantation show a more heterogeneous picture. Thus, while adynamic bone disease has also been described in these patients, most studies show decreased bone formation and prolonged mineralization lag-time faced with persisting bone resorption, and even clear evidence of generalized or focal osteomalacia in many patients. Thus, the main alterations in bone remodeling are a decrease in bone formation and mineralization up against persistent bone resorption, suggesting defective osteoblast function, decreased osteoblastogenesis, or increased osteoblast death rates. Indeed, recent studies from our laboratory have demonstrated that there is an early decrease in osteoblast number and surfaces, as well as in reduced bone formation rate and delayed mineralization after transplantation. These alterations are associated with an early increase in osteoblast apoptosis that correlates with low levels of serum phosphorus. These changes were more frequently observed in patients with low turnover bone disease. In contrast, PTH seemed to preserve osteoblast survival. The mechanisms of hypophosphatemia in these patients appear to be independent of PTH, suggesting that other phosphaturic factors may play a role. However, further studies are needed to determine the nature of a phosphaturic factor and its relationship to the alterations of bone remodeling after transplantation.

It has been well established that a rapid bone mass loss occurs in the first six to 12 months after a successful renal transplantation [1–6], and persists, albeit at a lower rate, for many years. Thus, previous studies from our and other laboratories have shown a low bone mineral density even in patients with long-term renal transplantation (3 to 10 or more years) and normal renal function [4–7]. Several studies indicate that the bone lesions observed in these patients are heterogeneous and suggest that the pathogenic mechanisms underlying these alterations are multifactorial.

The interest in the bone alterations underlying the post-transplant bone loss has increased during the last decade, not only because of the associated increase in morbidity and the augmented risk of fractures, but also because a better understanding of its histopathology and the pathogenic mechanisms involved are fundamental in designing effective strategies for prevention and control.

Key words: post-transplant bone disease, post-transplant osteoblast apoptosis.

ALTERATIONS OF BONE REMODELING

Studies by Julian et al [3] showed that six months after transplantation, patients exhibited a low bone mass, decreased mineral apposition rate, and delayed mineralization consistent with a pattern of adynamic bone disease. It has been argued that in the majority of the patients studied the pre-transplant bone lesions were not severe, consisting of mild secondary hyperparathyroidism, and that the high doses of glucocorticoids required early after transplantation explain the high prevalence of low bone turnover [7].

Studies performed in patients with long-term renal transplantation (more than 3 years) and relatively normal renal function are discrepant. Thus, while Velasquez-Forero et al [6] showed that the main alterations were consistent with adynamic bone disease and increased deposition of iron on the mineralization front, other studies have shown decreased bone formation and prolonged mineralization lag-time in the presence of persisting bone resorption [4–6]. Our studies in patients with normal renal function after a mean of 7.5 years of renal transplantation [5] showed a mixed lesion characterized by an increase in bone resorption in the majority of patients, while bone formation rate was low and mineralization lag-time prolonged. These lesions were more severe in patients having less time after transplantation, but improved with time, finally approaching normal values after 10 years of transplantation. In a similar study in patients with long-term renal transplantation, Cueto-Manzano et al [8] have also shown increased osteoclastic resorption and decreased osteoblastic function with delayed mineralization and bone formation rate, suggesting a mixed bone disease in the majority of patients, whereas adynamic bone disease and secondary hyperparathyroid-

ism were observed in fewer cases. More recently, Monier-Faugere et al [7] showed low bone turnover, decreased bone formation, and prolonged mineralization in the majority of patients, whereas an increase in erosion surface was observed in less than a quarter of the patients. A striking finding of the study was the presence of generalized or focal osteomalacia in many patients [7]. The authors suggested that these findings are likely to be present in the entire population of patients after transplantation.

Although there are discrepancies among the findings described in different studies, it seems evident from the majority of them that the main alterations in bone remodeling after renal transplantation are a decrease in bone formation and mineralization in the face of persistent bone resorption. This produces an imbalance in remodeling favoring resorption [5–8]. Therefore, it is possible that the defective bone formation may be a consequence of either alterations in osteoblast function, decreased osteoblastogenesis, or increased osteoblast death rates.

Based on these observations, we recently examined the possible role of an early increase in osteoblast apoptosis and alterations in osteoblastogenesis, as well as the influence of pre-existing bone disease in the histomorphometric alterations in bone after transplantation [9]. The studies were performed shortly after renal transplantation, a period in which patients receive high doses of glucocorticoids, and in which some of the pre-existing alterations of bone metabolism may still be present. Patients were subjected to bone biopsy the day of transplantation and within 21 to 120 days after transplantation. The main alterations in bone histology were a decrease in osteoid and osteoblast surfaces and adjusted bone formation rate, while the mineralization lag-time was prolonged. Resorption and osteoclast surfaces remained above the normal range [9]. In addition, almost half of the patients showed early osteoblast apoptosis in post-transplant biopsies and lower osteoblast surfaces and number [9]. Osteoblast apoptosis was more frequently observed in patients with adynamic bone disease, osteomalacia, and mixed bone disease than in patients with high bone turnover, suggesting a pathogenic role of the pre-existing bone disease. Since apoptosis is a short-lasting phenomenon, the fact that it was observed in some post-transplant biopsies, but not in pre-transplant biopsies, suggests that there is an increase in the proportion of cells undergoing apoptosis in these patients. Another observation of this study was a change in osteoblast morphology, demonstrating a marked shift toward quiescence, or inactive form from the cuboidal morphology of active osteoblasts, even in the presence of elevated osteoid thickness in many patients, suggesting defective mineralization [9].

PATHOGENESIS OF POST-TRANSPLANT BONE DISEASE

The pathogenesis of post-transplant bone disease is multifactorial. There seems to be no doubt that pre-transplant osteodystrophy plays an important role in the maintenance or development of post-transplant alterations of bone remodeling. Indeed, most transplant patients suffer different forms of pre-existing bone disease that may persist after transplantation. Thus, in patients with nonsuppressible nodular parathyroid hyperplasia, the persistently elevated PTH levels after restoration of the normal renal function may play a primary role in maintaining a high bone turnover. In addition, some patients may develop de novo secondary hyperparathyroidism resulting from progressive functional alterations of the transplanted kidney [1, 10, 11]. However, in many studies the bone histopathologic findings are heterogeneous, ranging from high bone turnover to low bone turnover [4–8] without apparent correlation to post-transplant serum PTH levels [5–8], suggesting that other factors that begin to operate after transplantation play a central role in the development of the bone alterations observed in these patients.

Finally, we found a positive correlation between osteoblast surface and the serum levels of pre- and post-transplant PTH, suggesting an important role of the hormone in preserving osteoblast number and activity after transplantation [9]. Indeed, previous studies by Jilka et al [25] indicate that in mice, PTH increases the lifespan of mature osteoblasts by preventing apoptosis. These findings are also in agreement with the fact that post-transplant apoptosis was rare in patients with pre-transplant secondary hyperparathyroidism

Several studies suggest that post-transplant immunosuppressive therapy constitutes a major factor in the pathogenesis of post-transplant bone disease [8, 12, 13]. The possible role of cyclosporine has been controversial. Studies in animals and humans have shown that cyclosporine causes high bone turnover [12–14]. However, other studies have failed to demonstrate an effect of the drug on mineral and bone metabolism in renal transplant recipients [5, 7, 15]. It should be considered, however, that the role of cyclosporine in transplant patients has been difficult to evaluate because its effects on bone turnover may be masked by glucocorticoids.

In the studies of Julian et al [3] and Monier-Faugere et al [7], glucocorticoids appear to be the sole determinant of bone volume and turnover. Thus, the cumulative and mean prednisone doses correlated negatively with bone turnover, whereas there was no correlation with cyclosporine cumulative dose, and serum PTH [7]. Since neither immunosupressive therapy nor biochemical and hormonal parameters, including PTH, calcitriol, and serum phosphorus correlated with delayed mineralization,

they concluded that post-transplant bone disease is mainly a consequence of glucocorticoid therapy [7].

The mechanisms by which glucocorticoids may affect bone metabolism are multifactorial. These drugs increase osteoclastic resorption and decrease osteoblastic activity [16]. In addition, glucocorticoids may indirectly affect bone metabolism by decreasing intestinal calcium absorption, leading to an increase in PTH secretion. There is evidence suggesting that, under normal conditions, an important number of osteoblasts undergo apoptosis [17]. Furthermore, studies in mice indicate that glucocorticoids promote osteoblast and osteocyte apoptosis and inhibit osteoblastogenesis, resulting in the defective bone formation observed in glucocorticoid-induced osteoporosis [18]. Therefore, it seems possible that continuous use of glucocorticoids represents an important pathogenic factor in the development and maintenance of post-transplant bone disease. Interestingly, we found a negative correlation between cumulative doses of glucocorticoid and post-transplant osteoblast surface early after transplantation [9]. Since this is a period of maximal glucocorticoid dose, it seems possible that glucocorticoids may play a role in the increased osteoblast apoptosis seen in our patients. However, we could not demonstrate a correlation between glucocorticoids and the number of apoptotic osteoblasts. In contrast, patients showing post-transplant osteoblast apoptosis had significantly lower serum phosphorus levels compared with those without evident apoptosis [9]. Furthermore, post-transplant serum phosphorus correlated negatively with the number of apoptotic osteoblasts and positively with the number of active osteoblasts, suggesting a role of phosphorus in the mechanisms leading to post-transplant bone disease. Indeed, hypophosphatemia has been associated with severe alterations in bone turnover that include a decrease in osteoblast activity, leading to rickets and osteomalacia [19, 20].

Hypophosphatemia, a frequent disorder after renal transplantation [19, 21–23], may occur as a consequence of inappropriate phosphaturia due to persistently elevated PTH levels, glucocorticoids, and relatively low levels of $1,25(OH)_2$ vitamin D_3. However, recent studies strongly suggest the presence of a circulating humoral factor (phosphatonin) that induces phosphaturia in chronic renal failure and early transplantation [23, 24]. This factor increases the fractional excretion of phosphate by inactivation of the Na/Pi co-transporter, leading to inhibition of phosphate transport from the cell membrane to the cytosol [24]. The persistence of this factor after transplantation would induce phosphaturia and hypophosphatemia in these patients. Recent evidence from diseases characterized by phosphaturia and hypophosphatemia, such as X-linked hypophosphatemic rickets, autosomal hypophopshatemic rickets, and oncogenic osteomalacia have identified fibroblast growth factor 23 (FGF23) as one of the possible candidates that fulfills the criteria for phosphatonin [25–27]. This factor induces phosphaturia, hypophosphatemia, and osteomalacia in mice. However, further studies are needed to determine the nature of a phosphaturic factor and its relationship with the alterations of bone remodeling after transplantation.

In summary, although the alterations of bone remodeling after transplantation are heterogeneous, most studies reflect a decrease in bone formation in the face of persistently elevated bone resorption. This imbalance in remodeling favoring resorption leads to a progressive loss of bone mass and an increased risk of fracture. The mechanisms involved in these alterations include preexisting conditions, such as the predominant state of bone turnover prior to transplantation. But post-transplant events such as the effects of glucocorticoids and the occurrence of hypophosphatemia seem to be fundamental for the alterations of bone remodeling.

ACKNOWLEDGMENTS

The present study was supported by Grant G97-000808 from the Fondo Nacional de Investigación Científica, Tecnología e Innovación (FONACIT), Venezuela.

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