cells in the paravascular zones when the optimum degree of occupation is achieved in zones far from the vessels.

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Calcium-Binding Properties of Plasma Proteins in Patients with Aseptic Necrosis of Bones after Cadaveric Kidney Transplantation

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Corticosteroid therapy is believed to be responsible for osteopathies developing in the long term after cadaveric kidney allotransplantation and manifested mainly in aseptic necrosis of the caput femoris [4,5]. The mechanisms by which corticosteroids disrupt osseous metabolism and cause focal necrosis in bones are still unknown [6,8]. Impairment of many tissue processes is known to be related to an elevated intracellular calcium concentration [5]. Previously we demonstrated that after cadaveric kidney allotransplantation patients show a high incidence of disorders of plasma protein Ca-binding properties which create a tendency toward hypercalcemia and toward an increase of calcium transport from the blood to tissue cells [2].

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We studied the calcium-binding properties of plasma proteins in aseptic necrosis of the bones after cadaveric kidney allotransplantation. Our task was to reveal the mechanism and quantitative parameters of calcium binding by plasma proteins in patients with aseptic necrosis developing after cadaveric kidney allotransplantation during prednisolone and azathioprine immunosuppression.

MATERIALS AND METHODS

Fifteen patients aged 15 to 48 who had undergone cadaveric kidney allotransplantation followed by two-component immunosuppressive therapy (prednisolone and azathioprine) and 36 healthy controls were examined. Eight of the fifteen patients presented with manifest clinical and x-ray signs of aseptic necrosis of one or both femur heads (group 1) and 7 had no signs of bone system involvement (group 2). The posttransplantation period was

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TABLE 1. Blood Plasma Protein Ca Binding Parameters by Consecutive Joining to Protein Centers in Cadaveric Kidney Recipients (Mean±SEM)

Parameter	Controls	Kidney recipients (n=12)	
		group 1	group 2
	Ca^{2+} = 1.00 mmol/liter		
β_{sp}	1.18±0.26 (8)	0.80±0.27* 5)	0.67±0.15* (6)
CaPr/A	1.47±0.37 (8)	1.64±0.21 (5)	1.66±0.40 (6)
Albumin, mmol/liter	0.62±0.06 (36)	0.53±0.06* (8)	0.52±0.09* (7)
β_{pr}	0.75±0.15 (8)	0.42±0.10* (5)	0.34±0.12* (6)
CaPr, mmol/liter (8)	0.93±0.22 (5)	0.87±0.12 (6)	0.82±0.09
	Ca^{2+} = 1.40 mmol/liter		
β_{sp}	1.05±0.19 (26)	0.47±0.13* (4)	0.80±0.59 (5)
CaPr/A	2.04±0.40 (26)	1.89±0.23 (4)	0.92±0.88*,** (5)
β_{pr}	0.64±0.12 (26)	0.27±0.06* (4)	0.41±0.26 (5)
CaPr, mmol/liter	1.24±0.15 (26)	1.07±0.18 (4)	0.99±0.17* (5)

Note. One asterisk: $p<0.05$ vs. controls; two asterisks: $p<0.05$ between groups 1 and 2; in parentheses: number of cases observed.

80±48.7 months in group 1 and 21.2±14.1 months in group 2 ($p<0.05$). The periods of osteopathy development after allotransplantation in group 1 are unfortunately unknown. Renal transplant function was satisfactory in group 1 and moderately reduced in group 2 (the plasma creatinine concentrations were 0.1±0.03 and 0.17±0.04 mmol/liter, respectively). The total prednisolone dose that had been administered to group 1 patients at the time of investigation was 41.1±30.2 g, while in group 2 it was 13.8±2.3 g ($p<0.05$); the azathioprine doses were 188.7±194.9 and 77.1±38.8 g, respectively. The mean monthly dosed of both drugs calculated as the quotient resulting from dividing the total drug dose by the duration of the postoperative period at the time of investigation were similar in both groups, being approximately 0.524±0.13 and 2.28±1.1 g in group 1 and 0.61±0.26 and 2.86± ±0.91 g in group 2. It should be mentioned, however, that the prednisolone doses administered to groups 1 and 2 during the first few months after the operation were much higher than the calculated mean monthly doses.

Quantitative characteristics of the ability of plasma proteins to bind calcium were obtained for each individual in experiments with *in vitro* induced hypo- and hypercalcemia with ionized calcium concentrations of 0.5 to 1.5 mmol/liter. Based on concentrations of ionized calcium (Ca^{2+}), total protein, albumin, and protein-bound calcium (CaPr) experimentally established in a series of plasma samples, we estimated the concentrations of standardized Ca^{2+} [3], CaPr, and the specific concentration of Ca bound to 1 mmol of albumin (CaPr/A) at pH 7.4 [1]. The mechanism of calcium binding by proteins was established in Langmuir and Scatchard's coordinates [7]. Parameters of calcium binding by plasma proteins for consecutive joining of Ca ions: the association constant (K_a), number of binding centers (n), and the specific buffer capacity of proteins (β_{sp}) and CaPr/A at Ca^{2+} 1.0 and 1.4 mmol/liter were calculated by routine methods [7]; total buffer capacity of proteins (β_{pr}) and CaPr values at Ca^{2+} 1.0 and 1.4 mmol/liter were calculated by multiplying the respective β_{sp} and CaPr/A values by the albumin concentration. The technique of the *in vitro* experiments, methods of chemical analysis, calculation methods, and formulas were described previously [1,2]. The Student test and χ^2 method were used for statistical processing of the results.

RESULTS

The cooperative mechanism of Ca binding by plasma proteins in hypocalcemia typical of 78% of healthy subjects was detected in only 3 of the 15 patients ($p<0.05$), and equally frequently in both groups of patients. The binding parameters in the cooperative mechanism were not analyzed because

very few cases were detected. During consecutive calcium joining to centers not interacting with each other on the protein, a reduction of the number of binding centers (3.51±1.36) and a K_a increase (1.22±0.6) were revealed in group 1 patients in comparison with normal controls (7.98±2.11 and 0.28±0.1, respectively; $p<0.001$), whereas in group 2 these parameters did not differ from the norm (11.3±14.4 and 0.93±0.79, respectively). The β_{sp} and β_{pr} values in hypocalcemia (Ca^{2+}=1.00 mmol/liter) were reduced in both groups, the β_{pr} decrease being caused not only by the β_{sp} reduction, but also by hypoalbuminemia. No changes in CaPr/A and CaPr in hypocalcemia were detected in either of the groups. In hypercalcemia (Ca^{2+}=1.4 mmol/liter) a marked reduction of β_{sp} and β_{pr} was observed only in group 1. In contrast to the case with hypocalcemia, in hypercalcemia CaPr/A and CaPr were reduced in group 2. Hence, the Ca-binding properties of plasma proteins in patients undergoing cadaveric kidney allotransplantation were violated during immunosuppression with prednisolone and azathioprine whatever the duration of the posttransplantation period or the total doses of the drugs. However, there were certain peculiarities of the disorders in patients with aseptic necroses (groups 1 and 2) detected in hypercalcemia, namely, a β_{sp} reduction caused by a reduced n and an increased K_a, and a drastic reduction of β_{pr} as a result of β_{sp} reduction and hypoalbuminemia.

Hence, Ca binding by plasma proteins in patients with aseptic necrosis of the femur heads was characterized by the following features: loss of the cooperative binding mechanism in the majority of patients and a sharp reduction of β_{sp} and β_{pr} in hypercalcemia in the presence of a noncooperative binding mechanism. Such grave disorders of the Ca-binding capacity of plasma proteins may be caused by prolonged corticosteroid therapy. The reduction of the total buffer capacity of proteins (β_{pr}) detected in patients with aseptic necroses inevitably creates a tendency toward hypercalcemia during increased calcium supply to the blood and eventually creates the prerequisites for an increased calcium uptake by the tissues. This mechanism may, along with others, bring about an increase of the calcium concentration in the bone cells, disturb their function, impair the metabolism, and contribute to the development and advancement of a pathological process in the bones. Moreover, an increase of intracellular calcium may occur in the myocytes of arteries and arterioles feeding the bone, which may result in their increased tone and eventually in local bloodstream disorders, which could possibly be the major factor in the origin of aseptic necrosis [4].

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