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Renal cortical calcification in syngeneic intact rats and those receiving an infrarenal thoracic aortic graft: possible etiological roles of endothelin, nitrate and minerals, and different preventive effects of long-term oral treatment with magnesium, citrate and alkali-containing preparations

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Abstract Renal cortical nephrocalcinosis (C-NC) is a rare disorder of uncertain etiology. Using highly inbred (syngeneic) male Lewis rats, we describe the spontaneous occurrence of histologically detectable C-NC in sham operated control rats (Sham; n = 12), its aggravation following grafting of the ascending thoracic aorta from a donor rat to the infrarenal aorta of a recipient (ATx; n = 12), and differences in C-NC inhibition after 12 weeks of oral administration of magnesium (Mg), citrate and alkali. C-NC is characterized by Kossa-positive areas located in cells of the proximal tubule close to blood vessels and also, to a lesser extent, within glomeruli. After ATx there was vascular overproduction of endothelin (ET-1) but decreased production of nitrate; in renal cortical tissue there was an excess of calcium over Mg and phosphorus and oxalate over citrate. In plasma there was an increase in calcium and creatinine within the normal range. Calcification of tubular cells was eliminated by a preparation containing potassium, sodium and bases (from citrate degradation and bicarbonate) in addition to Mg. Less effective than the latter was Mg-potassium citrate and least effective, Mg citrate. The former treatment also normalized calcemia and urinary nitrate, but only incompletely suppressed ET-1 and had no significant effect on glomerular calcification or tissue and urinary oxalate. Urinary ET-1 excess appeared directly related to the cortical tissue calcium/Mg ratio, and urinary excretion of Mg, citrate and total protein appeared to be inversely related to the severity of C-NC. It was concluded that (1) the highly inbred rat is prone to precipitation of calcium phosphate in the renal cortex; (2) this type of C-NC occurs in close proximity to and within renal vascular tissue and is associated with an imbalance of vasoconstrictors and vasodilators of endothelial origin; (3) effective inhibition of C-NC can be achieved by an alkalinizing combination of Mg, potassium, sodium and citrate, underscoring its utility in the prophylaxis of pathological calcium phosphate deposition. The significance of these findings for the etiology and treatment of clinical disorders with renal and vascular calcification is uncertain and requires further investigation.

Keywords Syngeneic rat · Aortal graft · Renal-cortical calcification · Renal-cortical minerals · Oxalate · Citrate · Endothelin and nitrate · Prophylaxis by magnesium

Introduction

The deposition of calcium phosphate in soft tissue is frequently seen in the kidney, as nephrocalcinosis (NC), and in blood vessels as a component of atherosclerosis. At present it is not known whether calcification in these organs is etiologically linked. NC has a predilection for the functionally most highly developed medullary region, and affects tubular cells and interstitial tissue [2]. It frequently occurs in association with systemic mineral metabolic abnormalities such as hyperparathyroidism and vitamin D excess but also with hypomagnesemia and dyslipidemia of dietary origin [2, 29, 30]. Vascular calcification is typically located in the subendothelial...
region and the media of arteries, but it is not clear whether calcification occurs in renal arterioles and capillaries. If yes, then it would be of interest to know whether there is a link between NC and renal vascular calcification, and whether both can be ascribed to vascular or to local factors such as altered tissue minerals, or whether both factors are involved. The current literature contains no information on this.

Current research on atherosclerosis is focused on genetic and environmental risk factors. In addition, vascular injury resulting, for example, from hypertension, dyslipidemia or surgery is under discussion as possibly initiating a cascade of events ultimately leading to vascular calcification [27, 39]. In the case of vascular injury caused by surgery, transplantation of the juxta-cardial artery ascending to the infrarenal aorta in the rat is followed by calcification of the graft endothelium and subendothelial media [22]. Working with a highly inbred rat strain, we observed that aortic graft calcification could be inhibited by magnesium (Mg) administered in the drinking water [34]. Further investigations revealed alterations of renal mineral concentrations, mainly in the cortical region, containing glomerular arterioles and post-glomerular capillaries. It was therefore hypothesized that the presence of calcifications in the kidney might be another characteristic of this tissue transplantation model [22, 34].

In the present work we illustrate our findings in intact and graft-bearing rats in more detail, in particular with regard to the renal cortical mineral content, to the accompanying histological changes of the kidney, and to two indicators of vascular endothelial and one of vascular connective tissue metabolic activity. In addition, the anti-calcification effects of several Mg preparations were evaluated. The combined data permit the assumption that in genetically uniform (syngeneic), intact rats, and especially in graft-bearing specimens, renal tubular and glomerular structures are prone to calcify, with simultaneous alteration of tissue minerals and vascular effectors, and that calcifications can be prevented by treatment with alkalinizing Mg preparations.

**Materials and methods**

**Animals**

The investigations described were approved by an ethics committee, as currently required by the German law for the protection of animals. Male rats of the syngeneic Lewis strain (Wiga, Sulzfeld, Germany), weighing 220–240 g, were housed individually in conventional cages under a 12-h light/12-h dark cycle. During a 1-week acclimatization period prior to the study they had free access to tap water. Before surgery food, but not water, was withdrawn for 12 h.

**Surgery**

The animals were anesthetized by ether, then tracheotomized, intubated and treated with analgesics in a standardized manner (air-oxygen mixture, isoflurane). The donor operation, lasting 2–3 min, comprised a laparo-thoracotomy followed by the clamp-

**Experimental groups**

Animals were fed normal rat chow (Altromin, code 1000, Lage, Germany) with a calcium to phosphorus ratio of 1:1 and 0.9% Mg content (for other components of this standard diet [29, 30]) ad lib, and were given deionized water with or without additives to drink (see below). The observation period was 12 weeks ± 2 days. Five groups were used.

**Group I**

Sham operation (Sham), n = 12; these rats served as an intact-aorta control group.

**Group II**

Aortic graft (ATx), n = 12; animals received no further treatment.

**Group III**

ATx, n = 12; the animals received deionized water containing 13 g/l (29 mmol/l) water-soluble tri-magnesium di-citrate (MgC; Boehringer, Ingelheim, Germany), corresponding to 58 mmol citrate, 87 mmol Mg, 174 milliequivalent bases (from metabolic degradation of citrate to bicarbonate).

**Group IV**

ATx, n = 12; the animals received deionized water containing 4.3 g/l MgC and 12.0 g/l (39 mmol) neutral potassium citrate (PC; Fluka, Ulm; Germany), corresponding to 66 mmol citrate, 29 mmol Mg, 117 mmol potassium, 198 milliequivalent bases.

**Group V**

ATx, n = 12; the animals received deionized water containing 4.3 g/l MgC, 9.0 g/l PC, and 2.52 g/l sodium bicarbonate (SB; Fluka, Ulm; Germany), corresponding to 44 mmol citrate, 29 mmol Mg, 85 mmol potassium, 28 mmol sodium, 160 milliequivalent bases.

In the following groups I–V are alternatively named Sham, ATx, MgC, MgPC, MgPCSB, respectively. The intention to treat the latter three groups with magnesium, citrate and alkali was prompted by information obtained from previous work of our own in which an oral supply of Mg and bases proved to be an effective prophylaxis against cortico-medullary NC in rats [29]. This treatment prevented calcium oxalate crystallization in the postprandial urine of humans forming calcium-containing renal stones [31]. For the present experiments it was further assumed that, provided there was similar fluid intake in groups III–V, these rats would receive bases in amounts varying by a maximum of 20 per cent, while the supply of Mg, sodium and potassium would vary by several orders of magnitude. Differences in calcification would therefore be ascribable to differences in the amount of cations supplied, rather than to the amount of bases.

**Procedures and collection of samples**

Weekly arterial blood pressure measurement via the tail cuff method yielded mean values of between 120 and 140 mm Hg, but