Antigen-Specific Induction of Thrombin-Cleaved Form of Osteopontin Contributes to Inhibit Renal Stone Formation

Introduction and Objective: Osteopontin (OPN) is known to play a crucial role in the formation of renal calcium crystals; however, the molecular mechanism by which OPN regulates crystal formation has not yet been elucidated. We have previously shown that the impaired RGD sequence of osteopontin inhibits renal crystal formation by using OPN-transgenic mice and OPN-knockout mice. Renal crystals in mice are sporadically detected in renal tubular cells of the corticomedullary junction where thrombin-cleaved OPN expression is coincidentally localized; however, the expression of full-length OPN is widely distributed in the whole kidney. Here, we investigated the effects of an anti-murine OPN antibody (35B6-Ab) that specifically reacts with the ^{162}SLAYGLR^{168} sequence, which is exposed by thrombin cleavage and is located adjacent to the RGD sequence, on renal crystal formation.
Materials and Methods: Monoclonal anti-OPN antibody 35B6 (IgG1) was obtained by immunizing mice with the synthetic peptide VDVPNGRGDSLAYGLR corresponding to the internal sequence of murine OPN. The ability of 35B6-Ab to alter the adherence of calcium oxalate monohydrate (COM) crystals to MDCK cells in vitro was evaluated. To examine whether intraperitoneal administration of 35B6-Ab (250, 500, and 1,000 μg per mouse) exerts a prophylactic effect on renal crystal formation in mice, renal crystal formation induced by glyoxylate injection was demonstrated by polarized light optical microphotography, scanning electron microscopy (SEM), and transmission electron micrographs (TEM).
Results: In an *in vitro* experiment analyzing radiolabeled oxalate data, 35B6-Ab significantly inhibited the attachment of renal crystals to renal tubular culture cells. 35B6-Ab also inhibited renal crystal formation in a dose-dependent manner in a mouse model. Scanning electron microscopy showed that crystals were cracked and became fine. The density of crystals was low in 35B6-Ab-treated mice, in contrast to the high density of crystals having a radial pattern of growth (rosette petal-like crystals) in untreated mice. Control mice without 35B6-Ab showed collapsed mitochondria in the flattened cytoplasm of renal tubular cells, when compared with the corresponding structures in 35B6-Ab-treated mice, in which renal tubular cell injury was inhibited in a dose-dependent manner.
Conclusion: We concluded that thrombin-cleaved OPN plays an important role in the formation of renal calcium crystals, and that 35B6-Ab significantly suppresses crystal formation. (2159)