Renoprotective effect of alpha-1-acid glycoprotein, an acute-phase protein, against renal fibrosis via anti-inflammatory action and its contribution in the functions of vitamin D and vitamin A

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Chronic kidney disease (CKD) is defined as kidney damage or glomerular filtration rate < 60 mL/min/1.73 m² for 3 months or more, irrespective of cause. The prevalence is 10% of the population worldwide. Renal fibrosis is the main feature of CKD progression and the final common pathway of kidney injury. Fibrosis is characterized by the formation of extracellular matrix, activation of myofibroblasts and multiple inflammatory cells represented by macrophages. Here we focus on alpha-1-acid glycoprotein (AGP), the second abundant serum protein and one of the major acute-phase proteins. Produced mainly in the liver and in some extra-hepatic tissues, AGP is known for anti-inflammatory and immunomodulatory properties, and exogenously administered AGP is reported to be beneficial in acute renal injury. In addition, several studies reported that vitamin D and vitamin A also have renoprotective effects and possess potential to induce AGP production. In this study, we hypothesize that AGP may have an anti-fibrotic and anti-inflammatory effect against renal fibrosis, and attempt to clarify (1) the role of exogenous AGP on renal fibrosis (2) the contribution of endogenous AGP on renal fibrosis (3) the involvement of endogenous AGP in the renoprotective effects of vitamin D or vitamin A, thus elucidating the role of AGP in the anti-fibrotic strategy.

1. AGP protects against mouse model of renal fibrosis similar with vitamin D

Renal fibrosis is associated with unremitting renal inflammation. Although it is reported that 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), the active form of vitamin D, elicits an anti-renal fibrotic effect, its molecular mechanism is unrevealed. In this study, renal fibrosis and inflammation (α-SMA, Col1a2, IL-6 and IL-1β) observed in the kidney of unilateral ureteral obstruction (UUO) mice were reduced by the treatment of 1,25(OH)2D3. The plasma protein level of AGP, a downstream molecule of 1,25(OH)2D3, was increased following 1,25(OH)2D3 injection. Additionally, increased mRNA expression of ORM1, an AGP gene, was observed in HepG2 hepatocytes and THP-1-derived macrophages that treated with 1,25(OH)2D3. To investigate the involvement of AGP, exogenous AGP was administered to UUO mice, resulting in attenuated renal fibrosis and inflammation. We also found the mRNA expression of CD163, a monocyte/macrophage marker with anti-inflammatory potential, was increased in THP-1-derived macrophages under stimulus from 1,25(OH)2D3 or AGP. Moreover, AGP prevented lipopolysaccharide-induced macrophage activation. Thus, AGP could be a key molecule in the protective effect of 1,25(OH)2D3 against renal fibrosis. Taken together, AGP may replace vitamin D to function as an important immune regulator, offering a novel therapeutic strategy for renal inflammation and fibrosis.

2. Generating AGP-knockout mice

To elucidate the role of AGP, we attempt to generate AGP-knockout (AGP-KO) mice. The
process was started from the sperm with a heterozygous C57BL/N-Orm\textsuperscript{2\textit{m}1(KOMP)Vclg} origin, in which ORM1-ORM2-ORM3 genes are very tightly linked, thus all AGP subtypes (ORM1, ORM2 and ORM3) would be knocked out in the mouse line once homozygous. Firstly, we generated the AGP flox heterozygous mice, then crossed these pups to acquire AGP flox homogeneous mice before applying cell-permeable Cre treatment to finally obtain AGP-KO mice. As a result, we successfully obtained the first ORM1-ORM2-ORM3 triple knockout mice.

3. The contribution of endogenous AGP in renal fibrosis and the connection with vitamin D

To clarify the role of endogenous AGP on renal fibrosis model, UUO treatment was subjected to wildtype (WT) mice and AGP-KO mice. AGP-KO showed increased expressions of α-SMA, Col1a2, IL-1β and the macrophage specific antigen, F4/80, in the kidney than the WT mice, suggesting that endogenous AGP plays an anti-fibrotic role during the development of UUO-induced renal fibrosis, and macrophage infiltration could be suppressed by AGP in the pathologic process. In addition, 1,25(OH)\textsubscript{2}D\textsubscript{3} was administered to UUO-treated WT mice and UUO-treated AGP-KO mice. Interestingly, 1,25(OH)\textsubscript{2}D\textsubscript{3} suppressed the mRNA expression of α-SMA, Col1a2 and IL-1β in the UUO-treated kidneys both in WT mice and AGP-KO mice to the same degree. This result suggested that 1,25(OH)\textsubscript{2}D\textsubscript{3} may play its anti-fibrotic and anti-inflammatory role in an AGP-independent way.

4. Am80, a retinoic acid receptor agonist, protects against renal fibrosis via AGP induction

Retinoids, derivatives of vitamin A, are involved in embryonic development and cell differentiation. Clinically used for acute promyelocytic leukemia, retinoids are attracting attention for renoprotective effects. All-trans retinoic acid (atRA), a natural retinoic acid that binds to retinoic acid receptors (RAR-α, RAR-β and RAR-γ) and Am80, a synthetic retinoid that binds to RAR-α and RAR-β, but not RAR-γ, are used in our study. atRA or Am80 were administered to the UUO-treated mice. Am80 showed equivalent level of anti-fibrotic and anti-inflammatory effects with atRA against UUO-induced renal fibrosis. The adverse effect of body weight loss in Am80-treated mice was significantly suppressed compared with those treated with atRA. In the same experimental condition, indicating that Am80 possesses stronger AGP-inducing activities compared with atRA. Furthermore, the anti-fibrotic effect of Am80 demonstrated in WT UUO-treated mice was not observed in AGP-KO UUO-treated mice, while atRA-treated AGP-KO mice still showed certain renoprotective effect. Collectively, these results suggested that Am80 protects against UUO-induced renal fibrosis model via the function of AGP.

To conclude, we have demonstrated that endogenous AGP has renoprotective effects against renal fibrosis. In addition, exogenously administered AGP and its inducers, 1,25(OH)\textsubscript{2}D\textsubscript{3}, atRA and Am80, protected against UUO-induced renal fibrosis. According to our findings, the AGP-dependency of the anti-fibrotic and anti-inflammatory effects could be described as Am80>atRA>1,25(OH)\textsubscript{2}D\textsubscript{3}. This research provides knowledge on the impact of AGP on renal fibrosis model, and highlight the possibility of AGP as a novel anti-fibrotic therapeutic.