Renal accumulation of macrophages in experimental polycystic kidney disease is reduced by bindarit

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Studies from the past two decades both in human and experimental polycystic kidney diseases (PKD) have raised interest in renal interstitial inflammation as a possible determinant of cyst growth and renal impairment. PKD are a group of cystic genetic disorders characterized by progressive growth of multiple fluid-filled cysts that originate from the tubule wall, damage the surrounding renal parenchyma, and eventually lead to end stage renal disease \[1, 2\]. Autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) are caused by mutations in the \(Pkd1/Pkd2\) and \(Pkhd1\) genes, respectively, which encode the proteins polycystin 1/2 and fibrocystin. Mutation or absence of these proteins may lead to abnormal cell signaling, promoting the proliferation of tubular epithelial cells and fluid secretion, altered interactions between mutated epithelial cells and extracellular matrix, and alternatively activated interstitial macrophages that participate in cyst formation and expansion \[3\]. Early clinical studies showed that CD68-positive macrophages accumulated in the renal interstitium of ADPKD patients with either early or advanced renal failure \[4\]. Recently, large numbers of macrophages expressing the M2 marker CD163 were detected in kidneys of patients with both ADPKD and ARPKD \[5\]. These macrophages were found in interstitial areas closely apposed to cysts or occasionally in the cystic epithelium \[5\]. Accumulation of macrophages in the renal interstitium of different rodent models of PKD has been reported [reviewed by \[6\]], and monocyte chemoattractant protein-1 (MCP-1)/CCL2 was suggested as a key driver of inflammatory cell recruitment. In the ADPKD model of Han:SPRD rats, ED-1 positive macrophages were present in the renal interstitium, accompanied by the overexpression of MCP-1 in the cystic epithelium \[7\]. Increased levels of MCP-1 were excreted in the urine of PKD patients which were strictly connected with renal function worsening \[8\]. Moreover, high MCP-1 levels were measured directly in cyst fluids removed from ADPKD kidneys \[8\]. There is also in vitro evidence that \(Pkd1^{-/-}\) tubular...
cells expressed significantly higher levels of MCP-1 mRNA than Pkd1/fl/fl cells [9].

Bindarit is an indazolic derivative with proven anti-inflammatory activity that selectively inhibits the production of the monocyte chemotactic protein subfamily of CC inflammatory chemokines (MCP-1/CCL2, MCP-3/CCL7, MCP-2/CCL8) [10]. Bindarit acts by down-regulating the NF-kB pathway, by reducing the phosphorylation of IkBα and p65 and the activation of NFκB dimers and subsequent decreased nuclear translocation and DNA binding [11]. Bindarit is devoid of any immunosuppressive activity and of effects on arachidonic acid metabolism [10]. This molecule exerted potent anti-inflammatory effects in several experimental diseases, such as arthritis [12], pancreatitis [13], coronary in-stent stenosis [14]. In mice with lupus nephritis bindarit reduced MCP-1 upregulation, limited the infiltration of macrophages in the kidney, retarded the development of proteinuria and renal damage, and prolonged animal survival [15, 16]. Moreover, there is clinical evidence showing that bindarit also decreased urinary MCP-1 levels and albuminuria in patients with acute proliferative lupus nephritis [17].

We elected to study the effect of bindarit in PCK rats, a PKD model characterized by spontaneous development of renal and hepatic cysts. It has many features that mimic human ADPKD, although the pattern of inheritance is autosomal recessive [18]. We first evaluated whether PCK rats were representative of the enhanced pathway of MCP-1 and macrophage infiltrates in PKD [19]. We showed that MCP-1 mRNA was upregulated in the kidneys of PCK rats at 5 weeks of age, the time when bindarit treatment started, and further increased at 15 weeks in comparison with age-matched control Sprague-Dawley rats. In parallel, MCP-1 protein expression was enhanced in epithelial cells of dilated tubules and cysts, interstitial inflammatory cells, and also at glomerular level in few podocytes. Treatment with bindarit reduced MCP-1 upregulation and its chemotactic effects limiting to a significant extent the number of ED-1 positive monocytes/macrophages that infiltrated the renal interstitium and the glomeruli of PCK rats. These data emphasize the anti-inflammatory effect of bindarit in renal disease and uphold the pivotal role of MCP-1 in the recruitment of inflammatory cells in PKD. However, cyst growth of PCK rats was not affected by bindarit. This could possibly be ascribed to the incomplete depletion of interstitial macrophage accumulation after therapy, so that residual macrophages could still contribute to the cyst proliferation.

It has been recognized that in kidney disease MCP-1 acts deleteriously in ways that may accrue beyond its capability to attract macrophages and foster inflammation [20-22]. In PCK rats, the anti-MCP-1 therapy, besides having a remarkable anti-inflammatory effect, ameliorated renal function and significantly reduced proteinuria [19]. Because of the pivotal role of podocyte injury in the development of proteinuria and progressive kidney disease [23, 24], we examined the impact of bindarit treatment on podocyte structure in PCK rats. The anti-MCP-1 therapy ameliorated podocyte damage in that it restored the defective expression of nephrin, a key component of the slit diaphragm acting to maintain slit pore integrity and renal filtration, reduced podocyte foot process effacement, and increased slit pore frequency in comparison with untreated-PCK rats [19]. Then we performed in vitro experiments exposing cultured murine podocytes to a high concentration of albumin to determine whether the amelioration of podocyte structure and the proteinuria-lowering effects of bindarit were due to inhibition of the podocyte release of MCP-1/CCL2 and its deleterious effects on podocytes. Previous studies on podocytes in culture indeed reported that MCP-1/CCL2, upon binding to the cognate receptor CCR2, caused podocyte motility, actin cytoskeleton rearrangement, and increased permeability to albumin [20, 21]. MCP-1 also reduced nephrin expression in podocytes via a CCR2-Rho-kinase-dependent mechanism [22]. We found that bindarit, by reducing the excessive MCP-1 production by podocytes in response to albumin load, limited podocyte dysfunction, almost preventing reorganization of F-actin fibers and inhibiting podocyte motility [19]. Translating these findings in cultured podocytes to the in vivo condition, it is conceivable that anti-MCP-1 therapy with bindarit may affect cell-cell interaction and cell matrix adhesion to the GBM, counteracting the podocyte detachment and the disruption of the glomerular filtration barrier [19]. Thus in PKD, bindarit, through its anti-inflammatory and antiproteinuric activities, could represent a complementary intervention to therapies explicitly aimed at blocking renal cyst growth.

References

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