Macromolecular Dexamethasone Prodrug Prevents And Resolves Lupus Nephritis With Reduced Systemic Toxicities

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ABSTRACT SUMMARY
Macromolecular dexamethasone prodrug (P-Dex) was found to be able to prevent and resolve lupus nephritis much more effectively than dose equivalent free Dexamethasone (Dex) in a lupus-prone (NZB×NZW)F1 mouse model, with significantly reduced systemic toxicities. The comprehensive mechanistic analyses of the prodrug’s action suggests that the outstanding performance of P-Dex may be ascribed to the Extravasation through Leaky Vasculature and subsequent Inflammatory cell-mediated Sequestration (ELVIS) [1, 2] mechanism, leading to its passive targeting and retention in the inflammatory kidneys.

INTRODUCTION
Systemic lupus erythematosus (SLE) is an autoimmune disease, in which autoantibodies are produced against nuclear antigens, including double stranded DNA (dsDNA). In ~50% of lupus patients, renal deposition of anti-dsDNA IgG containing immune complexes leads to nephritis, a major cause of morbidity and mortality in lupus patients. Renal immune complex deposits induce renal inflammation and immune cell infiltration. If unresolved, this inflammation leads to renal injury, dysfunction, and failure. Nephritis is treated with GCs, which are suboptimal because they frequently cause off-target toxicity. Because lupus patients often take GCs continuously for many years, they are at high risk for developing the adverse side effects associated with GC treatment, including osteoporosis and immunosuppression.

Macromolecular prodrug approach showed a great potential in targeting therapeutic agents to inflammation. In the present work, we synthesized P-Dex (Mw: 34 kDa, PDI: 1.3), and investigated its prophylactic and therapeutic effects in (NZB×NZW)F1 mice at different stages of nephritis. An efficient and long-lasting preventative and therapeutic efficacy by monthly administration of P-Dex was observed. In addition, the GCs associated secondary osteoporosis side effect following a 12-wk preclinical study was not found in the P-Dex treated mice. The accumulated evidences in the mechanism studies indicated that these observations were most likely attributed to the enhanced kidney cells (e.g. proximal tubular epithelial cells) uptake and retention of P-Dex followed by suppression of pro-inflammatory pathways in the kidney.

EXPERIMENTAL METHODS
Female (NZB×NZW)F1 mice (16-wk-old in prophylaxis study, and 22-wk-old mice with developed nephritis in therapy study) were treated with Dex phosphate (1.32 mg/kg, daily i.p. injections) and P-Dex (250 mg/kg, monthly i.v. injections) with saline as controls. The overall doses of P-Dex and Dex phosphate are equivalent in terms of Dex content. Albuminuria was measured weekly using Albustix. Mean arterial pressure (MAP) was measured by the tail cuff method every 4 wks. Serum was isolated from blood collected from the saphenous vein every 4 wks. Levels of anti double-stranded DNA (anti-dsDNA) antibodies were measured using ELISA. Mice were sacrificed 1-2 wks after cessation of treatment. Kidneys were harvested for histological assessment. The femurs were isolated for bone mineral density (BMD) analysis and bone histomorphometry analysis, respectively. The renal targeting and retention of Alexa 488- and IRDye 800 CW-labeled P-Dex (P-Dex-Alexa and P-Dex-IRDye) were assessed by confocal microscopy, flow cytometry and near infrared
RESULTS AND DISCUSSION

In the 8-wk prophylaxis study, monthly P-Dex treatment successfully prevented lupus nephritis at the endpoint as evidenced by 0% incidence of albuminuria, which is significantly lower than the daily Dex phosphate treatment (47%) and saline group (100%). To determine if P-Dex can ameliorate established nephritis, the 12-wk therapy study was performed in (NZB×NZW)F1 mice with sustained albuminuria. Over the entire experimental time course, albuminuria not only persisted in 100% of the mice in the saline treated group, but also increased in severity in most of these mice (93%). In the Dex phosphate group, albuminuria likewise continued in 100% of the mice, and 23% of these mice showed intensified albuminuria, indicating that Dex treatment could only partially halt the aggravation of renal dysfunction. By contrast, albuminuria resolved in 78% of the mice treated with P-Dex, and none of the remaining 22% of mice showed intensified albuminuria, suggesting P-Dex to be a more effective treatment than dose equivalent Dex in resolving established albuminuria associated with lupus nephritis.

As mentioned before, osteoporosis is a major adverse side effect of long-term use of GCs. As expected, by the end of the 12-wk treatment study, the mean bone mineral density (BMD) and trabecular bone volume/tissue volume (BV/TV) in the femurs of Dex treated mice were significantly lower than that observed in the saline group (P < 0.05). Interestingly, in the P-Dex group, mean femoral BMD, BV/TV and trabecular number are significantly better than that of the Dex group (P < 0.005), and did not differ significantly from the mean values in the saline group. Thus, unlike Dex, P-Dex did not negatively affect BMD or microarchitecture of the bone.

To elucidate the mechanism underlying P-Dex’ outstanding performance, optical imaging was performed to evaluate the in vivo distribution of P-Dex. P-Dex-IRDye preferentially accumulated (48 hrs) and was retained (7 days) in inflammatory kidneys of (NZB×NZW)F1 mice, but not in healthy kidneys of NZW controls. Flow cytometry revealed that ~61% of kidney cells from (NZB×NZW)F1 mice were P-Dex-Alexa⁺ whereas less than 20% of kidney cells from NZW mice were P-Dex-Alexa⁺. Those P-Dex-Alexa⁺ cells in the kidney of (NZB×NZW)F1 mice were most abundant in proximal tubules of the renal cortex, more specifically, proximal tubule epithelial cells, which are also an important contributor to the inflammation development and progression in lupus nephritis by secreting chemokines and inflammatory cytokines in response to albumin and immune complexes.

Interestingly, P-Dex did not attenuate nephritis by reducing serum anti-dsDNA IgG or glomerular immune complex deposition, but rather by suppressing the downstream pro-inflammatory pathways (i.e. inhibiting tubulointerstitial TLR9 expression as well as the recruitment of FcR-bearing macrophages to the tubulointerstitium) in response to deposition of dsDNA-containing immune complex.

CONCLUSION

P-Dex could effectively prevent and treat lupus nephritis with reduced systemic side effects. This outstanding performance may be explained by its passive targeting and retention in the inflammatory kidneys via ELVIS mechanism.

REFERENCES


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