Triptolide Reduces Cyst Formation in Pkd1 Murine Models of ADPKD

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Background: Triptolide is a natural product derived from the medicinal herb, Lei Gong Teng, previously shown to have anti-cancer and anti-inflammatory therapeutic effects. In studies examining triptolide’s mechanism of action we discovered an agonistic effect on polycystin-2 mediated calcium release. Additionally, in vitro experiments in murine kidney epithelial Pkd1+/− cells showed that triptolide treatment led to growth arrest and up-regulation of p21. We therefore began a series of experiments in Pkd1+/− mouse models to determine if triptolide could reduce cyst progression by restoration of polycystin-2 calcium signaling. Methodology: The two models of ADPKD that were used are as follows: 1). An in utero Pkd1 null mouse model, where pregnant mice were injected i.p. with either triptolide or DMSO vehicle control from embryonic day E10.5 until birth. 2). A postnatal mouse model where Pkd1 deletion was restricted to the kidney. Neonates were treated with triptolide or DMSO via i.p. injection and/or lactation (maternal i.p. injection) over the course of twelve days. Following completion of all experiments, body and kidney weights were collected and kidneys were subject to histological examination. Using image analysis software (Image J, NIH), kidney cysts were counted, the total and cystic areas were measured and the cystic burden calculated. Results: Pkd1−/− animals do not survive past birth, however we were able to collect and examine kidneys for cyst formation. Triptolide was maternally well tolerated up to 0.25 mg/kg/day, however, we found 0.07 mg/kg/day triptolide to be the maximum tolerated dose to safely maintain the pregnancy. This concentration did not adversely affect the kidneys or developmental progression of wild-type or heterozygous mice as assessed upon birth. Cystic Pkd1−/− animals treated with DMSO had numerous fluid filled cysts and the average cystic burden was 34%. Cystic animals treated with triptolide showed a significant reduction in cystic burden with an average of 15%. Additionally, triptolide treated animals had fewer cysts (53±11) compared to DMSO control (146±28), although the average cyst area was unchanged. We next determined whether this result could be reproduced in a slightly older animal model where Pkd1 deletion was limited to the kidney and triptolide administration could be controlled on an individual basis. We assessed cyst progression in this aggressive disease model over multiple time courses beginning with a lactation model from day P1 to P4. Cystic animals receiving DMSO had numerous fluid-filled cysts and an average cystic burden of 17%. In contrast, triptolide treatment led to a significant reduction in the number of cysts and cystic burden at this early time point. While the overall cyst number was reduced by two-thirds, we specifically observed the most significant reduction in the number of the smallest cysts. We next explored longer time courses and direct i.p. injections of triptolide to neonates through day P12. At day P8, triptolide treated animals maintained fewer cysts and had better renal function than DMSO control treated animals, however the disease progressed. By day P12, again the number of cysts remained lower than DMSO control but cyst progression and fluid secretion had deteriorated renal function in both experimental groups. Conclusion: We have completed studies in two different Pkd1 animal models of ADPKD to assess if the antiproliferative small molecule triptolide can reduce cystogenesis. In both in utero and neonatal models, triptolide effectively reduced cyst progression as compared to vehicle control. While the overall percentage of cystic burden was reduced, it was striking that the number of cysts formed was significantly lower with triptolide administration. Results obtained from these murine models are encouraging and future studies will continue to examine triptolide as a novel cystogenesis inhibitor for the treatment of ADPKD.