Vitamin K3 Inhibits Hepatic Cystogenesis *In Vitro* and *In Vivo*: A New Therapeutic Approach for Treatment of Polycystic Liver Diseases

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Background

- In Polycystic Liver Diseases (PCLDs), hyper-proliferation of cholangiocytes plays a predominant role in hepatic cystogenesis.
- We have shown that in the PCK rat (an animal model of one of the PCLDs, ARPKD) growth of liver cysts is associated with increased cholangiocyte proliferation. *(Gastroenterology, 2006)*
- Accelerated cell proliferation in many cell types is related to alterations in cell cycle.
- The cell division cycle 25A (Cdc25A) protein is an important cell cycle regulator and is involved in the G1/S and G2/M transitions.
- CDC25A is upregulated in a number of cancers and is currently considered as a potential therapeutic target.
- We recently showed that Cdc25A is over-expressed in cystic cholangiocytes of the PCK rat (Figure 1) and in patients with cystic liver diseases. (Figure 2, *JCI, 2008)*
- Several potent inhibitors of Cdc25 phosphatases and, in particular, the synthetic congener of natural Vitamin K, Vitamin K3 (menadione) have been identified and successfully used to suppress hyper-proliferation in different cell types. Vitamin K3 directly binds to and inhibits Cdc25A activity.

Hypothesis

- Cdc25A suppression decreases cholangiocyte proliferation and inhibit hepatic cyst growth.

Aims

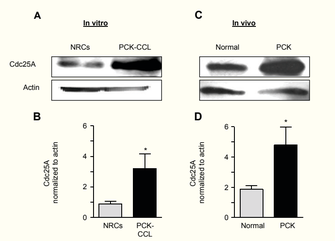
- To assess the effect of Vitamin K3 (VK3) on cyst growth *in vitro.*
- To evaluate the effect of VK3 on hepatic and renal disease progression *in vivo* in the PCK rat.
- To examine the effect of VK3 on the expression of down-stream targets of Cdc25A.

Experimental Models and Approaches

- *In vitro*, changes in areas of PCK cystic bile ducts grown in 3-D culture for 5 days were assessed in the presence/absence of different doses (50, 100 and 200 μM) of VK3.
- *In vivo*, PCK rats received VK3 (0.15 g dissolved in 1 L of water every other day) in drinking water for 8 weeks; control rats received only water. The following parameters were analyzed: liver and kidney weights, renal and hepatic cyst volumes and fibrosis, serum biochemistry.
- Effect of VK3 treatment on expression of the cell cycle proteins (i.e., Cdc25A, Cdk2, 4 and 6, cyclins E and D) and their down-stream targets (Rb and Fox01) was examined by western blot.
- Rate of cholangiocyte proliferation was determined by PCNA expression and confocal microscopy.

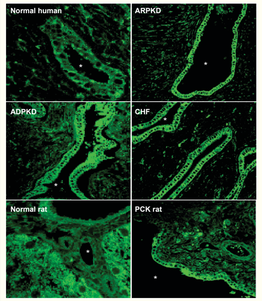
Results

Figure 1



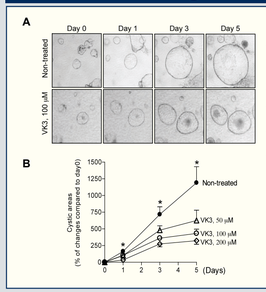
Cdc25A expression is increased in PCK cystic cholangiocytes. By western blot, levels of Cdc25A protein expression were significantly higher in cultured PCK cholangiocytes (PCK-CCL) and cholangiocytes freshly isolated from the PCK rats compared to cultured normal rat cholangiocytes (NRCs) and freshly isolated cholangiocytes of normal rats, respectively. (n=3), *P<0.05.

Figure 2



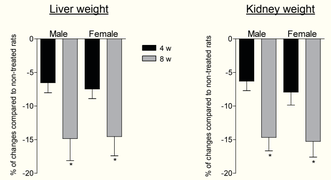
Cdc25A is overexpressed in cystic cholangiocytes of the PCK rat and in patients with cystic liver diseases. Levels of Cdc25A were increased in cholangiocytes lining rat and human liver cysts compared to normal cholangiocytes. Original magnifications: x100 (normal human and normal rat) and x63 (ADPKD, ARPKD, CHF, PCK rat). Asterisk indicates bile duct or cyst lumen. Data are representative of 3 independent experiments.

Figure 3



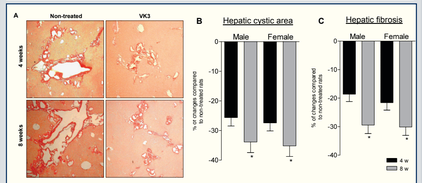
VK3 treatment halts cyst expansion *in vitro*. The effect of VK3 on cyst growth was studied in 3-D culture. In the absence of VK3, cystic structures derived from PCK bile ducts progressively enlarged exhibiting a 5-fold increase in size by day 5 as was assessed by light microscopy (A) and quantitation analysis (B). Addition of VK3 to culture media inhibited cyst growth in dose-dependent manner.

Figure 4



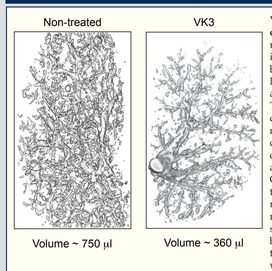
***In vivo*, in the PCK rats, liver and kidney weights are decreased after VK3 treatment.** In male and female PCK rats, VK3 treatment significantly reduced liver and kidney weights in time-dependent manner – longer treatment resulted in more significant reduction. *P<0.05 compared to 4 weeks of treatment.

Figure 5



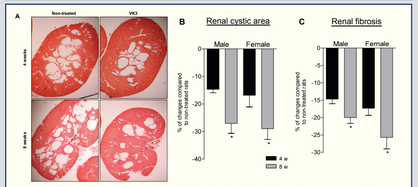
VK3 inhibits hepatic cystogenesis and fibrosis *in vivo* in a time-dependent manner. (A) Representative liver sections of non-treated or VK3-treated PCK rats. Magnification: x4. (B and C) In male and female PCK rats, VK3 significantly inhibited hepatic disease progression as reflected by a reduction in cyst volume (B) up to 35% and fibrosis (C) up to 31%. VK3 had a time-dependent effect – longer treatment resulted in more significant reduction of hepatic cyst volume and fibrosis. *P<0.05 compared to 4 weeks of treatment.

Figure 6



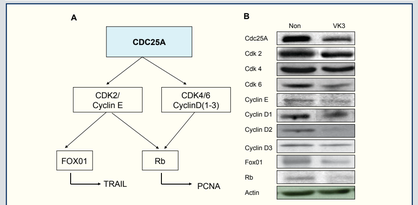
VK3 inhibits hepatic cystogenesis. The representative reconstructed images of the intrahepatic biliary tree of the whole left lateral lobe of non-treated and VK3-treated PCK rat. To reconstruct in three-dimension the biliary trees of normal and PCK rats under conditions that preserve the original morphology, we used a technique involving micro-CT scanning. The biliary tree in non-treated PCK rats undergoes significant multiple segmental and saccular dilatations and focal buddings. In contrast, in VK3-treated PCK rat biliary tree was affected less.

Figure 7



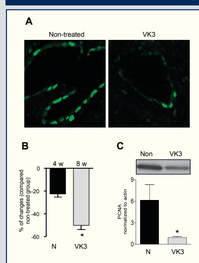
VK3 inhibits renal cystogenesis and fibrosis in a time-dependent manner. (A) Representative kidney sections of non-treated or VK3-treated PCK rats. Magnification: x4. (B and C) In male and female PCK rats, VK3 significantly inhibited renal disease progression as reflected by a reduction in cyst volume (B) up to 29% and fibrosis (C) up to 25%. VK3 had a time-dependent effect – longer treatment resulted in more significant reduction of renal cyst volume and fibrosis. *P<0.05 compared to 4 weeks of treatment.

Figure 8



VK3 treatment inhibits expression of Cdc25A down-stream targets. (A) Scheme of Cdc25A pathway. (B) Representative western blots using cholangiocytes isolated from non-treated and VK3-treated PCK rats indicate that expression of down-stream targets of Cdc25A was decreased in response to VK3.

Figure 9



Proliferation of cystic cholangiocytes is decreased in response to VK3 treatment. (A) Representative images show the expression of PCNA (green) in livers of non-treated or VK3-treated PCK rats. (B) Quantitative assessment of PCNA-positive nuclei demonstrates that the mitotic indices were significantly decreased in response to VK3. Longer treatment results in more significant reduction of the PCNA-positive nuclei. (C) Levels of PCNA were decreased in cholangiocytes treated with VK3. *P<0.05.

Summary

- *In vitro*, VK3 suppresses hepatic cyst growth;
- *In vivo*, VK3 treatment:
  - decreases liver and kidney weights,
  - suppresses hepatic and renal cyst volume,
  - inhibits hepatic and renal fibrosis,
  - decreases expression of Cdc25A and its down-stream targets,
  - inhibits cholangiocyte proliferation.

Conclusions

The present pre-clinical study provides a strong rationale for assessing the potential value of VK3 in the treatment of PCLDs.

Authors have no financial relationships to disclose