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Combination therapy of All-trans Retinoic Acid With Ursodeoxycholic Acid in Patients with Primary Sclerosing Cholangitis – A Human Pilot

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Abstract

GOALS—To perform an exploratory pilot study of all-trans retinoic acid (ATRA) combined with ursodeoxycholic acid (UDCA) in patients with primary sclerosing cholangitis (PSC).

BACKGROUND—PSC is a progressive disorder for which there is no accepted therapy. Studies in human hepatocyte cultures and in animal models of cholestasis indicate that ATRA might have beneficial effects in cholestatic disorders.

STUDY—ATRA (45 mg/m²/day, divided and given twice daily) was combined with moderate-dose UDCA in patients with PSC who had incomplete response to UDCA monotherapy. The combination was administered for 12 weeks, followed by a 12-week washout in which patients returned to UDCA monotherapy. We measured alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin, cholesterol, bile acids, and the bile acid intermediate 7 α -hydroxy-4-cholesten-3-one (C4) at baseline, week 12, and after washout.

RESULTS—Fifteen patients completed 12 weeks of therapy. The addition of ATRA to UDCA reduced the median serum ALP levels (277 $\pm$ 211 to 243 $\pm$ 225 U/L; $P=.09$) although this, the primary endpoint, did not reach significance. In contrast, median serum ALT (76 $\pm$ 55 to 46 $\pm$ 32

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U/L; $P=.001$) and C4 (9.8 ± 19 to 7.9 ± 11 ng/mL; $P=.03$) levels significantly decreased. After washout, ALP and C4 levels non-significantly increased while ALT levels significantly increased (46 ± 32 to 74 ± 74 ; $p=0.0006$), returning to baseline.

CONCLUSIONS—In this human pilot study, the combination of ATRA and UDCA did not achieve the primary endpoint (ALP), however it significantly reduced ALT and the bile acid intermediate C4. ATRA appears to inhibit bile acid synthesis and reduce markers of inflammation, making it a potential candidate for further study in PSC. NCT01456468.

Keywords

Primary Sclerosing Cholangitis; FXR; RXR; Clinical trial; All-trans retinoic acid

Introduction

Primary Sclerosing cholangitis (PSC) is a chronic, non-suppurative cholestatic liver disorder for which there is no accepted therapy¹. A significant number of patients develop progressive liver disease requiring transplantation or eventually succumb from liver failure. Therefore, there is an urgent need for new medical therapies in this disease^{2,3}.

All-trans retinoic acid (ATRA) is an evolutionarily conserved, permissive activator of the nuclear receptor complex FXR/RXR^{4,5}. Studies in HepG2 cells and isolated human hepatocytes demonstrated that ATRA inhibited CYP7A1 mRNA expression, the rate-limiting enzyme in bile acid synthesis from cholesterol⁵. Based on these findings, several cholestatic animal models, including bile-duct ligation (BDL) and alpha-naphthyl isothiocyanate (ANIT) treatment in rats, and the Mdr2^{-/-} mouse model that mimics sclerosing cholangitis, were treated with ATRA with and without ursodeoxycholic acid (UDCA) and compared with untreated cholestatic animals^{6,7}. The most striking findings were observed in the two-week bile duct obstruction model⁶. Animals treated with ATRA with or without UDCA had marked reduction in necrosis, decreased hepatic fibrosis and markers of inflammation associated with a 50% decrease in the bile acid pool size and decreases in bile duct proliferation. Interestingly, the most beneficial effects in the BDL injury model were found with the combination of ATRA and UDCA. Similar, albeit less dramatic, findings were observed in the other two animal models⁷.

Based on these animal studies, we embarked on a 12-week exploratory pilot study of ATRA combined with moderate-dose UDCA (15-23 mg/kg/day) in PSC patients with persistent cholestasis despite UDCA monotherapy. We measured changes to serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and the bile acid intermediate 7 α -hydroxy-4-cholesten-3-one (C4), a marker of bile acid synthesis.

Methods

Patients between 18 and 80 years of age, with a confirmed clinical diagnosis of PSC by imaging, cholangiography or biopsy and with persistent serum alkaline phosphatase (ALP) elevation of at least 1.5 x the upper limit of normal (ULN) while on moderate-dose (15-23 mg/kg/day) UDCA for at least 6 months were eligible for enrollment. Patients with

hepatobiliary cancer or any other malignancy, with evidence of decompensated cirrhosis within the past 6 months or an estimated need for liver transplantation within 12 months were ineligible. Patients with prior intolerance to UDCA, ATRA, or any vitamin A related compound, patients with any evidence of recent coronary artery disease, or patients who were or planning to become pregnant, were also ineligible. Subjects were enrolled and followed at two separate study sites, at Yale and Mayo Liver Clinics. The study was reviewed and approved by the institutional review boards at each institution, and registered in clinicaltrials.gov (NCT 01456468). Informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by each institution's human research committee.

This was an investigator-initiated study. An investigational new drug (IND) application was reviewed and approved by the Food and Drug Administration so that ATRA capsules could be locally compounded by each institution's research pharmacy. The oral dose of ATRA, 45 mg/m²/day divided twice daily taken with meals, is FDA approved for the treatment of acute promyelocytic leukemia⁸. Review of the literature revealed that this dose was well tolerated in a human pilot study of chronic hepatitis C infection [9]. We approximated this dose by compounding ATRA into single 20, 30 or 40 mg capsules that were dispensed twice daily according to the subject's body surface area. After the initial two patients were enrolled, gradual ATRA introduction over 7 days was performed in order to decrease the incidence of transient headaches. The dose of UDCA used during the preceding 6 months before enrollment was maintained throughout the pilot.

Combination therapy was administered for 12 weeks to all enrolled subjects in a single-arm fashion, followed by a 12-week washout period in which patients returned to UDCA monotherapy. Baseline blood tests including ALP, ALT, bilirubin, serum cholesterol including LDL, HDL and triglyceride levels, bile acids and C4 were compared to 12-week levels and again after the washout period. Serum lipid and bile acid levels were available only in the sub-group of patients enrolled at Yale (N=7). Although this was an exploratory human pilot, the pre-determined primary outcome of interest was a 30% reduction in serum ALP after 12 weeks of combination therapy with UDCA. It was estimated that enrollment of 30 subjects would achieve 90% power to detect a 30% improvement in ALP at alpha of 0.05 using a two-sided one-sample t-test. This target number would allow for dropout of up to 20% in subjects while still maintaining statistical power to detect a 30% improvement in ALP. The study was eventually terminated due to recruitment futility.

Statistical analysis was performed using signed-rank test for comparing medians. Two-sided tests were performed at the $\alpha=0.05$ -level of significance using SAS 9.3 (Cary, NC). The authors had access to the study data, and reviewed and approved the final manuscript.

Results

Twenty-one patients with PSC were screened and 19 were enrolled (**Figure 1**). The median age of the cohort was 45±11 years (**Table 1**). The majority were Caucasian (74%) and male (74%). Sixty-three percent had large duct PSC while 37% had small-duct PSC. Seventy-nine

percent had inflammatory bowel disease (2 with Crohn's Disease and 13 with Ulcerative Colitis) including 4 of the 6 patients with small-duct PSC. The median dose of UDCA was 16 ± 6 mg/kg/day.

Fifteen subjects completed 12 weeks of combination therapy with ATRA and UDCA. The median ALP declined after 12 weeks compared to UDCA monotherapy but this did not reach statistical significance (277 ± 211 to 243 ± 225 U/L, $p=.09$), **Figure 2**. Expressed as multiples of the ULN, there was a 14% reduction in the median pre vs. post-treatment ALP but this also did not achieve statistical significance (2.54 ± 1.82 vs. 2.18 ± 1.95 , $p=.09$). Three of the 15 subjects (20%) achieved 30% reduction in serum ALP, and nearly half of the group (47%) achieved 20% ALP reduction at 12 weeks. There was no significant difference in the demographic parameters or the baseline blood tests between those whose ALP decreased >20% and those who did not. There was no significant difference in response according to small vs. large-duct PSC. After washout, there was a non-significant increase in the median ALP level (243 ± 225 vs. 314 ± 190 U/L, $p=.15$) back to the pre-combination treatment baseline. Similar to ALP, the median serum bilirubin level after 12 weeks of combination therapy decreased but was not statistically significant (0.8 ± 1.32 vs. 0.6 ± 0.62 mg/dL, $p=.076$).

In contrast to ALP, the median serum ALT significantly declined after 12 weeks of combination therapy (76 ± 55 vs. 46 ± 32 U/L, $p=.001$), **Figure 3A**. Furthermore, after 12 weeks of washout following ATRA discontinuation the median ALT level significantly increased compared to the end of treatment level (46 ± 32 to 74 ± 74 ; $p=.0006$), returning to the pre-combination therapy baseline despite UDCA continuation. This result suggested an anti-inflammatory and reversible effect of ATRA therapy as measured by this secondary endpoint.

In accordance with pre-clinical findings in animal models of cholestasis^{6,7}, we observed inhibition of bile acid synthesis after ATRA and UDCA combination therapy. Specifically, the median serum level of the bile acid intermediate C4 significantly decreased after 12 weeks (9.8 ± 19 vs. 7.6 ± 11 ng/mL, $p=.03$), **Figure 3B**. This indicated that ATRA can inhibit bile acid synthesis in patients with PSC, a result which was previously demonstrated in isolated human hepatocytes⁵. Following ATRA washout, there was a non-significant return of serum C4 levels to baseline. In addition, serum bile acid concentrations were measured in a subset of subjects from one of the institutions ($N=7$) and there was a non-significant decrease at week 12 (24 ± 27 vs. 9 ± 45 μ mol/L, $p=.2$), **Figure 3C**.

As anticipated, effective inhibition of CYP7A1 resulted in a trend toward increased cholesterol levels in the subset of subjects where it was measured ($N=7$). The median LDL non-significantly increased (112 ± 60 vs. 151 ± 51 mg/dL, $p=.1$) while the median HDL significantly decreased (56 ± 21 vs. 41 ± 11 mg/dL, $p=.03$) after 12 weeks of combination therapy (**Figure 4A, 4B**). Further, the median serum triglyceride level significantly increased at 12 weeks (79 ± 31 vs. 136 ± 45 mg/dL, $p=.02$), **Figure 4C**. Following the 12-week washout period post-ATRA discontinuation, each serum lipid test returned to pre-treatment levels. These reversible findings represent further evidence of a physiologic effect of ATRA in

humans with cholestasis, by reducing bile acid synthesis and concomitantly increasing cholesterol concentration.

The frequencies of reported adverse effects are listed in the **Table 2**. The two most common adverse effects attributed to ATRA were headache (63%) and tinnitus (26%). Gradual ATRA introduction over 7 days was initiated after the first two subjects enrolled in the study by initiating therapy at a quarter of the final dose and increasing the dosage by 25% every 2 days. This method significantly decreased the incidence of headaches and improved tolerability. Notably, pruritus was not a reported adverse effect in this pilot. Four of the 19 enrolled subjects did not complete 12 weeks of combination therapy. Two subjects withdrew due to persistent ATRA-related headaches, which resolved after drug discontinuation. One subject withdrew due to ATRA-related tinnitus, which also resolved after drug discontinuation. Finally, one subject was withdrawn due to jaundice from acute biliary obstruction due to a previously known stricture.

Discussion

This is the first human study to explore the use of ATRA in cholestasis and as a candidate therapy for patients with PSC. In this small human pilot we did not achieve the primary endpoint of a 30% reduction in serum ALP with the addition of ATRA to UDCA. However, serum levels of serum ALT and the bile acid intermediate C4 significantly decreased and ALP tended to improve with a median reduction of 14%. The finding of reduced bile acid synthesis and ALT may provide a basis for further investigating ATRA as a repurposed drug in chronic cholestasis.

The rationale for combining ATRA with UDCA was based on pre-clinical studies indicating a superiority of combination therapy in reducing hepatic inflammation and necrosis⁶. In order to control for the effect of UDCA, subjects were enrolled and given ATRA only after treatment with moderate-dose UDCA for at least 6 months. Following 12 weeks of combination therapy, ATRA was discontinued and subjects continued to receive UDCA monotherapy for 12 more weeks. This design permitted evaluation of ATRA effects and their reversibility after washout. Indeed, biochemistries such as serum ALT significantly rebounded after ATRA discontinuation and returned to baseline values.

Although the magnitude of the ALP reduction was modest and non-significant with combination therapy, it should be noted that this reduction occurred in addition to the effect of UDCA monotherapy. Despite increased attention to the need for surrogate markers in PSC, ALP reduction through pharmacotherapy has not been established as a clearly reliable endpoint for trials. Further, the marked improvement in other relevant serum parameters suggests that ATRA may have caused a beneficial effect on potential mechanisms of injury in PSC, consistent with an effect on inhibition of CYP7A1 as demonstrated in isolated human hepatocytes⁵. Chief among the positive effects was a significant reduction in the serum levels of C4, indicating that bile acid synthesis was substantially reduced. This latter finding is consistent with the effects of ATRA in the bile duct obstructed rat model (BDL) where a two-week treatment with ATRA and UDCA reduced the bile acid pool size by

50%⁶. Reductions of bile acid synthesis would be expected to reduce hepatic levels of bile acids and presumably lessen the degree of bile acid induced injury to the hepatocytes.

Equally important, the significant reduction in serum ALT during ATRA treatment is also consistent with such an effect, and removal of ATRA increased ALT levels to the pre-combination therapy baseline. Since ATRA is known to have stimulatory effects on the adaptive immune system¹⁰, an immunomodulatory response might be an alternative explanation for the reduction in ALT levels. Future investigations could evaluate ATRA-induced immune modulation in PSC. However, this is additionally relevant given animal studies which demonstrate retinoic acid's ability to facilitate imprinting of T-cells in the gut, which could allow for migration to the hepatobiliary system¹¹.

Adverse effects from ATRA therapy were consistent with described effects observed when used in the FDA-approved indication for acute promyelocytic leukemia⁸, and consisted primarily of headaches and tinnitus. Each of the adverse effects noted in this human pilot was reversible after ATRA discontinuation. However, temporary headaches were significant enough in two subjects to require removal from the study. A gradual dose escalation of ATRA over the first week significantly improved the tolerability of the drug. The dose of ATRA used in this pilot was based on the clinically approved dose for treatment of acute promyelocytic leukemia. It is not known if a lower dose of ATRA would have decreased the frequency of adverse effects while maintaining biochemical responses. Future studies should assess the efficacy and tolerability of ATRA at lower doses in the setting of cholestasis.

A notable effect of ATRA was a change in serum lipids with a significant increase in triglycerides and a reduction in HDL. These changes are to be expected with any treatment that results in inhibition of cholesterol metabolism to bile acids since this is the major degradative pathway for the elimination of cholesterol. Further, a similar effect was observed in recent trials of the potent FXR inhibitor obeticholic acid in primary biliary cirrhosis and non-alcoholic steatohepatitis^{12,13}. This could be presumably counterbalanced by initiating statin therapy if treatment were continued for longer periods. The long-term cardiovascular consequences of dyslipidemia from bile acid modulation are unclear and warrant further study. Interestingly, there were no reports of pruritus during this pilot, in contrast to the known effect of obeticholic acid in cholestasis¹². This raises the possibility that ATRA may not have the same adverse effect profile as obeticholic acid with respect to pruritus. However, a formal assessment of pruritus was not performed in this pilot.

Minor liver test abnormalities have been reported during ATRA treatment for acne¹⁴ but this was not observed in our study, nor was it described in a clinical pilot in patients with hepatitis C⁹. ATRA is a minor metabolite of vitamin A, and several studies examining the effects of vitamin A or ATRA in animal models and stellate cell cultures suggest that vitamin A has anti-fibrotic effects^{6,15-17}. At the same time, vitamin A intake in excessive doses (>20,000 IU/day) has also been reported to cause the syndrome of hypervitaminosis A and significant liver injury¹⁸.

This study has several limitations. First, this single-arm pilot study was designed such that only patients pre-treated with UDCA were enrolled for the addition of ATRA as part of

combination therapy. As such, this pilot was not designed to compare multiple groups, such as UDCA monotherapy, to combination therapy. Despite the clear superiority of ATRA combined with UDCA in animal models of cholestasis⁶, it is unknown whether ATRA monotherapy in PSC is effective. Further, the role of UDCA itself in PSC management is controversial. Therefore, it will be necessary in future studies to analyze the effect of ATRA monotherapy in patients with PSC. A second limitation is that a single dose of ATRA was evaluated (approximately 45 mg/m²/day). Although this dose is FDA-approved in the treatment of acute promyelocytic leukemia, and is much lower than the dose used in our pre-clinical animal studies, it was associated with frequent side effects such as headache. It is not yet known whether bile acid inhibition with reduced adverse events could be achieved using a lower dose. Third, despite there being no reports of colitis exacerbation during this 12 week pilot, a formal assessment of colitis was not performed and thus we cannot exclude the possibility that variability in ALP or bile acid reduction could be influenced by the degree of underlying bowel inflammation. Similarly, patient data on changes to pruritus and quality of life was not collected. Fourth, the clinical significance of ALT or C4 reduction (secondary endpoints) without statistically significant ALP reduction (primary endpoint) is not clear, and additionally fibrosis markers were not measured. However, there is currently no effective therapy nor accepted surrogate endpoint in PSC trials and these secondary findings directly relate to ATRA's mechanism of action. Finally, the long-term effects of ATRA therapy in PSC beyond 12 weeks are not known, and the short duration of this study was chosen specifically to look for an early efficacy signal in the context of an exploratory pilot. Further investigations are needed to answer each of the above questions, potentially through a formal phase 2a study focused on assessing the adverse effect profile of a lower dose of ATRA monotherapy in PSC patients.

In summary, this 12-week human pilot study of ATRA in combination with moderate-dose UDCA in PSC, while demonstrating only a borderline significant reduction in ALP, demonstrated modest improvement in serum ALT and also evidence of bile acid synthesis inhibition. The findings from this pilot warrant dose-response trials with longer duration of ATRA in PSC to evaluate its efficacy and tolerability.

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List of Abbreviations

PSC	primary sclerosing cholangitis
ATRA	all-trans retinoic acid
FXR	farnesoid X receptor
RXR	retinoid X receptor

HepG2	cell line of human liver carcinoma cells
CYP7A1	cytochrome P450, Family 7, Subfamily 1, Polypeptide 1
mRNA	messenger ribonucleic acid
BDL	bile-duct ligation
ANIT	alpha-naphthyl isothiocyanate
Mdr2	multidrug resistance protein 2 gene
UDCA	ursodeoxycholic acid
ALT	alanine aminotransferase
c4	7 α -hydroxy-4-cholesten-3-one
ALP	alkaline phosphatase
ULN	upper limit of normal
kg	kilogram
IND	investigational new drug
FDA	food and drug administration
LDL	low density lipoprotein
HDL	high density lipoprotein
ULN	upper limit of normal
U/L	units per liter
IU	international units

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Figure 1.
CONSORT Figure for the Pilot Study evaluating ATRA and UDCA in PSC.

Figure 2. The effect of ATRA and UDCA on serum ALP

The median serum ALP level decreased, but did not achieve significance, from a baseline of 277 ± 211 to 243 ± 225 U/L at week 12 of combination therapy, $p=.09$.

Figure 3. The combination of ATRA and UDCA reduced serum ALT and bile acid intermediate C4

Panel 3A: The median serum ALT level decreased from 76 ± 55 to 46 ± 32 U/L at 12 weeks of combined therapy, $p=.01$. After a 12-week washout post-ATRA and back to UDCA monotherapy, the median ALT significant increased compared to end of treatment levels (end of treatment 46 ± 32 vs. post-washout 74 ± 74 ; $p=0.0006$). **Panel 3B:** The median serum C4 level decreased at week 12 from baseline (9.8 ± 19 to 7.9 ± 11 ng/mL; $P=.03$). **Panel 3C:** The median serum bile acid level decreased at week 12 from baseline but did not achieve significance (24 ± 27 vs. 9 ± 45 umol/L, $p=.2$). Only 7 subjects from one of the enrolling sites had bile acid levels available for analysis.

Figure 4. The combination of ATRA and UDCA altered the serum lipid profile

Subjects from one of the enrolling sites (N=7) had lipid levels available for analysis.

Panel 4A: The median serum LDL level increased at week 12 but did not achieve significance (112 ± 60 vs. 151 ± 51 mg/dL, $p=0.1$). **Panel 4B:** The median serum HDL level significantly decreased at week 12 (56 ± 21 vs. 41 ± 11 mg/dL, $p=0.03$). **Panel 4C:** The median serum triglyceride level significantly increased at week 12 (79 ± 31 vs. 136 ± 45 mg/dL, $p=0.02$).

Table 1

Baseline demographic data for the study subjects.

	Enrolled in Study (N=19)	Completed Study (N=15)
Median age at enrollment, years $\pm$ SD	45 $\pm$ 11	49 $\pm$ 11
Male Sex, %	74%	73%
Median BMI, $\pm$ SD	25 $\pm$ 5	24 $\pm$ 5
Race		
Caucasian	14	11
African American	3	2
Other	2	2
PSC Classification		
Large-duct	12	9
Small-duct	7	6
Inflammatory Bowel Disease		
Ulcerative Colitis	13	11
Crohn's Disease	2	1
Median Mayo PSC Risk Score [*] $\pm$ SD	-0.03 $\pm$ 0.71	-0.05 $\pm$ 0.59
Median daily dose of UDCA, mg/kg $\pm$ SD	16 $\pm$ 6	16 $\pm$ 7

* Mayo PSC Risk Score Formula: $R = 0.03 (\text{age [y]}) + 0.54 \log_e (\text{bilirubin [mg/dL]}) + 0.54 \log_e (\text{aspartate aminotransferase [U/L]}) + 1.24 (\text{variceal bleeding [0/1]}) - 0.84 (\text{albumin [g/dL]})$.

Table 2

Reported adverse effects.

Adverse Effects	Frequency, %
Headache	63%
Tinnitus	26%
Diarrhea	16%
Dry skin/mucus membranes	10%
Myalgia	5%
Anemia	5%