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Ursodeoxycholic Acid Treatment of LPAC Presenting as Post Cholecystectomy Pain: Rationale and Design of a Randomised Trial

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Abstract

Introduction: Post cholecystectomy pain is a common clinical problem. Investigation and management of patients with recurrent episodes of biliary pain and normal imaging is challenging. Low Phospholipid Associated Cholelithiasis (LPAC) is a recently described syndrome that often presents with difficult to manage episodes of post cholecystectomy pain. Ursodeoxycholic Acid (UDCA) treatment of LPAC is supported by animal studies and case series but has not been assessed in a randomised trial.

Methods and analysis: This protocol presents the design and rationale for an investigator-initiated, prospective, randomised, placebo-controlled, double-blind, phase 3 crossover trial: Ursodeoxycholic acid in LPAC Treating Recurrent Abdominal pain (ULTRA Pain). The study population consists of 24 patients with difficult to manage post cholecystectomy pain diagnosed with LPAC with no or minimal changes on liver ultrasound imaging. Participants will be recruited through the gastroenterology clinic at Royal Melbourne Hospital and randomly assigned to receive UDCA 10 mg/kg daily for 1 year followed by a matched placebo, separated by a 2-week washout period or vice versa. The primary endpoint is a reduction in the number of episodes of biliary pain.

This trial will provide level 1 evidence on the efficacy of UDCA in treating LPAC presenting with post cholecystectomy pain.

Ethics and trial registration: The trial was granted ethical approval from Melbourne Health Human Research and Ethics Committee (HREC/55994/MH-2019). This trial is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12621000450819).

Keywords: Low phospholipid associated cholelithiasis; Adenosine triphosphate-binding cassette subfamily B; Randomised control trial; Ursodeoxycholic acid; Post cholecystectomy; Biliary pain

Introduction

Gallstone disease is common. The prevalence of gallstones in western countries is approximately 20% and about a fifth of these patients become symptomatic [1-3]. Cholecystectomy is the standard management for symptomatic gallstones [4-6]. However, post cholecystectomy pain occurs in up to 33% of operations [7]. Investigation and management of recurrent biliary pain with normal imaging can be challenging.

Low Phospholipid Associated Cholelithiasis (LPAC) is a recently described syndrome that is an under recognized and under diagnosed cause of post cholecystectomy biliary pain [8]. The prevalence of LPAC is not well defined but has been estimated to be about 1% of patients presenting for cholecystectomy [9].

LPAC is characterised by the reduced secretion of phosphatidylcholine into bile and is often associated with loss of

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function variants of the Adenosine triphosphate Binding Cassette, subfamily B, member 4 (*ABCB4*) gene.

ABCB4 is a phospholipid translocator located on the biliary canalicular membrane of the hepatocytes and transports phosphatidylcholine into bile. Phospholipids associate with simple bile salt micelles to form mixed micelles containing both phospholipids and bile salts. This association strongly reduces the concentration of toxic bile salt monomers and simple micelles protecting the hepatocyte membrane from dissolution. Variants of the gene encoding ABCB4 may impair transport of phosphatidylcholine reducing the concentration of phospholipids in bile. The unrestrained toxicity of bile acids leads to inflammation of hepatocytes and cholestatic liver disease. The formation of mixed micelles also increases the solubility of cholesterol in bile. Impaired phospholipid transport increases cholesterol saturation supporting the formation of cholesterol crystals and stones.

ABCB4 variants are associated with a broad overlapping spectrum of phenotypes of liver diseases related to the degree of impairment of phospholipid transport. These range from transient neonatal cholestasis, contraceptive induced cholestasis, Intrahepatic Cholestasis of Pregnancy (ICP), LPAC, adult cholestatic liver disease and cirrhosis and Progressive Familial Intrahepatic Cholestasis type 3 (PFIC type 3). LPAC is usually associated with heterozygous *ABCB4* genetic variants with mild to moderate severity on the clinical spectrum.

In 2001 Rosmorduc et al. investigated 6 patients with atypical intrahepatic stones and found low biliary phospholipid concentrations and variants of the gene encoding for ABCB4. The clinical syndrome of LPAC was defined in a subsequent case control study as the presence of at least two of the following clinical criteria:

- Biliary symptoms before the age of 40 years
- Recurrence of symptoms after cholecystectomy
- Detection of intrahepatic intrahepatic stones or echogenic foci

This is a very broad definition and includes all patients with post cholecystectomy pain under the age of 40 years. At the time these criteria were recognised as sensitive but not specific. Diagnostic certainty increases if typical imaging findings are seen on ultrasound or if genotyping identifies a known variant. Known variants are only identified in approximately 50% of cases of LPAC.

As more cases of LPAC have been reported, the diagnostic criteria have evolved. Additions that expand the initial diagnostic criteria include a family history of cholecystectomy before 40 years of age and a personal or family history of other phenotypes of *ABCB4* gene variants. ICP was reported in 42% of women with LPAC and a history of pregnancy.

More information has also been published about the spectrum of clinical features in LPAC. Compared with initial descriptions macroscopic intrahepatic stones are less frequent, 43% and a subgroup of 10%-15% of patients present with large uni or multifocal spindle-shaped dilations of the intrahepatic ducts filled with cholesterol and soft pigment stones. A small number of LPAC patients present with established chronic cholestatic liver disease and are at risk of progression to cirrhosis and end stage liver failure.

A subgroup with clinically milder LPAC has been more frequently recognized. These patients present with post cholecystectomy biliary pain with normal imaging or minimal changes on ultrasound. The episodes of biliary pain are often associated with transiently abnormal liver function tests. Participants in the trial will be selected from this subgroup.

Ursodeoxycholic acid (UDCA; 3α,7β-dihydroxy-5β-cholanoate), a native hydrophilic bile acid forming about 3% of the bile acid pool, was first used to dissolve cholesterol gallstones. UDCA has also been used to treat cholestatic liver diseases initially based on the hypothesis that treatment replaces endogenous cytotoxic bile salts with UDCA which is a much less toxic bile salt. Several other mechanisms of action have been described more recently. UDCA stimulates the secretion of bile acids and phospholipids by upregulating the expression of the apical membrane transporters like ABCB11 and ABCB4 on the hepatocytes. The increase in phospholipid concentration in bile facilitates the solubilisation of cholesterol in bile and eventually the dissolution of cholesterol crystals and stones. UDCA also boosts the innate immune system within the biliary tree which suppresses biliary inflammatory and prevents phospholipid degradation. UDCA inhibits bile acid induced apoptosis by stabilizing the mitochondrial and hepatocyte membrane, increasing the hepatocyte defenses against oxidative stress.

Animal studies support the role of UDCA in treatment of LPAC. In Abcb4 knockout mice UDCA treatment reduces liver damage and intrahepatic stone formation.

UDCA has been used to successfully treat other phenotypes of *ABCB4* gene variants including PFIC3, ICP and drug induced liver disease. PFIC3 is associated homozygous or compound heterozygous *ABCB4* gene variants and severe reductions in phospholipid levels in bile. Symptoms occur in the first year of life, cirrhosis in childhood and about 50% require liver transplantation. Treatment with ursodeoxycholic acid 20-30 mg/kg/day improves liver function and delays progression in some patients.

EASL advises UDCA treatment for LPAC but qualifies this advice with the recommendation that a randomized trial should be performed. UDCA treatment of more severe LPAC is well established. Case series report dissolution of intrahepatic stones and improvement in dilated intrahepatic ducts and chronic liver disease. Episodes of biliary pain are also reported to improve; however, pain is a more subjective outcome. The placebo response of a sham sphincterotomy in the EPISOD trial was 47%. The placebo response in earlier trials of UDCA in post cholecystectomy pain was between 30% and 52%. A randomized trial is the most reliable method to assess the efficacy of UDCA treatment of LPAC in patients presenting with episodes of post cholecystectomy biliary pain.

The Ursodeoxycholic acid in LPAC Treating Recurrent Abdominal (ULTRA) pain trial aims to determine whether ursodeoxycholic acid reduces episodes of biliary pain and improves quality of life, in post cholecystectomy patients with LPAC and minimal or no abnormalities on imaging. The primary endpoint is the number of episodes of biliary pain. Quality of life, as measured by validated questionnaires, is a secondary endpoint.

Materials and Methods

Study design

ULTRA pain study is an investigator-initiated, double-blind randomised, placebo-controlled, phase 3 crossover trial assessing the efficacy of UDCA treatment of post cholecystectomy pain in patients with LPAC. Patients presenting with difficult to manage episodes of

biliary pain and diagnosed with LPAC, meeting other eligibility criteria, will be invited to participate in the trial. After informed consent, participants will be randomly assigned to receive UDCA 10 mg/kg/day for 1 year followed by a matched placebo for 1 year, separated by a washout period of 2 weeks or vice versa. The study protocol was written in accordance to CONSORT guidelines (Figure 1). And was granted ethical approval from Melbourne Health Human Research and Ethics Committee HREC number HREC/55994/MH-2019. This trial is registered with the Australian New Zealand Clinical Trials Registry (ANZCTR) (ACTRN12621000450819).

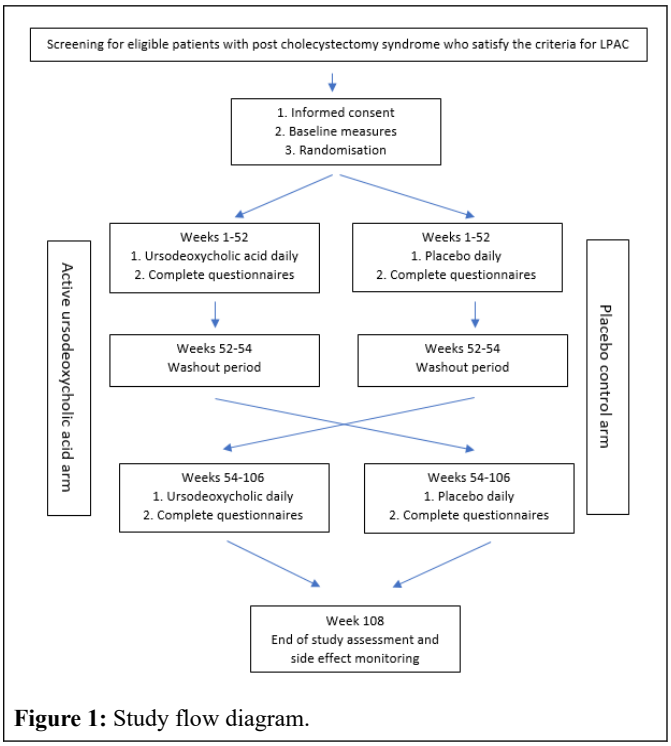

Figure 1: Study flow diagram.

Study setting and participants

Adult participants will be recruited from a specialised gastroenterology outpatient clinic at the Royal Melbourne Hospital and from referrals from other hospitals. Eligible patients will be initially seen and screened by a gastroenterologist involved in the research project, to discuss their potential involvement in the trial and be provided with a participant information. Informed consent will then be obtained by a medically qualified site investigator trained in good clinical practice.

Inclusion criteria: The study population is patients presenting with post cholecystectomy pain diagnosed with mild LPAC. Those with intrahepatic macroscopic stones or dilated intrahepatic bile ducts or chronic liver disease will be excluded. Entry criteria are as follows:

- Patients aged ≥ 18 years with a cholecystectomy, performed more than 6 months ago, presenting with 2 or more episodes of biliary pain in the last 12 months.
- Typical biliary pain is defined by the Rome IV criteria. These specify biliary pain is located in the epigastrium and/or right upper quadrant and is experienced at least 2-3 days per month. However, it is our experience that LPAC patients will not experience biliary pain this frequently hence the criteria have been modified to allow less frequent episodes (2 or more episodes per year).
- Two or more of the following criteria that are characteristic of LPAC: a) Age less than 40 years at onset of symptoms; b) Imaging features consistent with LPAC identified on a targeted liver ultrasound performed by an experienced hepatobiliary subspecialist radiologist: Intrahepatic echogenic foci in a position consistent with biliary tree, intrahepatic microlithiasis or sludge; c) Family history of cholecystectomy before 40 years of age, family history of other *ABCB4* gene variant phenotypes including ICP or a personal history of ICP, d) Evidence of transiently abnormal liver function tests corresponding with episodes of biliary pain.
- Access to a telephone.
- Must be able to speak, read and write English.
- Signed and dated informed consent.

Exclusion criteria

- Stone in the common bile duct on imaging.
- Intrahepatic macroscopic stones, dilated intrahepatic bile ducts and cholestatic liver disease.
- Known history of primary sclerosing cholangitis, primary biliary cholangitis, colitis, Crohn's disease, cystic fibrosis, chronic liver disease.
- Presence of a significant psychiatric disorder.
- Pregnancy women who are pregnant or are planning a pregnancy within 2 years at the time of screening.
- Any functional bowel disorders.
- Any use of regular narcotics.
- Inability to comply with study procedures and agents.
- Any significant medical illness that would interfere with study participation.
- Any condition that, in the investigator's opinion, makes the subject unsuitable for study.
- Recent treatment with UDCA.

Randomisation

Patients will be randomised by a predetermined schedule (in 4 blocks of 6) created prior to study commencement by the trial statistician. There will be no stratification. The sequence of treatment allocations will be concealed to staff undertaking the randomisation. Randomisation will correlate with a bottle number for each participant that links to the corresponding allocated study dose (blinded), in accordance with randomisation. All prescriptions will be dispensed from a clinical trials pharmacy.

Blinding

Both the participants and clinicians will be blinded to treatment, matching placebos will be used in the control arm. The treating physician will be aware of dose level of the study medication but not the treatment itself.

Emergency unblinding will only be performed if it is necessary to ensure the safety of the participant. Situations that may warrant unblinding include pregnancy and a serious adverse drug reaction.

Intervention and monitoring

Participants will be randomised to UDCA or placebo with a cross over at 1 year and change over period of 2 weeks between the cross over. The dose of trial medication 10 mg/kg daily may be titrated upwards for persisting episodes of biliary pain to a maximum of 20 mg/kg daily at the discretion of the treating clinician. Diarrhoea is an

occasional side effect. If diarrhoea is not responsive to regular treatment (fluids and anti-diarrhoea drugs) and is possibly related to the study drug, the trial medication can be down titrated to 5 mg/kg and slowly increased as tolerated.

Clinical review will be performed every 3 months and will include completion of quality of life questionnaires and blood tests. Participants will be given request forms for blood tests, to be performed within 24-48 hours of each episode of pain, including liver function tests, FBE, UEC, serum lipase and CRP. Episodes of biliary pain may be treated with Glyceryl Trinitrate (GTN) spray and hyoscine butylbromide (Buscopan) as required. Participants with severe refractory biliary pain will be assessed in the emergency department. Participants will also undergo liver US monitoring every 12 months (Table 1).

Testing variables	Baseline	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months
Informed consent	X								
Randomisation	X								
Medical history, surgical and family history	X								
Beck's depression inventory	X								
Rome criteria for FBSD and IBS	X								
Blood samples	X	X	X	X	X	X	X	X	X
Biliary pain assessment	X	X	X	X	X	X	X	X	X
Concomitant medication check	X	X	X	X	X	X	X	X	X
RAPID score	X	X	X	X	X	X	X	X	X
HADS	X				X				X
SF-36	X	X	X	X	X	X	X	X	X
EQ-5D-5L	X	X	X	X	X	X	X	X	X
Adverse events check	X	X	X	X	X	X	X	X	X
Liver US	X				X				X

Note: FBSD: Functional Biliary Sphincter Disorder; IBS: Irritable Bowel Syndrome; Blood samples include FBE, UEC, LFT, CRP, lipase, full coagulation profile; RAPID score: Recurrent Abdominal Pain Intensity and Disability score; HADS: Hospital Anxiety and Depression Scale; SF-36: 36-Item Short Form Survey; Liver US: Liver Ultrasound

Table 1: Schedule of assessments.

Adherence

Compliance with medication will be assessed by a pill count performed by pharmacy at each clinical review.

Primary outcome

The primary outcome is a reduction in the number of episodes of biliary pain.

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Secondary outcomes

Secondary outcomes include:

- Reduction in number of episodes of biliary pain associated with transiently abnormal liver function tests
- Reduction in number of episodes of biliary pain in participants with findings on ultrasound examination typical of LPAC
- Improvement in quality of life scores including the RAPID (Recurrent Abdominal Pain Intensity and Disability) score, the Hospital Anxiety and Depression Scale (HADS) and both SF-36 and EQ-5D-5L

Adverse events

UDCA is well tolerated with diarrhoea the major side effect, reported in up to 3% of patients. Nausea, vomiting and rarely urticaria have also been reported.

Study monitor and safety: An independent medical monitor has been appointed to monitor the safety of participants and oversee the adverse events, withdrawals and safety including adverse events. Adverse events will be reported as stipulated under the NHMRC safety monitoring and reporting and clinical trials involving therapeutic products. An independent monitor has been employed to regularly review the trial processes and data integrity.

Results

Data analysis

Sample size calculations and statistical method: We powered this study and based our sample size calculation on the number of patients needed to assess our primary outcome: Number of episodes of biliary pain per year. In our previous cohort of LPAC patients, it was noted that the mean frequency of biliary pain was 3 episodes per year, with a Standard Deviation (SD) of 1. Assuming that UDCA will reduce the frequency of biliary pain to a mean (SD) of 2 (1) episodes per year, 18 patients will be required to detect this between-treatment difference, with 80% power and a 2-sided alpha of 0.05. Cross-over studies typically require fewer subjects compared to parallel clinical trials given that inter-group variance is less. However, in the sample size calculation, it was conservatively assumed that inter-group variance was the same as would be observed for a parallel clinical trial. To allow for 30% drop-out in the study, a target of 24 patients will be recruited.

An intention-to-treat analysis will be performed in accordance with CONSORT guidelines to provide an assessment of the impact of the treatment.

Data management: Data will be entered for trial purposes to a secure validated clinical data management system (REDCapTM) with password protection. Only study clinicians will have the password and ability to access these files. All research data including the investigator site file will be kept in a secure, locked location on-site at the hospital and all electronic data will be kept on at the hospital server and REDCapTM for a minimum of 5 years. Participants will be identified by unique study identifier, date of birth and initials. All information collected including patient clinical, background and family history will be allocated a unique re-identifiable code.

Screening, baseline, trial entry, medical history and study discontinuation will be recorded in patient's medical records at their study site and this will form the source data. The data will be entered into the REDCapTM system by site staff. Follow up data will be collected by the site personnel *via* clinics or participant phone calls and correspondence with relevant healthcare providers. This information will be retained in clinical notes held at the research sites and this will form the source data.

All data will be handled, computerised and stored in accordance with the Data Protection Act 1998 (Figure 2).

1) On how many days in the last 3 months did you miss work or school because of your episodes of abdominal pain? _____ days
2) On how many days in the last 3 months was your productivity in work or school reduced by half or more because of your episodes of abdominal pain (Do not include days you counted in question 1 where you missed work or school)? _____ days
3) On how many days in the last 3 months did you not do household work because of your episodes of abdominal pain? _____ days
4) On how many days in the last 3 months was your productivity in household work reduced by half or more because of your episodes of abdominal pain (Do not include days you counted in question 1 where you did not do household work)? _____days
5) On how many days in the last 3 months did you miss family, social or leisure activities because of your episodes of abdominal pain? _____ days
6) On how many days in the last 3 months did you have episodes of abdominal pain (If the abdominal lasted more than 1 day, count each day)? _____ days
7) On a scale of 0-10, on average, how painful were these episodes of abdominal pain? _____

Figure 2: RAPID questionnaire.

Discussion

The ultra pain study is the first randomised trial to assess the efficacy of UDCA treatment of episodes of post cholecystectomy biliary pain in LPAC.

There is a broad spectrum of clinical severity in the LPAC syndrome. This trial will study patients with mild LPAC as judged by no or minimal changes on liver ultrasound imaging. Those with macroscopic intrahepatic stones, dilated ducts or chronic cholestatic liver disease will be excluded. This patient population typically presents post cholecystectomy with difficult to manage recurrent episodes of biliary pain.

Post cholecystectomy pain is a common clinical problem, the recently described LPAC syndrome is an under recognised and under diagnosed cause. The clinical presentation of LPAC is similar to that of functional biliary sphincter disorder, onset less than 40 years of age, predominantly females, seen several years after cholecystectomy with recurrent biliary pain often associated with transiently abnormal liver function tests. Making the distinction between these two diagnoses facilitates appropriate investigation and management. Level 1 evidence supporting the value of UDCA treatment would raise awareness of LPAC and the existence of an effective treatment.

The milder forms of LPAC generally have a benign clinical course. The major clinical problem, recurrent episodes of biliary pain, has been chosen as the trial primary endpoint. Distinguishing biliary from other causes of pain post cholecystectomy can be difficult. The Rome IV classification has been used to define biliary pain in this trial. The association of transiently abnormal liver function tests with pain confirms a biliary origin, although this is reported to occur in only about 47% of LPAC. Pain associated with transiently abnormal liver function tests is a secondary trial endpoint.

Improvement in quality of life, as measured by validated questionnaires, will also be a secondary endpoint. The RAPID score is a composite of pain severity and frequency and the impact of pain on function in primary roles. Emotional function will be assessed by the HADS is a 14 question tool comprising of seven questions for the assessment of depression in medical patients. Quality of life will be measured by both SF-36 and EQ-5D-5L. The inclusion of these questionnaires, also used in the EPISOD trial, will facilitate comparison of the two studies.

The diagnosis of LPAC syndrome has been defined using Rosmorduc's original 3 criteria with the addition of a family history of cholecystectomy before 40 years of age or a family or personal history of other ABCB4 phenotypes to make the definition more specific. Finding typical changes of LPAC on liver ultrasound examination strengthens the diagnosis however imaging may be normal. A secondary analysis will be performed on the subgroup of participants with characteristic findings of LPAC on ultrasound imaging.

The presence of a known variant of the ABCB4 gene will confirm the diagnosis of LPAC. However, genotyping is expensive and variants are identified in only approximately 50% of LPAC patients. Genotyping will not be performed prospectively in this trial.

After commencement of treatment the concentration of UDCA in the bile acid pool increases from 3% to 40%-60% over one week. Subsequent to the initiation of our randomised control study, evidence has shown the onset of the clinical effect of UDCA, a reduction in the frequency of episodes of biliary pain, begins a mean of 3.4 weeks after treatment commences. The assessment of the primary endpoint, the reduction in number of episodes of biliary pain, will begin 1 month after the beginning of treatment and continue over the next 11 months. With the changeover gap of 2 weeks this will provide an effective washout period of 6 weeks.

The results of this trial will not necessarily be applicable to all LPAC patients with biliary pain. Those with intact gallbladders with stones or macroscopic intrahepatic stones may have a different cause for their pain, including cholecystitis and cholangitis.

Conclusion

LPAC is an under diagnosed cause of post cholecystectomy pain. The results of this trial will provide level 1 evidence on the efficacy of UDCA treatment and will raise awareness of the importance of LPAC in the differential diagnosis of difficult to manage post cholecystectomy biliary pain.

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