







RESEARCH NOTE

REVISED **Differential response of the liver to bile acid treatment in a mouse model of Niemann-Pick disease type C [version 2; referees: 3 approved]**

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Abstract

Niemann-Pick disease type C (NPC) disease is a neurodegenerative lysosomal storage disease caused by mutations in the *NPC1* or *NPC2* genes. Liver disease is also a common feature of NPC that can present as cholestatic jaundice in the neonatal period. Liver enzymes can remain elevated above the normal range in some patients as they age.






We recently reported suppression of the P450 detoxification system in a mouse model of NPC disease and also in post-mortem liver from NPC patients. We demonstrated the ability of the hydrophobic bile acid ursodeoxycholic acid (UDCA) (3 α , 7 β -dihydroxy-5 β -cholanolic acid) to correct the P450 system suppression. UDCA is used to treat several cholestatic disorders and was tested in NPC due to the P450 system being regulated by bile acids. Here, we compare the effect of UDCA and cholic acid (CA), another bile acid, in the NPC mouse model. We observed unexpected hepatotoxicity in response to CA treatment of NPC mice. No such hepatotoxicity was associated with UDCA treatment. These results suggest that CA treatment is contraindicated in NPC patients, whilst supporting the use of UDCA as an adjunctive therapy in NPC patients.


Keywords

Niemann Pick disease type C, NPC1, lysosomal storage disease, p450, bile acids, ursodeoxycholic acid, cholic acid, liver

Open Peer Review

Referee Status: 

	Invited Referees		
	1	2	3
version 2 published 04 Apr 2018		 report	
			
version 1 published 31 Aug 2017	 report	 report	 report

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- Stephen D Turley**, University of Texas, USA
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Federal University of Rio Grande do Sul, Brazil
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Author roles: **Nicoli ER:** Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Validation, Writing – Original Draft Preparation; **Huebecker M:** Formal Analysis, Investigation, Methodology, Visualization, Writing – Review & Editing; **Smith D:** Formal Analysis, Investigation, Methodology; **Morris L:** Formal Analysis, Investigation, Methodology; **Platt FM:** Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: FP is a co-founder and consultant to IntraBio.

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REVISED Amendments from Version 1

This updated version of our article has been edited according to the comments of the reviewer. [Figure 1](#) now contains quantification of cholesterol levels in the livers of mice treated with UDCA or CA. These data were generated by Mylene Huebecker who has therefore been added as a co-author. The text of the article has been edited to acknowledge other potential treatment modalities for the hepatic dysfunction in NPC, and to stress that this short paper should be considered in parallel to the accompanying paper relating to the effect of UDCA and CA in NPC patients.

See referee reports

Introduction

Niemann-Pick disease type C (NPC) is a rare progressive neurodegenerative lysosomal disorder, which affects 1:100,000 live births^{1,2}. In addition to the central nervous system, the liver also plays a role in this disease, either presenting in infancy as cholestatic jaundice or if these patients survive as elevated levels of liver enzymes suggestive of chronic liver dysfunction¹. Previous published studies of murine *Npc1*^{-/-} models identified other treatment modalities for hepatic dysfunction. These included the cholesterol absorption inhibitor, ezetimibe, and 2-hydroxypropyl- β -cyclodextrin (2HP β CD)³.

We recently discovered that the hepatic cytochrome P450 system is suppressed in the *Npc1* null mouse model (*Npc1*^{-/-}), in the feline model of NPC disease and in three NPC human liver samples (post-mortem)⁴. In NPC, the efflux of unesterified cholesterol from late endosomes/lysosomes to the endoplasmic reticulum (ER) is inhibited, leading to a shift in the spectrum of bile acids synthesized^{5,6}, which suppresses gene expression of the P450 system⁴. Treatment of *Npc1*^{-/-} mice with ursodeoxycholic acid (UDCA; 3 α , 7 β -dihydroxy-5 β -cholanic acid) rescued gene expression and P450 system enzyme activity⁴. In this study, we compared the effects of UDCA (soluble bile acid) and cholic acid (CA; hydrophobic bile acid) in the *Npc1*^{-/-} mouse and discovered hepatotoxic adverse effects on the liver in response to CA, but not to UDCA, suggesting UDCA is the bile acid of choice for clinical treatment of NPC patients.

Methods

Npc1 mouse model

Npc1 mutant (BALB/cNctr-*Npc1m1N1J*, *Npc1*^{-/-}) and control (*Npc1*^{+/+}) were generated from heterozygote breeding. Genotyping was performed as described⁷ and mixed sex groups analysed. All mice were maintained under a standard 12h light/12h dark cycle with water and food available *ad libitum*. All procedures were performed according to the Animals (Scientific Procedures) Act 1986 under a project license (PPL No. 30/2923) from the UK Home Office. Care was taken to minimise suffering through euthanizing the animals once liver enlargement was unambiguously detected by visual inspection (i.e. 3 weeks after initiation of treatment).

Bile acid supplementation

Npc1^{-/-} and *Npc1*^{+/+} mice (n = 9-17 per group) were fed either normal chow (RM1 maintenance diet; SDS, London, UK) or normal chow supplemented with UDCA (0.5%, w/w, Sigma-Aldrich) or CA (0.5%, w/w, Sigma-Aldrich) mixed with powdered diet. Treatment started at weaning (3 weeks of age) and mice were sacrificed at 6 weeks of age by intraperitoneal injection with an overdose of phenobarbital.

Quantification of cholesterol

Cholesterol was quantified using an Amplex Red Cholesterol Assay Kit (Thermo Fisher), according to manufacturer's instructions.

Histology

Animals were euthanized at 6 weeks of age and were transcardiac perfused with 4% paraformaldehyde (PFA); samples of liver were fixed in 4% PFA, dehydrated and processed to wax using standard procedures. The wax blocks were cut at 4- μ m-thick sections, mounted on SuperFrost Plus slides (Thermo Scientific, Waltham, MA, USA) and subjected to hematoxylin and eosin (H&E) staining by standard methods. Images were taken on a Nikon eclipse E200 microscope with a Leica digital camera system.

Statistical analysis

Two-way ANOVA with Tukey's multiple comparison was used to analyse the sets of data comparing *Npc1*^{+/+} and *Npc1*^{-/-} mice for [Figure 1 A and C](#). One-way ANOVA with Tukey's multiple comparison was used to analyse the data comparing the treatment groups in *Npc1*^{-/-} mice in [Figure 1D](#). Statistical analysis was performed with GraphPad Prism version 6.

Results

We investigated the effects of UDCA and CA treatment in the *Npc1*^{-/-} mouse model, relative to wild type (*Npc1*^{+/+}) mice. Mice were fed on a diet containing either 0.5% UDCA or CA from weaning (3 weeks of age). After 3 weeks of treatment there was gross abdominal distention observed in all CA treated mice, irrespective of genotype, but the degree of distension appeared greater in the *Npc1*^{-/-} mice. No distension of the abdomen was observed in UDCA treated *Npc1*^{-/-} and *Npc1*^{+/+} mice. On necropsy, it was apparent that the abdominal distension in the CA treated group was due to liver enlargement ([Figures 1A and B](#)), and there was a statistically significant increase of liver weight in the *Npc1*^{+/+} and *Npc1*^{-/-} relative to UDCA and untreated mice ($p < 0.0001$). No significant changes in liver volume were observed in the UDCA treated mice irrespective of genotype ([Figures 1A and B](#)). *Npc1*^{-/-} mice have a lower body weight than wild type control mice⁸, and so the ratio of liver/body weight was calculated ([Figure 1C](#)). The liver enlargement in response to CA as a function of body weight was greater in the *Npc1*^{-/-} than in the *Npc1*^{+/+} mice. The liver-to-body weight ratios were the same in untreated *Npc1*^{+/+} and *Npc1*^{-/-} mice and in the UDCA treated mice, irrespective of genotype. Bile acids have previously been shown to affect intestinal absorption of cholesterol, with UDCA and CA demonstrated to

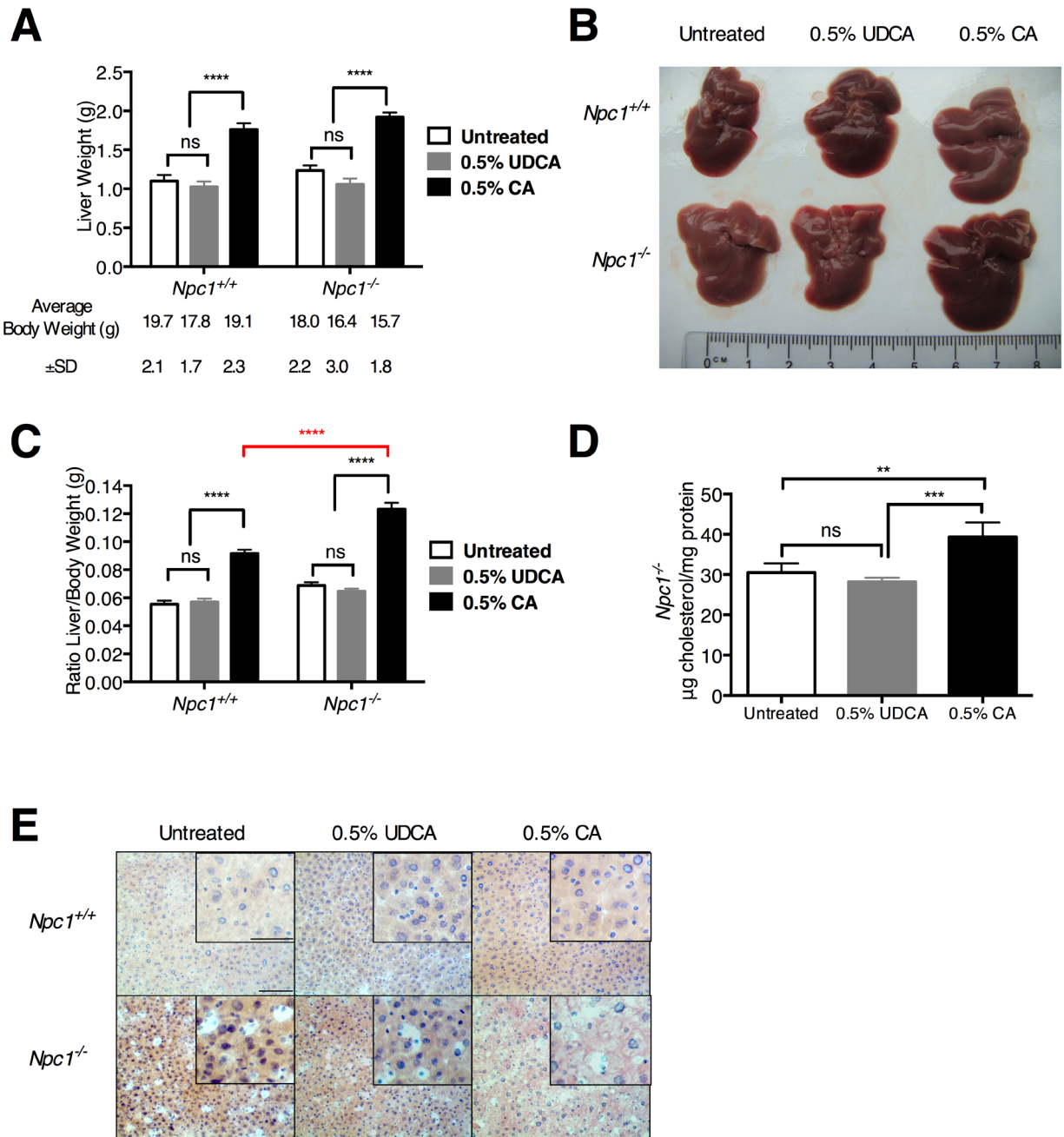


Figure 1. Effects of cholic acid (CA) and ursodeoxycholic acid (UDCA) on 6-week old *Npc1* mouse liver. *Npc1*^{+/+} and *Npc1*^{-/-} mice were treated with 0.5% UDCA or CA as admix with powdered diet from three weeks of age. The mice were euthanized at 6 weeks of age when the *Npc1*^{-/-} mice exhibited abdominal distension due to liver enlargement. **(A)** Liver weights of *Npc1*^{-/-} mice untreated, treated with 0.5% UDCA and 0.5% CA; average body weight ± SD of the mice are shown beneath each bar for each group. **(B)** Gross morphology of the liver. **(C)** Ratio of liver weight to mouse body weight. Data are presented as mean ± SEM, n = 9–17 animals per group/genotype, **** *p* < 0.0001 calculated using two-way ANOVA with Tukey's multiple comparison test. Red line indicates comparison between CA treated wild-type and *Npc1*^{-/-} mice. **(D)** Quantification of cholesterol levels in the liver of untreated, UDCA-treated and CA-treated *Npc1*^{-/-} mice. Values are adjusted for sample protein concentration. Data are presented as mean ± SEM, n = 3–4 livers per group, ** *p* < 0.01, *** *p* < 0.001 calculated using one-way ANOVA with Tukey's multiple comparison test. **(E)** H&E histopathology of liver sections, bars represent 10µm for high magnification inset panels and 5µm for the main panel.

decrease and increase absorption respectively⁹. Quantification of liver cholesterol levels revealed that cholesterol levels in the UDCA treated mice were unchanged relative to untreated *Npc1*^{-/-} ($p=0.4025$), whilst CA treatment was associated with a statistically significant ($p<0.01$) increase (29.2%) in liver cholesterol relative to the untreated group. (Figure 1D).

Histopathological analysis of liver (Figure 1E) showed the typical vacuolated appearance of the liver characteristic of *Npc1*^{-/-} mice relative to wild type mice. UDCA treatment had no impact on the histopathology of wild type and *Npc1*^{-/-} mice, whereas CA treatment led to hepatocyte enlargement and a foamy appearance indicative of lipid accumulation that often displaced the nucleus to the pole of the cell.

Discussion

NPC disease cells display a complex cellular pathophysiology, including impaired movement of LDL-derived cholesterol from late endosomes/lysosomes to the ER¹⁰ and the generation of non-enzymatically generated oxysterols¹¹. As a consequence, the bile acid profile is altered in NPC disease^{5,6}, and this change in bile acid composition leads to a secondary suppression of the P450 gene family, as they are bile acid regulated⁴. The expression of the P450 system genes could be rescued in *Npc1*^{-/-} mice by administering the bile acid UDCA, leading to clinical benefit⁴.

In another study (complementary to the murine work detailed here), UDCA was trialed in four clinical NPC cases with improvements in liver function (including reduced aspartate aminotransferase (AST) and alanine transaminase (ALT)) in those patients with elevated liver enzymes at baseline¹².

In this study, we investigated whether CA, another bile acid used in routine clinical practice for bile acid disorders¹³, would also be beneficial. Therefore, we treated *Npc1*^{-/-} mice with UDCA or CA to investigate this further. However, three weeks into treatment the CA treated mice presented with gross abdominal distension and were subsequently culled to determine the basis for this adverse finding. It was clear upon gross necropsy that the livers were significantly enlarged in CA treated wild type and *Npc1*^{-/-} mice. When adjusted for body weight it was apparent that the liver enlargement was greater in the *Npc1*^{-/-} mice relative to wild type mice. CA treated wild type mice had an increased liver volume (post-adjustment for body weight) of 165.5% relative to their untreated counterparts, whereas the CA treated *Npc1*^{-/-} mice exhibited a 179.1% increase in liver volume when compared with untreated *Npc1*^{-/-} mice.

Histopathology revealed lipid-laden distended hepatocytes in the CA treated *Npc1*^{-/-} mice. These data suggest CA is more hepatotoxic in mice than UDCA, consistent with the differential chemical properties of these two bile acids. The NPC1 protein, deficient in most cases of NPC disease, is a conserved member of a protein family, the RND permeases¹⁴. In bacterial systems they serve to efflux multiple classes of substrates across the periplasmic space, allowing bacteria to thrive in hostile environments¹⁵. CA's hydrophobic chemistry suggest it may be a potential substrate for NPC1¹⁵, so it may itself be stored in the late endocytic system in NPC1 deficient cells and adversely affect the acidic compartment of the cell leading to further lipid storage. This hypothesis merits further investigation.

When four NPC1 patients were treated with UDCA, benefit was observed based on improved liver function¹². Very interestingly, one patient was switched from UDCA to CA and their clinical status declined but was recovered upon switching back to UDCA. This has striking parallels with this murine study.

When considered in light of the accompanying clinical study these data strongly suggest that whereas UDCA has potential as an adjunctive therapy to treat residual liver disease in NPC patients, CA is contraindicated due to acute hepatotoxicity that is most pronounced in an NPC1 deficient background.

Data availability

Raw data have been uploaded to the online data repository OSF: <http://dx.doi.org/10.17605/OSF.IO/5A2F9>¹⁶

Competing interests

FP is a co-founder and consultant to IntraBio.

Grant information

This work was supported by the Wellcome Trust [202834] to FP; Action Medical Research to ERN; Parkinson's Disease UK to MH; Niemann-Pick Research Foundation to DS and LM; Royal Society Wolfson Research Merit Award to FP.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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[Data Source](#)

Open Peer Review

Current Referee Status:



Version 2

Referee Report 19 April 2018

doi:[10.21956/wellcomeopenres.15584.r32737](https://doi.org/10.21956/wellcomeopenres.15584.r32737)



Stephen D Turley

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It is misleading to say “---the liver also plays a role in this disease” . What should be said is that “--- although neurodegenerative disease is the principal cause of morbidity and mortality in NPC1 deficiency, pulmonary dysfunction or liver failure are the cause of death in subsets of NPC1 patients (add at least three supporting refs here). While 2-hydroxypropyl-beta-cyclodextrin is so far the most effective agent for managing neurodegeneration in NPC1 patients , in both mouse and feline models of NPC1 disease this compound has been shown also to significantly blunt or reverse hepatic unesterified cholesterol sequestration and dysfunction (add two ref here, these being Vite et al. in Science Translational Medicine Vol 7 Issue 276 276ra26, 2015 AND Ramirez et al. in Pediatric Research 68: 309-315 , 2010). Other agents evaluated in NPC1 mouse models suggest that ezetimibe, a potent cholesterol absorption inhibitor (Beltroy et al.--- Ref 3 cited in revised submission), and more recently the HDAC inhibitor, Vorinostat (Munkacsi et al. J Biol Chem 292:4395-4410,2017).

What the current study by Nicoli et al. is about is essentially to address the question of how changing the intestinal bile acid pool composition by feeding high levels of two different bile acids may affect liver cholesterol content and health in an NPC1 mouse model. Here it should be stated that, based on the definitive studies of Wang et al (cited in the revised submission), feeding cholic acid (which promotes cholesterol absorption and increases hepatic chol levels) versus ursodeoxycholic acid (which has the opposite effects) to an NPC1 mouse model would hypothetically have very different effects on hepatic cholesterol content and liver health in general. What is shown by Nicoli et al. is that when cholesterol absorption is impeded in the NPC1 mice by making ursodeoxycholic acid a major BA in their intestines, cholesterol absorption falls, just as it does with very low doses of ezetimibe in the diet of the NPC1 mouse. The opposite happens in NPC1 mice given cholic acid.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 12 October 2017

doi:[10.21956/wellcomeopenres.13461.r26337](https://doi.org/10.21956/wellcomeopenres.13461.r26337)



Roberto Giugliani  1,2,3

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³ Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil

This report is based in an interesting experiment, and the conclusions are supported by the data presented. The research problem was adequately addressed, and the paper is well written. The experiment was designed after the discovery that the hepatic cytochrome P450 system is suppressed in NPC animal models and also in NPC human liver (post-mortem analyses). As previously described, the inhibition of the efflux of unesterified cholesterol from the lysosomal system to the endoplasmic reticulum changes the spectrum of bile acids synthesized, and this eventually suppresses gene expression of the P450 system. It was already demonstrated that treatment of the NPC models with ursodeoxycholic acid (UCDA) rescued P450 activity. In this study, therapy with UCDA was compared with therapy with cholic acid (CA), being hepatotoxic adverse effects observed with CA but not with UCDA, which could be further explored as a potential adjunctive therapeutic agent in NPC.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 02 October 2017

doi:[10.21956/wellcomeopenres.13461.r26339](https://doi.org/10.21956/wellcomeopenres.13461.r26339)



Stephen D Turley

Southwestern Medical Center, University of Texas, Dallas, TX, USA

Although the rationale for this study is clearly explained, the relevance of the data obtained to the management of liver disease in NPC1 deficiency is questionable for reasons relating mainly but not fully to the methodological approaches that were used. The following points warrant close consideration.

1. Although the dietary level of BA supplementation employed is in line with what is often used in animal models, the changes in bile acid pool and composition that ensue when the amount of dietary BA is set at 0.5% w/w are beyond extreme and result in shifts in cholesterol metabolism at multiple levels that would never occur in humans taking approved doses of any specific BA. At a dietary level of 0.5%, the dose used in these studies was the equivalent of around 800 mg/day /kg bw which far exceeds what most human subjects taking oral BAs consume during a 24 hour period. When BA pool size and composition change in extreme ways there are invariably marked shifts in intestinal cholesterol absorption and this in turn has a major impact on the amount of chylomicron cholesterol reaching the liver from the small intestine (and therefore in the amounts of unesterified chol –UC—becoming subsequently trapped in the late /E/L compartment when NPC1 or NPC2 are mutated). There is an excellent study by Wang *et al.* (2003)¹ showing how a range of BAs including UDCA and CA (added to the diet at a level of 0.5%) affect intestinal cholesterol absorption in a normal mouse model (see Fig 3 in particular). While a BA pool enriched with UDCA drives down intestinal cholesterol absorption, the opposite effect occurs with CA. Another example illustrating how BA pool size and composition are major determinants of cholesterol absorption is seen in a study by Schwarz *et al.* (1998)² –see solid histograms for WT mice given hyodeoxycholic acid vs cholic acid in Fig 5. Anyone who knows about the properties of CA and who also understands the biochemical consequences of NPC1 deficiency would view the effects of CA described in the current report as “totally predictable” especially given the massive dose that was fed to the mice. It would have been better to use two different doses of UDCA.
2. The bottom line in all this is that the differing impacts on liver “health” of UDCA vs CA supplementation in NPC1^{-/-} mice shown here are likely fully explainable on the basis of major differences in the amount of intestinally derived cholesterol trapped in the livers of the KOs given these two particular BAs. Although no data are presented for hepatic total chol concs (mg/g), one might predict from other studies in the NPC1 mouse model that a several fold greater level of unesterified chol (UC) would be seen in the livers of the CA supplemented mice vs those given UDCA. There is no question that it is the cytotoxic effects of the entrapped UC in all organs, especially the liver, that has the most damaging impact in NPC1 (or NPC2) dysfunction.

While it is recognized that this is a preliminary report, it is essential that more informed conclusions be reached about what UDCA supplementation might conceivably be doing in the human NPC1-deficient liver. To achieve this, definitely the liver UC and EC concentrations in these mice, along with their plasma/serum ALT and AST levels should be measured. Such measurements are needed if these mouse model experiments are to provide any credible guide as to how UDCA supplementation in human NPC1 patients might be acting. The benefit most likely has nothing to do with putative improvement in functionality of the P450 detoxification system.

3. As it is currently written, this report will likely attract criticism because of its lack of acknowledgement of significant published work documenting the beneficial impact of other types of treatment modalities for improving liver function in NPC1 deficiency, mostly based on *in vivo* experiments using primarily the NPC1^{-/-} mouse model. In 2007 Beltroy *et al.* showed that the cholesterol absorption inhibitor, ezetimibe, given in the diet from the time of weaning, at doses far below those of other treatments (ie 20 or less mg /day/kg bw), reduced hepatomegaly and lowered liver cholesterol content (nearly all UC) by almost 50% (Fig 4)³. The benefit of ezetimibe in this disease is largely in the liver. After that, a series of definitive publications from a number of labs

attesting the potency of subcutaneously administered 2HPBCD in diminishing hepatic UC content and markedly improving liver function tests (and increasing lifespan) in NPC1 mouse models helped establish this agent as one of great potential for managing multisystem disease in NPC1 deficiency^{4,5}.

Although this preliminary report will be of interest to many working in the field of NPCD management, it should not generate the impression that UDCA has a clear potential to become a treatment modality for liver disease in NPCD patients. Citation of the earlier papers noted above documenting the decisive benefits of ezetimibe and 2HPBCD in NPC1 mice will allow readers to gain a better perspective of what these initial data in the UDCA treated NPC1 mouse model are telling us.

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Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Referee Expertise: Control of cholesterol metabolism in the liver and other major organs in lysosomal cholesterol storage diseases resulting from mutations in the LIPA, NPC1 or NPC2 genes

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 22 Mar 2018

Fran Platt, University of Oxford, UK

Although the rationale for this study is clearly explained, the relevance of the data obtained to the management of liver disease in NPC1 deficiency is questionable for reasons relating mainly but not fully to the methodological approaches that were used. The following points warrant close consideration.

1. Although the dietary level of BA supplementation employed is in line with what is often used in animal models, the changes in bile acid pool and composition that ensue when the amount of dietary BA is set at 0.5% w/w are beyond extreme and result in shifts in cholesterol metabolism at multiple levels that would never occur in humans taking approved doses of any specific BA. At a dietary level of 0.5%, the dose used in these studies was the equivalent of around 800 mg/day /kg bw which far exceeds what most human subjects taking oral BAs consume during a 24 hour period. When BA pool size and composition change in extreme ways there are invariably marked shifts in intestinal cholesterol absorption and this in turn has a major impact on the amount of chylomicron cholesterol reaching the liver from the small intestine (and therefore in the amounts of unesterified chol-UC—becoming subsequently trapped in the late /E/L compartment when NPC1 or NPC2 are mutated). There is an excellent study by Wang *et al.* (2003)¹ showing how a range of BAs including UDCA and CA (added to the diet at a level of 0.5%) affect intestinal cholesterol absorption in a normal mouse model (see Fig 3 in particular). While a BA pool enriched with UDCA drives down intestinal cholesterol absorption, the opposite effect occurs with CA. Another example illustrating how BA pool size and composition are major determinants of cholesterol absorption is seen in a study by Schwarz *et al.* (1998)²—see solid histograms for WT mice given hyodeoxycholic acid vs cholic acid in Fig 5. Anyone who knows about the properties of CA and who also understands the biochemical consequences of NPC1 deficiency would view the effects of CA described in the current report as “totally predictable” especially given the massive dose that was fed to the mice. It would have been better to use two different doses of UDCA.

We thank the reviewer for their comments. We have dealt with comment 1 & 2 together, with our response given below.

1. The bottom line in all this is that the differing impacts on liver “health” of UDCA vs CA supplementation in NPC1^{-/-} mice shown here are likely fully explainable on the basis of major differences in the amount of intestinally derived cholesterol trapped in the livers of the KOs given these two particular BAs. Although no data are presented for hepatic total chol concs (mg/g), one might predict from other studies in the NPC1 mouse model that a several fold greater level of unesterified chol (UC) would be seen in the livers of the CA supplemented mice vs those given UDCA. There is no question that it is the cytotoxic effects of the entrapped UC in all organs, especially the liver, that has the most damaging impact in NPC1 (or NPC2) dysfunction. While it is recognized that this is a preliminary report, it is essential that more informed conclusions be reached about what UDCA supplementation might conceivably be doing in the human NPC1-deficient liver. To achieve this, definitely the liver UC and EC concentrations in these mice, along with their

plasma/serum ALT and AST levels should be measured. Such measurements are needed if these mouse model experiments are to provide any credible guide as to how UDCA supplementation in human NPC1 patients might be acting. The benefit most likely has nothing to do with putative improvement in functionality of the P450 detoxification system.

We thank the reviewer for their helpful comments. With regards to a more thorough investigation of liver function we refer the reviewer to a publication (to which this mouse study acts as a supplement) that investigates the effect of UDCA in a small number of NPC patients. The Evans et al clinical case report investigated the effects of UDCA on liver function in NPC patients¹.

Treatment with UDCA (at between 10 and 30mg/kg/day) was associated with a marked improvement in liver function parameters (including aspartate aminotransferase (AST) and alanine transaminase (ALT)) in patients with elevated liver enzyme levels at baseline. We judged that the existence of human data demonstrating the benefit of therapeutic doses of UDCA on NPC patient liver function obviated the need for extended murine studies. The separation of murine and human studies into separate publications was an editorial requirement of Wellcome Open Research. However, the two studies should be considered together.

Importantly, this NPC patient case study (Evans et al) also suggested that cholic acid is deleterious in NPC patients¹. One of the patients detailed in the case study was commenced on UDCA from 4 weeks of age due to cholestatic jaundice of uncertain aetiology. A bile acid profile at 2 months of age indicated a potential bile acid synthesis defect, and cholic acid treatment was initiated (at 4.5 months) with the aim of suppressing bile acid synthesis. CA treatment was associated with elevations in aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase, bilirubin and total cholesterol levels. Upon discontinuation of CA and resumption of UDCA treatment there was quantitative improvement in multiple parameters of liver function.

*We have additional data demonstrating that the beneficial effect of UDCA is not due to effects on liver cholesterol levels. There is no significant difference in the levels of liver cholesterol in the UDCA-treated *Npc1*^{-/-} mouse (relative to untreated *Npc1*^{-/-} mouse, new Figure 1D in the revised manuscript). Whilst CA treatment is associated with a statistically significant elevation in *Npc1*^{-/-} liver cholesterol levels (relative to UDCA-treated *Npc1*^{-/-}) the extent of this elevation is less than 1.5 fold. These data have been added to the paper, and the text of the results section expanded accordingly.*

*This study should also be considered in light of our previous published study which fully characterised the beneficial effect of UDCA treatment on body weight, motor function, tremor and was consistent with restoration of the P450 system in *Npc1*^{-/-} mice mediating the therapeutic benefit².*

1. Evans, W. R. H., Nicoli, E.-R., Wang, R. Y., Movsesyan, N. & Platt, F. M. Case Report: Ursodeoxycholic acid treatment in Niemann-Pick disease type C; clinical experience in four cases. *Wellcome Open Res.* **2**, 75 (2017).
2. Nicoli, E.-R. et al. Defective Cytochrome P450-Catalysed Drug Metabolism in Niemann-Pick Type C Disease. *PLOS ONE* **11**, e0152007 (2016).

3. As it is currently written, this report will likely attract criticism because of its lack of acknowledgement of significant published work documenting the beneficial impact of other types of treatment modalities for improving liver function in NPC1 deficiency, mostly based on *in vivo* experiments using primarily the NPC1^{-/-} mouse model. In 2007 Beltroy *et al.* showed that the cholesterol absorption inhibitor, ezetimibe, given in the diet from the time of weaning, at doses far below those of other treatments (ie 20 or less mg /day/kg bw), reduced hepatomegaly and lowered liver cholesterol content (nearly all UC) by almost 50% (Fig 4)³. The benefit of ezetimibe in this disease is largely in the liver. After that, a series of definitive publications from a number of labs attesting the potency of subcutaneously administered 2HPBCD in diminishing hepatic UC content and markedly improving liver function tests (and increasing lifespan) in NPC1 mouse models helped establish this agent as one of great potential for managing multisystem disease in NPC1 deficiency^{4,5}.

Although this preliminary report will be of interest to many working in the field of NPCD management, it should not generate the impression that UDCA has a clear potential to become a treatment modality for liver disease in NPCD patients. Citation of the earlier papers noted above documenting the decisive benefits of ezetimibe and 2HPBCD in NPC1 mice will allow readers to gain a better perspective of what these initial data in the UDCA treated NPC1 mouse model are telling us.

We thank the reviewer for their comments. We agree that it would be beneficial to acknowledge the published work relating to other types of treatment modalities for hepatic dysfunction in NPC. We have amended the text of the paper accordingly. The intended purpose of this study was to demonstrate that there is a differential response of the Npc1^{-/-} mouse to treatment with UDCA and CA, despite both bile acids being widely used in the treatment of cholestatic disorders. CA treatment was deleterious in both Npc1^{-/-} mice and NPC patients¹ and hence is contraindicated for NPC1^{-/-}.

We would suggest, based on observed benefits to hepatic and neurological function (from this publication, the accompanying publication relating to NPC patients and previously published data with Npc1^{-/-} mice), that UDCA has the potential to be a valuable addition to combination therapy for NPC^{1,2}.

1. Evans, W. R. H., Nicoli, E.-R., Wang, R. Y., Movsesyan, N. & Platt, F. M. Case Report: Ursodeoxycholic acid treatment in Niemann-Pick disease type C; clinical experience in four cases. *Wellcome Open Res.* **2**, 75 (2017).
2. Nicoli, E.-R. *et al.* Defective Cytochrome P450-Catalysed Drug Metabolism in Niemann-Pick Type C Disease. *PLOS ONE* **11**, e0152007 (2016).

Competing Interests: No competing interests were disclosed.

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The article by Nicoli *et al* entitled “**Differential response of the liver to bile acid treatment in a mouse model of Niemann-Pick disease type C**” reports the effect of bile acid treatment on the liver pathology in a mouse model of NPC disease. Although, NPC is primarily considered a neurodegenerative disease, defects in systemic organs including liver have been seen in many patients. The authors previously reported suppression of cytochrome P450 detoxification system in the liver of NPC mice and limited set of human samples. Cytochrome P450 enzymes are also involved in the synthesis of bile acids from cholesterol. The authors hypothesize that exogenously supplied bile acid could potentially alleviate liver disease in NPC. In the study, they used a mouse model to test the hypothesis. The study comes from a lab that holds expertise in NPC and actively advancing the NPC research since last many years. The study was elegantly designed, results are clearly presented and the manuscript is well written and discussed. The conclusions drawn are based on the results reported. The manuscript is suitable to be published in the current form, however if the authors addressed the following comments, it would further enhance the quality of this publication.

1. In NPC, many systemic organs including liver show elevated inflammation. One would expect decreased cholesterol burden after UDCA treatment would reduce infiltration of inflammatory cells such as macrophages and neutrophils. It would be nice to have results of a couple of inflammatory marker analysis included in the liver of UDCA treated mice. In addition, it would also be interesting to see how liver enzymes (such as ALT & AST) in mice respond to UDCA treatment.
2. In a separate manuscript (submitted for peer review, ref #10), the authors report the clinical data of four NPC patients treated with bile acid. The results presented in ref#10 show significant improvement in the liver function in UDCA-treated patients. The submission is a useful extension of this study and shows how findings in an animal model translate to clinics. I would have preferred if both manuscripts were combined. This would allow readers to have a better understanding of the effect of bile acid treatment in NPC in the mouse model as well as in clinics without going through two separate publications. Nevertheless, this manuscript on its own reports interesting findings.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
