A randomised pilot trial of a steroid-free immunosuppressant regimen in paediatric liver transplantation.

Short Title: A pilot trial of paediatric liver transplantation without steroids.

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Sponsor – Birmingham Children's Hospital NHS Foundation Trust

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1. ABSTRACT

Objectives.

The overall objectives were to investigate whether a steroid free immunosuppressive regimen is as safe and effective as a steroid containing regimen following paediatric liver transplantation and whether it promotes tolerance.

Methodology.

Open label randomised controlled pilot trial.

Subjects.

14 children undergoing liver transplantation at Birmingham Children's Hospital.

Study protocol.

Children were randomised to receive one of two immunosuppression regimens which were identical except for the presence of steroid treatment.

Outcome measures.

Primary endpoint: The development of histologically proven acute rejection.

Secondary endpoints:

1. The development of steroid resistant acute rejection
2. The expression of tissue and circulating markers of immune tolerance in first year post transplant
3. The incidence of infection in the first year post transplant

Results

Survival free of rejection was higher in the steroid free group, but this was not statistically significant; 75% (95% CI 44.4 – 100) in the steroid free group compared to 33.3% (95% CI 0-71.7) in the steroid containing group (P = 0.24).

There was no difference in the incidence of infections or adverse reactions between the 2 groups.

Conclusion.

Steroid free immunosuppression is feasible in paediatric liver transplantation, but larger studies will be necessary to demonstrate a definitive benefit.

2. BACKGROUND

2.1 Treatment

Corticosteroid treatment has been an almost universal component of immunosuppressive regimens since the introduction of solid organ transplantation. This continues to be the case in paediatric liver transplantation. The side effect profile of corticosteroid treatment is well known and includes hypertension, obesity, growth impairment, hyperlipidamia and osteopenia. There is uniform agreement that the corticosteroid dose should be minimised and if possible discontinued. This has led to the concept of steroid free transplantation. [1;2;3;4;5] The realisation of this concept has been facilitated by the introduction of new potent immunosuppressants and particularly the class of IL2 receptor inhibitors. The rationale for steroid avoidance as opposed to withdrawal is that in addition to avoidance of steroid side effects steroid avoidance may

(a) maintain steroid sensitivity, so that if rejection does occur steroid boluses are more likely to be effective, and
(b) promote long term tolerance induction which is an important end point following paediatric solid organ transplantation.

Despite marked improvements in transplant immunobiology, tolerance is still not reliably achieved. Recent studies have shown that active cellular regulation is required to modulate immune responses and induce or maintain tolerance. In particular, regulatory T cells (Treg) have emerged as major elements of the apparatus that controls immunity and tolerance. Among the regulatory T cells that have been described are anergic CD4+CD25+ Treg.[6] These cells express the Foxp3 transcription factor, which is to date the most specific marker for cells that possess the Treg suppressive function. Their expression and cytokine profile can be measured in circulating lymphocytes or in liver biopsy tissue.[6;7]

In addition assays of lymphocyte function and cytokine expression have recently been developed that allow serial measurements of donor-specific immune responsiveness and global functional immunosuppression.[8] The effect of steroid avoidance on the expression of tolerance markers and on donor-specific responsiveness have not been described.

2.2 Research question

Is it possible to avoid the use of corticosteroids in paediatric liver transplantation?

2.3 Trial objectives

This was a pilot study to

(i) investigate to what degree a steroid free immunosuppressive regimen is as safe and effective as a steroid containing regimen following paediatric liver transplantation, and
(ii) investigate the effect of a steroid free immunosuppressive regimen on lymphocyte function and donor-specific immune responsiveness following paediatric liver transplantation
(iii) investigate the effect of a steroid free immunosuppressive regimen on expression of tissue markers of tolerance following paediatric liver transplantation

2.4 Investigational medicinal product

Intravenous methylprednisolone, intravenous hydrocortisone and oral prednisolone.

3. TRIAL DESIGN

3.1 Introduction

Design: Open label randomised controlled pilot trial.

Planned population to be studied: 30 children undergoing primary, isolated liver transplantation at Birmingham Children's Hospital.

Current therapy: For both groups this was according to the contemporary liver transplant protocol (see Appendix 1a and 1b) in use at Birmingham Children's Hospital with the exception of steroid treatment. One group received the current steroid regimen and the other group only received steroids if they develop rejection

3.2 Inclusion criteria

Children undergoing primary isolated hepatic transplantation.

Age <=18 years

Ability to provide informed consent

3.3 Exclusion criteria

- Children undergoing retransplantation.
- Transplantation for Intestinal failure associated liver disease.
- Multi-organ transplantation.
- Transplantation for autoimmune liver disease.
- Transplantation for extra hepatic malignancy.
- Pre-existing need for oral steroids, or high dose inhaled steroids sufficient to require a steroid warning card.

3.4 The Research Setting

The research was carried out in Birmingham Children's Hospital Ward 8, ITU and the Hepatology outpatient department at Birmingham children's Hospital NHS trust.

Figure 1 Study flow chart

Actions	When planning transplant assessment	At transplant assessment	Day of transplant	Weekly while in patient	One month post transplant	Two months post transplant	Three months post transplant (+/-14 days)	Six months post transplant (+/-14 days)	One year post transplant **(End of study)** (+/-28 days)
Study information to families	X								
Informed consent		X							
Randomisation			x						
Demographic data			x						
Clinical and laboratory assessment		X	x	x	x	x	X	x	x
Adverse events				x	x	x	X	x	x
Lymphocyte function and cytokine expression		X		x	x	x	X	x	x
Liver biopsy									x

3.5 Recruitment

Ethical approval was granted by South Birmingham National Research Ethics committee. Participants were identified from among children being considered for primary, isolated liver transplantation at Birmingham Children's Hospital.

Children and their families were approached about the study when an admission for liver transplantation was considered. Information booklets were provided at that time.

Recruitment occurred after the inpatient transplant assessment at a time when children had already been accepted onto the waiting list. This was usually some weeks after the initial approach. Where children and their families agreed, informed consent was obtained while waiting for liver transplantation. Randomisation was done at the time of transplantation**.**

3.6 Patient/ guardian and carer information leaflet

The conduct of the trial was in accordance with the Principles and Conditions of Good Clinical Practice. The parent's written informed consent to participate in the trial and the child's informed consent or assent as appropriate given the child's competence were obtained before randomisation and after a full explanation had been given of the treatment options and the manner of treatment allocation.

Information leaflets appropriate for older children and young people who were competent to give informed consent, for younger children and for parents were used where appropriate. The patient's GP/ shared care consultant were notified, with the parent's consent.

3.7 Randomisation

Randomisation to either group was by block randomisation of 2 per block. The randomisation sequence was predetermined using SAMPSIZE V2 software and held in sealed envelopes. Envelopes were available to the transplant coordinating team and were opened at the time of transplantation. If the transplant did not occur on that occasion this same allocation was used for that subject subsequently.

3.8 Follow-up and outcome measures

Primary endpoint: The development of histologically proven acute rejection within 12 months.

Secondary endpoints:

1.The development of steroid resistant acute rejection 12 months
2. The expression of tissue and circulating markers of immune tolerance in first year post transplant
3. The incidence of infection in the first year post transplant.

Demographic data.

Date of birth, sex, height, weight, blood group and indication for Transplantation.

Clinical and laboratory assessments.

Anthropometric and routine clincal data and including any abnormal/unexpected finding on clinical examination. e.g Jaundice, Skin lesion, Lymphadenopathy, organomegaly

Laboratory tests; the result of serum bilirubin, hepatic transaminases (ALT/AST), alkaline phosphatase, GGT, albumin, creatinine, haemoglobin, white cell count, platelet count and Prothrombin time.

Assessment of lymphocyte function and donor-specific tolerance.

Serial blood samples will be analysed for lymphocyte phenotype, function and cytokine secretion. Blood were taken at the time of routine venepuncture during the first 12 months post transplantation and frozen at -70^0C prior to analysis at University of Birmingham..

Post transplantation monitoring.

This was according to the existing paediatric liver transplantation protocol. Suspected rejection was confirmed histologically.

Indications for liver biopsy include:

(i) In the first week post transplantation where AST or ALT are elevated to more than 20% above the previous days levels for 48 hours or to more than 40% above the previous days level at any time, without explanation

(ii) After the first week where AST, ALT and/or GGT show a rise to more than 20% above the upper limit of normal for 48 hours or to more than 40% above upper limit of normal at any time, without obvious explanation.

(iii) Unexplained post transplant fever (>38^0) or unexplained persistently abnormal biochemical liver function tests for more than 72 hours.

Liver biopsy assesssment.

This was reported as in normal clinical practice. Acute rejection was defined using established histological criteria—a combination of portal lymphocytic infiltrates, endothelialitis and bile-duct injury.

Diagnostic criteria

As per published BANF criteria[15].

Treatment for rejection: Methyl Prednisolone 15 mgs/kg IV daily for 3 days (maximum daily dose 400mg). If complete clinical and biochemical response occurs all subjects will be returned to scheduled treatment.

Steroid resistant rejection: No improvement in any of the laboratory or histological criteria after one course of steroids as above.

Subject censoring:

Subjects were censored at 1 year post transplantation or at;

Development of steroid resistant rejection,

Development of recurrent or chronic rejection,

Development of treatment related side effects requiring discontinuation of any agent

Death.

3.9 Other medication allowed

This was as included in the contemporaneous protocol for paediatric liver transplantation at Birmingham Children's Hospital (appendix 1a and 1b). Concomitant medication was any other medication prescribed which is not included in this protocol.

3.10 Statistical Analysis

Subject data was expressed as percentage or median (range) as appropriate. Baseline characteristics of the groups were compared using student's t test or Fisher exact tests as appropriate. Kaplan-Meier analysis was used for survival time free of acute rejection. Differences between groups will be compared by the log rank test and/or Cox regression.

Two recent studies using a similar protocol to Group 1` reported 12 months acute rejection free survival of 50%.[13;14] If there was no true difference between the groups in rejection free survival there was a 60% chance that the apparent difference would not exceed 16% using groups of 15 cases. This seemed acceptable precision in a pilot study. It was thought likely that 30 suitable cases could be recruited over a 2 year period.

4. RESULTS

Recruitment.

Subject recruitment commenced in May 2008 prior to the formal commencement of the study in December 2008. Recruitment was discontinued prematurely in August 2010 because of lack of availability of Daclizumab. The final subject underwent transplantation in July 2010 and the study was formally closed when one year follow-up on this subject was completed on 15th July 2011.

26 children and their families were approached and provided with information on the study. 20 of these consented to enrolment in the study of whom 15 were randomised. Five children were not randomised because they underwent transplant either before December 2008 (3) or after August 2010 (2).
Seven children were randomised to a steroid containing regimen and 8 to a steroid free regimen. One child, who had been randomised to a steroid containing regimen, developed primary non-function of the transplanted organ and died on the fourth post-operative day due to multiorgan failure. This was not related to the immunosuppressive regime or rejection and this case was excluded from further analysis. Two further children developed graft primary non-function requiring retransplantation after three and five days respectively. One of these children was randomised to each of the immunosuppression groups and in neither case was the non-function related to rejection or the immunosuppressive regimen. In both cases the allocated regime was continued after the second transplant. In total 14 children were included in the final analysis, six of whom had received a steroid containing regimen and eight a steroid free regimen. Baseline demographic features are shown in table 1.

Incidence of rejection.

In total six children (37.5%) developed 12 episodes of acute rejection at median 53 days (15 to 365) post transplant. Two children (25%) receiving steroid free regimen each had a single episode of acute rejection compared to four children (66.6%) receiving a steroid containing regimen who had in total eight episodes.
All but one were treated with corticosteroids with six showing a complete response. One child, receiving a steroid free regimen, developed histologically proven acute rejection. By the time the result became available his biochemistry was improving and no extra treatment was given. The other child receiving steroid free regimen who developed rejection was treated successfully with corticosteroids for three days only. One child, who was receiving a steroid containing regimen, developed steroid resistant rejection at 35 days post transplantation. He responded to a combination of high-dose steroids and the introduction of sirolimus.

Survival free of rejection was higher in the steroid free group, but this was not statistically significant; 75% (95% CI 44.4 – 100) in the steroid free group compared to 33.3% (95% CI 0-71.7) in the steroid containing group (P = 0.24).

Microbiologically confirmed infections.

Nine children (6/8 receiving steroid free regimen and 3/6 receiving steroid containing regimen) had a total of 14 microbiologically confirmed infections at a median of 32.5 days (1-365) post transplantation. Eight were bacterial, five viral (1 each respiratory syncytial virus, rotavirus, varciella, parainfluenza and Epstein Barr virus) and 1 fungal.

Outcome after one year.

All randomised children are alive and well. Biochemical tests of liver function were normal in 7, slightly abnormal (< twice upper limit of normal) in 5 and significantly abnormal in 2. In one case this was due to persistent rejection and in the other it was a transient abnormality, presumably due to an intercurrent illness.

13 children underwent a liver biopsy close to one year after transplantation. In one child this was not undertaken because of persistent biliary dilatation. In 10 cases the biopsy was normal or showed minor post transplant changes. The child with previous steroid resistant rejection showed signs of persistent acute rejection. His immunosuppression was increased. Two children showed evidence of chronic hepatitis of the allograft, one in each study arm. The child receiving corticosteroids was successfully managed by introducing azathioprine. The child randomised to receive no steroids who developed chronic hepatitis of the allograft was treated successfully with low-dose prednisolone (0.1 mg/kg/d).

For the 10 children who had a formal Chromium EDTA measure of glomerular filtrate rate (GFR) pre and post transplant GFR were 125 and 112 ml/min/.73m^2 respectively, with no difference between the groups.

Current immunosupression.

At last follow up immunosuppression had been modified in 5 cases. Tacrolimus had been discontinued in 2 cases; in one case cyclosporine was substituted because of cholestasis and in another Sirolimus was introduced because of severe rejection. Mycophenolate had been discontinued in 3 cases; once because of the introduction of Sirolimus and in 2 cases because of diarrhoea. All children randomised to steroid containing regimen remained on low dose prednisolone. One case randomised to a steroid free regimen was commenced on low dose prednisolone while the remaining 7 do not receive steroids.

Markers of immune tolerance.

These results are not yet available and will be reported at a later time.

Adverse events.

There were no suspected unexpected serious adverse reactions. A total of 286 adverse reactions were reported of which 73 were deemed serious (Table 2). These were all within the expected effects of transplantation and there were no significant differences in numbers or types of adverse reactions between the study groups. Two adverse affects were provisionally attributed to the investigational medical product; one case of de novo diabetes requiring insulin treatment and an episode of gastrointestinal bleeding which resolved spontaneously without requiring transfusion.
Other adverse events attributed to the immunosuppressive regimen included two cases of prolonged diarrhoea which resolved on discontinuation of Mycophenolate and persistent cholestasis which resolved following substitution of Tacrolimus with Cyclosporin.

Discussion.

This small pilot study confirms that using steroid free immunosuppression is feasible for paediatric liver transplantation. The group randomised to the steroid free regimen had a lower rate of rejection and only one of eight children required the subsequent introduction of longterm corticosteroids. There were no obvious differences in infection rates or adverse event profiles between the groups.

Given that the toxicities of corticosteroid treatment in young children are well established, the advantages of steroid free immunosuppression are self evident if it is safe. This study suggests that the incidence of acute rejection is low and that where it does occur treatment appears straightforward. Another potential concern with a steroid free regimen is the development of chronic hepatitis of the allograft, which appears to be a variant of rejection[16]. It is reassuring to see that after 1 year the incidence of chronic allograft hepatitis is low in both groups, although long term graft surveillance will be necessary to address this issue.

Unfortunately the study had to be discontinued prematurely and hence was too small to demonstrate a statistically significant difference between the groups or to be definitive. However this data could be used to plan a definitive study of steroid free immunosuppression in paediatric liver transplantation. Such a study would need to be mutlicentric and probably multinational.

This study demonstrates some of the practical challenges of undertaking clinical research in areas of high dependency such as paediatric liver transplantation since the introduction of the EU Clinical Trials Directive 2001/20/EC. Adverse events are a frequent occurrence due to the nature of the clinical setting and may not necessarily be directly related to the investigational medical product. Keeping adequate research records and ensuring sufficient monitoring is both essential and resource demanding.

In conclusion steroid free immunosuppression is feasible in paediatric liver transplantation.

Trial registration data.

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EudraCT	2007-48222-06
MREC N°	07/H1207/262
UKCRN ID	5311

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The protocol is available at http://public.ukcrn.org.uk/search/

5. FINANCING

The trial was funded by an educational grant from Roche Products Limited. The funder had no input into the study analysis or into production of the final report.

6. REFERENCES

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Table 1. Demographic data.

	Steroid containing regimen (n=6)	Steroid free regimen (n=8)
Indication for transplantation	Biliary atresia 2 Progressive familial intrahepatic cholestasis 1 Glycogen storage disease type 1 non A 1 Cystic fibrosis liver disease 1 Tyrosinaemia 1	Biliary atresia 4 Progressive familial intrahepatic cholestasis 3 Primary hyperoxaluria 1
Sex	5 female 1 male	3 female 5 male
Age at transplantation	4 (0.5-17.5)	1.8 (0.5-12.1)
Weight at transplantation	17.8 (6.7-53)	15.5 (4.6-21)

Table 2. Adverse events.

Adverse event	Steroid containing	Steroid free
Hepatic		
Abnormal liver functions tests	25	19
Ascites	7	5
Bile leak	4	2
Vascular thrombosis	0	3
Primary non function	1	1
Gastroenterological		
Diarrhoea	16	20
Abdominal pain	5	7
Vomiting	5	6
Constipation	4	2
Stomal prolapsed	6	0
Infection related		
Fever	4	7
Lower respiratory tract infection	7	8
Upper respiratory tract infection	5	8
Intra abdominal infection	1	2
Wound infection	0	3
Bleeding	9	3
Hypertension	5	6
Hypertension	5	6
Metabolic acidosis	2	6

Appendices :

1A: Guidance for management of liver transplant Birmingham children's hospital.

1B. Shared care protocol following liver transplantation.


Guidance for management of trans


Shared care protocol appendix1b.pdf