Clinical Trial HHSC/001

EudraCT 2005-001222-83

Title: A phase I/II safety and tolerability dose escalation study following the autologous infusion of expanded adult haematopoietic cells to patients with liver insufficiency

End of Study Report

Introduction

A phase I study was performed to determine the safety and tolerability of injecting autologous CD34+ cells into five patients with liver insufficiency. The study was based on the hypothesis that the CD34+ cell population in granulocyte colony-stimulating factor (G-CSF)-mobilised blood contains a subpopulation of cells with the potential for regenerating damaged tissue.

We separated a candidate CD34+ stem cell population from the majority of the CD34+ cells (99%) by adherence to tissue culture plastic. The adherent and nonadherent CD34+ cells were distinct in morphology, immunophenotype, and gene expression profile. Reverse transcription-polymerase chain reaction based gene expression analysis indicated that the adherent CD34+ cells had the potential to express determinants consistent with liver, pancreas, heart, muscle, and nerve cell differentiation as well as haematopoiesis. Overall, the characteristics of the adherent CD34+ cells identify them as a separate putative stem/progenitor cell population. In culture, they produced a population of cells exhibiting diverse morphologies and expressing genes corresponding to multiple tissue types.

Study Rationale

The only current treatment for liver failure is liver transplantation. Unfortunately, very few patients are transplanted due to organ donation shortages. The result of this situation is that the majority of un-transplanted patients die as there is no effective liver support machine that is comparable to a renal dialysis machine. For this reason it is necessary to develop new therapeutic modalities for patients with chronic liver disease. The use of stem cell therapy is promising. We have performed a pilot feasibility study to assess the side effects and tolerability of injecting stem cells into the liver of five patients with liver disease, under a phase I safety and tolerability study at the Hammersmith Hospital. This phase I study demonstrated good tolerability and the safety of this approach. We propose to perform a phase I/II study where it is intended to administer incrementally increasing concentrations of stem cells in order to reach the maximum tolerated dose to assess the safety of dose escalation and then to assess the safety and efficacy of this treatment approach at the maximum tolerated dose.

STUDY OBJECTIVES

The aim of this trial is to determine the maximum tolerated dose of expanded CD34+ autologous stem cells when infused into either the hepatic artery or the portal vein. The trial will also seek to determine clinical improvement or deterioration by measurement of clinical parameters such as liver function tests.

STUDY ENDPOINTS

Primary endpoints

To assess the safety of ascending doses of autologous adult stem cells when introduced into either the hepatic artery or the portal vein and to determine the maximum tolerated dose of stem cells. The route of delivery is determined by radiological opinion.

Secondary endpoints

To assess improvement in liver function as measured by serological and biochemical analysis and determine whether there are any symptomatic improvements as reported by the patients.

Safety endpoints

Clinical - Safety will be evaluated in terms of adverse events graded according to CTC toxicity criteria and laboratory test results. All adverse events will also be graded for relationship to treatment and as expected and unexpected. They will be reported to the stem cell group, the appropriate Ethics Committee and regulatory bodies as required as well as an independent safety monitoring committee.
Laboratory - If laboratory results are significantly different from those of previous samples, further investigations may be required. Patients may be withdrawn from the study if significant deterioration occurs.

The study will be stopped if serious and unexpected adverse events occur that are believed to be treatment-related.

Study Procedure

Patients (4 male and one female; Table 1) with chronic liver failure who fitted the study inclusion/exclusion criteria were admitted to the ward and were given 520 µg granulocyte-colony stimulating factor by subcutaneous injection for five consecutive days to increase the number of circulating CD34+ cells. Leukapheresis was performed on Day 5. The leukapheresis product was transferred to the GCP laboratory where CD34+ cells were immunoselected using the CliniMacs device (Miltenyi Biotech). The CD34+ cells were then transferred to the patient via the hepatic artery (2 patients) or portal vein (3 patients) in the Imaging Department. Patients were discharged after overnight bed rest. Patients returned to the clinic on Days 7, 15, 30, 45 and 60 after the infusion for liver function tests, full blood count, coagulation profile and α-fetoprotein assay.

Results

All patients experienced thrombocytopaenia as expected after leukapheresis. Platelets returned to baseline within 1 week. Patients were given $1 \times 10^6 – 2 \times 10^8$ CD34+ cells as a single bolus. There were no mortalities or side effects except for mild pain and discomfort at the site of the injection (Table 2). In particular, there was no bleeding or infection or significant deterioration in liver function. Post injection CT scans showed no evidence of focal liver lesions and duplex Doppler ultrasound scans showed patent portal veins with

hepatopedal flow. Patient 1 showed initial improvement in serum bilirubin but this reverted to baseline by Day 60. Patient 2 had normalisation of bilirubin and serum albumin increased. Patient 3 demonstrated no change in liver function. Patient 4 experienced an initial improvement in liver function, but this was disrupted due to a severe urinary tract infection that necessitated hospitalisation and treatment with antibiotics. Patient 5 had a dramatic improvement in bilirubin and an increase in serum albumin and disappearance of ascites.

Table 1: **Aetiology of Patients**

Criteria		**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**
Age		42	48	41	55	61
Gender		Male	Male	Male	Female	Male
Diagnosis						
	Cirrhosis	Yes	Yes	Yes	Yes	Yes
	Alcoholic	Yes	Yes	Yes	Yes	No
	Hepatitis B	Yes	No	No	No	No
	Hepatitis C	No	No	No	Yes	No
Symptoms						
	Jaundice	No	Yes	No	Yes	Yes
	Fever	No	No	No	No	No
	Weight loss	No	No	No	Yes	No
	Fatigue	Yes	Yes	No	Yes	Yes
	Abdominal pain	No	Yes	No	No	Yes
	Constipation	No	No	No	No	No
	Loose stools	No	Yes	No	No	No

Table 2: **Injection routes and cell concentrations plus post injection observations**

Injection	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**
Site	Portal Vein	Portal Vein	Portal Vein	Hepatic Artery	Hepatic Artery
Number CD34+ cells	1×10^6	2×10^8	1×10^6	2×10^8	1×10^6
Post Injection					
Fever	No	No	No	No	Yes
Pain	No	Yes	Yes	Yes	Yes
Nausea	Yes	Yes	Yes	Yes	Yes
Vomiting	No	No	Yes	Yes	Yes
Jaundice	No	Yes	Yes	Yes	Yes
Ascites	No	Yes	No	Yes	Yes
Bleeding	No	No	No	No	No

Discussion

The study demonstrated the safety of administering G-CSF followed by leukapheresis and reinfusion of CD34+ cells in patients with liver insufficiency. It was important to note that

patients could respond to G-CSF treatment and that their white cell increased in all cases given that this was perquisite for the study protocol. Cell yields were lower than expected when compared with haematology patients. There were no cases of hepatorenal syndrome and injection of the CD34+ cells into the hepatic artery or portal vein did not result in any thrombotic episode or bleeding after the percutaneous procedure.

Overall the results were encouraging for the future development of stem cell therapy for patients with liver insufficiency.

Publications

Gordon MY, Levicar N, Pai M, Bachellier P, Dimarakis I, Al-Allaf F, M'Hamdi H, Thalji T, Welsh JP, Marley SB, et al. Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. Stem Cells **24**(7):1822-1830 Jul 2006

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