

SINGLE DOSE OF GRANULOCYTE COLONY STIMULATING FACTOR IN LEUKOPENIA AFTER POST-LIVER TRANSPLANT

ALI AKBAR SHAYESTEH¹, KAMRAN B LANKARANI², SEYED ALIREZA TAGHAVI³,
SEYED ALI MALEK-HOSSEINI⁴, PAYAM PEYMANI²

¹Research Institute for Infectious Disease of Digestive System, School of Medicine, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran. ²Health Policy Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran. ³Department of Internal Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. ⁴Department of Surgery and Organ Transplantation Research Centre, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran. Email: Peymanip@sums.ac.ir

Received: 06 May 2014, Revised and Accepted: 29 May 2014

ABSTRACT

Immunosuppressive therapies are the main treatment modalities after transplantation to prevent rejection. One of the major side effects of potent immunosuppressant is leukopenia. Granulocyte colony stimulating factor (G-CSF) is a cytokine agent which is increasing life span and functional activity of mature neutrophils. G-CSF is a well-established treatment of chemotherapy-induced leukopenia, during hematopoietic stem cell transplantation and HIV infection neutropenia. There were several studies in animal models of liver diseases which showed hematopoietic stem cell mobilization into the injured liver and their differentiation to hepatocytes. There were a few clinical trials in human after development of neutropenia in post liver transplantation (LTx) periods. This study was designed as a pilot study to evaluate the safety of G-CSF on leukopenia in early post LTx periods. Seventeen leukopenic patients in 4 weeks after LTx entered to the study and randomized in one to one manner in this open-label study. Treatment group received 0.3 mg of G-CSF (PD - G-CSF) at the time of leukopenia ($\leq 3000/\text{mm}^3$) and short-term patient's and graft survival were determined. Data are reported as mean, and all data were analyzed using Chi-square and Student's t-test. (SPSS of ware, version 14) $p < 0.05$ were considered significant. 9 patients in the control group and 8 patients recruited in the treatment group. There were no significant differences in days of hospital admission ($p: 0.244$), microbiologic active cultures ($p: 0.30$), Oropharyngeal candidiasis ($p: 0.30$), acute cellular rejection (ACR) ($p: 0.437$), and day of desirable mycophenolate mofetil dosage achievement ($p: 0.691$) and episodes of ACR treatments ($p: 0.08$). Our open-labeled pilot study shows that single dose of G-CSF in leukopenic post liver transplant patients is safe. Although there was no statistically significant beneficial effect on hospital stay, opportunistic, and surgical site infections, but there was a trend toward less ACR episodes in the treatment group. Whether single dosage has a beneficial effect on liver function, survival, rejection, and hospital stay needs further research in another clinical trial.

Keywords: Granulocyte colony stimulating factor, Leukopenia, Post liver transplant.

INTRODUCTION

Immunosuppressive therapies are the main treatment modalities after transplantation to prevent rejection. One of the major side effects of potent immunosuppressant is leukopenia which in turn may predispose to infections.

Granulocyte colony stimulating factor (G-CSF) is a cytokine agent, produced by monocytes, macrophages, endothelial cells, and fibroblasts under the influence of endotoxins, interleukin 1, and tumor necrosis factor. (Reference) G-CSF primarily acts on late myeloid progenitors and enhances their functions and productions by receptors located on immature and mature granulocytes, and to a lesser extent on monocytes and macrophages. The major effects are increasing life span and functional activity of mature neutrophils, but some anti-inflammatory effects are reported as well [1].

G-CSF is a well-established treatment of chemotherapy-induced leukopenia [1-3]. It is also used widely during hematopoietic stem cell transplantation and HIV infection neutropenia. Furthermore, perioperative management of elective surgical patients with G-CSF (Filgrastim) reinforces innate immunity [4], enabling better prevention of infection [5]. There were several studies in animal models of liver diseases which showed beneficial effects of G-CSF on liver tissue after liver transplant. It seems to be related to hematopoietic stem cell mobilization into the injured liver of rats after partial liver transplant and differentiation to hepatocytes through hepatic oval cells and cholangiocytes [6] with subsequent improvement in the survival [7]. In addition to progenitor cell expansion and mobilization, immune

modulatory properties were also described for G-CSF [8]. Yannaki *et al.* experiment in CCL4 induced liver injury in mice revealed that improvement of survival and liver histology by G-CSF may be due to activation of endogenous repair mechanisms by oval cells [4]. On the contrary, Dirsch *et al.* advised against the use of G-CSF due to possible impairment of perfusion that may consequently provoke ischemic-induced biliary complications [9].

There were few clinical trials of G-CSF after liver transplantation (LTx) in humans. Trindade *et al.* reported beneficial effect of GM-CSF after neutropenia in pediatric orthotopic liver transplantation (OLT) with sever bacterial infections [10]. Winston *et al.* showed prophylactic administration of G-CSF had not beneficial effects on infection, rejection, and survival in recipients despite producing a substantial increase in white blood cell (WBC) counts after transplantation [11]. Turgeon *et al.* also showed that G-CSF was well-tolerated, and the effects were appropriate especially in patients who were receiving ganciclovir for the treatment of cytomegalovirus (CMV) infection [12]. Ishizone *et al.* reported that administration of recombinant human G-SCF restored leukocytes counts without any significant adverse effect [13]. G-CSF has also used in the treatment of mycophenolate mofetil (MMF) induced neutropenia [14].

Raising WBC may decrease the risk of infection, hospital stay while avoiding the dose reduction of immune suppressive agents which may lead to rejection especially in early post-transplantation period. This study was designed as a pilot study to evaluate the safety of G-CSF on leukopenia in early post LTx periods.

MATERIALS AND METHODS

Subjects and samples

This pilot study was carried out in patients undergoing LT at Nemazee hospital affiliated to Shiraz University of Medical Sciences (SUMS) Shiraz, Iran. Patients were randomized in one to one manner in this open-label study. The inclusion criteria were leukocyte count $\leq 3000/\text{mm}^3$ within the first 4 weeks after the first LT for cirrhosis. Patients were excluded if they had hepatocellular carcinoma, if their pretransplantation leukocyte count was below $4000/\text{mm}^3$. Patients with comorbid conditions such as congestive heart failure, chronic kidney disease, and chronic obstructive pulmonary disease were also excluded. Written informed consent was obtained from all patients. The protocol was approved by local Ethic Committees of SUMS.

G-CSF protocol and study design

Treatment group received 0.3 mg of G-CSF (PD - G-CSF) at the time of leukopenia ($\leq 3000/\text{mm}^3$). Short-term patient's and graft survival, as well as hospital stay, development of acute cellular rejection (ACR), oropharyngeal candidiasis, active infections, day of maximum MMF dosage achievement, and ACR treatments episodes for each transplanted liver were determined and compared in both groups.

The effect of G-CSF on leukocyte counts 24 hrs after administration was also determined.

ACR was defined on the basis history, physical examination, laboratory finding (fever, elevated transaminase), and response to high-dose corticosteroids. Liver biopsy was not considered necessary for this diagnosis. Active infection was positive culture from body fluids or tissues including ascites or fluid from JP vacuum, urine, blood, or CSF from patients during their hospital stay or after any episodes of fever.

Statistical analysis

Each patient corresponding data and information were recorded in separate but unified questionnaires. Data are reported as mean, and all data were analyzed using chi-square and Student's t-test (Statistical Package for the Social Sciences of ware, version 14) $p < 0.05$ were considered significant.

RESULTS

During the study period, 17 patients were recruited. 9 patients in the control group and 8 patients in the treatment group. Etiology of liver disease is illustrated in Table 1.

Demographic characters of patients were shown in Table 2 which was not different between two groups. Mean WBC counts were 2.0 and 2.4×10^3 at beginning in the treatment and control groups, respectively, which were not significantly different ($p: 0.113$).

Mean WBC count was increased to $11.05 \times 10^3/\text{mm}^3$ 24 hrs after injection in the treatment group with a mean increase of $9 \times 10^3/\text{mm}^3$ ($p: 0.006$, confidence interval 95%: $3.5-14 \times 10^3/\text{mm}^3$). There were no significant differences in days of hospital admission ($p: 0.244$), microbiologic active cultures ($p: 0.30$), oropharyngeal candidiasis ($p: 0.30$), ACR ($p: 0.437$), and day of desirable MMF dosage achievement ($p: 0.691$) and episodes of ACR treatments ($p: 0.08$).

Table 1: Demographic characters of donor and recipient

	G-CSF	Control	p value
Recipients mean age (Years)	28.0±11	28.9±14	0.879 (t-test)
Recipients sex (Female/Male)	4/4	2/7	0.247 (Fischer)
Harvesting time (h)	6	5.1	0.527 (t-test)
Donor weight (Kg)	60.7	64.0	0.790 (t-test)
Donor sex	1/7	1/8	0.735 (Fischer)
Donor age (Age)	31.6	25.5	0.244 (t-test)
Mean WBC ($10^3/\text{mm}^3$)	2.057	2.4	0.380 (t-test)

WBC: White blood cell, G-CSF: Granulocyte colony stimulating factor

However, there was a trend to have more ACR in the control group (Table 3).

DISCUSSION

The main concerns in the early post-transplantation of solid organs are graft preservation and prevention of infections. As the major immunosuppressors increase the risk of infection while preserving the liver form rejection, aligning these two counter actions are of utmost importance in the early phase of post-transplantation. G-CSF has been proposed as one of the cytokines with regenerative properties for the liver. In the study of Piscaglia AC, G-CSF promoted liver repair by triggering the endogenous Oval Cells, which represent G-CSF receptor and also by increasing the bone marrow (BM) derived liver repopulation [15]. Lemoli *et al.* showed tissue damage after OLT and liver resection induces increased serum levels of multiple cytokines especially G-CSF, but only ischemia/reperfusion injury associated with OLT results in the remarkable mobilization of BM stem/progenitor cells [16]. On the basis of these evidences, G-CSF may have beneficial effect on mobilization and differentiation of BM stem cells to hepatocytes especially in a damaged liver by ischemia and allogeneic antibodies.

It was anticipated that the restoration of WBC counts would eventually decrease hospital stay by rearranging one of the immune system's components resulting in decreasing susceptibility to microorganism's invasions. Furthermore, earlier achievement of desirable MMF dosage may prevent rejection in transplanted liver. Our findings did not support this idea that may be due to the low number of patients in the study. Besides this, we have had septicemia by multi-drug resistant *E.coli*, which result in earlier discharge of OLT patients in the ward and then decrease hospital stay of patients in control groups.

Although G-CSF has been used in the recipients of solid organ transplant especially in ganciclovir-induced leukopenia in past (reference), its use for MMF-induced leukopenia was not reported in the past. As this is usually in the first weeks of transplantation, there might be concerns of concerns of safety. In a study by Lodato *et al.* [17], G-CSF was used to treating neutropenia induced by Pegylated Interferon in liver transplant recipients treated for active HCV infection. There was no *de novo* autoimmune liver disease. Morris *et al.*, showed that G-CSF enhances production of LI-10-regulatory T-cells in donor, and may promote tolerance to transplanted organ in mice [18]. They also showed that single dose of pegylated G-CSF may prevent graft versus

Table 2: Etiology of end-stage liver disease

	G-CSF	Control
HBV	1	2
Autoimmune	3	1
PSC	2	1
Wilson disease	0	3
Cryptogenic	2	2
Total	8	9

HBV: Hepatitis B virus, G-CSF: Granulocyte colony-stimulating factor, PSC: Primary sclerosing cholangitis

Table 3: Patients character at post LTx

	G-CSF	Control	p value
Hospital stay	26	22	0.436 (t-test)
Microbiologic active cultures	5/7	3/8	0.205 (Fischer)
Oropharyngeal candidiasis	5/7	4/8	0.465 (Fischer)
ACR	5/7	6/8	0.455 (Fischer)
Day of maximum MMF dosage achievement	10±6	9±5	0.743 (t-test)
Treatment episodes for ACR	5/7	8/8	0.08 (t-test)

ACR: Acute cellular rejection, LTx: Liver transplantation, MMF: Mycophenolate mofetil

host disease more than standard G-CSF in the selected murine model of allogeneic hematopoietic stem cell transplantation. Our open-labeled pilot study shows that a single dose of G-CSF in leukopenic post liver transplant patients, is safe. Although there was no statistically significant beneficial effect on hospital stay, opportunistic, and surgical site infections, but there was a trend toward less ACR episodes in the treatment group. WBC counts increments and maintenance by single-dose injection of G-CSF indicated a transient event or events as ischemic reperfusion injury or splenic sequestration interference with leukocytes in circulation which may have a positive effect on graft survival. An important advantage of our study was using the minimum dosage of G-CSF, which exerted their effects by a single injection. Whether single dosage has a beneficial effect on liver function, survival, rejection, and hospital stay needs further research in another clinical trial [19,20].

The main limitations of this study were related to low number of patients and no placebo arm. In addition, we used the leukocyte count as an indication of treatment. This might be an indicator of absolute granulocyte count, however, monocytes, and lymphocyte counts were not monitored which on their own might have relevance to predisposition to infectious complications such as CMV and wound healing [7].

CONCLUSION

In conclusion, our pilot study showed that a single dose of G-CSF did not have short-term harmful effects on the outcome and function of the graft with a potential to reduce ACR episodes. This needs to be sought in larger placebo-controlled randomized trials.

ACKNOWLEDGMENT

We like to extend our thanks to the Ms Janghoraban for her help in gathering the data.

REFERENCES

1. Timmer-Bonte JN, de Boo TM, Smit HJ, Biesma B, Wilschut FA, Cheragwandi SA, *et al.* Prevention of chemotherapy-induced febrile neutropenia by prophylactic antibiotics plus or minus granulocyte colony-stimulating factor in small-cell lung cancer: A dutch randomized phase III study. *J Clin Oncol* 2005;23(31):7974-84.
2. Vogel CL, Wojtukiewicz MZ, Carroll RR, Tjulandin SA, Barajas-Figueroa LJ, Wiens BL, *et al.* First and subsequent cycle use of pegfilgrastim prevents febrile neutropenia in patients with breast cancer: A multicenter, double-blind, placebo-controlled phase III study. *J Clin Oncol* 2005;23(6):1178-84.
3. Kuderer NM, Dale DC, Crawford J, Lyman GH. Impact of primary prophylaxis with granulocyte colony-stimulating factor on febrile neutropenia and mortality in adult cancer patients receiving chemotherapy: A systematic review. *J Clin Oncol* 2007;25(21):3158-67.
4. Yannaki E, Athanasiou E, Xagorari A, Constantinou V, Batsis I, Kaloyannidis P, *et al.* G-CSF-primed hematopoietic stem cells or G-CSF per se accelerate recovery and improve survival after liver injury, predominantly by promoting endogenous repair programs. *Exp Hematol* 2005;33(1):108-19.
5. Schneider C, von Aulock S, Zedler S, Schinkel C, Hartung T, Faist E. Perioperative recombinant human granulocyte colony-stimulating factor (Filgrastim) treatment prevents immunoinflammatory dysfunction associated with major surgery. *Ann Surg* 2004;239(1):75-81.
6. Liu F, Pan X, Chen G, Jiang D, Cong X, Fei R, *et al.* Hematopoietic stem cells mobilized by granulocyte colony-stimulating factor partly contribute to liver graft regeneration after partial orthotopic liver transplantation. *Liver Transpl* 2006;12(7):1129-37.
7. Liu F, Pan XB, Chen GD, Jiang D, Cong X, Fei R, *et al.* Granulocyte colony-stimulating factor-mobilized autologous bone marrow stem cells promote the liver regeneration of partial liver transplant: An experiment with rats. *Zhonghua Yi Xue Za Zhi* 2005;85(47):3342-5.
8. Inderbitzin D, Beldi G, Sidler D, Studer P, Keogh A, Bisch-Knaden S, *et al.* Granulocyte colony-stimulating factor supports liver regeneration in a small-for-size liver remnant mouse model. *J Gastrointest Surg* 2007;11(3):280-5.
9. Dirsch O, Chi H, Ji Y, Gu YL, Broelsch CE, Dahmen U. Administration of granulocyte colony stimulating factor after liver transplantation leads to an increased incidence and severity of ischemic biliary lesions in the rat model. *World J Gastroenterol* 2006;12(31):5021-7.
10. Trindade E, Maton P, Reding R, de Ville de Goyet J, Otte JB, Buts JP, *et al.* Use of granulocyte macrophage colony stimulating factor in children after orthotopic liver transplantation. *J Hepatol* 1998;28(6):1054-7.
11. Winston DJ, Foster PF, Somberg KA, Busuttill RW, Levy MF, Sheiner PA, *et al.* Randomized, placebo-controlled, double-blind, multicenter trial of efficacy and safety of granulocyte colony-stimulating factor in liver transplant recipients. *Transplantation* 1999;68(9):1298-304.
12. Turgeon N, Hovingh GK, Fishman JA, Basgoz N, Tolkoff-Rubin NE, Doran M, *et al.* Safety and efficacy of granulocyte colony-stimulating factor in kidney and liver transplant recipients. *Transpl Infect Dis* 2000;2(1):15-21.
13. Ishizone S, Makuuchi M, Kawasaki S, Matsunami H, Terada M, Kitahara S, *et al.* Effect of granulocyte colony-stimulating factor on neutropenia in liver transplant recipients with hypersplenism. *J Pediatr Surg* 1994;29(4):510-3.
14. Nogueras F, Espinosa MD, Mansilla A, Torres JT, Cabrera MA, Martín-Vivaldi R. Mycophenolate mofetil-induced neutropenia in liver transplantation. *Transplant Proc* 2005;37(3):1509-11.
15. Piscaglia AC, Shupe TD, Oh SH, Gasbarrini A, Petersen BE. Granulocyte-colony stimulating factor promotes liver repair and induces oval cell migration and proliferation in rats. *Gastroenterology* 2007;133(2):619-31.
16. Lemoli RM, Catani L, Talarico S, Loggi E, Gramenzi A, Baccarani U, *et al.* Mobilization of bone marrow-derived hematopoietic and endothelial stem cells after orthotopic liver transplantation and liver resection. *Stem Cells* 2006;24(12):2817-25.
17. Lodato F, Azzaroli F, Tamè MR, Di Girolamo M, Buonfiglioli F, Mazzella N, *et al.* G-CSF in Peg-IFN induced neutropenia in liver transplanted patients with HCV recurrence. *World J Gastroenterol* 2009;15(43):5449-54.
18. Morris ES, MacDonald KP, Rowe V, Johnson DH, Banovic T, Clouston AD, *et al.* Donor treatment with pegylated G-CSF augments the generation of IL-10-producing regulatory T cells and promotes transplantation tolerance. *Blood* 2004;103(9):3573-81.
19. Dey P, Saha MR, Sen A. An overview on drug-induced hepatotoxicity. *Asian J Pharm Clin Res* 2013;6:53-6.
20. Uma MR, Ramanarayana RR, Vangoori Y, Mohana SJ. Evaluation of hepatoprotective (preventive & curative) activity of leaves extract of *ROSA Centifolia* on experimental animal models. *Asian J Pharm Clin Res* 2014;7:105-7.