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Stem Cell Mobilization is Life Saving in an Animal Model of Acute Liver Failure

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INTRODUCTION

Acute liver failure (ALF) affects over two thousand Americans per year and results in approximately four hundred liver transplants annually. Defined as the abrupt loss of hepatocellular function in patients with previously normal liver function, the most common etiologies are acetaminophen overexposure and acute viral hepatitis.[1] In the most severe cases, patients manifest a rapidly developing coagulopathy, and encephalopathy that progresses to cerebral herniation and death without prompt liver transplantation.[2] Fewer than half of patients with ALF will spontaneously recover with supportive care alone, yet at present, no pharmacologic or adjunct therapies have been shown to be of benefit in this clinical scenario. For these critically ill patients, liver transplantation frequently represents the only option for survival, however this limited resource may be inaccessible at the time at which it is emergently needed. Furthermore, the decision to proceed to transplantation is not always straightforward, since some patients will spontaneously recover but the ability to predict recovery is markedly limited. Under aggressive utilization of liver transplantation can result in devastating outcomes in potentially salvageable patients, while overly aggressive utilization of transplantation both commits patients who might have spontaneously recovered to a lifetime of immunosuppression, and also unnecessarily utilizes precious grafts that could be allocated to others in need. New therapies, particularly those that can promote spontaneous recovery and lessen the need for liver transplantation, are badly needed.

Mechanisms of hepatic regeneration after liver injury are well understood: mitotic division of mature hepatocytes promptly regenerates liver mass and function in sublethal settings.[3] When this mechanism is blocked or is inadequate, circulating bone marrow-derived hematopoietic stem cells(HSCs) participate in liver injury recovery.[4] Circulating HSCs are

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characterized by the cell surface marker CD34: a glycoprotein involved in cell-cell adhesion interactions that is also expressed on cells in the umbilical cord, mesenchymal stem cells, endothelial progenitor cells, and on mature endothelial cells.[5] It has recently been reported that levels of $CD34^+$ HSCs are increased in the peripheral circulation of patients after liver resection,[6] though the magnitude of the increase has not been demonstrated in other studies.[7] Certainly, while innate mechanisms that lead to stem cell mobilization may contribute to liver repair in some circumstances, this mechanism is inadequate in the setting of severe liver injury.

Pharmacologic mobilization of HSCs has emerged as the standard of care for patients with hematologic malignancies that require autologous bone marrow transplantation (BMT) after ablative chemotherapy.[8] Currently, patients with non-Hodgkin's lymphoma or multiple myeloma who are treated with autologous stem cell transplant undergo HSC mobilization with granulocyte colony stimulating factor (G-CSF), (Neupogen ®, Amgen) and plerixafor (Mozobil ®, Genzyme) for five days prior to plasmapheresis and cell collection.[9] This preparatory regimen results in a synergistic mobilization of large numbers of $CD34^+$ stem cells via antagonism of the CXCR4-SDF-1 interaction which anchors stem cells to their home in the bone marrow.[10] HSC mobilization protocols have thus been developed and optimized in the autologous BMT setting but have not yet been considered for treatment of acute liver injury, where they may be of benefit. Any pharmacologic intervention which improved outcomes in this difficult patient population by increasing the chance of spontaneous liver recovery, especially one which utilizes medicines already known to be safe and in current clinical use, would be of great benefit. We describe herein the use of stem cell mobilization to improve survival in an animal model of ALF.

METHODS

Rodent models of ALF

Female Lewis rats or C57BL/6 mice were administered various doses of carbon tetrachloride (CCl_4, Sigma) via intraperitoneal injection to determine the sublethal dose that would best recapitulate ALF in humans. CCl_4, was dissolved in mineral oil to a final of concentration of fifty percent for mice and twenty five percent for rates and was administered by single intraperitoneal injection at doses ranging from 2 ml/kg to 4.5 ml/kg under inhaled isoflurane anesthesia. To account for the inherent variability in survival and severity of injury after CCl_4, injection, each treatment group was comprised of eight to ten animals. For characterization of hepatic injury in response to CCl_4 injection, animals were sacrificed daily for measurement of serum transaminases (AST and ALT), and for histopathologic analysis of liver injury. All experimental regimens were repeated for survival analyses and animals were observed for up to 16 days.

All animals were housed in an approved microisolation facility with free access to food and water in a light-cycled room. All experimental protocols were approved by the Johns Hopkins University Institutional Animal Care and Use Committee.

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Mobilization of HSCs

For HSC mobilization, plerixafor and G-CSF were administered by subcutaneous injection at weight-based doses consistent with those that have proven effective in human clinical trials or as has been used in our laboratory previously (plerixafor, 2 mg/kg/dose; G-CSF 300 μg/kg/dose). Plerixafor and G-CSF were administered 12-hours following CCl_4 injection, in treatment groups comprised of eight to ten animals. Control treatment groups were administered an injection with an equivalent volume of saline.

Flow cytometric analysis of peripheral blood

Following HSC mobilization, animals were sacrificed at either one or six hours post treatment and blood was collected. Leukocyte suspensions (1×10^6) of peripheral blood were analyzed for CD34, Thy-1 and c-Kit expression. Nonspecific antibody binding was blocked with mouse and donkey serum (Sigma) for 30 minutes. The cells were incubated with Alexa Fluor 647 conjugated mouse anti-rat CD34 (1:10, Santa Cruz biotechnology), fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat CD90 (Thy-1, 1:100, BD Pharmingen), and rabbit anti-rat c-Kit (1:100, Santa Cruz biotechnology) for 45 minutes at 4°C, and followed by phycoerythrin (PE)-conjugated donkey anti-rabbit IgG (1:200, eBioscience) for 30 minutes at 4°C. The CD34, Thy-1 and c-Kit positive cells were counted by flow cytometry (fluorescence activated cell sorting [FACS]) using CELLQuest software (Becton-Dickinson).

Histology and Immunohistochemistry

Five μm serially cut, frozen sections were fixed with acetone at −20°C for 10 minutes and dried for 1 hour at room temperature. The streptavidin-biotin-peroxidase method with the DAKO Kit (Carpinteria, CA) was used to detect CD34 antigen. After the blocking of nonspecific binding of antibody, the specimens were reacted with goat anti-CD34 (1:100; R&D Systems, Inc) at 4°C overnight. Subsequently, the sections were incubated with Biotin-SP-conjugated bovine anti-goat IgG (1:200, Jackson Lab) at room temperature for 30 minutes. Diaminobenzidine tetrahydrochloride was used as the chromogen, and hematoxylin was used for counterstaining.

RESULTS

Carbon tetrachloride administration recapitulates ALF in rodents

Animals that were treated with increasing amounts of carbon tetrachloride showed a dose-dependent decrement in survival (Figure 1A). Groups of ten animals that were treated with 2 ml/kg routinely showed one or two mortalities per ten within the first one to two days after treatment. The large majority of animals in this group were transiently ill but rapidly regained vigor. Animals that received 3mls/kg showed higher mortality rates, typically 3 or 4 animals died but reliably more than half recovered spontaneously. A dose of 4 mls/kg would result in 60–100% mortality amongst the animals over the course of three to seven days. Slight adjustments in the dose (i.e. 4.5 mls/kg) at this level would have obvious effects in mortality with all animals succumbing within 24–48 hours. A dose of 4 mls/kg was eventually selected for the final model. Mortality with this dose was typically 80% around

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five days and was preceded by a significant elevation in serum transaminases. AST and ALT levels peaked at approximately 5,000 on day one to two after injury (Figure 1B). Histologic examination of rodent livers after injury revealed marked inflammation and necrosis which increased over the first three days (Figure 1C). Injured livers displayed penetrating inflammatory cells and increased hepatocyte vacuolization as well as apoptotic bodies consistent with severe injury.

There was low but persistent variability between groups of animals and batches of carbon tetrachloride based on baseline animal vigor at time of delivery to our institution, strength of drug, and efficacy of delivery by injection.

Plerixafor and G-CSF mobilize a population of CD34+ HSCs in rodent

Rats that received either plerixafor or G-CSF showed an increase in their peripheral WBC counts at one and 6 hours (Figure 2A). Animals receiving plerixafor alone had an average total WBC of 6,100 at one hour (n=3) and 7,300 at 6 hours (n=3). Animals receiving G-CSF alone had average total WBCs of 3,300 at 1 hour (n=3) and 7,800 at 6 hours (n=3). Animals receiving both drugs had an average total WBC count of 6,000 at 1 hour (n=3) and of 9,900 at 6 hours (n=3). The lymphocyte compartment, which is thought to contain the majority of HSCs showed similar changes (Figure 2A). Animals receiving plerixafor had total lymphocyte counts of 4,400 and 4,800 at one and six hours respectively. Animals receiving G-CSF had total lymphocyte counts of 2,000 and 4,100 at one and six hours respectively. Animals receiving both drugs had total lymphocyte counts of 3,600 and 5,200 at one and six hours respectively. CD34+ cells were increased in all three treatment groups (Figure 2B). Animals that received plerixafor alone had peripheral CD34+ absolute counts of 333 cells/ml and 135 cells/ml at one and six hours. Animals that received G-CSF alone had absolute CD34+ cell totals of 216 and 238 at one and six hours. Animals that received both drugs had peripheral CD34+ cells counts of 363 and 346 cells/ml. Though rats have a wider distribution of “normal” starting peripheral white blood cell counts the mobilization results shown closely mirrors that known to occur after administration of these agents in humans whom likewise show a robust increase at 6 hours (Figure 2C) which peaks at 9 hours[11]. Administration of both agents together resulted in the greatest increase in serum WBCs. Analysis of the mobilized population showed it to be enriched with CD34+ HSCs (Figure 2B) with the greatest absolute CD34+ numbers in the serum of animals treated with both plerixafor and G-CSF. This response is similar to that seen in humans after administration of plerixafor and G-CSF.

Stem Cell Mobilization improves survival in an animal model of ALF

Rodents (n=8) that were administered intraperitoneal CCl_4 (4mls/kg) displayed a high rate of mortality (75% at 6–7 days) consistent with our model. In contrast, animals (n=8) that received an identical injury (4mls/kg) but twelve hours later initiated plerixafor (2 mg/kg/d x 3d) and G-CSF (300 ug/kg/d x 3d) survived at a much higher rate (13% mortality) (Figure 3A). Examination of liver specimens from intentionally sacrificed animals from the treated and untreated groups after injury showed lesser histologic injury in the group that had received stem cell mobilization (Figure 3B).

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Animals that have undergone stem cell mobilization after liver injury show CD34+ HSCs infiltrating their livers

Animals that underwent CCL_4 injury (or control vehicle) and stem cell mobilization (or control saline injection) were sacrificed at days 1 or 3 and underwent hepatectomy. Thin slide microscopy after staining with antibodies specific for CD34 revealed the presence of significant periportal infiltrating HSCs at 24 hours (Figure 4A 4). Conversely, animals that had undergone injury only (Figure 4A 3), or had been treated with mobilizing agents but no injury (4A 2) displayed far fewer CD34+ cells in hepatectomy specimens. Animals that received neither injury nor stem cell mobilization showed no CD34+ staining at 24 hours (Figure 4A 1). Panel 4B shows increased CD34 staining over five days in animals that were injured and underwent stem cell mobilization.

DISCUSSION

Patients with ALF represent a challenging group. Untreated, they have high mortality rates, though if they survive they will return to normal functioning spontaneously. There is a life saving therapy available, but it is a resource badly needed by others who have no hope of spontaneous recovery and survival rates after transplant for ALF are known to be far inferior to that of liver transplant for other indications[12]. ALF patients are often critically ill and decisions must be made rapidly. Novel medical therapies that improve the rate of spontaneous recovery in these patients would be extraordinarily helpful.

Why the liver fails to utilize all possible recovery pathways, i.e. recruitment of endogenous marrow derived stem cells, in these scenarios of massive insult is unclear. It has been shown that G-CSF can be given safely and effectively to patients with chronic liver disease and that CD34+ HSCs can be mobilized effectively in this population.[13,14,15] Further it has been shown that patients with ALF have markedly lower serum levels of several stem cell mobilizing factors, like SCF (stem cell factor) and thrombopoietin. Interestingly, levels were lowest in the group of ALF patients that had the worst eventual outcomes.[16] Augmenting the release of marrow bound stem cells has been well studied and optimized for utilization in a very different group of patients who donate via pheresis for subsequent HSC auto transplantation after chemoablation of their lymphoma or myeloma. Meanwhile, efforts to augment liver recovery from injury with stem cells has thus far focused on provision of exogenous stem cells with success demonstrable in some animal models.[17] Here we have combined these lines of inquiry and utilize pharmacologic mobilization of endogenous hematopoietic stem cells in the setting of liver injury to show that these cells can be of therapeutic benefit. Our model shows survival advantage from the treatment provided but the mechanism of benefit remains undescribed. We have demonstrated an elevation of serum leukocytes and CD34+ cells in animals treated with plerixafor and G-CSF, and it is the mobilization of these hematopoietic stem cells that are key in the current oncologic uses of these drugs, though it is recognized that CD34 is an imperfect surrogate for the cell population that mediates recapitulation of the marrow after ablative chemotherapy. We have also shown here the rapid appearance of CD34+ cells in the livers of only the animals who undergo injury and stem cell mobilization, again suggesting a role for these cells in the treatment benefit but not necessarily a mechanism. These cells could conceivably

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differentiate into hepatocytes to directly support an animal with inadequate liver mass or perhaps supply some sort of paracrine support to mitigate injury or accelerate ongoing local repair mechanisms. Though the mechanism by which HSCs exert their benefit in this model remains undetermined we are undertaking experiments which will discriminate between an effect which lessens injury versus one which promotes recovery. Experiments which carefully assess levels of injury to the liver using CCl_4 as well as other toxins like acetominophen preliminarily show similar injury profiles in animals treated with stem cell mobilizing agents versus control animals suggesting a mechanism involving primarily enhancement of injury recovery (unpublished data).

Plerixafor was initially studied as an anti-HIV drug as it was found to be a small molecule antagonist of CXCR4, then known as an HIV co-receptor.[18] CXCR4 also binds Stromal derived factor 1 (SDF-1) and this interaction anchors HSCs to their niche in the bone marrow. Elevated levels of SDF elsewhere in the body, i.e. at the site of injured tissue, create a gradient which releases stem cells from the marrow, perhaps so that they may participate in tissue repair elsewhere in the body. Why this process is not more efficient in settings like massive liver injury is unclear. Plerixafor was not an effective anti-HIV agent *in vivo* but resulted in massive mobilization of HSCs to the periphery when given to healthy volunteers. Plerixafor acts synergistically with G-CSF in mobilizing HSCs in clinical trials as well as in our animals and thus allows for assessment of whether endogenous stem cells are able to participate in tissue repair under optimized conditions, whatever their exact role may be. To be clinically relevant in scenarios of acute liver failure, stem cell mobilizing agents would have to exert their beneficial effect on survival even when given well after injury has been detected. We are currently performing experiments to test how long treatment with G-CSF and plerixafor can be delayed after initial injury and still result in survival improvement. Plerixafor is also known to mobilize a more diverse population from the marrow beyond CD34+ cells. Whether its other actions may be mediating the benefit seen here remains to be clarified and is the subject of ongoing research in our laboratory and others.

Effective pharmacologic mobilization of marrow derived stem cells to liver may have application in other settings as well. Quicker recovery after surgical resection or larger surgical resections could be facilitated, for example. Transplant applications might include the ability to use ever more extended criteria grafts, or ever smaller living donor grafts, which would then serve as scaffolds for endogenous repopulation by mobilized host stem cells. Perhaps this pharmacologically driven graft repopulation by recipient cells might lead to an eventual conversion of the donor graft to recipient phenotype creating a novel type of “tolerance” protocol, a phenomenon described in animal models previously[19]. Applications with other tissues in other injury scenarios are possible as well. Benefit has already been reported from treatment with G-CSF after myocardial infarction and with acute kidney injury.[20,21,22] Clinical familiarity with G-CSF and plerixafor and their relatively benign side effect profiles make these protocols immediately attractive, especially for this challenging group of ALF patients that has few other options, suggesting the need for a clinical trial if results can first be repeated in a large animal preclinical model of liver injury.

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References

1. Stravitz RT, Kramer DJ. Management of acute liver failure. Nat Rev Gastroenterol Hepatol. 2009; 6:542–553. [PubMed: 19652652]
2. Lee WM, Squires RH, Nyberg SL, et al. Acute Liver Failure: Summary of a Workshop. Hepatology. 2008; 47:1401–1415. [PubMed: 18318440]
3. Michalopoulos GK, DeFrances MC. Liver regeneration. Science. 1997; 276(5309):60–6. [PubMed: 9082986]
4. Petersen BE, Bowen WC, Patrene KD, et al. Bone marrow as a potential source of hepatic oval cells. Science. 1999; 284(5417):1168–70. [PubMed: 10325227]
5. Simmons DL, Satterthwaite AB, Tenen DG, Seed B. Molecular cloning of a cDNA encoding CD34, a sialomucin of human hematopoietic stem cells. J Immunol. 1992; 148:267–71. [PubMed: 1370171]
6. De Silvestro G, Vicarioto M, Donadel C, et al. Mobilization of peripheral blood hematopoietic stem cells following liver resection surgery. Hepatogastroenterology. 2004; 51(57):805–810. [PubMed: 15143921]
7. Di Campli C, Piscaglia AC, Giulante F, et al. No Evidence of Hematopoietic Stem Cell Mobilization in patients Submitted to Hepatectomy or in Patients with Acute on Chronic Liver Failure. Transplantation Proceedings. 2005; 37:2563–2566. [PubMed: 16182744]
8. Hahn T, Wingard JR, Anderson KC, et al. The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of multiple myeloma: An evidenced-based review. Biol Blood Marrow Transplant. 2003; 9 (1):4–37. [PubMed: 12533739]
9. Flomenberg N, Devine SM, Dipersio JF, et al. The use of AMD3100 and G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. Blood. 2005; 106(5):1867–1874. [PubMed: 15890685]
10. Dar A, Kollet O, Lapidot T. Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. Exp Hematol. 2006; 34(8):967–75. [PubMed: 16863903]
11. Mozobil (Plerixafor injection). Cambridge, MA: Genzyme Corporation; 2009. [Product Monograph]
12. Farmer DG, Anselmo DM, Ghobrial RM, et al. Liver transplantation for fulminant hepatic failure: experience with more than 200 patients over a 17-year period. Ann Surg. 2003; 237(5):666–75. [PubMed: 12724633]
13. Gaia S, Smedile A, Omede P, et al. Feasibility and safety of G-CSF administration to induce bone marrow-derived cells mobilization in patients with end stage liver disease. Journal of Hepatology. 2006; 45:13–19. [PubMed: 16635534]
14. Lorenzini S, Isidori A, Catani L, et al. Stem cell mobilization and collection in patients with liver cirrhosis. Ailment Pharmacol Ther. 2008; 27:932–939.
15. Di Campli C, Zocco MA, Saulinier N, et al. Safety and efficacy profile of G-CSF therapy in patients with acute on chronic liver failure. Digestive and Liver Disease. 2007; 39:1071–1076. [PubMed: 17964871]
16. Okumoto K, Saito T, Onodera M, et al. Serum Levels of stem cell factor and thrombopoietin are markedly decreased in fulminant hepatic failure patients with a poor prognosis. Journal of Gastroenterology and Hepatology. 2007; 22:1265–1270. [PubMed: 17688667]
17. Liu F, Liu ZD, Wu N, et al. Transplanted Endothelial Progenitor Cells Ameliorate Carbon Tetrachloride-Induced Liver Cirrhosis in Rats. Liver Transplantation. 2009; 15:1092–1100. [PubMed: 19718641]
18. Uy GL, Rettig MP, Cashen AF. Plerixafor, a CXCR4 antagonist for the mobilization of hematopoietic stem cells. Expert Opinion on Biologic Therapy. 2008; 8(11):1797–1804.

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19. Sun Z, Zhang X, Locke JE, et al. Recruitment of host progenitor cells in rat liver transplants. Hepatology. 2009; 49(2):587–97. [PubMed: 18972402]

20. Ince H, Petzsch M, Dieter Kleine H, et al. Prevention of Left Ventricular Remodeling with Granulocyte Colony-Stimulating Factor after Acute Myocardial Infarction. Circulation. 2005; 112:I73–I80. [PubMed: 16159869]

21. Leone AM, Rutella S, Bonanno G, et al. Endogenous G-CSF and CD34+ cell mobilization after acute myocardial infarction. International Journal of Cardiology. 2006; 111:202–208. [PubMed: 16051386]

22. Stokman G, Leemans JC, Claessen N, et al. Hematopoietic stem cell mobilization therapy accelerates recovery of renal function independent of stem cell contribution. J Am Soc Nephrol. 2005; 16(6):1684–92. [PubMed: 15829714]

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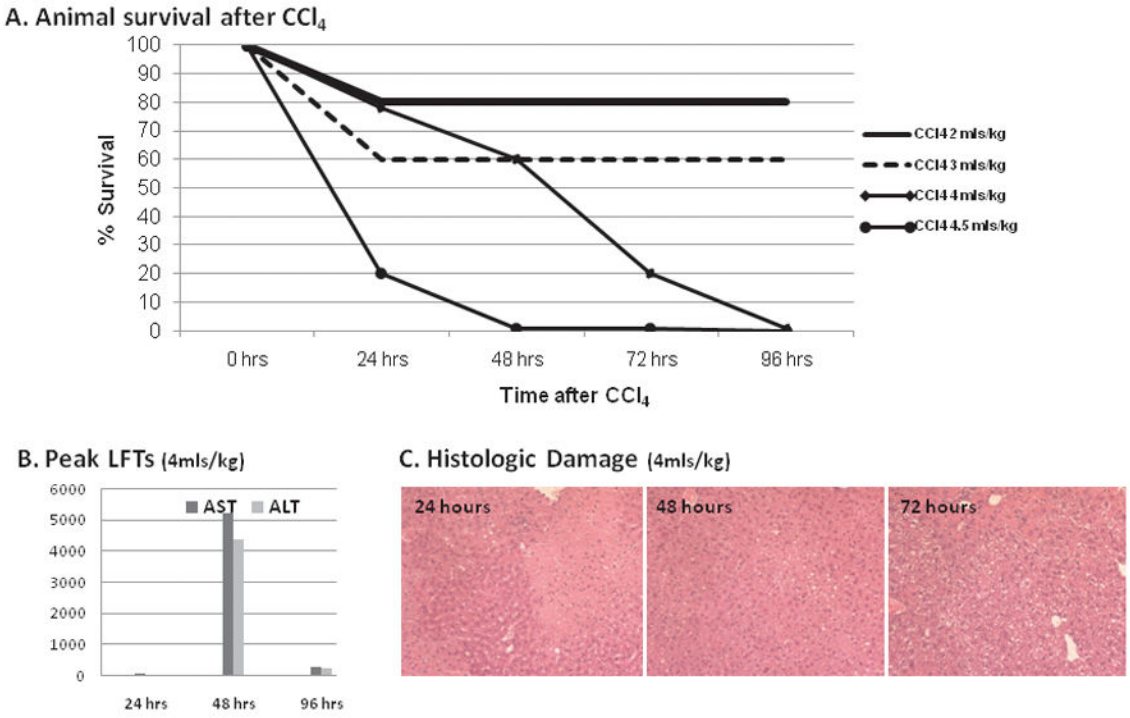

Figure 1.

Mortality and hepatic injury after injection of CCl_4. (A) Percent survival over four days among groups (n=10) of mice administered intraperitoneal injections of CCL_4 at doses of 2ml/kg (solid line), 3ml/kg (dashed line), 4 ml/kg (diamond markers), or 4.5 ml/kg (circle markers). (B) Serum AST (dark bars) and ALT (light bars) levels in mice (n= 2) 24 hrs and 96 hrs after intraperitoneal injection of CCL_4 at 4 ml/kg compared to baseline levels in uninjected animals (0 hrs). (C) Hematoxalin and eosin staining of mouse livers at 24 hrs, 48 hrs, and 72 hrs after intraperitoneal injection of CCl_4.

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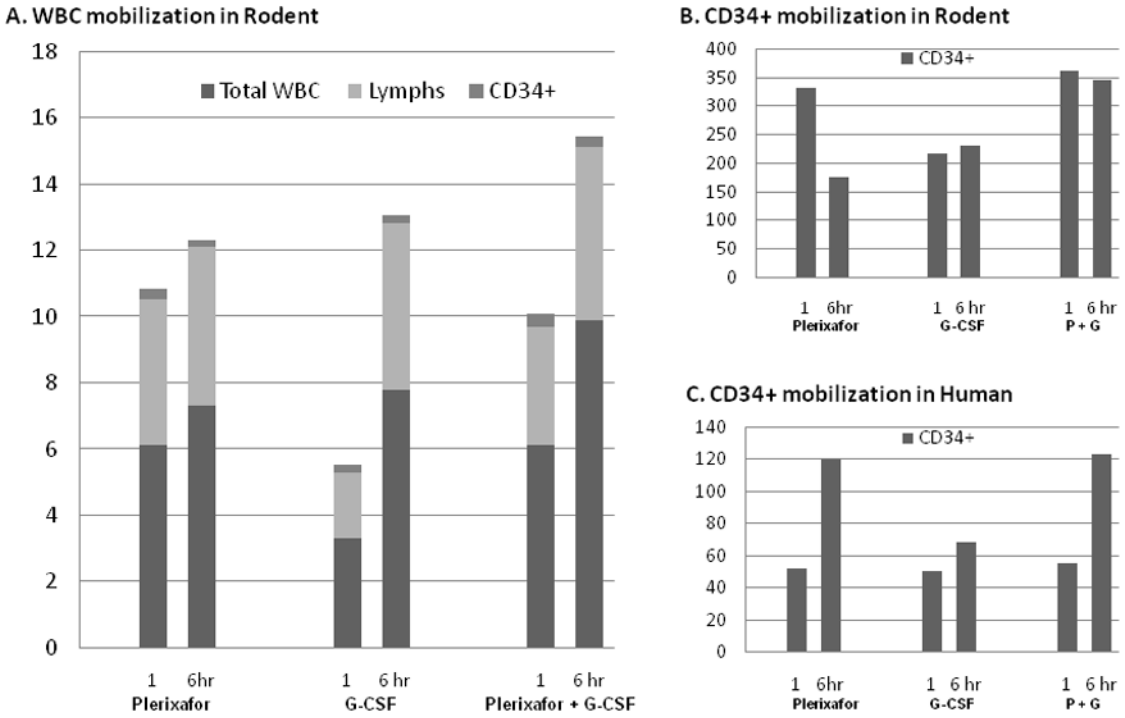

Figure 2.

Hematopoietic stem cell mobilization response in to plerixafor and/or G-CSF administration. Rats (n=3 per treatment group) were administered a single dose of plerixafor (2 mg/kg), G-CSF (300μg/kg) or both. (A) Peripheral total white blood cell (WBC) count, total lymphocytes, and $CD34^{+}$ cells were measured in serum by FACS analysis at 1 hr and 6 hrs after injection. (B) Increases in CD34+ cells in rodent in response to plerixafor and/or G-CSF. (C). Mobilization of CD34+ cells in human after administration of plerixafor and/or G-CSF (adapted from data provided in reference 11).

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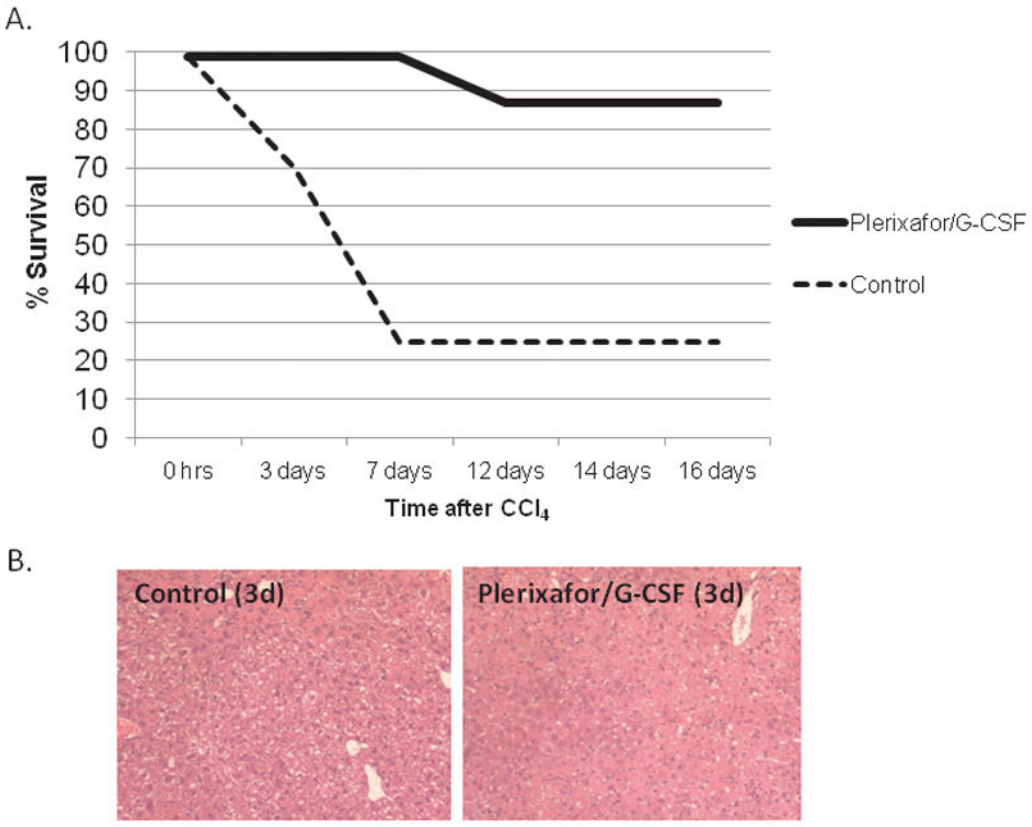

Figure 3.

Plerixafor and G-CSF administration improve survival and lessen hepatic injury after injection of CCl_4. (A) Percent survival over 16 days among groups (n= 8) of mice administered intraperitoneal injections of CCL_4 (4 ml/kg) and treated with 2 mg/kg/d plerixafor and 300 μg/kg/d G-CSF for 3 days(solid line) or saline control (dashed line). (B) Representative hematoxalin and eosin stained sections of liver from CCL_4 injected mice three days after treatment with saline control, or plerixafor and G-CSF.

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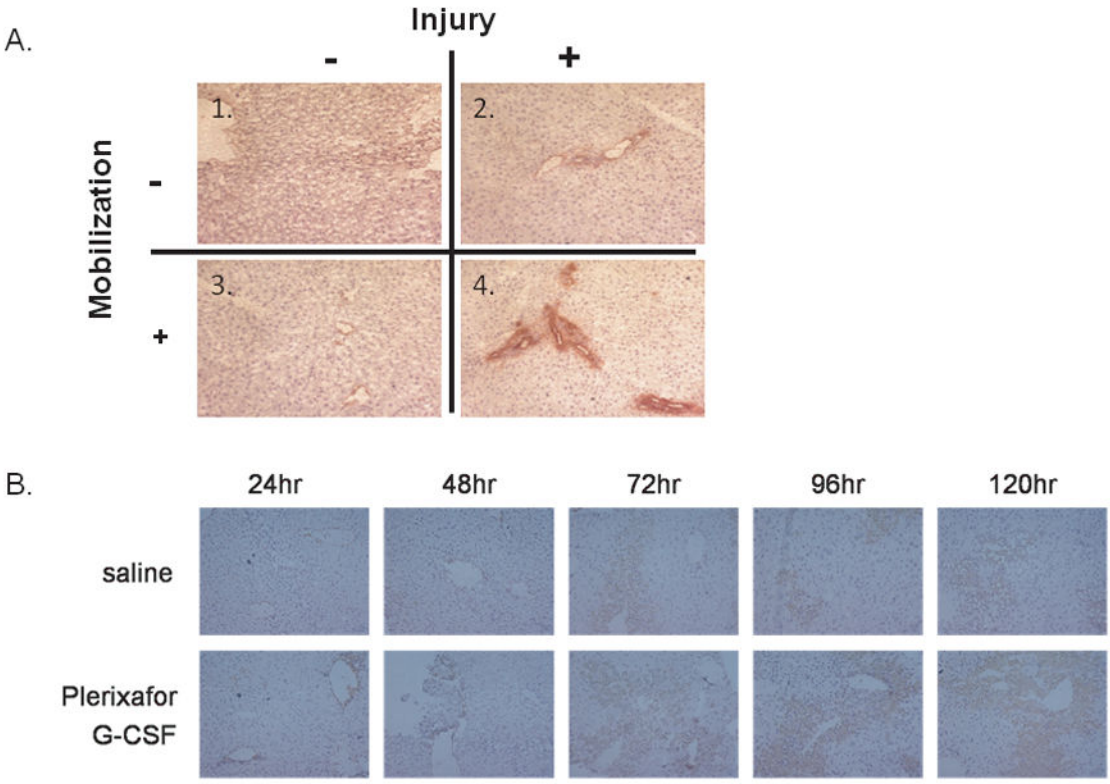

Figure 4.

CD34 immunostaining of rat livers 24 hours after stem cell mobilization. Representative images from (A 1) control uninjured animals administered neither CCl_4 nor plerixafor/G-CSF, (A 2) CCl_4 injected animals administered saline control, (A 3) uninjured animals administered plerixafor/G-CSF, and (A 4) CCl_4 injected animals administered plerixafor/G-CSF. (B) CD34 immunostaining over the first five days after injury in animals that were treated with stem cell mobilizing agents versus control.