Scientific Research and Essays Vol. 6(21), pp. 4646-4650, 30 September, 2011
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.1070
ISSN 1992-2248 ©2011 Academic Journals

Full Length Research Paper

Assessment effect of granulocyte colony-stimulating factor in experimental models of liver injury

Durdi Qujeq[1,2]*, Roya Abassi[2], Farideh Faeizi[3], Hadi Parsian[2], Alieh Faraji[3], Maryam Elmei[1,2], Mohsen Tatar[2], Sohrab Halalkhor [2] and Hassan Tahhery[4]

[1]Cellular and Molecular Biology Research Center (CMBRC), Faculty of Medicine, Babol University of Medical Sciences, Ganjafrooze Avenue, Babol, Iran.
[2]Department of Biochemistry and Biophysics, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.
[3]Department of Anatomical Sciences, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.
[4]Department of Internal Medicine, Ayatollah Rouhani Hospital, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.

Accepted 16 August, 2011

Previous investigators have suggested that granulocyte colony-stimulating factor (G-CSF) may accelerate the healing process of liver lesions. This hypothesis has been discussed by some researchers. The action mechanisms remains unclear and further studies are needed to clarify this mechanism. To test this, we establish a rat model of liver injury using dimethylnitrosamine (DMN), transplanted the G-CSF into the rats. To determine whether G-CSF could prevent liver injury in DMN-intoxicated rats, we employed the model of acute and chronic liver injury induced by DMN: our study was limited to the characteristic changes in biochemical and histological parameters seen in liver damage. The DMN injections were given twice weekly for four weeks to establish liver damage. Rat was treated with DMN by intraperitoneal (IP) injection. On the 1st, 7th or 28th days, rats were sacrificed and liver histology was examined. Serial 5 µm sections of the liver were stained with hematoxylin and eosin (H&E). The serial measurement of the end products of hepatic synthetic activity also was used to assess liver damage. Liver injury was determined by biochemical and histological examination. As it is evident from results after administration of DMN, in addition to histologically changes of liver tissue, central vein, liver cord, sinusoids and bile duct were not normal, the activities of the serum liver enzymes also rose. Administration of G-CSF caused a statistically significantly decrease in the liver enzymes activity and sever of biochemical synthesis disruption in the liver. The protective role of G-CSF was showed in this rat model of acute and chronic liver damage induced by DMN. Our results showed beneficial effects of G-CSF treatment after liver injury on liver function. Histological and biochemical assessment of liver function revealed that liver tissue have a unique regenerative capability.

Key words: Dimethylnitrosamine, damage, granulocyte colony stimulating factor, liver.

INTRODUCTION

Earlier studies document that DMN is a potent hepatotoxin (Haggerty, 1990). Recent studies have demonstrated that it is an N-nitroso compound present in processed meats and industrial products (George, 2001). Several experimental studies suggest that G-CSF is a cytokine able to regulate a number of functions in neutrophils (Bergley, 1986; Clark, 1987; Ichinose, 1990; Avalos, 1990). According to the recent work, G-CSF also causes a marked increase in the mobilization of hematopoietic stem cells into the peripheral circulation (Avalos, 1996; Link, 2000). There are some reports in the literature showing the effect of treatment with G-CSF consisted of a slight effect on the inflammatory reaction \

*Corresponding author. E-mail: d.qujeq@mubabol.ac.ir, dqujeq@hotmail.com. Tel: +98-111-2229591-5. Fax: +98-111-2226109.

Abbreviations: DMN, dimethylnitrosamine; **G-CSF,** granulocyte-colony stimulating factor, **IP,** intraperitoneal, **H&E,** hematoxylin and eosin.

(Ghanem, 2010). According to the recent work G-CSF used as a skeletal muscle regeneration therapy (Hara, 2011). It is established that G-CSF administration could significantly increase the survival rate in the rats (Zhang, 2011). As reported by many investigators, granulocyte-colony stimulating factor are shown to reduce liver injury (Takayama, 2011). It is now clear that G-CSF treatment activates serial events of inflammation-thrombosis circuitry (Wei, 2011; Lian et al., 2011).

The purpose of the present study was to utilize an established animal model of liver injury, and then to utilize G-CSF administrated to rats in order to observe the effects on liver function, and their kinetics using biochemical and histological criteria.

MATERIALS AND METHODS

Experimental animal model

Wistar rats were used at ages from 8 to 12 weeks weighing of 200 to 250 g. Rats were prepared and used at the Animal Center of Babol University of Medical Sciences. Babol, Iran. All the protocols involving animals were approved by the Babol University Animal Care and Use Committee. Effort was made to minimize the number of animals. Age-matched rats were used as control animals. The animals were housed in suspended bracket cages in a climate controlled room with temperature of 23 ± 3°C in a cycle of 12 h light and 12 h darkness with free access to food and water. All animals were carefully maintained under standard animal housing conditions. The animals were divided into four groups of six each as follows: Group I: control group, non-treated animals receiving only PBS buffer, Group II: administration of DMN followed by administration of PBS 2 h later. Group III: G-CSF administered and Group IV: administration of DMN followed by administration of G-CSF 2 h later.

Experimental model of liver damage

The experiments had started between 8.00 and 10.00 am and the animals were fasted for 12 h before any manipulation. The DMN, C2H6N2O was purchased from (Sigma, USA), and administered at a dose of 100 µl/kg body weight and G-CSF at 100 µg/kg body weight. Human recombinant G-CSF expressed in E. coli, (Sigma, USA) or PBS was administered by i.p. (intraperitoneal) injection in the respective groups of rats under light ether anesthesia (diethyl ether), (Sigma, USA).

Experimental model of acute liver damage

Acute liver damage on rats were induced by i.p. injection of a DMN 50% (v/v) solution in PBS (Sigma) at a dose of 100 µl/kg for two consecutive days. Control rats received i.p. PBS alone.

Experimental model of chronic liver damage

Chronic liver damage on rats were induced by intraperitoneal injection of a DMN 50% (v/v) solution in PBS at a dose of 100 µl/kg of DMN. The injection was given twice a week for 4 weeks to establish chronic liver damage. After 2 weeks of injury, all rats were submitted to blood tests for the evaluation of liver injury and function. Samples obtained from 6 normal rats referred to as control were used to define the normal range for each blood parameter analyzed. The specific values were: alanine aminotransferase (ALT), aspartate aminotransferase (AST). Only those animals presenting blood values different from the predefined range in all two parameters were used in the study.

Effect of G-CSF administration in healthy rats

To determine the effects of G-CSF in healthy animals, we selected six rats that each received daily i.p. injection of G-CSF at the dose of 100 µg/kg in PBS for seven consecutive days.

Effect of G-CSF administration after DMN damage

Following DMN injections, the animals received i.p. injections of G-CSF. The four groups of six rats were sacrificed under general anesthesia after 1, 7 and 28 days to collect blood and liver tissue samples. Immediately after exsanguinations, the livers were removed, cleaned and weighed. One portion of the livers was separated and immersed in buffered formalin solution and then paraffin-embedded for histological examination.

Histological grading and staining

Following sacrifice of animals by cervical dislocation, the liver was removed, rinsed with phosphate-buffered saline and weighed. Histological features were analyzed by a single histologist who was unaware of animal characteristics. All liver sections were fixed in 10% (volume/volume) phosphate-buffered formalin and embedded in paraffin wax. Sections were cut at 5 µm and stained with hematoxylin and eosin. All specimens were randomized and were given code number however the examiner was unaware of the group to which the specimen belonged. The morphologic changes produced in the liver by the i.p administration of DMN as well as those produced by G-CSF in DMN-administered animals were assessed. The histopathologic index was assayed as the number of cases observed per 10 high power fields (HPF) chosen at random.

Statistics

All data are expressed as mean ± standard error. Data were analyzed by unpaired student's t-test for comparison between two means. A statistical analysis was performed by running the SPSS Version 16.0 for Windows package. Probability values <0.05 were considered as statistically significant.

RESULTS

All animals survived the study without signs of illness .None of the rats receiving the G-CSF and DMN doses died. Representative pictures of hematoxylin and eosin stained liver sections are shown in Figures 1 and 2. There were no abnormalities or histological changes in the livers of control (Group I) rats (Figure 1). Central vein, liver cords and sinusoids, bile duct, parenchymal cells and kupffer cells were normal. Results from histological studies correlated with the level of biochemical parameters. No deaths were observed due to DMN and/or G-CSF treatment during the experiment. We have administered the G-CSF in healthy rats up to 7

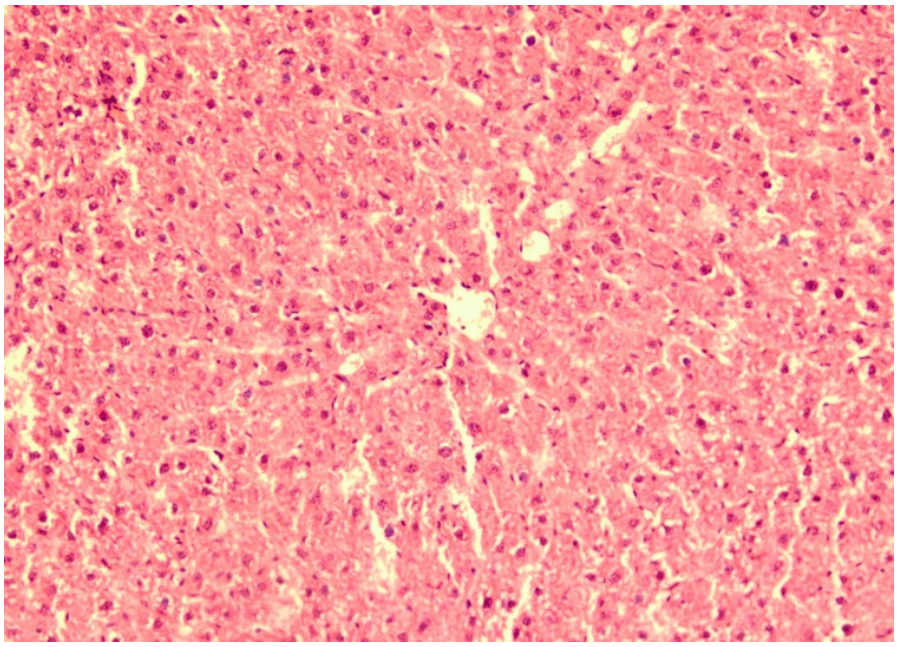


Figure 1. Histological analysis of liver sections from control rat (hematoxylin and eosin staining, original magnification 100××x).

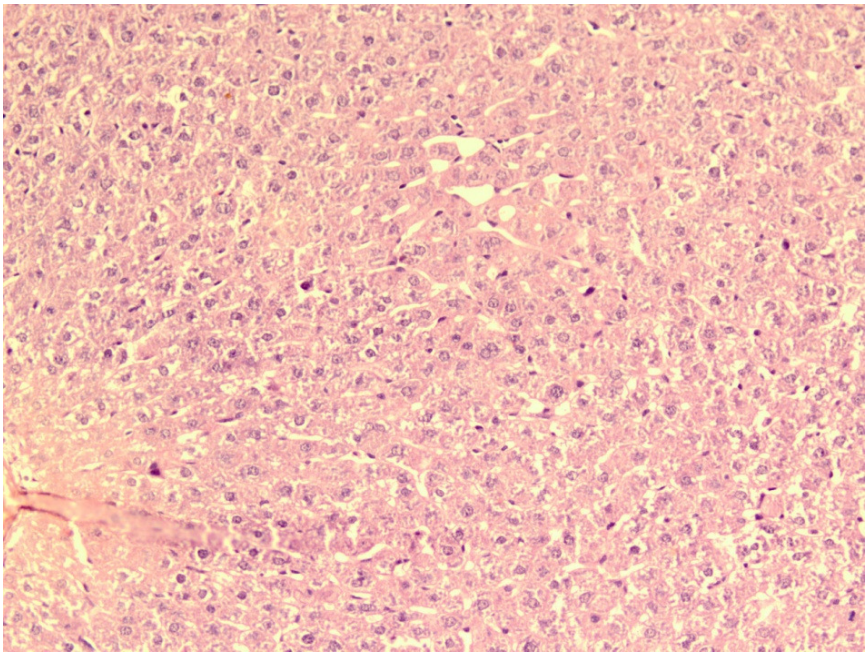


Figure 2. Histological analysis of liver sections after administration of G-CSF alone – treated group (hematoxylin and eosin staining, original magnification 100××x).

consecutive days without finding any evidence of tissue injury. There were no biochemical abnormalities, (data not shown) or histological changes in the livers of (Groups I and III) rats. Figure 2 shows degree of hepatic change in liver sections of rat treated with G-CSF. Our results showed that treated with G-CSF in DMN administered rat caused a significant rise in albumin and total protein compared with untreated group. Accompanied by a drop in the hydroxyproline level compared with untreated group, DMN administration

caused slight to moderate increased activities of serum hepatic enzymes compared to those observed treated group; aminotransferases are enzymes widely used to assess liver damage. Elevated aminotransferase activities suggest liver damage. Any injury to the liver that results in necrosis causes the liberation of transaminases enzymes. The measurement of these hepatic enzymes in the serum is used to assess the extent of liver damage.

DISCUSSION

In the present study, the DMN treatment caused liver damage in rats as evidenced by increased serum aminotransferase activities (data not shown) levels. A marked rise in the levels of aminotransferases activities caused by liver injury. Any injury to the liver that results in damaging liver causes the liberation of various biochemical parameters. The measurement of these liver parameters in the serum is used to assess the extent of liver injury. Low serum albumin levels due to liver damage were caused by destruction of liver tissue. The diminution in albumin content was paralled by fall in total serum protein. A decreased serum albumin maybe due to decreased liver protein synthesis. This phenomenon was confirmed by the histological changes. Histological and biochemical evidence from this study supports the effectiveness of G-CSF for treating liver damage by DMN. The histological sections of rat livers following treatment with G-CSF indicated reduced incidence of liver injury, hepatocyte swelling, leukocyte infiltrations and necrosis induced by DMN. Controversial experimental observations ascribe different roles to G-CSF. In this regard many investigators demonstrate that G-CSF decreases aminotransferases activity and subsequently enhance liver regeneration in chemical–induced liver injury in animal models (Theocharis et al., 2003). Other investigators report that G-CSF administration significantly improves survival of animals in the liver injury model induced by chemical liver injury (Yannaki et al., 2005). Similar results were obtained in the present study. This role of G-CSF in reducing the damaging effect of chemical toxin was challenged by other studies. G-CSF administration did not shown liver repair in chemical induce liver injury in such studies (Ono, 2004; Ogiso, 2007). These results differ from the data in the present study. It is difficult to explain the discrepancies between these studies. The mechanism by which G-CSF induce protective effects on liver injury are not well understood. The precise mechanism will require further basic and clinical studies. To explain the protective action of G-CSF in rat, we hypothesized that the reduction in transaminses activities and sever liver injury following the administration of G-CSF might be attributed to several mechanism: first, it facilitate improving of liver function with regard to hepatocyte function, a unique feature of liver tissue, ability to regenerate. Secondly, with regard to kupffer cells function, G-CSF may aid in the immune mechanism by removing chemical toxin or drug detoxification via reduction reactions. Thirdly, recruitment of stem cells from the bone marrow by the G-CSF is an attractive hypothesis.

G-CSF may induce liver homing of bone marrow cells to regenerate liver function. Hematopoiesis may occur in the rat liver in damage state. However, relatively little is known about the mechanism of it. Several, possible mechanisms for the association between G-CSF and liver ability to regeneration must be considered in understanding of its details. In future studies, it will be important to examine the possible roles of G-CSF and tissue injury. Further basic and clinical experimental investigations are needed to explore the role of G-CSF in acute and chronic liver injury.

Conclusions

Our present findings taken together with previous results demonstrated that G-CSF administration facilitate improvement of liver function in the experimental model of acute and chronic induced by DMN. Our findings suggest that administration of G-CSF could be an effective approach to the treatment of liver damage. This observation is in agreement with the results obtained by other investigators.

ACKNOWLEDGMENTS

We wish to express our gratitude to the staff of the Departments of Biochemistry and Anatomical Sciences, Babol University School of Medicine, Babol, Iran for their assistance in blood collection and liver tissue samples. This investigation was a collaborative work of the Cellular and Molecular Biology Research Center and the Faculty of Medicine. This investigation was supported by grants # 8929126 and # 381 from the Research Council of Babol University of Medical Sciences. We also wish to thank Mr. Shikhzadeh for his excellent technical assistance.

REFERENCES

Avalos B (1996). Molecular analysis of the granulocyte colony-stimulating factor receptor. Blood. 88: 761–777.

Avalos B, Gasson J, Hedvat C, Quan S, Baldwin G, Weisbart R, Williams R, Golde D, DiPersio J (1990). Human granulocyte colony-stimulating factor: biologic activities and receptor characterization on hematopoietic cells and small cell lung cancer cell lines. Blood.75: 851-857.

Bergley C, Lopez A, Nicola N, Warren D, Vadas M, Sanderson C, Metcalf D (1986). Purified colony-stimulating factors enhance the survival of human neutrophils and eosinophils in vitro: A rapid and sensitive micro-assay for colony-stimulating factors. Blood. 68: 162-166.

Clark SC, Kamer R (1987). The hsematopoietic colony-stimulating

factors. Science, 236: 1229–1237.
George J, Rao KR, Stern R, Chandrakasan G (2001). Dimethylnitrosamineinduced Liver injury in rats: the early deposition of collagen. Toxicol., 156: 129–138.
Ghanem LY, Dahmen U, Dirsch O, Nosseir MM, Mahmoud SS, Mansour WA (2010).Does granulocyte-colony stimulating factor administration induce damage or repair response in schistosomiasis? World J. Hepatol., 27: 2(12): 434-441.
Haggerty HG, Holsapple MP (1990).Role of metabolism in dimethylnitrosamine- induced immunosuppression: a review. Toxicol., 63: 1-23.
Hara M, Yuasa S, Shimoji K, Onizuka T, Hayashiji N, Ohno Y, Arai T, Hattori F, Kaneda R, Kimura K, Makino S, Sano M, Fukuda K (2011). G-CSF influences mouse skeletal muscle development and regeneration by stimulating myoblast proliferation. J. Exp. Med., 193(1): 1-8.
Ichinose Y, Hara N, Ohta M, Aso H, Chikama H, Kawasaki M, Kubota I, Shimizu T, Yagawa K(1990). Recombinant granulocyte colony-stimulating factor and lipo-polysaccharide maintain the phenotype of and superoxide anion generation by neutrophils. Infect Immunol., 58: 1647- 1652.
Lian WS, Lin H, Cheng Wt, Kikuchi T, and Cheng CF (2011).Granulocyte-CSF induced inflammation-associated cardiac thrombosis in iron loading mouse heart and can be attenuated by statin therapy. J. Biomed. Sci., 18(26): 1-16.
Link DC (2000). Mechanisms of granulocyte colony-stimulating factor-induced hematopoietic progenitor-cell mobilization. Seminar Hematol., 37(Suppl 2): 25–32.
Ogiso T, Nagaki M, Takai S, Tsukada Y, Mukai T, Kimura K, Moriwaki H(2007). Granulocyte colony-stimulating factor impairs liver regeneration in mice through the up-regulation of interleukin-1bq. J. Hepatol., 47(6): 816-825.
Ono M, Yu B, Hardison EG, Mastrangelo MA, Tweardy DJ(2004). Increased susceptibility to liver injury after hemorrhagic shock in rats chronically fed ethanol: role of nuclear factor-kappa B, interleukin-6, and granulocyte colony-stimulating factor. Shock, 21: 519–525.
Takayama H, Miyake Y, Nouso K, Ikeda F, Shiraha H, Takaki A, Kobashi H and Yamamoto K (2011).Serum levels of platelet-derived growth factor-BB and vascular endothelial growth factor as prognostic factors for patients with fulminant hepatic failure. J. Gastroenterol. Hepatol., 26: 116–121.
Theocharis SE, Papadimitriou LJ, Retsou ZP, Margeli AP, Ninos SS, Papadimitriou JD (2003).Granulocyte-colony stimulating factor administration ameliorates liver regeneration in animal model of fulminant hepatic failure and encephalopathy. Dig. Dis. Sci., 48: 1797–1803. (Please provide full names of authors).
Yannaki E, Athanasiou E, Xagorari A, Constantinou V, Batsis I, Kaloyannidis P, Prova E, Anagnostopoulos A, Fassas A (2005). 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., 33: 108-119.
Zhang L, Kang W, Lei Y, Han Q, Zhang G, Lv Y, Li Z , Lou S, Liu Z (2011) .Granulocyte colony-stimulating factor treatment ameliorates liver injury and improves survival in rats with d-galactosamine-induced acute liver failure. Toxicol. Lett., 204: 92–99.