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Effects of Combination of G-CSF and SCF One Week Prior to Liver Injury in Acute liver Damage Model Induced by Thioacetamide Administration

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Abstract

Background: There are many reports regarding to effects of Granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF)alone in liver repair .But conflicting data have been reported regarding the role of growth factors such as G-CSF and SCF in the liver regeneration system. Also, there is not such data regarding to effects of co-administration both of G-CSF and SCF in the liver damage condition.

Materials and Methods: An experimental model of rat liver damage induced by the thioacetamide. Five different groups of animals receiving 0.9% NaCl, TAA alone, TAA + G-CSF, TAA + SCF and TAA + (G-CSF+SCF).The activity of glutamate pyruvate transaminase (GPT/AlT)and glutamate oxaloacetate transaminase (GOT/AST) were measured after the thioacetamide (TAA) injection and the administration of combination of G-CSF +SCF for 12 weeks. Also histological tests were carried out at the end experiments.

Results: The pre-treatment of combination of G-CSF and SCF for 12 weeks reduced the degree of liver injury. The mean of GOT activity was 61.24 (U/L) in the G-CSF +SCF and versus 132.86 in the TAA-alone group. These differences in the GOT activity were statistically significant ($P<0.05$). Also, in the G-CSF +SCF and TAA group the mean of GPT activity (4.35 versus 11.79, respectively) were lower than in the TAA-alone group, this difference was statistically significant ($P<0.05$). Liver sections from a rat treated only with TAA, showing damage, but TAA and G-CSF + SCF no significant damage is present. On the other hand histological results revealed a very mild degree of inflammation were observed in the livers of the combination of G-SCF+SCF and TAA-treated rats compared to TAA only treated group.

Conclusion: Biochemical and microscopic analysis revealed that combination of G-CSF and SCF pre-treatment significantly enhances liver regeneration after TAA-induced liver injury.

Keywords: Damage; Granulocyte colony-stimulating factor; Stem cell factor; Thioacetamide; Glutamate pyruvate transaminase; Glutamate oxaloacetate transaminase

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Introduction

Recent studies from several laboratories have demonstrated that thioacetamide [TAA, CH3-C(S) NH2], induce liver injury. In addition, it has been found that after administration in animals, it undergoes further metabolism by the enzymes to acetamide (1-2). In this regard recently, several papers have been published on that reactive intermediates in this pathway strongly bind to liver biomolecules and eventually cause disruption of liver architecture (3, 4). Recent studies, however, have demonstrated that

thioacetamide-S-oxide is metabolized by several different pathways to further compounds, including the very reactive compound thioacetamide-S-dioxide, which increased disruption of tissue architecture (5-6). Based on these findings the binding of this metabolite to tissue macromolecules may be responsible for the induction of liver injury (7, 8-11). As previously reported by investigators it is established that G-CSF treatment after chemical agent administration could significantly increase the survival rate in the rats (12). A growing of evidence suggests that G-CSF can stimulate bone marrow progenitor maturation into granulocyte colonies (13). In this respect, investigators demonstrated that G-CSF stimulate the proliferation of progenitor cells of hematopoietics cells in the bone marrow into mature granulocytes (14). According to previous study SCF is a cytokine that plays important role in survival of precursor or progenitor cells (15). It has been suggested that cytokines that are candidate in liver-remodeling processes include stem cell factor, granulocyte colony-stimulating factor, these regulate bone marrow production of circulating red cells (16). Some researchers have reported that the SCF regulates the differentiation of CD34- positive stem cells, conversely whereas other factors, such as G-CSF, modulate the synthesis of more specific cell types (17). In respect to liver regeneration, the liver has three levels of cells that can respond to liver injury include mature hepatocytes, progenitor cells, and stem cells (18-19). Based on the mentioned studies, we designed this project in order to study the protective potential of co-treatment of G-CSF and SCF in liver injury. We utilized a rat model of TAA-induced liver damage leading to dysfunction. On the other hand the aim of our study was to examine the pre-treatment of G-CSF and SCF on liver repair in an experimental model of acute damage induced by the thioacetamide.

Materials and Methods

Materials

Granulocyte colony stimulating factor, human recombinant expressed in E.Coli. (G-CSF), product number G 0407; GOT/AST, 89003; GPT/ALT 89003; Stem cell factor(SCF), S7901-10UG; TAA,T 3057-25 g were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals

Male rats (180-230 g), were obtained from the Babol University animal center, and were kept in a temperature-controlled environment (22±5 ºC), and fed adlibitum with standard rat chow. Animals were kept in a 12:12-h light/dark cycle at constant temperature and humidity. It was carried out under the control of the guidelines for animal experiments. All animals were carefully maintained under standard animal house conditions. Furthermore, all protocols involving animals were approved by Babol University Animal Care and Use Committee. Effort was made to minimize the number of animals.

Experimental design

TAA was dissolved in 0.9% NaCl and a volume of 1 ml was injected intraperitoneally at a dosage of (175 mg/kg/rat in 5 ml/kg saline) twice weekly for two weeks. Dosage of materials was carried out as previously described (21-24). Five groups of ten rats each were treated as follows: First group received only 0.9% NaCl. Second group received only TAA. Third group received TAA plus G-CSF (125 µg/kg). Forth group received TAA plus SCF (75 µg/kg) .Fifth groups received TAA plus combination of G-CSF (125 µg/kg) +SCF (75 µg/kg) one week prior to liver injury by TAA injection (pre –treated). All the rats received the above mentioned substances for 12 weeks. End of experiments at four, eight and twelfth weeks after TAA treatment, blood samples (200-500 µl) were collected from tail of rats. Rats have been scarified after 12 weeks and liver samples were collected. Then one part of the liver each rat was sampled for histology.

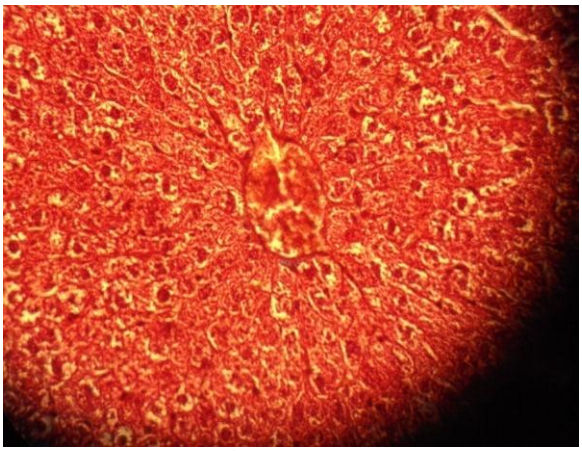


Figure 1. Liver section from a rat that received TAA (G-CSF +SCF) for 12 weeks. Note less nodular formation and fibrotic septa. (Hematoxylin and eosin; original magnification 40)

Evaluation of liver injury

Serum liver enzymes activity (glutamate pyruvate transaminase, GPT/AlT;glutamate oxaloacetate transaminase, GOT/AST) and liver histology were determined at four, eight and twelfth weeks following the first TAA injection.

Liver histopathology

The rats were sacrificed at the completion of the treatment protocols, their livers were removed .For

liver histopathology analysis, and one section of the liver was processed for light microscopy. This processing consisted of fixing the specimen in a 5% neutral formalin solution, embedding the specimens in paraffin, slicing sections 5 µm thick, and staining the sections with hematoxilin and eosin. The tissue slices were scanned and scored blindly by expert histothologists. The degree of damage was expressed as the mean of 10 different fields.

Liver enzymes

The activity of glutamyl oxaloacetic transaminase (GOT/AST) and glutamyl pyruvic transaminase (GPT/ALT) in the serum, were measured by using standard kit. All assays were performed according to the procedures described by the kit manufacturers, Sigma Chemical Co. (St. Louis, MO).

Ethics statements

The approval of the Ethics Committee of Babol University was also obtained (#: PJ30.3989, 90. 3.31).

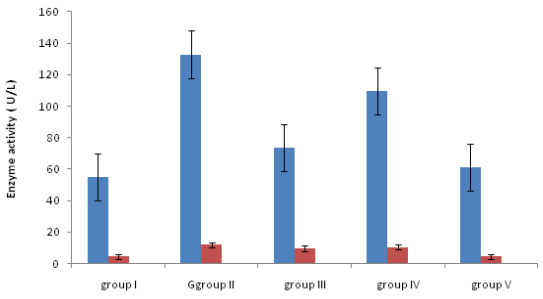
Statistical analysis

All data expressed as Mean ± SD. A One-Way ANOVA followed by a Tuky test was used for analysis of differences in multiple comparisons between the groups. In each case, the null hypothesis was rejected if the probability of no differences was less than 5%.

Results

Our data showed that histopathologic examination of liver specimens showed severe damage in the livers of the rats treated with TAA only (175 mg/kg). In contrast, no evidence of damage and a very mild degree of inflammation, less nodular formation and fibrotic septa, were observed in the livers of the combination of G-SCF+SCF and TAA-treated rats (Figure 1). The mean GOT activity was 61.24 (U/L) in the G-CSF +SCF and 132.86 versus in the TAA-alone group. These differences in the GOT activity were statistically significant (P<0.05).

Table1. Glutamate pyruvate transaminase, GPT; and glutamate oxaloacetate transaminase, GOT activity. Column 1, GOT activity; Column2, GPT activity.



In the G-CSF +SCF and TAA group the mean GPT activity (4.35 versus 11.79, respectively) were lower than in the TAA-alone group, this difference was statistically significant (P<0.05). Compared with the TAA-injury group, the blood GOT and GPT declined in the combination of G-CSF +SCF plus TAA-treated rat (Table1).

Discussion

It is well known that serum biochemical analyses such as branched amino acids to the serum content of tyrosine, urine sulfated bile acids and liver enzymes are used for the evaluation of liver damage condition. In this regard, in the present investigation, we have shown that treatment of rats with TAA induced liver damage associated with elevated levels of liver enzymes such as glutamate pyruvate transaminase and glutamate oxaloacetate transaminase and increased urine sulfated bile acids (data not shown). Theses biochemical parameters are useful indicators for evaluation of liver damage. In the present study, the fact that TAA caused the increment of serum glutamate pyruvate transaminase and glutamate oxaloacetate transaminaseactivity, agrees with previous studies in TAA administration (3-5). In contrast, in the rats treated with TAA and combination of G-CSF and SCF the activity of GOT and GPT were significantly decreased, compared to the animals which received TAA only. Our results showed that combination of G-CSF and SCF had more effect in reducing damage in our rat model compared to administration of only G-CSF, as previously reported (20-22). We suggest that the synergy observed between SCF and G-CSF is probably due to be a consequence of interaction between G-CSF and SCF.

Our hypothesed suggest that the up-regulation of liver repair with combination of G-CSF +SCF in pre-treatment manner might be attributed to: first, stimulates rat liver cell protect or to accelerate the development of liver repair process. Second, may be due to the role of these growth factors to migrate to liver tissue in response to damage to participate to liver repair. Third, the improved liver function may also be a consequence of interaction SCF and G-CSF. Forth, may be attributed to a number of intracellular pathways that mediated by biologic actions of SCF and G-CSF. Fifth may be attributed to the promotion of endogenous repair programs by G-CSF and SCF.

These findings of our experiments are in contrast with those reported by the other investigator (23). This discrepancy may be due to the dosage, experimental time and animal models used. Our findings agreement with previous report that they found G-CSF administration improves liver repair after injury (24).

Conclusion

Combination of G-CSF and SCF had beneficial effect in reducing damage in our rat model. We conclude that combination of G-CSF and SCF pre-treatment significantly enhances liver repair after TAA–induced injury.

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