Original Research Article

Assessing the effect of oleic acid on markers of hepatocyte transplantation in Wistar rat model of induced liver damage

Abstract

Aims*:* Hepatocyte transplantation is an alternative to liver transplantation for acute liver failure (ALF). Hepatocyte therapy is limited by several factors including limited homing of transplanted cells and liver functional improvement. Our aim was to study oleic acid effects on hepatocyte transplantation outcome.

Methodology: ALF was induced by acetaminophen (APAP) injection. Hepatocytes were isolated from male rats and transplanted intraperitoneally into female rats (ALF+HT group). Effect of oleic acid was assessed in rats fed an oleic acid rich diet (ALF+HT+OA group). Plasma levels of albumin (ALB), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) Alkaline phosphatase (ALP) were determined. Detection of Y-chromosome by PCR was used for homing assessment of transplanted hepatocytes. Finally, hematoxylin and eosin staining was used for histopathologic evaluation of liver.

Results: APAP injection resulted in an increase in levels of ALT, AST and ALP. ALT level was decreased to normal range only in ALF+HT+OA group. Oleic acid administration lowered the maximum amount of elevated AST levels compared to ALF+HT group. No significant difference was observed between ALF+HT group and ALF+HT+OA group in

ALP recovery. Plasma level of ALB was decreased after APAP injection which was only fully retrieved in ALF+HT+OA group. SRY detection by PCR confirmed successful engraftment of transplanted hepatocytes. H&E staining revealed that OA administration lead to an increase in the number of normal hepatocytes and reduced inflammation in the liver.

Conclusion: In conclusion, our findings suggest that dietary oleic acid may improve hepatocyte transplantation success via improvement of liver function.

Keywords: Hepatocyte therapy, cell therapy, Liver failure, oleic acid

Introduction:

The liver is a vital organ with many essential functions related to homeostasis, digestion, detoxification, protein and lipid metabolism, synthesis of albumin and coagulation factors, immunity, energy supply and glucose storage [1, 2] , while hepatocytes ability of self-renewal makes liver a regenerative organ [3, 4]. Liver disease remains among the top 12 leading causes of death globally [5]. Liver failure involves millions of people worldwide. Acute liver failure (ALF) is a condition in which liver dysfunction occurs rapidly following severe damage of hepatocytes and often results in rapid deterioration of mental status and potential multi-organ failure [6, 7]. Liver transplantation is the ultimate treatment for liver failure. Since the demand for whole liver transplant outruns the number of suitable donors, according to the United Network for Organ Sharing (UNOS) 40% of listed patients each year do not receive a liver transplant. Also, the 5-year survival following liver transplantation has only been 70%–80% [8-10].

While recent studies on cell based therapies on animal models of liver failure revealed hepatocytes regenerative ability in vivo [11-14] much attention has been paid to hepatocyte transplantation as an alternative to whole organ transplantation. Hepatocyte transplantation (HT) could be considered a potential minimally invasive treatment for liver failure [15, 16]. Primary isolated mature hepatocytes can be used as a source for cell transplantation procedures and delivered through portal vein [17].

Beneficial effects of plant derived natural compounds found in diet on human health and their therapeutic potential have widely been studied [18-24]. Monounsaturated fatty acids (MUFA) have been shown to possess health effects in cardiovascular diseases [25], diabetes [26], immunity, inflammation and etc. [27], [28]. Oleic acid (OA) has been found to act as an antitumor agent [29] and also affect liver metabolism and hepatocytes function [30-33].

Since oleic acid effect on the outcome of hepatic cell therapy has not been addressed, this manuscript studies effect of oleic acid administration on mature hepatic cell transplantation in rats with induced acute liver failure.

Materials and methods:

–Ethical approval and Animals

The studies were performed in accordance with guidelines established by the Research Animal Care and Use Committee of Tabriz University of Medical Sciences and all animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985).

8-week old male and female Wistar rats with an initial body weight ranging from 200-250 g were obtained from experimental Animal Unit of Tabriz University of Medical Sciences and maintained on a 12 hours light/12 hours dark cycle in a temperature-controlled environment (22°C). Animals had free access to food and water and were fed a standard rodent chow. Male and female rats were used as donors and recipients, respectively.

– Rat model of hepatic failure:

24 hours before hepatocyte therapy, acetaminophen (N-acetyl-p-aminophenol [APAP]) (Sigma, Germany) was administered in female rats in a single dose of 1 g/kg using intraperitoneal injection. It should be mentioned that four days before APAP injection animals received Phenobarbital at 350 mg/L in drinking water for 10 days (to increase APAP induced hepatotoxicity) [34].

– Oleic acid administration:

180 g oleic acid (Sigma, Germany) was fed to female rats per kilogram food pellet from day 0 until the end of the experiment (day 10).

–Hepatocytes isolation:

Hepatocytes were isolated from male rats by liver perfusion with collagenase as describe previously [35].

After Anaesthetization of rats by diethyl ether, collagenase was perfused through portal vein and finally liver digested in Hanks buffer. Isolated hepatocytes were separated by sedimentation. Cell viability of isolated hepatocytes was assessed by trypan blue uptake test after suspension in krebs- henseleit buffer [36].

All buffers were sterile and freshly prepared with pH 7.4.

– Determining the viability of isolated hepatocytes:

The viability of isolated hepatocytes was assessed by trypan blue exclusion assay. Isolated hepatocytes were stained with 0.2% trypan blue solution. After determining the viability, cells were suspended in buffer IV and prepared for transplantation to recipient rats. In our experiments cell viability ranged between 95-98 %.

–Cell transplantation procedure:

24 hours following APAP induced hepatotoxicity, a total of 10^7 hepatocytes were injected intraperitoneally into female rats. 40 female rats were separated into four groups as following: APAP group (n=10), control group (n=10), APAP+HT group (n=10), APAP + HT+ OA group (n=10).

–Sampling:

On day 0 and on days 1, 2, 3, 6 and 10 following hepatocyte transplantation, blood samples were collected from orbital sinus of female rats for biochemical analysis of liver enzymes.

–Assessment of transplanted cells engraftment:

Detection of the Y-chromosome in the liver tissue of female recipient rats can determine the efficacy of hepatocyte therapy [34]. Polymerase chain reaction (PCR) was used to identify male-specific SRY gene. On the 10th day of the experiment, all hepatocyte transplanted female rats were anesthetized by ether and liver samples were collected for SRY gene detection. DNA was extracted by TRIZOL and PCR was performed using following set of primers [35]

Forward: 5’AAGCGCCCCATGAATGCATT 3’

Reverse: 5’CAGCTGCTTGCTGATCTCTG3’

–Functional assessment by biochemical analysis:

Blood samples were centrifuged at 5000 rpm for 5 minutes and serum was separated in order to measure liver enzymes including Alanine transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline phosphatases (ALP) plus albumin (ALB) protein using Biochemistry Autoanalyzer (Alpha Classic At plus).

–Histopathologic evaluation of rat liver:

Rats were anesthetized with chloroform and the liver was removed and fixed in 10% formalin, and embedded in paraffin. Liver samples were sectioned serially with 50µm intervals and 5µm thickness. Sections of liver were stained with H&E and studied with light microscope. The 50µm interval was chosen based on the size of liver lobules.

–Data analysis:

Data in this report are presented as mean ± SEM and analyzed by analysis of variance (ANOVA) and student t-test. P value of 0.05 was considered statistically significant.

Results:

Plasma kevel of liver enzymes:

As shown in Figure 1, in the first 24 hours following APAP administration a marked increase was observed in plasma levels of ALT. Hepatocyte transplantation was not able to fully attenuate increased ALT levels and at the end of the experiment (day 10) ALT levels were still higher as compared with control group. On the other hand, oleic acid administration in combination with hepatocyte therapy seemed to be more effective and was able to restore ALT levels to the normal range compared to healthy rats.

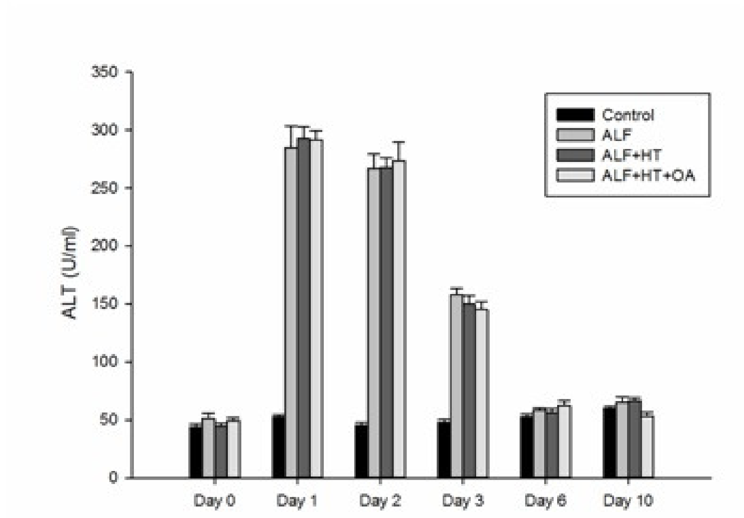

Figure 1 Serum levels of ALT in experimental groups compared to average serum levels of ALT in control group throughout the experiment period (10 days).

As shown in Figure 2, APAP administration resulted in significant increase in serum AST levels which was similarly attenuated in both ALF+HT group and ALF+HT+OA group, but was also recovered in ALF group with no hepatocyte transplantation or oleic acid administration. While it seems that in all 3 groups of rats AST level was recovered in a similar manner, a difference between these groups can be observed at day 1 (24 hours after APAP administration). Maximum level of AST was lower in ALF+HT+OA group compared to ALF and ALF+HT group.

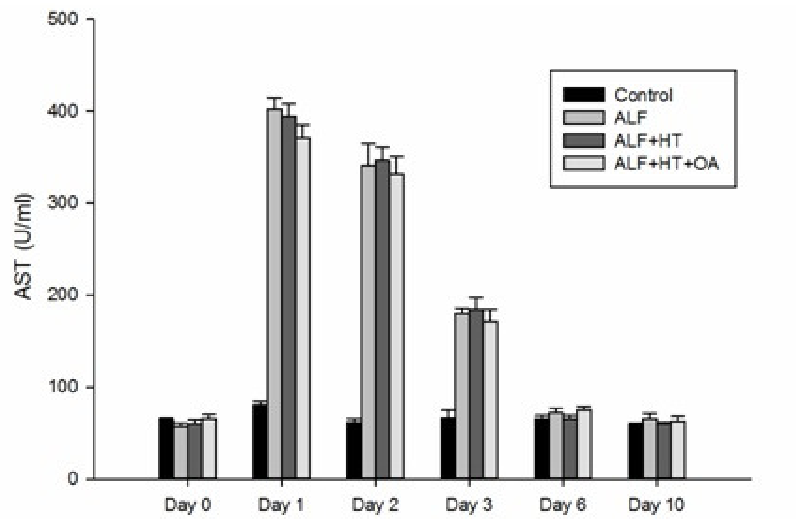

Figure 2 Serum levels of AST in experimental groups compared to average serum levels of AST in control group throughout the experiment period (10 days).

As shown in Figure 3, following APAP administration significant elevations in plasma ALP levels was observed which was fully recovered in all groups within 10 days of the experiment. No significant difference was observed between ALF+HT and ALF+HT+OA groups. However, hepatocyte transplantation seemed to delay the recovery process. As in ALF group (rats with no hepatocyte transplantation) already on day 6 after APAP administration there were no significant differences in plasma ALP levels as compared with healthy rats, while this was not observed in other two groups until the last day of the experiment (day 10).

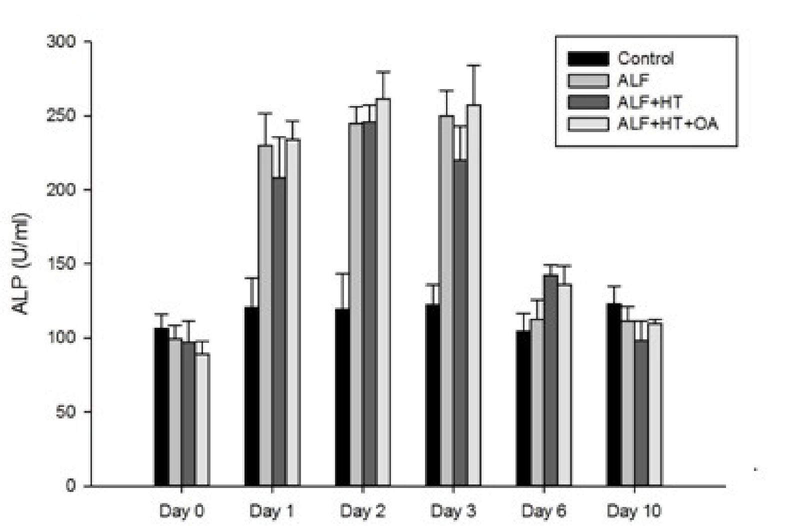

Figure 3 Serum levels of ALP in experimental groups compared to average serum levels of ALP in control group throughout the experiment period (10 days).

As shown in Figure 4, following APAP administration a drastic decrease in plasma albumin levels was observed with the maximal decrease seen at day 1 (24 hours after APAP injection) in all experimental groups. This decrease in albumin level was not recovered in ALF group throughout the experiment. Hepatocyte transplantation was mostly able to retrieve the decrease in plasma albumin levels at day 10. Oleic acid administration seemed to be more effective in returning albumin levels to normal range compared to hepatocyte transplantation alone. As beginning from day 3 after APAP administration the plasma albumin levels were not significantly lower as compared with healthy rats.

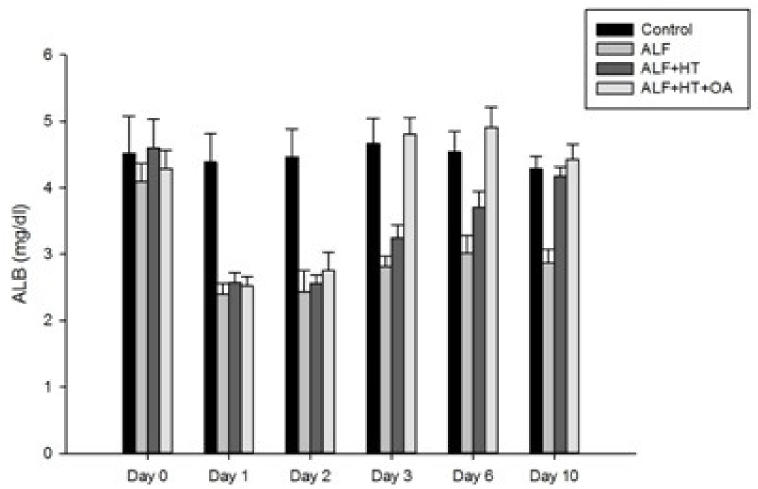

Figure 4 Serum levels of ALP in experimental groups compared to average serum levels of ALP in control group throughout the experiment period (10 days).

SRY detection in female rat liver:

Figure 5, represents gel electrophoresis detection of SRY gene following PCR in male and female rats after hepatocyte transplantation both in ALF+HT group and in ALF+HT+OA group, showing successful engraftment of transplanted hepatocytes in recipient rats.

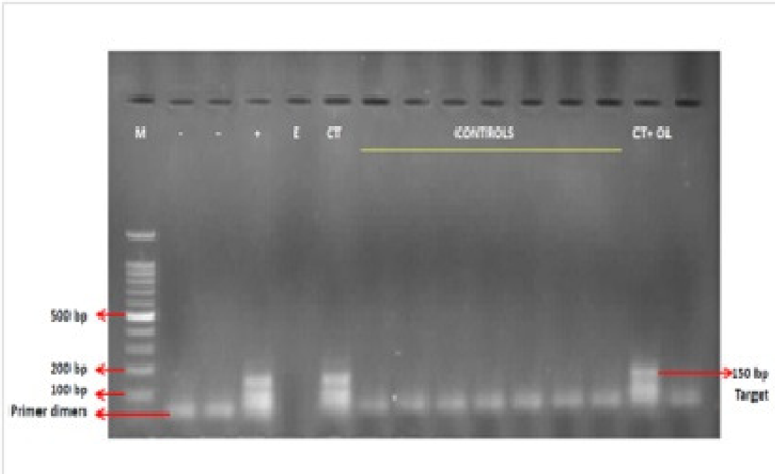

Figure 5 Gel electrophoresis of SRY gene. DNA ladder (M), primer controls (-), male donor rats hepatocytes (+), hepatocyte therapy group (CT) and hepatocyte therapy+oleic acid group (CT+OL).

Histopathologic evaluation of rat liver:

Figure 6 represents example images of H&E stained histological liver sections. In ALF group, some degree of inflammation and degeneration with the presence of lymphoid cells has been detected. Absence of hepatic cell's outline is observed near the central Vein. Also an increase in the number of hepatocytes with condensed nuclei in addition to sinusoidal congestion is apparent.

In ALF+HT+OA group typic hepatocytes with a clearer outline is recognized. Although venus congestion of the central vein is still present. But the presence of less lymphoid cells near central vein indicates decreased inflammation.

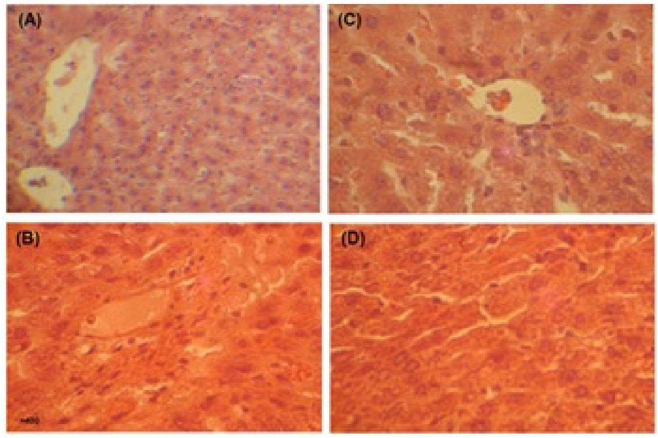

Figure 6 Histopathological evaluation of rat liver. (A) Control group, with normal hepatocyte, central vein and sinusoids. (B) ALF group, a mild inflammatory cell infiltration with fibrotic tissue near the central vein. (C), (D) ALF+HT+OA group with reduced inflammation, improved lobular structure and normal hepatocytes. Hematoxylin and eosin (H&E) staining.

Discussion:

Insufficient liver donors, immunological issues regarding the use of immunosuppressant after whole liver transplantation, constant post-operative care and etc. makes hepatocyte transplantation as one of the alternatives for orthotopic liver transplantation in patients with acute liver failure (ALF). Another advantage of hepatocyte transplantation is that a single liver donor could be used for multiple recipients [17, 37]. Despite considerable number of studies on hepatocyte transplantation since 1992, there are still some limitations to this technology as a treatment for liver failure. Some of these major limitations include the limited number and quality of liver tissues as cell sources, quality control evaluation of hepatocytes prior to transplantation, preconditioning treatments to enhance engraftment and proliferation of donor cells, tracking or monitoring cells after transplantation [38].

In this paper the effect of hepatocyte transplantation on the liver function after induction of acute liver failure by APAP injection and potential positive effect of oleic acid in the hepatocyte transplantation outcome and subsequent liver function was studied.

AST, ALT and ALP and ALB are useful biomarkers of liver injury [39-41]. Administrating APAP in experimental rats resulted in significant increase in the levels of ALT, AST and ALP as well as a decrease in the levels of ALB. In our experiment AST and ALT reached their highest levels 24 hours after APAP injection which is in accordance with previous reports [42]. These results suggest that after liver induced damage, ALT, AST and ALP levels increased within a short period of time which is indicative of liver function failure.

Our results showed that hepatocyte transplantation improved APAP induced ALF which was evident from functional improvement of the liver as indicated by lowering ALT, AST, ALP and increasing ALB levels in recipient rats. These findings suggest that intraperitoneal delivery of hepatocytes is a suitable root in hepatocyte therapy of liver failure. These findings are consistent with previous reports in rat [43].

Although hepatocyte transplantation improved induced ALF, our results show that OA administration in combination with hepatocyte transplantation can restore liver function to the normal level almost completely in 10 days and showed good therapeutic effect. The recovery of ALT, AST and ALB levels in the blood was better in the ALF+HT+OA group than in the ALF+HT group. ALB levels recovery was highly affected by OA as indicated by its fast return to normal levels on day 3, indicating that OA administration may improve synthesis and secretion of ALB by liver cells or alternatively, may have proliferating effect on engrafted hepatocytes.

Rodrigues D. and colleagues injected freshly isolated hepatocytes through portal vein in APAP induced ALF in Wistar rats. They reported ALT levels in days 0, 1 and 3. The highest level of ALT was shown in day 1 and returned to normal level on day 3 [34]. In comparison to our experiment this fast returning time to normal rate could be related to the rout of hepatocyte transplantation.

The engraftment of transplanted hepatocytes in recipient livers was investigated by PCR analysis for SRY in female organs. In both hepatocyte transplantation groups, SRY was positive in liver tissue. SRY detection was performed in day 10 following transplantation showing that hepatocytes migrated and remained in liver for 10 days.

Histopathological alterations were detected following APAP administration in liver tissue. Pre-administration of OA in combination with hepatocyte therapy had a positive effect on liver tissue injuries which was evident from increasing the number of normal hepatocytes and reduce inflammation near central veins. It also seemed to have a positive effect on hepatic lobular structure.

Further research is required on the condition of transplantation, optimizing the number of transplanted hepatocytes, delivery through portal vein and dose of administered OA to provide a definitive conclusion.

In conclusion, our findings suggest that dietary oleic acid may improve hepatocyte transplantation success

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