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. Since beneficial effects of monounsaturated fatty acids on liver function and metabolism have been reported previously, 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. 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 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 and inflammation [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 human 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).

Male and female Wistar rats (8-weeks old) 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 h light/12 h 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:

APAP (Sigma, Germany) was administered in female rats in a single dose of 1 g/kg using intraperitoneal injection 24 h before hepatocyte therapy. 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:***

Oleic acid (180 g) (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:

Following APAP induced hepatotoxicity (24 h), 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 min and serum was separated in order to measure liver enzymes including ALT, AST, ALP plus 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 h 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.

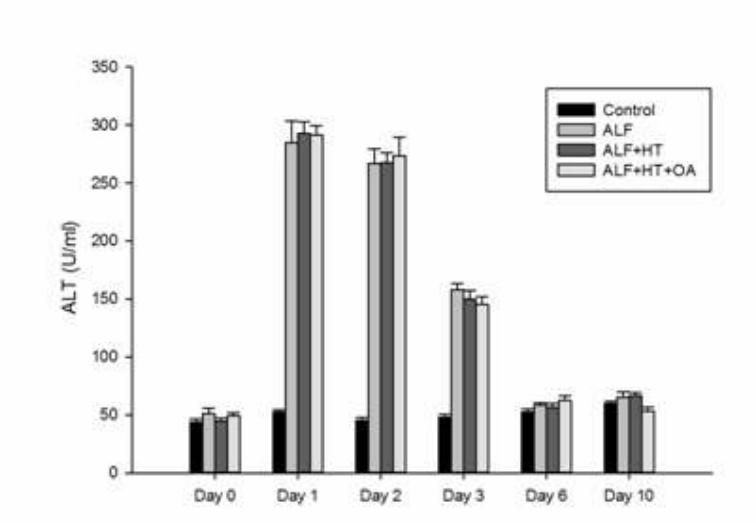

Figure 1 Serum levels of ALT in experimental groups compared to average serum levels of ALT in control group throughout the experiment period (10 days).

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 was observed at day 1 (24 h after APAP administration). Maximum level of AST was lower in ALF+HT+OA group compared to ALF and ALF+HT group.

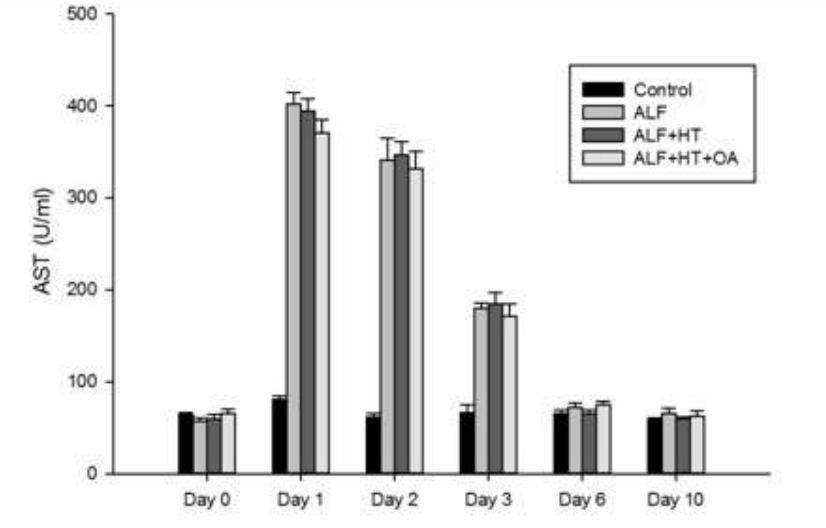

Figure 2 Serum levels of AST in experimental groups compared to average serum levels of AST in control group throughout the experiment period (10 days).

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).

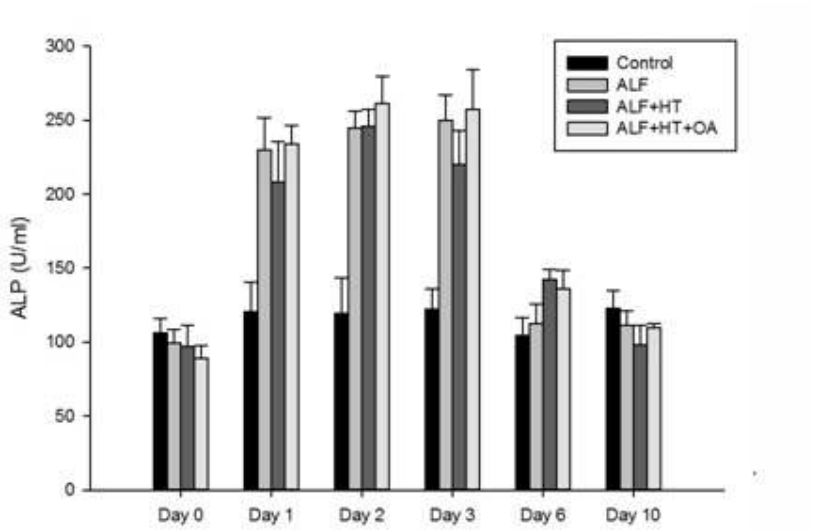

Figure 3 Serum levels of ALP in experimental groups compared to average serum levels of ALP in control group throughout the experiment period (10 days).

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 h 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. In ALF+HT+OA group however, from day 3 after APAP administration the plasma albumin levels were not significantly lower compared to healthy rats.

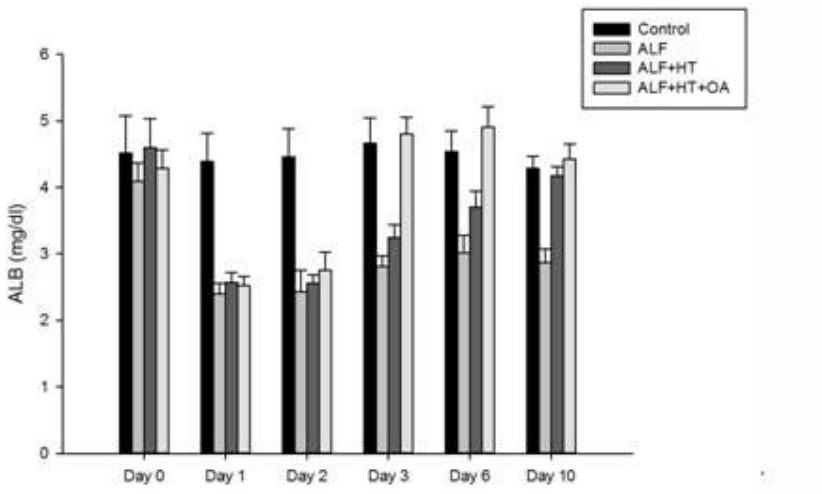

Figure 4 Serum levels of ALB in experimental groups compared to average serum levels of ALB in control group throughout the experiment period (10 days).

SRY detection in female rat liver:

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 was performed. The results showed successful engraftment of transplanted hepatocytes in recipient rats (gel electrophoresis picture not shown).

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 was observed near the central Vein. Also

an increase in the number of hepatocytes with condensed nuclei in addition to sinusoidal congestion was apparent.

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

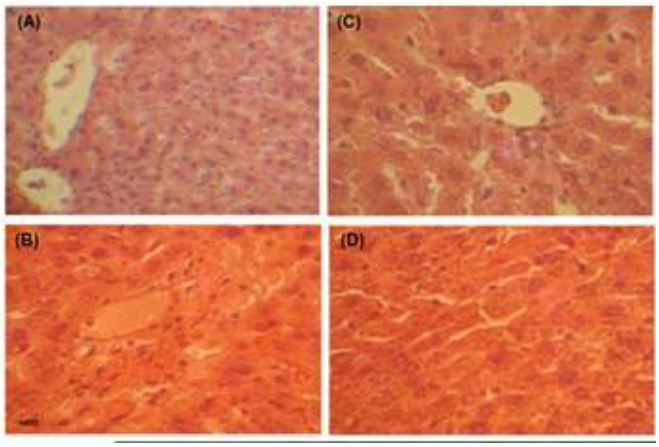

Figure 6 Histopathological evaluation of rat liver. (A) Control group, with normal hepatocyte, central vein and sinusoids. (B) ALF group, a mild inflammatory cell infiltration with fibrotic tissue near the central vein. (C), (D) ALF+HT+OA group with reduced inflammation, improved lobular structure and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

Discussion:

Insufficient liver donors, immunological issues regarding the use of immunosuppressants after whole liver transplantation and constant post-operative care makes hepatocyte transplantation as one of the alternatives for orthotopic liver transplantation in patients with

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

AST, ALT and ALP and ALB are common 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 h after APAP injection which is in accordance with previous reports [42]. Elevated levels of ALT, AST and ALP was observed within a short period of time following APAP injection which is indicative of liver failure. Hepatocyte transplantation improved APAP induced ALF by lowering ALT, AST, ALP, increasing ALB and improved liver function in recipient rats. These findings suggest that intraperitoneal delivery of hepatocytes is a suitable root in cell therapy of liver failure which is consistent with previous reports [43].

Although hepatocyte transplantation improved ALF, our results show that OA administration in combination with hepatocyte transplantation seemed to be more effective and restored liver function to the normal level almost completely in 10 days with good therapeutic effect. The recovery of ALT, AST and ALB levels was improved in the ALF+HT+OA group compared to ALF+HT group. ALB 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 proliferative effect on engrafted hepatocytes. Based on these results, Oleic acid administration seemed to be more effective in returning albumin levels to normal range compared to other tested biomarkers.

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 observed in day 1 and returned to normal level on day 3 [34]. In comparison to our experiment this fast return to normal levels 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 reduced inflammation near central veins. It also seemed to have a positive effect on hepatic lobular structure.

In conclusion, we have demonstrated that OA supplementation ameliorates liver failure and improves hepatocyte transplantation outcome in rat which was demonstrated by serum biomarkers of liver function and histological studies. OA effects may be due to improving liver function, metabolism and hepatocyte proliferation. However, further research is required on the condition of transplantation, optimizing the number of transplanted hepatocytes, available delivery roots and OA dosage to provide a definitive conclusion.

References:

1. Kuntz, E., Kuntz, Hans-Dieter in *Hepatology Textbook and Atlas*. 2008, Springer Berlin Heidelberg. p. 35-76
2. Gaw, A., *Clinical biochemistry : an illustrated colour text*. 4th ed. 2008, Edinburgh ; London: Churchill Livingstone. p.
3. Karp, S.J., *Clinical implications of advances in the basic science of liver repair and regeneration.* Am J Transplant, 2009. **9**(9): p. 1973-80.
4. Michalopoulos, G.K., *Liver regeneration.* J Cell Physiol, 2007. **213**(2): p. 286-300.
5. Kadyk, L.C., et al., *Proceedings: moving toward cell-based therapies for liver disease.* Stem Cells Transl Med, 2015. **4**(3): p. 207-10.
6. O'Grady, J.G., S.W. Schalm, and R. Williams, *Acute liver failure: redefining the syndromes.* Lancet, 1993. **342**(8866): p. 273-5.
7. Jalan, R., *Acute liver failure: current management and future prospects.* J Hepatol, 2005. **42 Suppl**(1): p. S115-23.
8. Yu, Y., et al., *Cell therapies for liver diseases.* Liver Transplantation, 2012. **18**(1): p. 9-21.
9. Alison, M.R., S. Islam, and S.M. Lim, *Cell therapy for liver disease.* Curr Opin Mol Ther, 2009. **11**(4): p. 364-74.
10. *Scientific Registry of Transplant Recipients*. Available from: Available at http://www.srtr.org.

11. Jamal, H.Z., T.C. Weglarz, and E.P. Sandgren, *Cryopreserved mouse hepatocytes retain regenerative capacity in vivo.* Gastroenterology, 2000. **118**(2): p. 390-4.
12. Ho, C.M., et al., *Transplantation Speed Offers Early Hepatocyte Engraftment in Acute Liver Injured Rats - A Translational Study with Clinical Implication.* Liver Transpl, 2015.
13. Koblihova, E., et al., *Hepatocyte transplantation attenuates the course of acute liver failure induced by thioacetamide in Lewis rats.* Physiol Res, 2015.
14. Kwon, Y.J., K.G. Lee, and D. Choi, *Clinical implications of advances in liver regeneration.* Clin Mol Hepatol, 2015. **21**(1): p. 7-13.
15. Fox, I.J. and J.R. Chowdhury, *Hepatocyte transplantation.* Am J Transplant, 2004. **4 Suppl 6**: p. 7-13.
16. Fox, I.J., et al., *Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation.* N Engl J Med, 1998. **338**(20): p. 1422-6.
17. Lee, L.A., *Advances in hepatocyte transplantation: a myth becomes reality.* J Clin Invest, 2001. **108**(3): p. 367-9.
18. Pandey, K.B. and S.I. Rizvi, *Plant polyphenols as dietary antioxidants in human health and disease.* Oxid Med Cell Longev, 2009. **2**(5): p. 270-8.
19. Pan, M.H. and C.T. Ho, *Chemopreventive effects of natural dietary compounds on cancer development.* Chem Soc Rev, 2008. **37**(11): p. 2558-74.
20. Fugh-Berman, A. and J.M. Cott, *Dietary Supplements and Natural Products as Psychotherapeutic Agents.* Psychosomatic Medicine, 1999. **61**(5): p. 712-728.
21. Tripoli, E., et al., *The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health.* Nutr Res Rev, 2005. **18**(1): p. 98-112.
22. Taghizadeh, B., et al., *Equol as a potent radiosensitizer in estrogen receptor-positive and -negative human breast cancer cell lines.* Breast Cancer, 2013.
23. Britton, G., S. Liaaen-Jensen, and H. Pfander, *Carotenoids Volume 5: Nutrition and Health*. 2009: Birkhäuser Basel.
24. Vitaglione, P., et al., *Dietary antioxidant compounds and liver health.* Crit Rev Food Sci Nutr, 2004. **44**(7-8): p. 575-86.
25. Schwingshackl, L. and G. Hoffmann, *Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies.* Lipids Health Dis, 2014. **13**: p. 154.
26. Ma, W., et al., *Prospective association of fatty acids in the de novo lipogenesis pathway with risk of type 2 diabetes: the Cardiovascular Health Study.* Am J Clin Nutr, 2015. **101**(1): p. 153-63.
27. Sales-Campos, H., et al., *An overview of the modulatory effects of oleic acid in health and disease.* Mini Rev Med Chem, 2013. **13**(2): p. 201-10.
28. Delgado-Lista, J., et al., *Olive oil and haemostasis: platelet function, thrombogenesis and fibrinolysis.* Curr Pharm Des, 2011. **17**(8): p. 778-85.
29. Carrillo, C., M. Cavia Mdel, and S.R. Alonso-Torre, *Antitumor effect of oleic acid; mechanisms of action: a review.* Nutr Hosp, 2012. **27**(6): p. 1860-5.
30. Mattson, F.H. and S.M. Grundy, *Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man.* J Lipid Res, 1985. **26**(2): p. 194-202.
31. Hao, L., et al., *Shifts in dietary carbohydrate-lipid exposure regulate expression of the non-alcoholic fatty liver disease-associated gene PNPLA3/adiponutrin in mouse liver and HepG2 human liver cells.* Metabolism, 2014. **63**(10): p. 1352-62.
32. Cascales, C., E.H. Mangiapane, and D.N. Brindley, *Oleic acid promotes the activation and translocation of phosphatidate phosphohydrolase from the cytosol to particulate fractions of isolated rat hepatocytes.* Biochem J, 1984. **219**(3): p. 911-6.
33. Salam, W.H., H.G. Wilcox, and M. Heimberg, *Effects of oleic acid on the biosynthesis of lipoprotein apoproteins and distribution into the very-low-density lipoprotein by the isolated perfused rat liver.* Biochem J, 1988. **251**(3): p. 809-16.

34. Rodrigues, D., T. Reverbel da Silveira, and U. Matte, *Freshly isolated hepatocyte transplantation in acetaminophen-induced hepatotoxicity model in rats.* Arq Gastroenterol, 2012. **49**(4): p. 291-5.
35. Moldeus, P., J. Hogberg, and S. Orrenius, *Isolation and use of liver cells.* Methods Enzymol, 1978. **52**: p. 60-71.
36. Niknahad, H. and P.J. O'Brien, *Mechanism of sulfite cytotoxicity in isolated rat hepatocytes.* Chem Biol Interact, 2008. **174**(3): p. 147-54.
37. Strom, S.C., J.R. Chowdhury, and I.J. Fox, *Hepatocyte transplantation for the treatment of human disease.* Semin Liver Dis, 1999. **19**(1): p. 39-48.
38. Gramignoli, R., et al., *Clinical hepatocyte transplantation: practical limits and possible solutions.* Eur Surg Res, 2015. **54**(3-4): p. 162-77.
39. McClatchey, K.D., *Clinical laboratory medicine*. 2nd ed. 2002, Philadelphia: Lippincott Wiliams & Wilkins. xiv, 1693 p.
40. Mengel, M.B. and L.P. Schwiebert, *Family medicine : ambulatory care & prevention*. 4th ed. Lange clinical manual. 2005, New York: Lange Medical Books/McGraw-Hill. xx, 790 p.
41. Gaw, A., *Clinical biochemistry : an illustrated colour text*. 5th ed. 2013, Edinburgh: Churchill Livingstone/Elsevier. p.
42. Shen, K., et al., *Depletion of activated hepatic stellate cell correlates with severe liver damage and abnormal liver regeneration in acetaminophen-induced liver injury.* Acta Biochim Biophys Sin (Shanghai), 2011. **43**(4): p. 307-15.
43. Kobayashi, N., et al., *Hepatocyte transplantation in rats with decompensated cirrhosis.* Hepatology, 2000. **31**(4): p. 851-7.