ORIGINAL ARTICLE

Effects of Sofosbuvir on Serum Lipid Profile and Serum Uric Acid in Albino Rats

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ABSTRACT

Objective: To determine the effects of sofosbuvir over serum lipid profile and serum uric acid level in albino rats.

Study Design: Experimental interventional Design

Setting: Department of Pharmacology Isra University Hyderabad and Department of animal husbandry, Sindh Agriculture University Tandojam.

Study Duration: 6 months following synopsis approval.

Sampling techniques: Non probability purposive sampling

Sample Size: 40 Albino Rats weighing 200-300gm on average

Methodology: Rats were selected based on inclusion and excluding criteria. Rats were divided into 4 equal groups control, A, B and C each having 10 rats. Control group was given Normal saline, powdered milk, and flour. A, B and C were treated with Sofosbuvir dose 5.7 mg/kg, 11.4 mg/kg, 17.0 mg/kg respectively. Blood samples were collected at day 1 before starting the treatment and then at the end of the study (after 6 weeks) by sacrifying the rats under international protocols. Blood samples were analysed for serum uric acid and lipid profile.

Results: There were significant changes in the total cholesterol between control and experimental group C, p-value 0.05, while no significant difference was noted between control and experimental group A and B. These findings show that the high dose of sofosbuvir drug changed the total cholesterol level. LDL showed a significant elevation in the experimental groups as compared to control with uses of sofosbuvir drug, the elevated difference was noted as in experimental groups A. P value = 0.006, similarly in group B p- value 0.008 and in group C p- value 0.008. No significant effects were found in TG and HDL levels in all experimental groups with controls. A significant difference was noted in the reduction of uric acid level between control and experimental group B and Experimental Group C, p- values were quite significant.

Conclusion: It was concluded that serum uric acid was significantly decreased; LDL and total cholesterol were elevated with use of sofosbuvir drug.

Key Words: Sofosbuvir Drug, Lipid Profile, Uric Acid

Introduction

Hepatitis C virus is a single stranded RNA virus. There are six genotypes of hepatitis C Virus (1-6). The most common genotype in Pakistan is genotype 3 (69%) sub type 3a accounts for 61.4%. Transmission of HCV through infectious blood. HCV is a significant wide-ranging medical issue as well as a major source of liver transplantation and hepatocellular carcinoma.[1] Approximately 180 million people are infected with HCV; prevalence of HCV in USA is about 1.6%, which compares to an expected 4.1 million infected people.[2] However, recent surveillance data recommended that HCV infection has expanded to 5.2 million individuals in the U.S.[3] Transmission of HCV occurs by means of introduction to infectious blood. Then population at highest risk for HCV transmission is intravenous drug users. Hepatitis C virus (HCV) among Pakistani population is quite high with estimated prevalence of

6.8%. Uses of the interferon-based treatment have made HCV extermination challenging, particularly for cases infected by HCV genotype I. Combination treatment of peg interferon and ribavirin induces only around 50% of HCV infected cases of genotype I at the higher load of virus to achieve the sustained virological response "SVR", though around 80% of HCV genotypes II and III infected cases achieve SVR.[4,5] The appearance of direct-acting antiviral agents (DAAs), which specifically target HCV proteins, has provided insights into the current situation. "Pegasys, Roche" and ribavirin provided SVR rates of 68% to 75% in treatment-naïve patients with genotype 1.[6,7] However, the regimen had significant limitations due to contraindications and intolerance to interferon therapy. However, the regimen had huge impediments because of contraindications and intolerance to interferon treatment, added substance unfriendly impacts of anaemia from ribavirin, a low genetic boundary to the advancement of resistance innate to protease inhibitors, and regular dosing intervals.[8,9] Many direct-acting antiviral (DAA) agents are being tested to address the requirement for free of interferon, all-oral treatments. Sofosbuvir treatment has a big barrier to the resistance, once-daily dosing with pan-genotypic activity. This direct-acting antiviral drug can be utilized without peg-interferon.[10] Sofosbuvir (Sovaldi, Gilead Sciences) is an inhibitor of NS5B polymerase, an enzyme crucial for the HCV replication as well as it is nucleotide analogue. FDA approved this regimen in the therapy of chronic HCV infection.[10] Sofosbuvir-containing treatment have achieved an extremely higher rate of sustained virological response. Since treatments including sofosbuvir result less complicated events in contrast to interferon-based regimens, sofosbuvir treatment has taken the central role in the treatment of hepatitis C.[4] Lipid profile is a blood test penal use for medical screening initially to see the abnormalities in the lipid. The components of lipid profile are high density lipoprotein (HDL), Low density lipoprotein (LDL), TAG and total cholesterol. Lipoproteins are VLDL, IDL, HDL, & LDL. They are transporting the cholesterol, TAG and phospholipids between the body cells. Changes in lipid profiles in patients infected with hepatitis C virus (HCV) during direct-acting antiviral therapy have been reported in recent years.[11] Recently, a similar association of serum LDL-cholesterol level with treatment outcome was reported in combination therapy with SOF and RBV.[12] In studies observe that during treatment with DAAs, the serum lipid profile may reflect not only recovery from the disruption of lipid metabolism induced by HCV, but also the pharmacological effects of DAAs.[13] There was no clear data found regarding effects of only sofosbuvir over serum lipid profile and serum uric acid level and by studies suggested further investigations are needed to elucidate the effect of DAAs on serum lipid profiles.[13] Therefor this study has been conducted to evaluate the effects of sofosbuvir over serum lipid profile and serum uric acid level in albino rats.

Methodology

This cross-section study was conducted Isra University Research Lab at Pharmacology Department, and Animal House Agriculture University, TandoJam. The duration of the study was 6 months

40 Albino Rats were taken as sample size according to international studies (Charan J, Kantharia,2013. Fitts DA. Ethics and animal numbers 2011.) [85,86].

The Rats were divided into 4 groups each having 10 Albino Rats.

Group A: control group given powdered Milk and Normal saline.

I. Group B: Sofosbuvir dose 5.7 mg / kg
II. Group C: Sofosbuvir dose 11.4 mg / kg
III. Group D: Sofosbuvir dose 17.0 mg / k

Through aseptic measures Rats were trapped in Rat chamber tail were cleaned with sprit swab, samples of blood were drawn.

Rats were sacrificed after the cervical dislocation blood sample was collected through cardiac puncturing. Blood was placed in jell tubes and send laboratory for chemical analysis.

Inclusion Criteria

- Normal,and healthy Rats between the weight of 200grams to-300grams,only male was taken by the animal house Agriculture University Tandojam.

Exclusion Criteria

- The Weight less than 200grams

- Female Rats(Due to hormonal changes during the reproductive period)
- Diseased Rats.

Collection of Data and Experiment Rats

All Rats were kept at animal's house, Agriculture University Tandojam. All Rats were, weighed, tag and were kept in separate cages. Sofosbuvir was purchase form pharmacy it was grind in a mortar, than the powder was dissolved in distilled water and Rats handle with safety measures, the drug was given orally through the metallic tube at the different doses.

Control Group: It was given normal saline, powdered milk and flour.

Group A:5.7mg/kg/body weight

Group B:11.4mg/kg/body weight

Group C:17.0mg/kg/body weight

The baseline sample was taken from each group on day-0 and the experimental sample was taken after 6 weeks

Statistical Analysis: After collection of samples the data was analysed by using software statistical package for social science (SPSS) version 21. For quantitative variables like; age, total cholesterol, HDL-C, LDL-C, TAG and serum uric acid, Mean ±SD were calculated. For the gender frequency percentage was calculated. T-test was applied to compare the mean of total cholesterol, HDL-C, LDL-C TAG and serum uric acid baseline measurements and after 6 weeks, measurements were done between control and experimental groups.

The dosage of Sofosbuvir to experimental groups according to their body weight

400mg of Sofosbuvir dissolved in 80ml distilled water (D/W)

1ml D/W =100 units(Insulin Syringe) contain 5mg of Sofosbuvir

0.5ml D/W =50 units (Insulin Syringe) contain 2.5mg of Sofosbuvir

0.4ml D/W= 40 units (Insulin Syringe) contain 2mg of Sofosbuvir

0.3ml D/W=30 units (Insulin Syringe) contain 1.5mg of Sofosbuvir

0.2ml D/W =20 units (Insulin Syringe) contain 1mg of Sofosbuvir

0.1ml D/W=10 units (Insulin Syringe) contain 0.5mg of Sofosbuvir

The normal adult human dose of sofosbuvir is 400mg/70kg

400/70= 5.71mg/kg

1st experimental group was given 5.71mg/kg of Sofosbuvir.

2nd experimental group given 11.4mg/kg (2 times the normal dose) of Sofosbuvir.

3rd experimental group given 17mg/kg (3times the normal dose) of Sofosbuvir

Results

In this study 40 animals were selected to see the effects of sofosbuvir drug on lipid profile and uric acid level. In all the animals' lipid profile was found without a significant difference on baseline assessment.

Table: I

Table I: Baseline lipid profile Comparison between control and experimental groups n=40

	Study groups		P value
	Control	Experimental	
cholesterol mg/dl			
Group-A	124.7±18.83	129.08±15.26	0.57
Group-B	124.1±18.83	129.57±11.77	0.69
Group-C	124.7±18.83	126.09±11.79	0.84
HDL mg/dl			
Group-A	55.25±7.87	55.77±8.43	0.88
Group-B	55.25±7.87	54.38±6.38	0.789
Group-C	55.25±7.87	55.13±7.43	0.972
LDL mg/dl			
Group-A	59.18±11.27	67.78±8.52	0.07
Group-B	59.18±11.27	65.29±16.59	0.34
Group-C	59.18±11.27	64.35±16.84	0.43
TGs mg/dl			
Group-A	110.05±22.93	113.48±11.25	0.67
Group-B	110.05±22.93	111.86±13.89	0.83
Group-C	110.05±22.93	108.57±20.39	0.88

After 6 weeks treatment of sofosbuvir significant changes in the total cholesterol were observed between control group and experimental groups B and C P-value 0.05, and 0.03, while no significant difference was noted between control group and experimental group A. These findings show that the high dose (2-3 times more than the human dose) of sofosbuvir changed the total cholesterol level.(Table: II)

Table II: After 6wks lipid profile Comparison between control and experimental groups (n=40)

	Study groups		P-value
	Control	Experimental	
cholesterol mg/dl			
Group-A	122.9+17.26	131.24+22.15	0.36
Group-B	122.9+17.26	141.41+18.90	0.03
Group-C	122.9+17.26	140.12+0.10	0.05
HDL mg/dl			
Group-A	56.26+8.17	56.34+8.40	0.98
Group-B	56.26+8.17	55.08+6.36	0.72
Group-C	56.26+8.17	57.25+7.23	0.77
LDL mg/dl			
Group-A	58.85+11.62	74.57+11.08	0.006
Group-B	58.85+11.62	73.61+10.49	0.008
Group-C	58.85+11.62	73.54+9.66	0.007
TGs mg/dl			
Group-A	108.53+24.29	111+10.31	0.55
Group-B	108.53+24.29	08.13+39.38	0.99
Group-C	108.53+24.29	107.32+22.30	0.91

In this study no effects were found of sofosbuvir drug on HDL levels in all experimental groups with controls p-values 0.77 group A with control, 0.72 group B with control and 0.98 group C with control. Table: II

LDL-C shows significant elevation in the experimental groups as compared to control with uses of sofosbuvir drug, the elevated difference was noted in experimental groups A 74.57±11.08 and control 58.85±11.62 p value = 0.006, similarly in group B LDL-C was 73.61±10.49 p- value 0.008 and in group C 73.54±9.66 p- value 0.008. Table: II

No significant effects were found in TG levels in all experimental groups with controls p-values 0.91 group A with control, 0.99 group B with control and 0.26 group C with control after uses of sofosbuvir drug. Table: II.

On baseline basement of the uric acid level, there was no difference in both groups i.e. experimental and controls before use of the drug. Table: III

Table III: Baseline uric acid Comparison between control and experimental groups (n=40)

Baseline Uric acid	Study groups		P-value
	Control	Experimental	
Group-A	1.36±0.03	1.36±0.08	0.95
Group-B	1.36±0.03	1.35±0.04	0.61
Group-C	1.36±0.03	1.35±0.08	0.95

After use of the drug no significant difference was found in serum uric acid level between control and experimental Group A, while a significant difference was noted in serum uric acid level between control and experimental group B and Experimental Group C, P- value 0.001 and 0.0001 respectively. Table: IV

Table V: After 6wks S uric acid Comparison between control and experimental groups n=40

After treatment uric acid mg/dl	Study groups		P-value
	Control	Experimental	
Group-A	1.40+0.15	1.27+0.15	0.08
Group-B	1.40+0.15	0.98+0.10	0.001
Group-C	1.40+0.15	0.80+0.15	0.0001

Discussion

The animal study on sofosbuvir based regimen was conducted to see the effects of sofosbuvir on serum lipid profile, because in many human studies it is reported that dyslipidemia is associated with chronic HCV, and many reported that abnormalities occur after the treatment of HCV. In this series total cholesterol was significantly increased after sofosbuvir administration at high doses that is consistent to study by Hashimoto S et al[14] showing increases in serum TC during HCV therapy with Daclatsavir and sofosbuvir combination (DCV/SOF). Similarly results of studies by Pedersen MR et al[15] and Chang ML et al[16] were also consistant with our results who declared that treatment with Direct Acting Antiviral drugs increases the serum total cholesterol levels. In contrast to current study Bernuth S et al[17] reported that total cholesterol showed a decrease under therapy and after the end of treatment of sofosbuvir. In another study of Meissner EG et al[18] reported that total cholesterol did not change

significantly during therapy. These effects of Sofosbuvir may be due to alteration in HMG-COA reductase activity, inhibition of this enzyme decreases serum cholesterol and its stimulation increases the serum cholesterol levels. SREBP-2 (Sterol Regulatory Binding Protein) control cholesterol homeostasis may also get affected.

According to our findings LDL was found significantly increased after administration of sofosbuvir drug that is consistent to a human study by Meissner EG et al[18] who reported that the rapid increase in LDL levels following oral antiviral therapy (Ribavirin/Sofosbuvir) which likely shows a transfer of lipid metabolism during the process of inhibition of replication of HCV. Similarly, Sheridan DA et al[19], Felmlee DJ et al[20], Petta S, et al[21], Pederse MR et al[15] and Bernuth S et al[17] also reported that LDL increases during and after treatment with sofosbuvir.

According to current study LDL-C was found significantly increased after administration of sofosbuvir drug. In a human study of Meissner EG et al[18] in 2015 mentioned that the rapid elevation in LDL and dimension in VLDL found early in therapy, Hepatitis C Virus changes in the pathway of intra hepatic cholesterol biosynthesis to stimulate viral replication. Chronic HCV diseased is associated with disturbed metabolism, also with dyslipidemia, and more it is examined that alteration in lipid profiles with sofosbuvir and ribavirin, in patients with chronic infection of HCV genotype.1.[20] Similarly Hashimoto s et al.[14] in 2016,demonsterated that elevation in serum LDL HCV treatment were strongly dependent on the type of HCV treatment. Sofosbuvir may increase the synthesis of LDL by stimulating the MTTP Gene responsible for LDL synthesis. Sofosbuvir may inhibit the uptake of LDL-C at receptor level (LDL Receptor) that may be due to down regulation Of LDL receptor.

This study shows that there was no effect found on HDL-C among all experimental groups receiving sofosbuvir drug that was consistent to results of Meissner EG et al[18] who also reported that HDL did not change significantly during therapy (Ribavirin/Sofosbuvir). Studies by Pedersen MR et al[15] and Chang ML et al[16] found that treatment with DAA results in increases in HDL levels that is in contrast to our findings. While a study by Bernuth S et al[17] reported that HDL-CL decreases with sofosbuvir treatment.

According to the findings of the present study, no effect was found on TAGS in any experimental groups receiving sofosbuvir. Pedersen MR et al[15] found that treatment with DAA resulted in a decrease in TGs while Chang ML et al[16] and Bernuth S et al[17] found significant post-therapeutic increases in TGs. All were in contrast to our finding whereas, we could not find any consistent study in this regard. In contrast of our study Pedersen MRet al[15] 2016 shown that medication with DAA caused in elevation in HDL. A recent study by Chang ML et al[16] in 2014 found significant increases in HDL and TGs. Pedersen MR et al[22] in 2015 found that DAA treated patients, resulted in an elevation in serum HDL as well as a decrease in serum TGs. In the current study we found a significant decrease in serum uric acid level with sofosbuvir treatment. No national or international animal studies were found regarding this in literature possibly sofosbuvir may have some inhibitory effect on xanthene oxidase or it may have some impact on xanthene and hypo-xanthene production that needs further exploration. While in human study of combine treatment of sofosbuvir and ribavirin showed contrast findings as *hyperuricemia*.[23]

Conclusion

It was concluded that sofosbuvir treatment increases the serum LDL at all doses. Total cholesterol levels are increased significantly at higher doses and does not effect on low doses. Sofosbuvir does not affect serum HDL and TGs at any dose. There is significant decrease in the serum uric acid levels following Sofosbuvir therapy at higher doses while it has no effect on serum uric acid on normal human doses.

RECOMMENDATIONS

More studies are needed to further confirm the consequences of sofosbuvir drug.

Human studies in HCV patients on lipid profile and serum uric acid levels are recommended.

Studies are also recommended to explore the reduction in serum uric acid and elevation in LDL level.

Physicians should consider these aspects of Sofosbuvir drug while treating patients of HCV.

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