Original Article
Sofosbuvir and ribavirin induced changes in some haematological and biochemical parameters in normal rats

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ABSTRACT

Background: Hepatitis C virus infection represents a serious epidemic problem in Egypt, it has the highest presence of the infection globally. Liver cirrhosis and hepatocellular carcinoma is mainly resulted from chronic infection with Hepatitis C Virus. Many direct antiviral agents have been utilized for treatment, but the hepatotoxicity of those drugs still rarely recorded.

Objectives: We conducted this study to evaluate the drug-induced toxicity of sofosbuvir and ribavirin.

Methods: Thirty two female albino rats were divided into four equal groups; Group one included rats which were administered orally with sofosbuvir (0.01 mg/kg body weight/day) suspended in distilled water. Group two included rats which were administered orally with ribavirin (0.0227 mg/kg body weight/day) suspended in distilled water. Group three included rats which were administered with a combination of sofosbuvir and ribavirin (0.01 and 0.023 mg/kg body weight/day, respectively) suspended in distilled water. Group 4 consisted of untreated animals and were served as control group.

Results: The present study found a significant decrease in mean corpuscular volume and mean corpuscular haemoglobin values and a significant elevation of red blood corpuscles distribution width percentage in groups treated with ribavirin. Moreover, a significant elevation of aminotransferases, alkaline phosphatase, ammonia, total bilirubin and malondialdhyde were found in the treated groups as well.

Conclusions: Sofosbuvir plus ribavirin can induce some hepatotoxicity in normal rats characterized by elevations in aminotransferases and microcytic hypochromic anaemia.

Introduction

Hepatitis C virus (HCV) is a global serious problem, Egypt specifically has the highest prevalence worldwide. Chronic viral hepatitis, is recognized as a serious healthcare burden.

Hepatitis C virus (HCV) is considered the main reason of chronic hepatitis and it may cause cirrhosis, failure of the liver and eventually hepatocellular carcinoma. Chronic HCV cases are administered treatment with oral therapy of ribavirin and interferon-α (IFN-α), which is effective in only 40% of these cases.

Ribavirin (1-b-D-ribofuranosyl-1H-1, 2,4-triazole-3-carboxamide) (RBV) is a water soluble synthetic guanosine analog, after intracellular phosphorylation, ribavirin exhibits antiviral effect against DNA and RNA viruses. Current studies indicate that combination therapy with RBV and IFN is usually caused higher rates of sustained virological, biochemical, and histological response compared to monotherapy with IFN. The major adverse effect of RBV treatment is the occurrence of a hemolytic anaemia in a lot of treated patients.

Sofosbuvir is a nucleotide analog that inhibits HCV NNSB polymerase and used for chronic hepatitis C (CHC) infection as one of a combination antiviral treatment regimen. The nonstructural (NNSB) polymerase inhibitor, sofosbuvir (SOF), was approved for treating patients with chronic hepatitis C virus (HCV) infection since 2013. In the 2nd and 3rd phase of clinical trials leading up to the approval of SOF, subjects with genotype 1 HCV received different regimens: SOF with pegylated interferon (Peg-IFN) and ribavirin (RBV), SOF with RB V, and the purine (guanidine) nucleotide analog polymerase inhibitor, GS-0938, which was formerly in development as a potential complementary oral antiviral agent for use with SOF. Although rates of response were high in these trials, a number of patients did not get sustained virological response (SVR). Sofosbuvir is considered a new medicine which has been created recently and
prevent proliferation of the virus by a direct act on the virus life cycle. The aim of the current study is to test the single and combined effect of Sofosbuvir and ribavirin on some hematological and biochemical parameters in normal rats.

**Materials and Methods:**

**Experimental animals:**

Thirty two female albino rats (weight range 180 ± 10 g) were used were bought from the animal house of the National Research Center (Giza, Egypt). The experimental rats were housed in the animal house in Zoology Department, Faculty of Science, Damietta University, New Damietta, Egypt. They were placed in plastic cages under controlled temperature and fed on standard pellet diet. All experiments were done according to research protocols approved by the animal care committee of the National Research Center, Egypt.

**Chemicals and doses:**

Sofosbuvir and ribavirin were obtained from National liver institute, Egypt. The suitable doses (Sofosbuvir= 0.01 mg/kg body weight and ribavirin =0.0227 mg/kg body weight) were calculated by using the following equation: Animal dosage (mg/kg) = clinical standard doses/(animal weight in kg/human weight in kg) 0.33. Where human weight was assumed to be 70 kg and rats were assumed to be 0.2 kg.

**Experiment Design:**

Rats were categorized into 4 groups. Group one included rats which were administered orally with sofosbuvir (0.01 mg/kg body weight/day) suspended in distilled water. Group two included rats which were administered orally with ribavirin (0.0227 mg/kg body weight/day) suspended in distilled water. Group three included rats which were administered with a combination of sofosbuvir and ribavirin (0.01 and 0.023 mg/kg body weight/day, respectively) suspended in distilled water. Group 4 consisted of untreated animals and were served as control group. The experiment was maintained for 12 weeks, all rats were weighed regularly and the rate of weight change was calculated.

By the end, all rats were sacrificed for two blood collections, one with antiocoagulant agent for hematological measurements and one for serum preparation. Blood collected was allowed to coagulate at ambient temperature (20-25°C) for 30 minutes. Serum was separated by centrifugation at 3000 r.p.m. for 15 min and used for the estimations of GGT, ALT, AST, ALP, MDA, creatinine, uric acid, urea, total bilirubin, creatinine, cholesterol, triglycerides, total protein, albumin, total lipid, and cholesterol.

**Haematological parameters:**

The haperinized blood samples were used for enumeration of the blood corpuscle (RBCs), determination of the haemoglobin content (Hb), and haematocrit percentage (HCT), while other haematological indices including mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated using RBC, Hct and Hb using the following formulae: MCV (fl) = haematocrit (%) * 10/ RBCs (millions/µl blood), MCH (pg) = HB/RBCs* 1013and MCHC = (Hb × 100)/ haematocrit (%).

White blood cells count was determined using haemocytometer chamber according to the method described by Dacie and Lewis. Differential count of WBCs (neutrophils, eosinophils, lymphocytes and monocytes) were conducted on Giemsa-stained blood smears.

**Biochemical parameters:**

The activity of ALT, AST, GGT, ALP and SOD, and the concentrations of total protein, albumin, ammonia, uric acid, urea, total bilirubin, creatinine, cholesterol, triglycerides, MDA and gamma interferon in the serum were measured using GENWAY spectrophotometer. All chemicals, reactants, and procedures are listed in table (1).

**Statistical analysis:**

XLSTAT program was used herein for descriptive statistics, graphing data and comparative statistics according to spreadsheet programs. Data were expressed as mean ± S.E. of different treated groups and control ones. Normal distribution of all metabolic parameters were tested.

The results were analyzed using one way analysis of variance (ANOVA) followed by Tukey (HSD) test to compare groups with each other and Dunnett two sided test to determine which treated group was significantly different from control. A of probability (p) less than 0.05 was considered significant.

**Results:**

The current results showed that the rate in the body weight change was lower in all group treated groups than control group, Sofosbuvir and ribavirin combination groupshowed the most significant decrease rate (Figure 1).

Haematological results are shown in table 2. From the statistical analysis, Sofosbuvir/ribavirin treated group showed decreased HCT and Hb than control group. Moreover, MCV was significantly lower in group two and 3 than control group (P=0.005, and 0.008, respectively), ribavirin treated group exhibited significant decrease in MCH than control group (P=0.027), while both group two and 3 showed a significantly elevated RDW% than control group (P=0.001and 0.0001, respectively).

Means and ranges of all assayed biochemical parameters are listed in table 3. MDA level elevated significantly in group 2 and 3 (P=0.011) than control group. ALT was higher in all treated groups than control group, but the most significant increase was found in sofosbuvir treated group (P<0.001), similarly GGT increased significantly in ribavirin group than control group (P<0.05). Moreover, ALP levels increased significantly in all treated group rather than control group (P<0.001). Only group one exhibited a higher AST activity than other groups.
Ammonia concentration reached significantly high levels in all treated groups rather than control rats (P = 0.037, 0.027 and 0.021 in group one, group 2 and group 3, respectively). Serum total bilirubin level elevated significantly in group one more than control group (P = 0.034), it was 3.25±0.26 and 1.93±0.23 mg/dL, respectively.

No significant changes were found in superoxide dismutase activity, total protein, albumin, uric acid, urea, creatinine, cholesterol, triglycerides and gamma interferon levels.

Table: Methods used for biochemical assays.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unite</th>
<th>Method (Test kit/reagent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (MDA)</td>
<td>nmol/ml</td>
<td>Colorimetric method (bio-diagnostic)</td>
</tr>
<tr>
<td>Superoxide Dismutase (SOD)</td>
<td>u/ml</td>
<td>Colorimetric method (bio-diagnostic)</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>u/l</td>
<td>Kinetic method (ELITech)</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>u/l</td>
<td>Kinetic method (ELITech)</td>
</tr>
<tr>
<td>γ-Gamma-Glutamyl transferase</td>
<td>u/l</td>
<td>Enzymatic Kinetic method (ELITech)</td>
</tr>
<tr>
<td>(GGT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>u/l</td>
<td>Enzymatic Kinetic method (ELITech)</td>
</tr>
<tr>
<td>Total protein (TP)</td>
<td>g/dl</td>
<td>Quantitative Colorimetric (Stanbio)</td>
</tr>
<tr>
<td>Albumin (ALB)</td>
<td>g/dl</td>
<td>Quantitative Colorimetric (Stanbio)</td>
</tr>
<tr>
<td>Ammonia (AMM)</td>
<td>mg/dl</td>
<td>Enzymatic Kinetic method (Salucea)</td>
</tr>
<tr>
<td>blood urea (UREA)</td>
<td>mg/dl</td>
<td>Enzymatic colorimetric method (Diamond)</td>
</tr>
<tr>
<td>uric acid (UR AC)</td>
<td>mg/dl</td>
<td>Enzymatic colorimetric method (ELITech)</td>
</tr>
<tr>
<td>Total Bilirubin (TB)</td>
<td>mg/dl</td>
<td>Total and direct (Diagnostics)</td>
</tr>
<tr>
<td>creatinine (CREA)</td>
<td>mg/dl</td>
<td>Colorimetric Kinetic method, (Diamond)</td>
</tr>
<tr>
<td>cholesterol (CHOL)</td>
<td>mg/dl</td>
<td>Enzymatic colorimetric (spinreact)</td>
</tr>
<tr>
<td>triglycerides (TRI)</td>
<td>mg/dl</td>
<td>Enzymatic colorimetric (spinreact)</td>
</tr>
<tr>
<td>Gamma interferon (IFN-γ)</td>
<td>pg/ml</td>
<td>Sandwich-ELISA method (Ebioscience)</td>
</tr>
</tbody>
</table>
Table 2: Haematocrite (HCT %), haemoglobin (Hb), haematimetric indices, Erythrocytes (RBCs), Thrombocytes (TC), Leucocytes (WBCs), and differential count of WBCs of female albino rats treated with sofosbuvir, ribavirin and ribavirin/sofosbuvir mixture for a period of 12 weeks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ribavirin</th>
<th>Sofosbuvir</th>
<th>Sofo-Rib</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SE</td>
<td>Min - Max</td>
<td>Mean±SE</td>
<td>Min - Max</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>38.39±0.82</td>
<td>33.7-40.4</td>
<td>38.69±1.85</td>
<td>35.7-45.4</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.93±0.26</td>
<td>10.7-12.6</td>
<td>11.96±0.42</td>
<td>11.2-13.5</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>53.25±0.67a</td>
<td>50-56</td>
<td>49.4±0.51a</td>
<td>48-51</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>16.56±0.27a</td>
<td>15.3-17.7</td>
<td>15.30±0.22a</td>
<td>14.7-16</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.05±0.30</td>
<td>29.7-31.9</td>
<td>30.94±0.46</td>
<td>29.7-32.3</td>
</tr>
<tr>
<td>Erythrocytes (10⁶/µl)</td>
<td>71.51±1.82ab</td>
<td>62.3-77.9</td>
<td>78.42±3.83b</td>
<td>70.6-92</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>13.71±0.20b</td>
<td>13.1-14.7</td>
<td>15.28±0.46b</td>
<td>14.5-16.8</td>
</tr>
<tr>
<td>Thrombocytes (10³/µl)</td>
<td>476.38±45.32a</td>
<td>228-648</td>
<td>542.80±63.99a</td>
<td>316-602</td>
</tr>
<tr>
<td>Leucocytes (10³/µl)</td>
<td>7.14±1.16a</td>
<td>2.9-13.5</td>
<td>6.62±1.17a</td>
<td>4.1-10.5</td>
</tr>
<tr>
<td>Lymphocyte (10³/µl)</td>
<td>5913±996.50a</td>
<td>2200-11200</td>
<td>5240±1151.80b</td>
<td>2300-8800</td>
</tr>
<tr>
<td>Monocytes (10³/µl)</td>
<td>725±106.49a</td>
<td>400-1300</td>
<td>560±120.80b</td>
<td>200-900</td>
</tr>
<tr>
<td>Granulocytes (10³/µl)</td>
<td>500±88.64a</td>
<td>300-1000</td>
<td>960±292.57a</td>
<td>200-1900</td>
</tr>
</tbody>
</table>

Values are means ± S.D. Values with different superscript letters within each row are significantly different (analysis of variance, P<0.05).

Table 3: Serum biochemical parameters of female albino rats treated with sofosbuvir, ribavirin and sofosbuvir/ribavirin mixture for a period of 12 weeks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ribavirin</th>
<th>Sofosbuvir</th>
<th>Sofo-Rib</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>Mean±SE</td>
<td>Min - Max</td>
<td>Mean±SE</td>
<td>Min - Max</td>
</tr>
<tr>
<td></td>
<td>4.62±0.43a</td>
<td>3.67-5.79</td>
<td>5.51±1.32b</td>
<td>5.60-14.36</td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td>268.91±39.73</td>
<td>158.49-362.88</td>
<td>296.00±38.65</td>
<td>109.12-370.67</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>13.30±2.60a</td>
<td>3.49-17.94</td>
<td>23.02±3.86b</td>
<td>15.7-29.7</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>15.36±3.90b</td>
<td>5.24-27.94</td>
<td>23.05±3.80b</td>
<td>13.9-31.4</td>
</tr>
<tr>
<td>GGT(U/L)</td>
<td>23.50±7.30b</td>
<td>1.55-63.71</td>
<td>79.64±40.03b</td>
<td>1.6-335.66</td>
</tr>
<tr>
<td>ALP(U/L)</td>
<td>27.18±4.44a</td>
<td>16.99-374</td>
<td>251.92±34.1b</td>
<td>163.1-353.3</td>
</tr>
<tr>
<td>TP (g / dL)</td>
<td>10.81±1.5</td>
<td>7.93-18.78</td>
<td>8.23±0.32</td>
<td>7.26-9.51</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>5.64±0.15</td>
<td>5.08-5.96</td>
<td>5.50±0.26</td>
<td>4.65-6.67</td>
</tr>
<tr>
<td>Ammonia (mg/dl)</td>
<td>1220±62.4a</td>
<td>873-1513</td>
<td>3477.7±975b</td>
<td>1240.7-9447</td>
</tr>
</tbody>
</table>
Figure 1: The rate of weight change/day in female albino rat treated with sofosbuvir and/or ribavirin for 12 weeks. () significant from control group.

Discussion:

Many studies showed SVR rates higher than 90% with administration of sofosbuvir and ribavirin combinations. So far, drug toxicity has not been noticed during administration of these two direct antiviral agents. This study describes some hematotoxicity effects induced by administration of dual therapy, sofosbuvir and ribavirin. The mechanism beneath these drug reactions is still unclear.

Haematological parameters:

During complete blood cell count (CBC), cell distribution width (RDW%), mean cell haemoglobin (MCH) and the volume of each erythrocyte (MCV) can be measured. These parameters provide a good indication of dispersion in distribution. Changes in hematological parameters can be taken as a good indicator for chemicals and drug toxicity. Our results show that ribavirin induced serious haematological changes identified by significant decrease in MCV and MCH, and a significant increase RDW% in group treated with ribavirin only or with sofosbuvir.

These results indicate a presence of microcytic hypochromic anemia characterized by low MCV and MCH. Both low MCV and low MCH are an indicator of iron deficiency. Previous studies showed that ribavirin induced anemia is usually associated to red blood cells (RBC) membrane oxidation which cause elevated erythropagocytic extravascular removal. Ronzoni et al. found that treatment with peg-IFNα and ribavirin combination also cause suppression of bone marrow. Fellay et al., also proved that ribavirin induced anemia is associated to asingle nucleotide polymorphisms (SNPs) within inosine triphosphatase (ITPA) gene, which could be used as a marker suggesting the degree of anemia in HCV patients having DAA therapy including ribavirin.

Moreover, RDW% elevated significantly in sofosbuvir plus ribavirin treated group as well as ribavirin group. In the same context RBC size has been shown to be a good indicator for diagnosis of erythropoiesis pathology. RDW % is a direct effect of the heterogeneity of peripheral RBCs. Therefore, the RBC distribution width is usually contained in heterogeneous RBC populations in different maturation stages and differing ages. Variations in RBC size distribution indicate a variation in between RBC production and RBC age, RDW% might improve our ability to understand mechanisms underlying anemia development such as different RBC maturation abnormalities or RBC destruction due to hemolysis or bleeding. Recently, increased RDW has been used as an indicator of death in many non-hematological disorders, such as inflammation and circulatory diseases.

In addition to previous haematological changes, platelets number decreased significantly in sofosbuvir plus ribavirin group this is in line with another study in using ribavirin as prophylactic treatment after liver transplantation. On contrary, in some HCV patients, thrombocytopenia b e elevated significantly early during treatment with dual therapy of sofosbuvir plus ribavirin.

The effect of Direct Antiviral Agents (DAAs) on white blood cells is important to enhance our knowledge about the mechanism underlying DAAs induced changes. In our study, white blood cells, lymphocytes, granulocytes, and monocytes number increased significantly in sofosbuvir and ribavirin treated group than other groups, while ribavirin group had lower leucocytes, lymphocytes, and monocytes count than other groups.

Other studies found low white blood cell count, low neutrophils and low monocyte number during treatment with somedirect antiviral agents. Low leucocytes was also found during administration of ribavirin as a prophylactic agent after liver transplantation. Moreover, ribavirin administration is usually induceinfections and might be an immune suppressive which might in crease viral and bacterial infection.

<table>
<thead>
<tr>
<th>Urea (mg/dl)</th>
<th>59 ± 2.99</th>
<th>46.2-66.7</th>
<th>66.91 ± 4.31</th>
<th>50.00-78.24</th>
<th>62.25 ± 1.35</th>
<th>57.06-66.18</th>
<th>61.1 ± 3.4</th>
<th>45.00-72.06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>4.29 ± 0.31</td>
<td>3.41-5.24</td>
<td>5.22 ± 0.52</td>
<td>3.47-6.76</td>
<td>5.00 ± 0.34</td>
<td>3.90-6.00</td>
<td>4.90 ± 0.3</td>
<td>4.14-6.21</td>
</tr>
<tr>
<td>T. Bilirubin (mg/dl)</td>
<td>0.90 ± 0.09</td>
<td>0.68-1.12</td>
<td>1.03 ± 0.11</td>
<td>0.88-1.41</td>
<td>0.92 ± 0.11</td>
<td>0.68-1.27</td>
<td>0.89 ± 0.11</td>
<td>0.49-1.32</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>95.2 ± 1.36</td>
<td>82.7-153.4</td>
<td>127.4 ± 26.7</td>
<td>75.34-308.90</td>
<td>216.78 ± 85.73</td>
<td>56.16-491.78</td>
<td>123.52</td>
<td>92.97</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>203.4 ± 0.3</td>
<td>135.1-254.5</td>
<td>194.6 ± 17.7</td>
<td>119.37-269.11</td>
<td>242.15 ± 35.71</td>
<td>145.55-387.43</td>
<td>189.1 ± 28.5</td>
<td>122.51-312.04</td>
</tr>
<tr>
<td>Y-interferon (pg/mL)</td>
<td>18.0 ± 4.06</td>
<td>9.33</td>
<td>18.13 ± 3.86</td>
<td>5.35</td>
<td>12.33 ± 2.28</td>
<td>9.23</td>
<td>10.29 ± 1.32</td>
<td>6.16</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. Values with different superscript letters within a row are significantly different (analysis of variance, P<0.05)
Both monocytes and granulocytes count increased significantly in the group treated with both sofosbuvir and ribavirin. It is known that granulocytosis and mononcysis are a usually indicator of viral infection, parasitic infection, chronic inflammation severe depression and stress.

2. Biochemical Parameters:

Drugs induced oxidative stress has been determined during almost all clinical and experimental conditions of the chronic liver diseases both in vivo and in vitro. Ribavirin and a combination of sofosbuvir and ribavirin increased MDA in our study. Many studies showed elevated MDA levels in the blood and liver of chronic hepatitis C patients. Although the previous studies showed a decrease in MDA levels, but it still was higher than the normal level, this may indicate that the drug itself can induce oxidative stress which was confirmed during this study. No one showed the relationship between administration of Sofosbuvir and ribavirin on lipid peroxidation, but it could be explained that elevated MDA may be due to inflammation which might be confirmed with increased leukocytes count.

administration of sofosbuvir and/or ribavirin caused a significant elevation ALT and AST, our result in agreement with a case study where six patients out of 45 (13%) maintained elevated ALT during the treatment. In another patient, and during treatment, ALT remain elevated and normalized only after treatment termination. Serum ALT and AST had a transient increase with antiviral drugs. On the other hand, liver function parameters including aminotransferases improved in the majority of patient during antiviral agents. ALT normalized in about 88% of patient within the first four weeks of treatment with DAAs. Although direct antiviral agents such as sofosbuvir enhance aminotransferases levels in HCV patients but increased their levels in normal cases. This increase in ALT and AST activities usually indicates damage to the cytosol and mitochondria.

The level of serum hepatic ALP associates mainly to cholestasis, bile salts and to a lesser extent when liver cells are damaged. In this study, the results of serum ALP activity showed significant increase after 12-weeks treatment period with ribavirin, sofosbuvir, ribavirin/sofosbuvir mixture compared with control. Similar results were obtained in other studies. Blood alkaline phosphatase increase is found among people who take sofosbuvir, especially for patient who administered the drug for six months.

Sofosbuvir only or combined with ribavirin induced significant increase in total bilirubin concentration compared to the control group. These high bilirubin levels were correlated to transaminases elevations. Our result in agreement with an elevated serum bilirubin in HCV subject treated with peg-INF and ribavirin. Ribavirin usually causes hemolysis and may increase hyperbilirubinemia.

Many patients complain from having elevated ammonia level shortly after starting sofosbuvir and ribavirin therapy. (http://www.medhelp.org/posts/Hepatitis-C/Does-Ribavirin-cause-anemia-when-used-with-Sovaldi/show/2088605), this is in line with our findings where serum Ammonia level significantly increased in all treated groups. This significant increase in ammonia level is an indicator of acute acid-base imbalance from moderate acidosis. Abnormal Ammonia level is found among patients who administered Sovaldi, and who have been taking the drug form more than a month, combined with Ribavirin.

In conclusion, ribavirin is responsible of some serious long term adverse effect. These effects may not happen shortly after starting treatment because ribavirin takes about four weeks to reach its full level in the body. When ribavirin side effects appear though, they can stay longer or be dangerous than side effect from the other drugs. Onereason for this is ribavirin takes a relatively long time to be excreted. In fact ribavirin can stay in the body tissues for up to six months after end the treatment. Future studies should be carried out to better define the effects of direct antiviral agents combined with ribavirin on erythropoiesis to enhance the hematological outcome of chronic HCV patients having coadministration therapy.

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