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Decreasing cytotoxic effect of sofosbuvir treatment by vitamin B12 in hepatitis C virus infected cells *in-vitro*

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KeyWords
Sofosbuvir, Cytotoxicity, vitamin B12, HCV infection, Proinflammatory cytokines, HepG2 cells, In-vitro

ABSTRACT

Hepatitis C virus (HCV) belongs to the family *Flaviviridae* and contains a positive-sense single-strand RNA assembled within a small core protein. Sofosbuvir, traditionally named Sovaldi (SOV), is a new medication for treating patients with chronic hepatitis C induced by HCV. Here, we investigated the possible cytotoxic effect of SOV treatment in hepatocellular carcinoma using HepG2 cell line and normal hepatocytes. Moreover, we tested the potential impact of vitamin B12 in combination with SOV to avoid the cytotoxic events and improved its ability to block HCV replication. **O**ur findings revealed that SOV treatment decreases the cell viability rate of both cancer and normal cells parallel with a high production level of lactate dehydrogenase (LDH) from treated cells. However SOV treatment showed significant inhibition of HCV replication in infected cells, the production of proinflammatory cytokines including IL-6 and IL-8 was dramatically increased in a time and dose-dependent manner. Importantly, the combination of vitamin B12 and SOV in equal concentration resulted in a competent inhibition of HCV replication indicated by fold changes of HCV-NS5A gene expression. Furthermore, the level of produced IL-6 and IL-8 was strongly decreased in infected HepG2 cells that were pretreated with the combination of vitamin B12 and SOV indicated by ELISA assy. **In conclusion, w**e hypothesis that the combination between sofosbuvir, as a sufficient anti-HCV drug, and vitamin B12 is resulted in increasing its safety profile and improving its antiviral activity *in-vitro* in infected HepG2 cells.

Introduction

Hepatitis C is a liver infectious disease caused by HCV, which belongs to the genus *Hepacivirus* within the family *Flaviviridae*, meaning that the genetic material of the virus is a positive single-strand RNA [1]. HCV infection causes a chronic hepatitis C in almost 80% of infected patients and triggers a chronic inflammatory disease process that normally developed into liver fibrosis, cirrhosis, and hepatocellular carcinoma [2]. The genetic material of HCV contains approximately 9,600 nucleotides encoding a single polyprotein of 3,010 amino acids which is processed into three structural proteins (core, E1, and E2) and seven nonstructural (NS) proteins called p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B [3]. Importantly, the genome of HCV is known with a high degree of genetic variability and mutation rates mainly within E1 and E2 regions [4]. Meanwhile, the untranslated regions of terminal segments are known with the highest degree of conserved sequence among various strains [5,6].

Sofosbuvir, traditionally named sovaldi (SOV), is a new medication which can disturb all genotypes of HCV reproduction [7]. As the first interferon-free drug, SOV can successfully reduce the risk of long-term complications of HCV when added to pegylated interferon and ribavirin. Mechanistically, sofosbuvir a nucleotide analogue inhibitor of HCV NS5B polymerase that can block the polymerase enzyme required for viral replication [8]. In addition, pegylated interferon stimulates cellular immune response, while ribavirin used as antiviral medication acting in different way [9,10]. Noteworthy, treating peoples co-infected with human immunodeficiency virus (HIV) and HCV with sofosbuvir showed the same response and the same side-effects similar to those of HIV-negative infected persons [11]. Importantly, SOV can be used at all different stages of liver fibrosis and cirrhosis; nevertheless it is more recommended for people with less liver damage. Although SOV treatment showed few adverse-effects, there have been accumulating evidence reported serious drug-induced hepatotoxicity [12]. In addition, SOV-induced cytotoxic effect includes glutathione depletion, mitochondrial membrane damage, mitochondrial reactive oxygen species (ROS) formation, lysosomal membrane collapse, and necrotic actions [13].

On the other hand, vitamin B12 or corrinoid complex contains a corrin macrocycle in its structure. In living cells, the vitamin B112 complex is taken up by the receptor-mediated endocytosis followed by digestion process by lysosomal proteases. The digestion event of vitamin B12 complex facilities releasing of B12 inside the cells [14]. Noteworthy, vitamin B12 is usually presented in unnatural and chemically stable from known as cyanocobalamin (CN-B12). CN-B12 is usually used for human nutritional supplements which synthesized in living cells into the coenzyme forms of cobalamin, methylcobalamin (CH_3-B12), and 5'-deoxyadenosylcobalamin (AdoB12) [15]. The function of AdoB12 as a coenzyme is characterized in catalyzing of the isomerization of *R*-methylmalonyl-CoA to succinyl-CoA in the catabolism of amino acid and odd-chain fatty acid in mammalian cells [16]. This structure and function of vitamin B12 supports its role in so many biological processes such as DNA methylation, histone acetylation, gene expressing, and protein folding.

In this work, we sought to monitor the levels of proinflammatory cytokines in HCV-infected HepG2 cells as the main regulators of inflammatory events during sofosbuvir treatment. Furthermore, we investigated the biological effects of vitamin B12 when combined with sofosbuvir during HCV treatment *in-vitro.*

Subjects and Methods

Cell lines

Hepatocellular carcinoma (HepG2 cells) were grown in CO_2 incubator at 37°C under using RPMI media that complemented with 4 mM L-glutamine, 4 mM sodium pyruvate, and 5% of heat-treated bovine serum albumin (BSA). The normal hepatocytes were grown in RPMI media complemented with 4 mM L-glutamine and 10% BSA using CO_2 incubator [17–19]. The imaging of cultured cells was determined by using inverted microscopy with a Zeiss A-Plan 10X.

Cytotoxic concentration 50% (CC_{50})

The purified SOV and vitamin B12 was tested for its cytotoxic effect and the potential CC_{50} was calculated on HepG2 cells and normal hepatocytes. Therefore, the cells were cultured in 96-well plates in a density of $10X10^3$cells/well and were incubated in CO2 incubator at 37°C. The cells were exposed to different concentrations of purified agents (0-40mg/ml) followed by overnight incubation. The cell viability rate and cytotoxic concentration were monitored by using MTT cell growth assay kit (Sigma-Aldrich, Germany), based on the amount of formazan dye which has been measured by measuring absorbance at 570nm.

Lactate dehydrogenase (LDH) production

LDH assay kit (Abc-65393) was used to assess LDH production in the fluid media that collected from cultured-treated cells. Accordingly, 100µl of lysed cells was incubated with 100µl LDH reaction mix for 30min at room temperature. LDH activity was quantified by a plate reader at OD450nm. The relative produced level of LDH was detected by the fold change of mean values of treated cells that divided on the mock values [20].

HCV preparation and infection

Sample from patients with HCV was obtained from Ain Shames Specialized Hospital, Egypt. The genotype 4a was identified and purified from collected samples by adding equal volume of phosphate buffer saline (PBS) to 500µl of the blood samples, and then the mixture was centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a clean eppendorf tubes and was purified using 0.22 µm filters. For infection, HepG2 cells were seeded in 6-well plates with a density of hundred thousand cells per well and incubated overnight. The cells were treated with 200 µg/ml or 400 µg/ml of SOV and/or vitamin B12 and incubated for 2h prior infection. Then treated cells were infected with MOI of 1 of HCV (Multiplicity of infection =1) and incubated for 3 days.

Enzyme-linked immunosorbent assay (ELISA)

ELISA assay was used for the quantification analysis of the released interleukin-6 (IL-6), IL-8, and transforming growth-beta (TGF-β) using human ELISA kits (Abcam 178013, Abcam 214030, and Abcam 100647, respectively). HepG2 cells and normal hepatocytes cultured in 96-well plates were overnight incubated. Then the cells were incubated with either 200 µg or 400 µg/ml of each candidate for (0, 6, 12, 24, 48, and 72 hrs). On the time point, the cells were lysed using 1X cell lysis buffer (Invitrogen, USA) then 100 µl of the lysed cells were reloaded into the ELISA plate reader and were incubated for 2 hrs at RT with 100 µl control solution and 50µl 1X bio-

tinylated antibody. Then 100 µl of 1X streptavidin-HRP solution was added to each well of samples and incubated for 30 min in the dark. 100 µl of the chromogen TMB substrate solution was added to each well of samples and incubated for 15 min at RT away from the light. Finally, 100 µl stop solution was added to each well of samples to stop the reaction. The absorbance of each well was measured at 450 nm [21,22].

Quantitative real time PCR (qRT-PCR)

The quantification analysis of genes expression was detected by using qRT-PCR in which the cellular total RNA was obtained using TriZol (Invitrogen, USA) and was purified by using RNA purification kit (Invitrogen, USA). Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using M-MLV reverse transcriptase (Promega, USA). The quantification analysis of mRNA expression of HCV-NS5A, IL-6, IL-8, and TGF-β was achieved using QuantiTect-SYBR-Green PCR Kit (Qiagen, USA) and the specific primers listed in table 1. The housekeeping glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression level was used for normalization in the real-time PCR data analysis. The PCR reaction system conatined 10 µl SYBR green, 0.25 µl RNase inhibitor (25 U/µl), 0.2 µM of each primer, 2 µL of synthesized cDNA, and nuclease-free water up to a final volume of 25 µL. The following PCR conditions were used; 94°C for 5 min, 35 cycles (94°C for 30 sec, 60°C for 15 sec, 72°C for 30 sec) [20,23].

Data analysis

All histogrames and charts were prepared by Microsoft Excel. Delta-Delta Ct analysis was used in the quantification analysis of mRNA delivered from qRT-PCR assay based on the following equations: (1) delta-Ct = Ct value for gene- Ct value for GAPDH, (2) (delta–delta Ct) = delta Ct value for experimental –delta Ct for control), (3) Quantification fold change = ($2^{-\text{delta-delta ct}}$) [21,24]. The student's two-tailed t-test was used for statistical analysis by which the resulted P-value ≤ 0.05 was considered significant.

Table 1: Oligonucleotides sequences used for mRNA quantification of indicated genes

Description	Primer sequences 5'-3'
NS5A-Forward	ATTCGTTCGTAGTGGGATCCA
NS5A-Reverse	AAGAGTCCAGTATTATCACCTT
IL-6-Forward	TGAACTCCTTCTCCACAAGCG
Il-6-Reverse	TCTGAAGAGGTGAGTGGCTGTC
IL-8-Forward	AAGAGAGCTCTGTCTGGACC
Il-8-Reverse	GAT ATT CTC TTG GCC CTT GG
TGF-β-Forward	CCCAGCATCTGCAAAGCTC
TGF-β -Reverse	GTCAATGTACAGCTGCCGCA
NF-kB1-sense	GAAATTCCTGATCCAGACAAAAAC
GAPDH-sense	TGGCATTGTGGAAGGGCTCA
GAPDH-antisense	TGGATGCAGGGATGATGTTCT

Results

Cytotoxic consequences of SOV on cancer and normal hepatocytes independent of infection

Chemical-induced liver toxicity refers to toxic chemicals in the environment, such as organic chemicals and solvents which are considered as a common cause of toxic hepatitis. The direct contact can occur through ingesting a chemical, breathing, or exposure to the skin. Furthermore, drug-induced hepatitis may cause liver toxicity or hepatitis dependent of the drug treatment such as diclofenac, statins, antirheumatic drugs, and antiviral medications [25]. The cytotoxic concentration 50% (CC_{50}) of the extracts or agents is the minimum concentration that can cause death to 50% of viable cells in the host [26]. The CC_{50} of the SOV on HepG2 cells and the normal hepatocytes was detected independent of HCV infection using MTT assay kit. Accordingly, the cells were seeded in 96-well/plate in a density of 10000cells/well and were incubated overnight. Then the cells were treated overnight with different concentrations of SOV agent (0.2, - 40 mg/ml). Interestingly, the cell viability rate of HepG2 cells was being interrupted at a low concentra-

tion of SOV treatment (200ug/ml) and revealed 50% inhibition at the concentration of 600ug/ml. Meanwhile, the cell viability rate of the normal hepatocytes treated with SOV revealed the same toxic effect at the same concentrations of SOV treatment (Figure 1A and B). Further, the CC_{50} of SOV agent on the normal cells was 900 µg/ml, indicating that the SOV agent can disturb the cancer and normal hepatocytes at the low concentration. Moreover, the relative LDH production was significantly increased up to 3 fold in HepG2 cells that were treated with 200ug/ml of SOV and was gradually increased in dose-dependent when compared with the normal hepatocytes (Figure 1C). This result firstly confirms the cytotoxic effect of SOV agent on HepG2 cells indicated by cell morphology, number of survived, and the production level of LDH upon treatment in comparison with the normal cells.

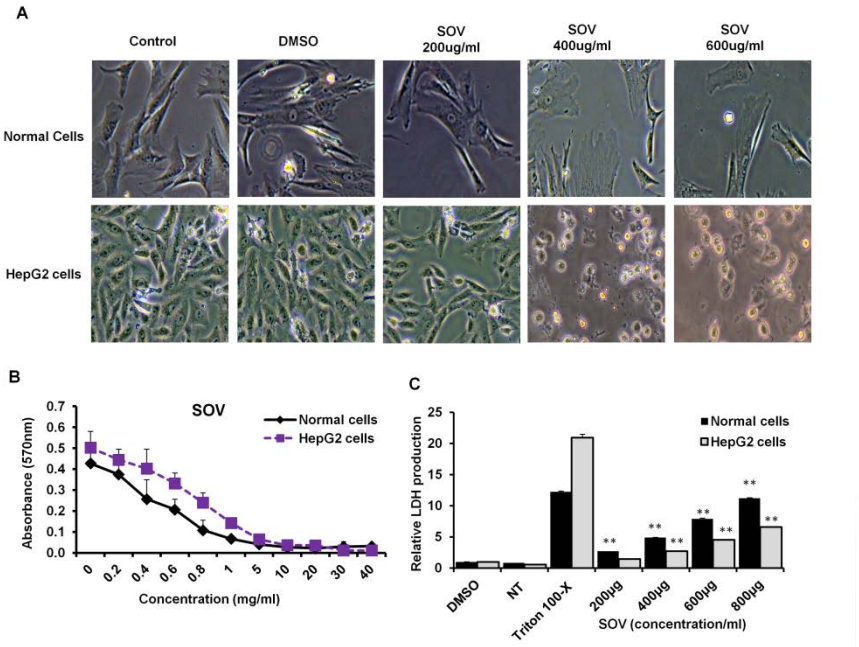

Figure 1: **The cytotoxic potential of the anti-HCV, SOV agent in HepG2 cells and normal hepatocytes**
(A) The representative inverted microscope images of normal cells and HepG2 cells in 48 hrs upon treatment with the indicated concentration of SOV compared with DMSO treated cells **(B)** Cell viability rate of treated HepG2 cells and normal hepatocytes with different concentrations of SOV that revealed its CC_{50} using MTT assay. **(C)** Relative LDH production from treated cells in comparison with Triton 100-X treated cells. Error bars reveal the standard deviation (SD) of three different replicates. Student's two-tailed t-test was used to determine *P* values and significance of cell viability and LDH production level. **P*<0.05 was considered statistically significant and ***P*<0.01 as highly significant.

SOV treatment prevents HCV replication and increases inflammation in infected cells

Nucleotide compounds like SOV, has verified as a potent orally available anti-HCV treatments. This drug showed high efficiency and a wide therapeutic index against HCV infections. SOV contains molecules with an exclusive and a high specificity pharmacology for the HCV ribonucleic acid polymerase such as NS5A [27]. Accordingly, HepG2 cells were treated with either 200µg/ml or 400µg/ml of SOV and then were infected with HCV genotype 4a (MOI = 1) and incubated for two days. HCV replication was monitored in treated HepG2 cells via the fold changes in NS5A gene expression using qRT-PCR. Meanwhile, the relative expression of proinflammatory cytokines produced from treated and infected cells were monitored to state the potential toxic effect of SOV in treated cells. As shown in figure 2A, the relative expression of HCV-NS5A was significantly decreased in a dose-dependent manner of SOV treated cells. While the expression profile of IL-6 and IL-8 were significantly upregulated in a dose-dependent manner of treated cells compared to control-treated cells (Figure 2B and C). In contrast, the fold changes in TGF-β gene expression was significantly decreased in cells that were treated with 400 µg/l, but not in the cells treated with 200 µg/ml (Figure 2D). These data indicate that SOV treatment can prevent HCV replication and TGF-β production in infected cells while increasing inflammatory mediators IL-6 and IL-8.

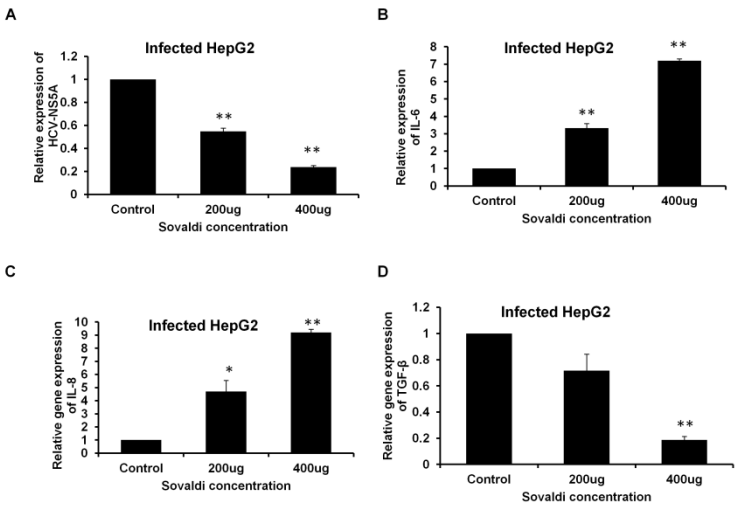

Figure 2: Quantification analysis of NS5A, IL-6, IL-8 and TFG-β expression in HCV infected cells that were pretreated with SOV

(A) Quantification analysis of NS5A-mRNA indicated by fold changes in infected HepG2 cells subjected to 200µg or 400µg of SOV compared with DMSO-treated cells. **(B)** Steady-state mRNA of IL-6 indicated by fold changes in infected cells that pretreated with SOV in comparison with DMSO-treated cells. **(C)** Steady-state mRNA of IL-8 indicated by fold changes in infected cells treated with SOV compared with DMSO-treated cells. **(D)** Steady-state mRNA of TFG-β indicated by fold changes in infected cells treated with SOV compared with DMSO-treated cells. Levels of GAPDH-mRNA were used as an internal control. Error bars indicate the SD of three independent experiments. A student two-tailed t-test was used for significance analysis of cycle threshold (Ct) values. Asterisks (*) indicates *P-values* ≤0.05 and (**) indicates the *P* ≤0.01.

SOV mediates the produced cytokines and TFG-β in treated cells

To detect the level of produced cytokines, the fluid medium of treated and infected cells was investigated using the ELISA assay. Accordingly, HepG2 cells and normal hepatocytes were seeded in 96/wells plate with a density of hundred thousand cells per well. The cells were treated 2 hrs before infection with either 200 µg or 400 µg SOV, then the treated cells were infected with HCV (MOI = 1) and incubated for different time points, cells treated with DMSO were served as control treatment. As shown in Figure 3A, the mean level of IL-6 was increased in a dose and time-dependent manner of SOV-treated cells, while its level was markedly decreased in control-treated cells. Likewise, the mean level of IL-8 was markedly increased in infected HepG2 in a dose and time-dependent manner of SOV treatment compared with control-treated (Figure 3B). Whereas the mean level of TGF-β was markedly decreased in treated and infected HepG2 cells in comparison with control-treated cells (Figure 3C). In normal hepatocytes, the production of IL-6 was increased in a dose and time-dependent manner of SOV-treated cells in comparison with control-treated cells (Figure 3D). Similarly, the mean concentration of produced IL-8 was markedly increased in infected cells in a dose and time-dependent manner of SOV treatment compared with control-treated (Figure 3E). Once more the mean level of TGF-β was markedly decreased in treated and infected cells when compared with untreated cells or DMSO-treated cells (Figure 3E). These findings further confirm that SOV treatment stimulates IL-6 and IL-8 production and triggers the inflammatory and necrotic events in treated cells.

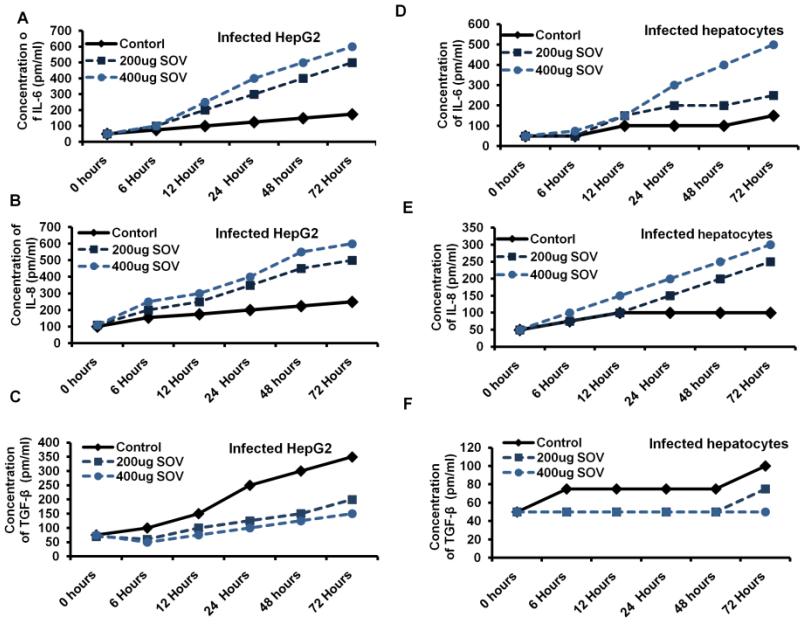

Figure 3: Levels of proinflammatory cytokines produced in HCV infected and treated cell

(A, B and C) The concentration of produced IL-6, IL-8 and TFG-β (pm/ml) in the fluids media of infected HepG2 cells that were subjected to either 200 or 400 µg/ml of SOV for the indicated time points compared with DMSO-treated cells (Control)**. (C, D and F)** The concentration of produced IL-6, IL-8 and TFG-β (pm/ml) in the fluids media of infected normal cells that were subjected to either 200 or 400 µg/ml of SOV for the indicated time points compared with DMSO-treated cells (Control)**.**

HepG2 cells and normal hepatocytes can be safely subjected to vitamin B12

Vitamin B12 supports iron impact in the metabolic cycle and facilitates folic acid role in choline synthesis. Thus, B12 metabolic pathway is cross-linked with folic acid metabolism and its deficiency causes pernicious anemia, megaloblastic anemia, and neurologic lesions. Importantly, vitamin B12 is a chemical cofactor of methionine synthase (MTR) in the synthesis of methionine, the originator of S-Adenosylmethionine (SAM), the common methyl donor in epigenetic alternation [28,29]. To investigate whether vitamin B12 can be safely used *in-vitro*, HepG2 cells and normal hepatocytes were subjected to 600µg/ml and incubated for two days. The cell morphology, cell viability rate, and LDH production were assessed in treated cells to achieve the possible cytotoxicity of vitamin B12. Interestingly, vitamin B12 treatment showed a comparable morphology statement in both HepG2 cells and normal hepatocytes when compared with control-treated cells (Figure 4A). Likewise, the cell viability rate of both cancer and normal cells was partially increased in a time-dependent manner of vitamin B12 treatment (Figure 4B). In the same way, the production of LDH showed neglected variety between vitamin B12 treated cells compared with control-treated cells (Figure 4C). These data indicate that vitamin B12 has no cytotoxic effect on HepG2 cells and normal hepatocytes when used up to 600µg/ml. Furthermore, this finding indirectly provides evidence for the safe and possible utilizing of vitamin B12 as a supplement material with the main drug treatment.

Vitamin B12 improves the efficiency of SOV treatment in HCV infected HepG2 cells

The biological influence of vitamin B12 and its combination with SOV was investigated in HCV-infected cells. Accordingly, HepG2 cells were subjected to 400µg/ml of vitamin B12 or 400µg of SOV or equal concentration of both of them. The treated cells were then infected with HCV (MOI =1) and incubated for two days. The relative gene expression of HCV-NS5A, IL-6, and IL8 was detected by using qRT-PCR. Moreover, the level of produced IL-6 and IL8 from infected and treated cells was monitored using ELISA assay. Interestingly, the relative gene expression of NS5A was dramatically decreased in HepG2 cells that were subjected to the combination of vitamin B12 and SOV when compared with vitamin B12 or SOV treated cells (Figure 5A). Furthermore, the relative expression of both IL-6 and IL-8 was strongly interrupted in cells treated with an equal concentration of vitamin B12 and SOV, while their expression level was significantly stimulated in cells treated with SOV (Figure 5B). Similarly, the level of produced IL-6 and IL-8 was depleted in HepG2 cells that were treated with both vitamin B12 and SOV in a time-dependent manner, while their concentration was increased in cells treated with SOV only (Figure 5C and D). These data confirm that vitamin B12 can improve the efficiency of SOV in facing HCV replication and can adjust the production of IL-6 and IL-8 which play a major role in the inflammatory event and cytotoxic effect SOV.

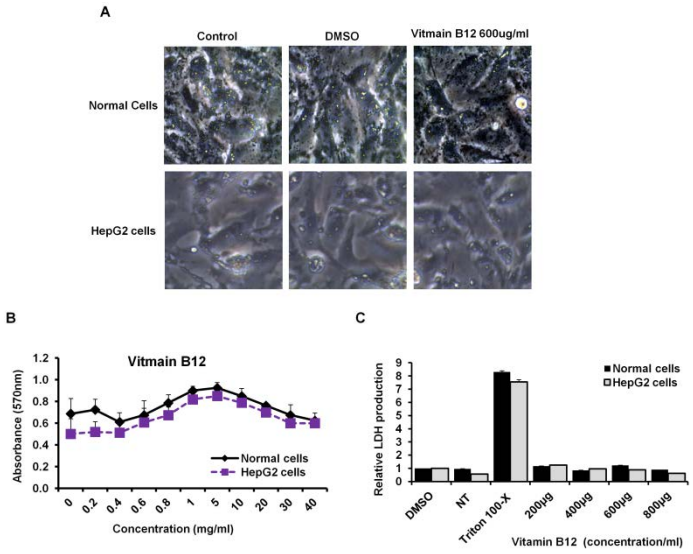

Figure 4: **The cytotoxic potential of vitamin B12 in HepG2 cells and normal hepatocytes independent of HCV infection**
(B) The representative cell morphology images of normal hepatocytes and HepG2 cells in 48 hrs upon treatment with the indicated concentration of vitamin B12 compared with DMSO treated cells. **(B)** Cell viability rate of treated HepG2 cells and normal hepatocytes with different concentrations of vitamin B12 that revealed its CC_{50} using MTT assay. **(C)** Relative LDH production from treated cells in comparison with Triton 100-X treated cells. Error bars reveal the SD of three different replicates. Student's two-tailed t-test was used to determine *P* values and significance of cell viability and LDH production level.

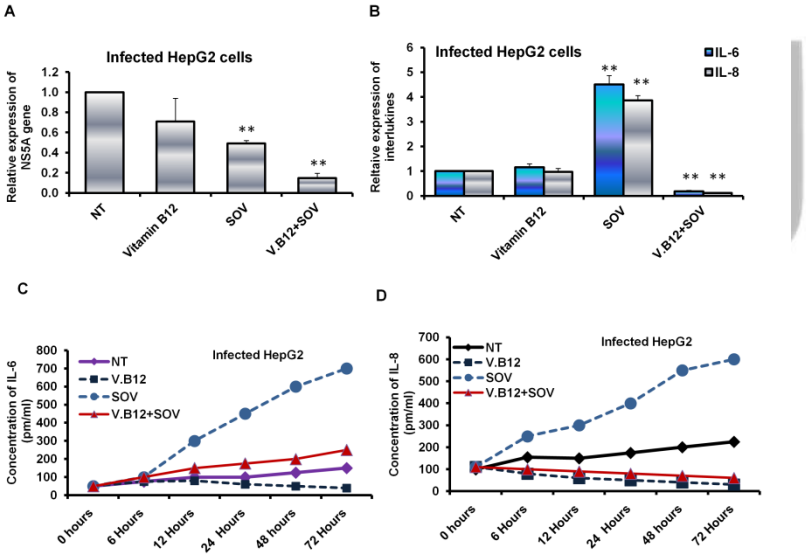

Figure 5: Biological activity of vitamin B12 in combination with SOV in HCV replication and HepG2 cellular immune response
(A) Quantification analysis of NS5A-mRNA indicated by fold changes in infected HepG2 cells subjected to 400µg/ml of vitamin B12, SOV or equal concentration from both of them compared with DMSO-treated cells. **(B)** Steady-state mRNA of IL-6 and IL-8 indicated by fold changes in infected HepG2 cells that pretreated with 400µg/ml of vitamin B12, SOV or equal concentration from both of them in comparison with DMSO-treated cells. Levels of GAPDH-mRNA were used as an internal control. Error bars indicate the SD of three independent experiments. A student two-tailed t-test was used for significance analysis of cycle threshold (Ct) values. Asterisks (*) indicates *P-values* ≤0.05 and (**) indicates the *P* ≤0.01. **(C and D)** The concentration of produced IL-6 and IL- (pm/ml) in the fluids media of infected HepG2 cells that were subjected to 400µg/ml of vitamin B12, SOV or equal concentration from both of them for the indicated time points compared with DMSO-treated cells (NT)**.**

Discussion

During the current work, we reported that SOV treatment stimulates the proinflammatory cytokine secretion parallel with the disturbance efficiency of HCV replication in both infected liver cancer cells and normal hepatocytes. Interestingly, the combination of SOV and vitamin B12 in equal concentration (400µ/ml) can adjust the production of IL-6 and IL-8 and improve the ability to disturb HCV replication in treated cells. IL-6 and IL-8 are rapidly and immediately produced in response to infections and tissue injuries; participate in host defense via the stimulation of acute phase reaction, hematopoiesis, and innate immune response. The expression of IL-6 and IL-8 is strictly regulated by transcriptional and posttranscriptional mechanisms, therapy dysregulated continual synthesis of IL-6 plays a pathological role on autoimmune diseases and chronic inflammation [30]. Therefore, vitamin B12, as an anti IL-6 and Il-6 production, is expected to be effective for many inflammatory events and intractable immune-mediated diseases. Importantly, the level of produced IL-6 and IL-8 from host cells mechanically activates the expression of nuclear factor-kappa B (NF-kB) which consequently maintain cell division, inflammatory events, and tumorigenesis [31,32]. The up-regulation of NF-κB is associated with several distinct stimuli, the canonical stimuli, such as cytokines and growth factors. While the non-canonical stimuli of the NF-κB includes cellular autophagy, tumor necrosis factor receptor (TNFR) super-family such as CD40 (cluster differentiation 40) and the receptor activator of NF-kB (RANK) [31,33,34]. Based on this, we were interested to prevent the production of both IL-6 and IL-8 in SOV-treated cells by using vitamin B12 which revealed a competitive inhibition of interleukin production and increased the antiviral activity of SOV.

Notably, vitamins B9 (folate) and B12 play a critical role in the generation of methyl groups required for DNA synthesis, amino acid homeostasis, antioxidant production, and epigenetic alteration through the preservation of one-carbon metabolism [35]. The imbalance in this pathway has implicated in different types of cancer [36]. A systematic study of B vitamins reported that the increased intake of vitamin B2 and vitamin B12 is associated with esophageal cancer risk, while vitamin B6 and B9 contribute in an opposite way [37]. Furthermore, vitamin B12 plays a major role in the epigenetic changes that occur in host cells as it is a cofactor in the methionine synthase (MTR) to form methylcobalamin and stimulate the conversion of homocysteine to methionine. During such process, the methyl group is transferred from methyltetrahydrofolate to methionine in which vitamin B12 in association with methionine synthase participates in the DNA methylation pathway through the synthesis of the methyl donor S-adenosylmethionine (SAMe), and the synthesis of purine and pyrimidine groups through generation of tetrahydrofolate [38]. In this way, we hypothesis that vitamin B12 treatment prevents interleukins production may via controlling the epigenetic changes and regulating gene expression profile.

Conclusion

In the current work, we investigated the cytotoxic effect of SOV treatment in HepG2 cell line and normal hepatocytes. Addtionally, we achived the possible impact of vitamin B12 to avoid the cytotoxic events induced by SOV treatment. Interestengly, we found that SOV treatment decreases the cell viability rate of both cancer and normal cells accompanied by high secresion level of proinflammtory cytokines and LDH production from treated cells in a time and dose-dependent manner. Notably, the combinations of vitamin B12 and SOV in equal concentration can inhibit HCV replication and adjest the level of produced IL-6 and IL-8 from infected HepG2 cells. Therfore, we hypothesis that the combination between sofosbuvir, as a sufficient anti-HCV drug, and vitamin B12 resulted in increasing its ability to inhbit HCV replication without any detectable toxic effect.

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Authors' contributions

Mohamed F. Fanny**,** Khaled Elawdan and assisted in performing the experiments. Sabah Farouk helped conceptualize experiments and interpreted data. Hany Khalil designed the research plan, supervised overall research, provided and interpreted data, prepared, wrote and revised the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

All authors declare that there are no conflicts of interest.

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