IJBPAS, September, 2018, 7(9): 1777-1785

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COUNTENANCE OF INTERFERON ALPHA (IFN-α) INDUCED HEPATIC INSULT AND POTENTIAL ROLE OF VITAMIN-D IN RAT MODEL

MALIK A[a*], ASIF S[a], AMIN G[b], WAQUAR S[a], SHAFIQUE H[a], ZAHID A[a] AND ALI Q[a]

a Institute of Molecular Biology and Biotechnology (IMBB), University of Lahore-Pakistan.

b The University College of Medicine and Dentistry, The University of Lahore-Pakistan

***Corresponding author: Arif Malik (Ph.D): Email: arifuaf@yahoo.com; Mobile number: 0321-8448196**

Received 17th March. 2018; Revised 4th April 2018; Accepted 9th May 2018; Available online 1st Sept. 2018

https://doi.org/10.31032/IJBPAS/2018/7.9.4547

ABSTRACT

By the twentieth century more than 170 million hepatitis C Virus (HCV) patients were reported and which was further believed to increase 3 to 4 million cases every year. Interferon therapy show significant decrease in the incidence of hepatocellular carcinoma in HCV patients. Aims of the current study were to evaluate the antiviral and anti-oxidative role of IFN in rats with induced hepatic insult. Hepatotoxicity was induced through administration of CCl_4 by intraperitoneal route. Nine groups of rats (10 in each) were administered different therapeutic treatment singular and in combination of Interferon-α (IFN-α), ribavirin, vitamin-D and thyroxin. Various biochemical perimeters (i.e., ALT, AFP, 8OHdG, TNF-α, IsoP-2α, MDA and COX-2) were measured to evaluate their role in progression of hepatic insult. The biochemical parameters (ALT, AFP, 8OHdG, TNF- α, IsoP-2α, MDA and COX-2) of rats that received IFN-α, ribavirin, Vit-D and thyroxin (group I) were significantly ameliorated (31.29±2.56 IU/L, 18.26±3.29 ng/L, 5.26±1.08 pg/mL, 21.29±1.09 ng/mL, 28.26±1.99 pg/mL, 1.86±0.65 nmol/mL, and 0.99±0.0061 ng/mL) as compared with the CCl_4 induced rats (group B). Present study concludes that the levels of ALT, AST were increased in rats with induced hepatic insult. Moreover, there was an increased level of MDA, 8OHdG and IsoP-2α that might be responsible for the induction of lipid peroxidation and DNA damage. Treating the rats with IFN-α decreased the levels of liver profile and stress markers but somehow, it was responsible for the induction of thyroid dysfunction.

Therapeutic combination treatment of vit-D along with IFN-α remained promising factors in the case of oxidative stress and liver damage.

Keywords: IFN, ALT, 8OHdG, COX-2, TNF-α, hepatitis C Virus

INTRODUCTION

At the time of discovery interferon (IFN) was believed to be a member of cytokine family having small molecular weight around 15KDa to 21KDa [1]. Interferon (IFN) was primarily classified into three major classes (i.e., IFN-α, IFN-β, and IFN-γ). Furthermore, recently a new class has been discovered known as IFN-λ [2]. All of these classes show significant antiviral effects on the biological system having variable extent of activity [3]. These are synthesized in the biological cells and are secreted in response to several viral, synthetic and biological inducers. IFN induces antiviral activity against viral infection by inhibiting antiviral state and through the modulation of immune response. Among all classes of IFNs, IFN-α plays a significant anti-viral role against the viral hepatitis that is the major cause of liver cirrhosis and hepatocellular carcinoma [4]. Liver cirrhosis occurs due to chronic injury and can be characterized by the presence of excessive extracellular matrix proteins that are synthesized specifically by hepatic stellate cells and acquire a presence of mayofibroblast in the damaged area of liver. Severe liver damage leads to the liver cirrhosis that might results in the hepatic cellular carcinoma which increases the production of ROS in the biological system [5]. Liver cirrhosis and oxidative stress are highly linked with each other as reported in experimental and clinical trials [6]. Depletion of antioxidants and increase in ROS leads to the accumulation of matrix and collagen over liver. In vitro studies suggest that antioxidant and IFN treatment leads to the recovery of liver damage and inhibit the accumulation of collagen on hepatocytes [7].

Among all types of IFNs, PEGylated IFN is superior in terms of achieving significant antiviral response and tolerability against viral hepatitis [8]. Upon treatment with IFN-α as an antiviral drug, it significantly stimulates the immune system via development of cytotoxicity within the cell and is responsible for significant antiviral and antineoplastic activity [9]. Moreover, induction of IFN-α induces antifibrotic effects against viral hepatitis. It reduces the levels of ROS in the biological system of patients infected with hepatitis C virus (HCV) [10]. Anti-viral response induced by

IFN- α activates JAK/STAT pathway, including double-stranded RNA-activated protein kinase (PKR) and 2959-oligoadenylate synthethase (2959-OAS) respectively. A third critical anti-viral protein is a dynamin-like large guanosine triphosphatase (GTPase) and myxovirus resistance gene A (MxA), which are up-regulated by IFN-α in various species [11].

Along with the beneficial aspects, IFNs show severe side effects as well, stimulation of immune response through the induction of IFN leads to the development of thyroiditis, blood related and neuropsychiatric defects in the HCV patients, due to such side effects there is around 14% depletion in the usage of IFN worldwide during the last decade [12]. Relationship between IFN-α and thyroid diseases was initially recognized in 1985 by critical symptoms of carcinoid tumours and breast cancer [13]. As the IFN receptors (IFNR) are present on thyroctes, IFN binds with IFNR and inhibits the production, synthesis and metabolism of thyroid hormones leading to the condition of thyroiditis [14] it can be further identified by the increase in thyroid antibodies, usually around 15% of IFN treated HCV patients are diagnosed with thyroid dysfunction as hypothyroidism, Grave's thyrotoxicosis and thyrotoxicosis. As aforesaid that IFN decreases the levels of ROS in the biological system that is also a major culprit of induction of thyroid diseases, as hydrogen peroxide (H_2O_2) is required for the production of thyroid hormone that gets depleted due to induction of IFN. Thyroid peroxidase immediately uses the H_2O_2 and oxidized iodine by catalysing the peroxidation reaction [15]. However, present research was performed on the animal model by induction of CCl_4 and evaluates the antiviral and anti-oxidative effect of IFN induced through intraperitoneal injection.

MATERIALS AND METHODS

Present study was conducted on total ninety (n=90) albino rats with body weight around 80-100g. They were further divided into nine groups having ten (n=10) rats in each group receiving different treatments (Table-1). Carbon tetrachloride (CCl_4) was introduced via intraperitoneal route to albino rats at rate of 1 ml/kg B.Wt. every week for three months to induce hepatic injury. After the period of time rats were slaughtered by injecting ketamine injection intraperitoneal followed by drawing 5 ml blood. Levels of ALT, AFP, 8OHdG, TNF-α, IsoP-2α were measured through ELISA kits provided by Abcam, Cambridge, MA, USA. Whereas, lipid peroxidation in the blood samples were

measured calorimetrically by estimating the thiobarbituric acid reactive substances (TBARs) by the protocol described by Ohkawa [16]. Briefly, after labelling test tube add 0.2 ml serum sample then 0.2 ml of 8.1% SDS, 1.5 ml of acetic acid (20%) and 1.5 ml of TBA (0.8%), n-Butanol (4ml) and centrifuged at 4000rpm for 10 min and absorbance was taken at 532nm against blank. Levels of MDA were measured in nmol/mL against standard curve. All the chemicals and reagents required for the biochemical assays were purchased from Sigma Chemical Co. (St Louis, MO, USA). Ethical approval has been taken from the Institutional Review Board of The University of Lahore (Approval No: USM/Animal Ethics approval/2009/[45] [140]). One way ANOVA test was performed for statistical analysis with *p*-value of ≤0.05 was considered as statistically significant.

Table 1: Experimental design

GROUPS	TREATMENTS
A	Control
B	CCl_4
C	IFN-α
D	Ribavirin
E	CCl_4+ IFN-α
F	CCl_4+Ribavirin
G	CCl_4+ IFN-α+ Ribavirin
H	CCl_4+ IFN-α+ Ribavirin+Vit-D
I	CCl_4+ IFN-α+ Ribavirin+Vit-D+Thyroxin

CCl_4 @ (1 ml/kg B.Wt. per week for three months), IFN-α @ (2.5µg/ Kg B.Wt./week SC), Ribavirin @ (14.28mg/Kg B.Wt./ day), Vit-D @ (60 µg/Kg B.Wt/week), Thyroxin @ (1.7µg/Kg B.Wt/day) for six months.

RESULTS

Significant restoration of the investigated biochemical variables in rat groups that received the IFN-α treatments were seen (Table 2). The biochemical parameters (ALT, AFP, 8OHdG, TNF- α, IsoP-2α, MDA and COX-2) of rats that received IFN-α, ribavirin, Vit-D and thyroxin (group I) were significantly (p=0.006, 0.028, 0.031, 0.002, 0.014, 0.000 and 0.023) ameliorated (31.29±2.56 IU/L, 18.26±3.29 ng/L, 5.26±1.08 pg/mL, 21.29±1.09 ng/mL, 28.26±1.99 pg/mL, 1.86±0.65 nmol/mL, and 0.99±0.0061 ng/mL respectively) compared with the control group. Moreover, levels of COX-2, ALT, 8-OHdG, AFP, IsoP-2a, TNF-α and MDA were significantly amended as compared with the healthy control rats that were administered normal diet (0.74±0.0019 ng/mL, 17.26±2.19 IU/L, 2.08±0.56 pg/mL, 6.29±1.99 ng/L, 21.29±3.19 pg/mL, 19.65±2.18 ng/mL and 1.60±0.019 nmol/mL) respectively.

DISCUSSION

HCV can induce chronic as well as acute infections; the chronic type of

infection causes liver cirrhosis and hepatocellular carcinoma (HCC) [17]. It not only induces hepatic carcinoma but also associated with other disease such as oral mucosa, skin, kidney, brain, sweat gland and stomach by the detection of HCV-RNA in these tissues [18]. From last 50 years IFN-α was believed to be the best treatment against HCV but due to its severe side effects, now ribavirin is administered along with IFN-α in combination for reduces the progression of thyroiditis induces due to IFN-α [19]. In present study rats were induced hepatic injury by CCl_4 injection, it has been observed that there was an increased production of ROS in the body of CCl_4 induced rats by measuring the levels of MDA that is a significant marker of lipid peroxidation. Increase in the level of MDA clearly depicts that there was increase production of ROS, resulting in the depletion of antioxidants leading to oxidative stress. Increase MDA levels from the cell membrane moves inside the nucleus and damages the DNA by synthesizing DNA adducts known as 8OHdG (MDA vs 8OHdG, r=0.748**).

Moreover CCl_4 also induces the hepatic inflammation as well, that can be measured by the levels of COX-2, which is a significant signal of inflammation. TNF-α increases the production of prostaglandins and COX-2 as shown in the table-3 (COX-2 vs TNF-α, r=0.642]. Increase production of ALT also increases the levels of COX-2 (ALT vs COX-2, r=0.816**) that indicate hepatic insult induced due to CCl_4 leads to inflammation of liver as well. CCl_4 induced the liver toxicity artificially by the inhibition of B-oxidation and induction of lipid peroxidation [20]. Inside the hepatocyte CCl_4 is converted into $CCl_3^{\bullet}$ and $CCl_3OO^{\bullet}$. CCl3 inhibits B-oxidation and increase the production of TNF-α that induces the production of COX-2 and inducible Nitric oxide synthase (iNOS) that increase the production of NO resulting in liver injury. On the other hand ROS that were released due to $CCl_3OO^{\bullet}$ also increases the production of COX-2 and eventually leads to liver injury, increase production of ROS also leads to lipid peroxidation resulting in depletion of antioxidants (GSH, SOD, CAT) and increase of MDA that induces liver injury [21]. Treatment of IFN-α initiate JAK/STAT pathway that induces γ-IFN activating sequence (GAS) that increases the production of IFN-γ and decreases the levels of ROS in liver compromised rats [22, 23]. Exogenous IFN-α injection activates immune response by acting on the antigen presenting cell (APC) and activates neive T

cells (Th_o) that activates Th1 and Th2 cells that are responsible for production of cytotoxic t lymphocytes (CTL) and antibodies respectively. CTL increases the production of granzyme and perforin, perforin damages the thyroctes and built a path for the transportation of granzyme, inside the thyroctes granzyme activates different classes of caspases leads to apoptosis resulting in thyroid dysfunction.

Table 2: Biochemical response of biochemical variables in a rat model following CCl_4 induced liver insult

GROUPS	MEAN±SD (n=10)						
	ALT (IU/L)	AFP (ng/L)	8-OHdG (pg/ml)	TNF-α (ng/mL)	IsoP-2α (pg/mL)	MDA (nmol/mL)	COX-2 (ng/mL)
A	17.26±2.19	6.29±1.99	2.08±0.56	19.65±2.18	21.29±3.19	1.60±0.019	0.74±0.0019
B	102.26±4.26	91.65±5.32	31.29±4.47	65.29±4.26	102.29±9.65	6.35±1.02	3.29±0.027
C	90.26±5.26	68.26±7.16	28.26±3.27	75.26±3.29	104.28±3.29	5.29±2.09	2.49±0.426
D	86.26±7.16	65.23±6.33	27.26±6.37	34.30±7.09	91.65±8.26	6.66±1.03	2.38±0.131
E	81.26±8.16	47.26±7.65	18.69±2.65	31.25±4.23	78.39±5.66	4.19±1.08	2.09±0.231
F	76.29±6.25	40.26±5.81	16.35±2.19	27.26±2.19	67.26±2.19	3.08±0.96	1.56±0.532
G	61.35±6.35	38.26±4.66	22.26±21.22	26.35±3.16	47.26±3.65	3.00±0.85	1.44±0.013
H	51.29±4.26	23.25±2.99	16.35±3.24	22.18±3.26	31.65±2.99	2.69±0.46	1.08±0.056
I	31.29±2.56	18.26±3.29	5.65±1.08	21.29±1.09	28.26±1.99	1.86±0.65	0.99±0.0061
LSD (0.05)	8.16	4.56	5.16	1.65	1.019	0.49	1.08
p-VALUE	0.006	0.028	0.031	0.002	0.014	0.000	0.023

Table 3: Pearsons' correlation coeeficients of different variables in liver tissues of rats under CCl_4 stress receiving IFN-α

VARIABLES	AFP	8-OHdG	TNF-α	IsoP-2α	MDA	COX-2
ALT	0.756*	0.652*	0.742*	0.711**	0.688*	0.816*
AFP		0.589*	0.845*	0.741*	0.610*	0.815*
8-OHdG			0.646*	0.644*	0.748**	0.741**
TNF-α				0.651*	0.456*	0.642*
IsoP-2α					0.674*	0.642*
MDA						0.741*
COX-2						

** Correlation is significant at the 0.01 level (Two-tailed)

CONCLUSION

Results of present study concludes that injection of CCl_4 leads to the induction of liver injury by the increase in ALT, AFP, ROS and lipid peroxidation levels. Moreover CCl_4 also leads to the DNA damage (ALT vs 8OHdG, r=0.652*) and induces hepatic inflammation (ALT vs COX-2, r=0.816**). Induction of IFN-α as an antiviral drug might play a significant role in reduction of liver damage and ROS production but because of having lethal side effects such as thyroid dysfunction reduces its singular use. Present study concluded that combination therapeutic treatment of Vit-D and IFN-α result in significant reduction in the stress levels and liver profile with decreased side effects.

ACKNOWLEDGEMENTS

The authors are grateful for the valuable contribution of Prof. Dr. M. Arslan, Director Centre for Research in Molecular Medicine (CRiMM), University of Lahore-Pakistan

for the financial support and for critically reviewing the manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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