ORIGINAL ARTICLE

Protective Role of Alpha Tocopherol Against Ribavirin Induced RBC Membrane Damage in Rat Model

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ABSTRACT

Objective: To assess the red blood cell membrane physiology in ribavirin induced hemolysis in rats and possible protective role of alpha tocopherol (vitamin E) against ribavirin induced hemolysis in RBCs of rats.

Subjects and Methods: This experimental study was conducted Isra University Hyderabad with collaboration of at Animal house of department of Animal Husbandry and Veterinary Sciences Sindh Agriculture University Tando Jam. Total 30 albino Wistar rats was selected and were divided into 3 groups like A (controls), and experimental groups B given ribavirin only for 4 weeks, Groups C were given Ribavirin and alpha tocopherol. All drugs were used for 4 weeks as an oral dose daily. Erythrocyte osmotic fragility test was performed with NaCl solutions of difference osmotic concentrations. Data was collected via study proforma.

Results: Total 30 rates were studied by divding different groups. Body weight of all animals was found to be statistically insignificant. Average of the red blood cells was found significantly decreased in only ribavrion administrated group as compared to control group and ribavirin+vitamin E administrated group (p-0.045). Mean of MCV, MCHC and HCT were higher in ribavirin+vitamin E administrated group as compared to only ribavrion administrated group.

Conclusion: It was concluded that alpha tocopherol (vitamin E) have protective role against ribavirin induced hemolysis in RBCs of rats.

Keywords: Ribavirin, Hemolysis, Lutein

INTRODUCTION

Ribavirin (also called tribavirin) is frequently being used for treating the individuals infected with Hepatitis C virus (HCV).Despite the fact that its modes of action are yet unknown, ribavirin is effective in attaining virological response as well as reducing the virological relapse rate following therapy termination.[1] The liver metabolizes ribavirin, and metabolites of ribavirin such as ribavirin-5 triphosphate (RTP) and ribavirin-5 monophosphate (RMP) have toxic effects. Ribavirin-5 triphosphate has mutagenic effects against RNA virus because it templates the absorption of uridine and cytidine with comparable efficiency in vitro.[2] Ribavirin, on the other hand, can cause negative effects, resulting to termination of early treatment. Howe Ribavirin is linked to dose-limiting effect called hemolytic anemia, which necessitates hemoglobin monitoring on treatment.[3] Ribavirin, which is nucleoside analog, is phosphorylated in the cells to produce monophosphate-, diphosphate-, and triphosphate-ribavirin (RMP, RDP and RTP respectively). RTP buildup in erythrocytes (RBCs) in substantially higher amount than the other types of cells due to the absence of dephosphorylating enzymes in RBCs.[3] RTP causes oxidative damage to membrane by lowering the levels of adenosine triphosphate (ATP) and impairing ATP-dependent transport mechanisms.[3,4] Patients are frequently given supplemental erythropoietin or their antiviral dosage may be decreased to minimize or avoid ribavirin-provoked hemolytic anemia.[5] While both techniques may be beneficial, as both have their own set of drawbacks. Treatment with erythropoietin is expensive and takes many weeks to show results.[5]

Though vitamin E (fat-soluble) is mostly found in plasma membranes and significantly contributes in non-enzymatic fatty acid protection from oxidative damage.[6] Previous research has shown that maintaining high-dose tribavirin through mitigating hemolytic anemia with high antioxidant supplemental doses such as alpha-tocopherol (vitamin-E) and ascorbic acid (Vitamin-C) can result in a sustained virological response (SVR) rate through tribavirin and pegylated IFN therapy, implying that tribavirin dose significantly contributes in successful combination therapy.[7,8] The antiviral drug tribavirin or ribavirin(RBV) is being extensively used now a days with interferons for chronic disease of liver (CLD) hepatitis and hemolytic anemia has been observed the main side effects of ribavirin. However thiss tudy has been condcuted To assess the red blood cell membrane physiology in ribavirin induced hemolysis in rats and possible protective role of alpha tocopherol against ribavirin induced hemolysis in RBCs of rats.

MATERIALAND METHODS

This was a Quasi-Experimentalstudy and was condcuted at Isra University Hyderabad in the collaboration of atanimal house of Sindh AgricultureUniversity, Tandojam. Total 30 healthy male Albino Wistar rat with Body weight of 200 to 250 grams were sslected for the study. All the un-healthy rats, Rats not feeding properly and moribund rats were excluded. Rats6were fed on chow6to both experimental and controls6groups, having a scientifically6approved composition consistent with instructions6of veterinary specialists. All the selected animals were divided in three grpous group A, grpup B And group C. Animals of group A

(control) were kept on 0.9% normalsaline asplacebo. Animals of group B were taken on Ribavirin alone4mg/Kg/day and animals of group C were taken on ribavirin 4mg/Kg/day+alpha tocopherol 100 mg/kg/day.

The animals6were handled and6housed as per NIH6Guide for the6Use and6Care of6Laboratory Animals. Rats6were housed6in stainless6steel cages (with6saw dust6bedding). The cages6were equipped with6stainless steel6feed containers6and plastic drinkers6with stainless6nozzles. The animals6were housed6under hygienic and6well ventilated6environment. Rats were6provided6food (lab-chow) mixed with tablet lutein and ribavirins, capsule alpha tocopherol and tap water6ad libitum. The6light/dark cycle was6maintained on612 hour6intervals. All6animal procedures were conducted6under an animal6protocol approved6by Sindh Agriculture6University, Tando6Jam. The cages6of rats of control6and experiment6groups were labeled6as exhibiting different6parameters.

Erythrocyte osmotic fragility test: This assay was carried out with NaCl6solutions of different6osmotic concentrations such6as. 0.11N NaCl6solution, 0.21N NaCl6solution, and likewise.

Osmotic fragility test (OFT): This assay was the first6method applied for6screening of thalassaemia6and was6introduced as a simple6approach to detect thalassaemia carriers by6Silvestroni and6Bianco in the 1940s. This fast6and simple5method has been applied as a6screening assay in6large populations. The6availability of electronic6counters for the6measurement of MCV6and MCH6has decreased6the use of6OFT. It is6still used6in low6resource nations to6screen large6rural or tribal populations. Several variations6of the basic6method have been proposed. The most6used test at6present is NESTROFT, the acronym6for Naked6Eye Single6Tube Red6cell Osmotic6Fragility Test.[9]

Blood CP: Was done using SYSMAX XN 550 Analyzer

Peripheral Blood Smear staining and Preparation:

Peripheral smears were dried in air and stained using leishman's stain.

Staining of peripheral blood smear:

- Smear was placed on the staining rack.
- On dried smears Leishman's stain was poured.
- Leave the stain for 2-3 min.
- On the slides, Buffered water is added for 10 min.
- Then slides are washed in tap water and dried in air.

Peripheral smear Morphology: Stained blood smears were Morphologically observed using microscope with 40 X power lens (Olympus, Japan).

All the data was collected via self-made proforma and analyzed on SPSS version 22.0

RESULTS

In this experimental study to 30 rates were studied by divding different groups. Body weight of all animals was found to be statistically insignificant as shown in table.1

Average of the red blood cells was found significantly reduced in only ribavrion administrated group in comparison to controls and ribavirin+vitamin E administrated group (p-0.045)as showen in tables 2.

Average of MCV, MCHC and HCT were higher in ribavirin+vitamin E administrated group as compared to only ribavrion administrated group as shown in Table 3

Table 1: Discriptive statistics of animal weight in grams (n=30)

Study groups	Mean	SD	F-value	P-value
Group A	203.50	4.69	1.77	0.734
Group B	158.4	6.60		
Group D	204.42	204.42		

Group A = Controls, Group B= Ribavirin 4mg/kg/day, Group C= ribavirin 4mg/Kg/day+alpha tocopherol 100 mg/kg/day

Table 2. Osmotic fragility in various animal groups exhibiting % hemolysis (n=30)

	Group A	Group B	Group C
% NaCl	% Hemolysis	% Hemolysis	% Hemolysis
0	99	100	100
0.1	96	100	100
0.2	81	100	100
0.3	72	99	97
0.35	54	96	94
0.4	51	87	89
0.45	46	81	79
0.5	41	51	48
0.55	26	41	41
0.6	23	38	40
0.65	19	31	32
0.75	16	32	31
0.85	11	22	24
0.9	4	16	19

Table 3: Mean RBC count, MCHC, MCH and HCT of rats (n=30)

Parmetyers	Study groups	Mean±SD	P-Value
Mean RBC count	Group A	4.65±1.01	0.045
	Group B	3.29±0.22	
	Group C	5.46±0.27	
Mean MCHC	Group A	53.4±3.60	0.001
	Group B	37.3±1.52	
	Group C	52.6±3.05	
Mean MCH	Group A	19.4±0.6	0.084
	Group B	18.7±0.1	
	Group C	221.3±00.4	
Mean HCT	Group A	34.2±2.1	0.074
	Group B	25.7±1.4	
	Group C	35.3±3.2	

Group A = Controls, Group B= Ribavirin 4mg/kg/day, Group C= ribavirin 4mg/Kg/day+alpha tocopherol 100 mg/kg/day

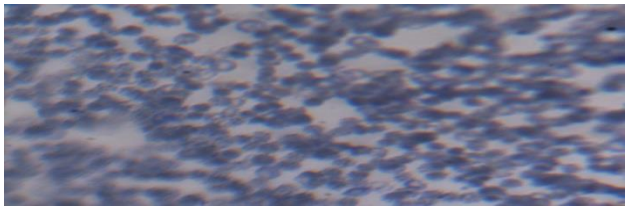

Fig-1: (Group C) Showing Normal RBC Morphology with minute RBC breakdown Normocytic , Normochromic (100x)

DISCUSSION

As for as we know, in this study reported the6effects of alpha-tocopherol therapy on the red blood cell osmofragility by seeing the osmotic fragility, peripheral blood smear and blood Cp in Ribavirin induced experimental6rat model. This6study reports increased6osmofragility with ribavirin use and a6reduction was6noted by use alpha tocopherol.

In present study, the RBCs of most experimental6rats exhibited >90% hemolysis6at60.45% NaCl6concentrations and >695% hemolysis6at60.35% NaCl concentrations.

hemolysis6was noticed significantly in ribavirin treated6animals, while the oral usage of alpha-tococpherol showed a decline of osmofragility in ribavirin treated animals. Howevevr, increased hemolysis (%) was seen in all rats of experimental group in comparison to controls. Cumulative % hemolysis of RBC in experimental groups was noticeable at 0.45% NaCl concentration in experimental rats. Uydu6et al[10] studies the effects6of ribavirin6drug therapy6on rheological6characteristics of erythrocyte membrane, serum6lipid profile6and oxidative6status in cases with dyslipidemia. Osmofragility6has been reported by6Zahediasl[11] in6experimentally induced6hyperthyroid rats6from University of6Brussels, Belgium. Though, findings of6above study were6inconclusive and6not comparable to6our present6study. A recent6case report has6reported toxic6epidermal necrolysis6and rhabdomyolysis by6Ribavirin in6human being. In this study average of the red blood cells was found significantly decreased in only ribavrion administrated group as compared to control group and ribavirin+vitamin E administrated group (p-0.045). In the favor of our findingds Assem M et al[7] administarted the high-dose supplementation of vitamin E in chronic HCV patients on Ribavirin-provoked hemolytic anemia and they reported that the anemia significantly reduced in combined therapy group (8.5%) in comparison to controls (21.5%). Previous studies suggested that administration of erythropoietin would improve anemia that results from ribavirin and peginterferon therapy and also in improving the quality of life, erythropoietin is more effective as compared to dose reduction in treatment.[7,11] Inconsistently, Saeian K et al[12] observed that supplementation of vitamin E alone during ribavirin + alfa-interferon standard therapy appears to have not affect on minimizing ribavirin-provoked haemolysis. On the other hand, Kawaguchi Y et al[13] concluded that the high-dose supplementations of alpha-tocopherol (vitamin-E) and ascorbic acid (Vitamin-C) helped preventing ribavirin-provoked hemolytic anemia in the course of IFN alfa-2b and ribavirin combination therapy in chronic HCV patients as they found in their case control study that the decline in the levels of hemoglobin was significantly blocked in the alpha-tocopherol and ascorbic acid group in comparison to the controls (*P*-0.029). Previously also reported that the alpha-tocopherol might protect RBC membranes against oxidative damage because of ribavirin. Though, several researchers suggest the potential advantages of antioxidants (such as vitamin E), on Ribavirin-provoked hemolysis. However, no systematic study has been documented.[7,14] This study was the animal study containing small number of animals, therefore large scale multicenre humen studies are suggested on this subject.

CONCLUSION

Ribavirn causes significant red blood cells hemolysis, as assessed through peripheral blood smear and RBC indices. Alpha-tocopherol6have a protective role against ribavirin induced hemolysis in rats. Howevevr more large scale studies should be done to conform the findings to use the alpha-tocopherol6combine therapy to prevent the anemia in ribavirin treating patients.

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