ORIGINAL ARTICLE

Evaluation of Protective Role of Lutein Against Ribavirin Induced RBC Membrane Damage in Rat Model

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ABSTRACT

Objective: To determine the protective role of lutein against RBC cell membrane damage assessed by RBC osmofragility test, Peripheral blood smear and blood CP.

Subjects and Methods: Present experimental study was carried out at Isra University, Hyderabad and Department of AHVS (Animal Husbandry) Agriculture University,Tando Jam. 30 albino Wistar rats was selected and were divided into 3 groups namely A (controls), and experimental groups B which was given ribavirin only for 4 weeks and Group C was given Ribavirin as inducing agent and lutein. 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 analyzed by SPSS version 21.

Results: Hemolysis was higher 81% in group B as compared to group C as 70%. Mean of RBC count in the groups B was markedly decreased as compared to group A and C4.65, 3.29, and 5.13 (P=0.036). Mean of MCV in the grops A,B,C was noted as 53.4, 37.3 and 55.6 respectively (P=0.0001). Mean of MCH in the groups A,B,Cwas notedas 19.4, 18.7,and19.1 respectively (P=0.09), while mean of HCT in the groups A,B,C was noted as 34.2 , 25.7 and 34.2 (P=0.004)

Conclusion: It was concluded that lutein have protective role against ribavirin induced hemolysis in RBCs of rats

Keywords: Ribavirin, Hemolysis, Lutein

INTRODUCTION

Ribavirin, also known as tribavirin, is an antiviral medication used to treathepatitis C,therapy withRBV leads to hemolytic anemiadue to accumulationof the drugin RBCs, Ribavirin-induced hemolysis is passiveand non inflammatory intervened.[1,2] Ribavirin-inducedhemolysis is also dose-relatedand sustained. The reductionin hemoglobin levels appears to correlatedirectly with thedegree of hemolysis and inversely withthe erythropoetic abilityof the bonemarrow.[3,4]The potential side effect of medical care with RBV is the incidence of reversible anemia caused by hemolysis in a large share of the patients treated. The mechanism behind that is unclear. Studies of RBV's steady pharmacokinetics indicate that RBV's erythrocyte level surpasses plasma levels, and that RBV is a distributed within human erythrocytes by nucleoside transporter. RBV is intracellularly transformed into the associated triphosphate resulting in a significant ATP deficit within erythrocytes.[5-7]The decreasing ATP level may indirectly impact the antioxidants defense mechanisms by reducing the synthesis of hexokinase-mediated G6PD, a prevalent main substrate for shunting pentosephosphate and glycolysis. In addition, RBV is a dehydrogenase inhibitor (IMPDH), that precipitates xanthosine oxidations of inosinic acid (AMP), with corresponding transformation of nicotinamide (NAD) to thionicotinamide (Thio-NADP).Since this response is a rate-limiting phase of biosynthesis in guanine nucleotide, inosine (IMPDH) enzyme is targeted by chemotherapy against virus. The blend of such events representing pro-oxidant occurrence can stimulate premature senescence of the erythrocyte as well as phagocytic elimination. Erythrophagocytosis of marrow macrophages and also aggregation of granular pigmentand erythrocyte residues have been reported in spleen, liver, and marrow phagocytes were reported in RBV-administered monkeys, without any indication of intravascular erythrocytic degradation.[8,9] lutein, a phytochemical categorized as a carotenoid. Phytochemical compounds are derived from plants thatare unnecessary nutrients to sustain life.As they frequently havebitter taste, fragnance or pigments, they are considered to contribute as protective against external threats,for examplepathogens, ultravioletlight,andplants eating creatures.[5] Thephytochemicals with fundamental structure of C40H56 are the member of carotenoid group. Thesecompounds actasantioxidants.Theycontain several double bonds, which react with reactive oxygen species (ROS) to scavenge radicals.[6]Luteinare sole carotenoids in primate retina's macula [such as, macular pigments], where they exist in ~500-fold higher concentrations than inother body tissues (e.g, serum) andare thought tobe protective through their roles as blue-light filters and antioxidants.[7,10] Lutein depositesin humaneye (lens, outer rod segments, and retina) and other sitesof body such ascervix, skin, breast and brain.[11,12] Because lutein is modestly soluble in aqueous medium, it is general located in or tied with proteins within central core of plasma membranes.[13,14]It was thus speculated that (RBV) causes oxidative membrane impairment, which supports a premature extravascular red cell lysis identical to that reported in hemolytic anemia.[9]Lutein is the anti-oxidants which show promising effects as individual therapy, so to see weather they have any combined/synergistic effect on RBV Membrane stress or not was the aim of study.

MATERIALAND METHODS

StudyDesign: Quasi-Experimentalstudy

StudySetting: The studywas performed atanimal house Isra University Hyderabad and Departmentof AHVS, Sindh AgricultureUniversity, Tandojam.

SAMPLE SELECTION

Inclusion Criteria: Healthy Male Albino Wistar rat with Body weight of 200 – 250 grams

Exclusion Criteria: Un-healthy sick rats and female Rats

Animal Groups: Ratswere categorized into3 groupsas;

GroupA (n=10): Controlrats–receive0.9% normalsaline asplacebo

Group B (n=10): Ribavirin alone4mg/Kg/day

Group C (n=10): ribavirin4mg/Kg/day+lutein150mg/Kg/day

ANIMAL PROTOCOL & HOUSING

The animalswerehandled andhoused as per NIHGuide for theUse andCare ofLaboratory Animals at Department of AHVS, Sindh Agriculture University, Tando Jam

Ratswere housedin stainlesssteel cages (withsaw dustbedding). Animalswere housedunder hygienicandwell ventilatedenvironment. Rats wereprovidedfood (labchow) mixed with tablet lutein, ribavirinsand tap waterad libitum. light/dark cycle wasmaintained on12 hourintervals. Allanimal proceduresweree conductedunder an animalprotocol approvedby Sindh AgricultureUniversity, TandoJam. The cagesof rats of controland experimentgroups were labeledas exhibiting differentparameters.

After 4 weeks Ratsfrom each ofcontrol groupA and experimentalgroups B and Cwereselected forblood collection. Blood samples were collected in Blood CP bottles containing anti coagulant.

Erythrocyte Osmotic Fragility Test

Thisassay was carried out with NaClsolutions of differentosmotic concentrations suchas. 0.1NNaClsolution, 0.2N NaClsolution, and likewise.

- **Blood CP:** Wasdone using SYSMAX XN 550 Analyzer.
- **Principle of the analyzer SYSMEX XN 550:**

Hydro dynamically focused impedance measurement:

RBC and Platelets are measured by this method. Inside detector sample nozzle is placed in front of aperture in the center. Then sample move fromnozzle toward conical chamber. When diluted sample has passed through aperture, the sample is then sent to recovery tube.

Flow cytometry method by semiconductor laser:

Chemical and physiological aspects of cell are analyzed by flow cytometry. Here the blood specimen is aspirated, measured and diluted. Then the sample is placed in flow cell via sheath flow mechanism.

Laser beam (semiconductor) that passes through flow cell is directed on to blood cells.

Three types of light are captured by photo iodides, which are converted into electrical pulsesto obtain blood cell formation which are as follows:

Forward Scattered Light: This light give information about cell size and material properties.

Side Scattered Light: It provides information on the cell interior (size of the nuclues)

Side Flourescent Light:It provides information about blood cell labelling.

Peripheral Blood Smear: Preparation and staining of peripheral blood smear:

Peripheral smears were made, air dried and stained with 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.
- Buffered water was added on slides for 10 min.
- Then were washed in running tap water and air dried.

Morphology of peripheral smear: Morphology of stained blood smears were observed under the microscope (Olympus, Japan) under 40 X power lens.

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

RESULTS

According to the animal body weight group B showed decrease in body weight as compared to group A and C as bodies weightsof study groups of A,B and C was 203.50±5.59,183.4±5.70 and206.00±5.54 grams respectively(P=0.68).Table 1

Mean of RBC count was decreased in group B 3.29±0.22incontrast to group A and C as 4.65±1.01 and 5.46±0.27, (P=0.036).Table2

Mean MCV was normal in group A as 53.4±3.60flwhich was significantly higher as compared to Group B as 37.3±1.52fl and insignificant as compared to group C as 55.6±2.08fl.Table2

MCHC was significantly decreased in group B as 37.3+1.52incontrast to group A and group C as 53.4±3.60 and 55.6±2.08 (p-0.001)Table2

Mean MCH and haemocrit were also significantly lowered in only ribviron consumed group B as compared to control group A and Ribavirin + Lutein consumed group C (p-value 0.004. Table2

Table 1: Body weight of rats in grams (n=30)

Study groups	Mean	SD	F-value	P-value
Group A	203.50	5.59	1.56	0.685
Group B	183.4	5.70		
Group D	206.00	5.54		

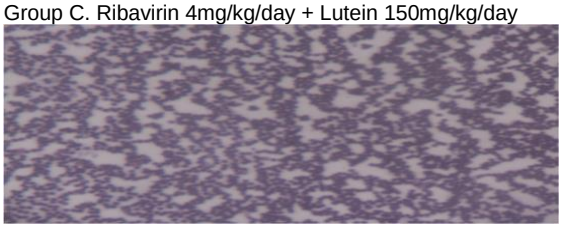
Group A. Controls, Group B. Ribavirin 4mg/kg/day
Group C. Ribavirin 4mg/kg/day + Lutein 150mg/kg/day

Table 2: Mean RBC count, MCHC, MCH and HCT of rats (n=30)

Mean RBC count	Mean	SD	F-Value	P-Value
Group A	4.65	1.01	3.90	0.036
Group B	3.29	0.22		
Group C	5.13	0.91		
Mean MCHC			19.27	0.0001
Group A	53.4	3.60		
Group B	37.3	1.52		
Group C	55.6	2.08		
Mean MCH			2.38	0.09
Group A	19.4	0.6		
Group B	18.7	0.1		
Group C	19.1	0.3		
Mean HCT			7.55	0.004
Group A	34.2	2.1		
Group B	25.7	1.4		
Group C	34.2	2.6		

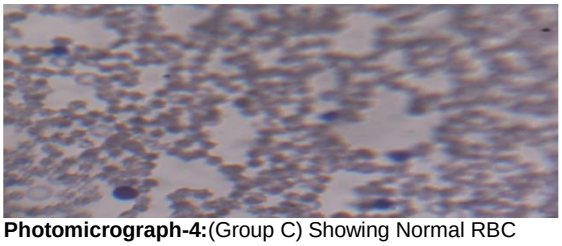
Group A. Controls, Group B. Ribavirin 4mg/kg/day

Group C. Ribavirin 4mg/kg/day + Lutein 150mg/kg/day



Photomicrograph-1: (Group A) Showing Normal RBC Morphology Normocytic , Normochromic (100x)

Photomicrograph-2:(Group B) Showing Abnormal RBC Morphology Anisocytosis, Polychromasia , Fragmented Red Blood Cells (100x)



Photomicrograph-4:(Group C) Showing Normal RBC Morphology with minute RBC breakdown Normocytic , Normochromic (100x)

DISCUSSION

Ribavirin is known drug used worldwide for treatment of viral hepatitis. It has shown marked reductions in viralloadwhich is eveident through numerous studies worldwide, but also leads to hemolytic anemia. Thisstudy reports increasedosmofragility with ribavirinuse and areduction wasnoted by concomitantuse of lutein.

In present study, hemolysiswas noticed significantin ribavirin treatedanimals GroupsB, while in lutein administration with ribavirin animals of GroupC showed lower hemolysis. Uydu et al studied the effectsof ribavirindrug therapyon rheologicalcharacteristics of erythrocytemembrane, serumlipid profileand oxidativestatus in patientswith dyslipidemia.[15] Similallry Soumaya et al[16] conducted a study on Rat blood observing the effects of different antioxidants on osmotic fragility they showed Vitamin C, L-carnitine and Curcumin have positive effects on the osmotic fragility on stored blood, In present study we used different antioxidants in form of alpha tocopherol& lutein and analysed then for osmotic fragility the study is consistent in terms of antioxidants agents on osmotic fragility.[4]Vidya et al in a study a human blood treated with lutein and solution containing lutein showed minimum hemolysis indicating lutein as a protective agent against hemolysis the finding of this study are consistent with our study as we also observed reduced hemolysis in lutein group.[17]Assem et al conducted study on HCV positive patients taking ribavirin therapy.They added vitamin E to the regime & saw effects on hemolytic anemia as compared to the control group taking ribavirin only they saw significant improvement hemolytic anemia by observing improved results in MCV,MCH,Hct,RBC count levels which is consistent with our study as in our groups all parameter were improved with alpha tocopherol. [18] Garbe et al has also reported ribavirin induced hemolysis in human beings Lutein and alpha-tocopherolis synthesized bysimilar HMGCo A pathwayof cholesterol.[19]

In this animal body weight group B showed decrease in body weight as compared to group A and C. These findings were similar to Shaimuna et al 2012 reported weight loss in patients taking ribavirin the findings are consistent with our study as ribavirin groups showed loss of weight in wistar rats.[20]Harisaet alhas reporteda study on thehuman erythrocyteas a potential carrierof Pravastatin, whichis a HMGCoA reductaseinhibitor similar toribavirin. Itwas an invitro study conductedon human erythrocyteusing electronmicroscope.[21]Possible reasons ofcontradictory results canpossibly be; different studypopulation, differentdrug agent – Pravastatinvs. Ribavirin. Bothare HMG co Areductase inhibitor, butmolecular structure isdifferent, studydesigns, methodologybias and moreoverlaboratory facilities andinstrumentation.

CONCLUSION

Ribavirn causes significant red blood cells hemolysis as assessed through peripheral blood smear and RBC indices.lutein have a protective role against ribavirin induced hemolysis in rats.lutein combination as an add on therapy to prevent the anemia in ribavirin treating patients.

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