Original Article

Effect of Propranolol on Hepatic Blood Flow for Reduction of the Hepatotoxicity of Rifampicin in Rabbits

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ABSTRACT

Objective: The study was undertaken in rabbits to investigate the effect of propranolol to reduce hepatoxicity of rifampicin (RIF).

Study Design: Experimental study.

Place and duration of study: The study was conducted in Animal House of Baqai Medical University, from March 2015 to August 2015.

Materials and Methods: Animals were divided into three groups; control, RIF 100mg/kg for 28 days as single daily dose in oral solution and RIF plus propranolol (30 mg/Kg for 28 days) treated group. Liver function test and histological evaluation by H and E staining was carried at the end of dosing by using standard procedures.

Results: RIF caused significantly ($P<0.05$) elevated the serum levels of ALT, ALP, γGT and bilirubin as compared to control. These levels were also higher in RIF plus propranolol treated group but when comparing the levels in between group B and C, it was illustrated that propranolol provide significant protection to the RIF induced damage. Histology of liver sections also supported these results. Liver damage induced by RIF expressed as central vein dilation, infiltration of inflammatory cells, portal vein dilation and damage of hepatocytes. All of these changes successfully turned to normal by combined administration of propranolol.

Conclusion: Propranolol is non cardioselective beta blocker used to treat various cardiac and non-cardiac diseases including arrhythmia, hypertension, and portal hypertension and oesophageal varices. It was disclosed from above results that propranolol offer significant protection against RIF induced hepatotoxicity by decreasing the hepatic blood flow.

Key Words: Oesophageal varices, hepatotoxicity, portal hypertension, H and E staining, central vein, hepatocytes, inflammatory cells.

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INTRODUCTION

Pharmaceutical agents and herbal products should always be considered as a possible cause of liver injury [1].Rarely hepatitis and death due to liver failure have been observed in patients who received RIF. RIF may cause cholestatic jaundice and strongly induce cytochrome P450 which increases the elimination of several other drugs [2].

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Chronic liver disease, alcoholism and old age appeared to increase the incidence of severe hepatic problem when RIF is given alone or concurrently with isoniazid[3]. Weight reduction during antitubercular treatment was the most considerable risk factor for drug induced hepatotoxicity imposing interruption of anti-TB treatment [4]. The pathogenesis ranges from hepatic adaptive changes to hepatocellular damage [5].

Propranolol is effective in the prevention of esophageal variceal bleeding[6]. This effect of propranolol is due to reduction in portal blood flow[7-9]. As propranolol reduces the portal blood flow, that might be effective in reducing the hepatotoxicity of RIF. Therefore, present study was designed to focus the effect of reduced hepatic blood flow induced by propranolol in reduction of hepatoxicity of RIF.

MATERIALS AND METHODS

Animals: In the present study rabbits were selected as experimental animals due to the similarity in hematological biochemistry to human beings [10]. Thirty healthy male rabbit of weight 1200 to 1400 grams were

recruited from the animal house of Baqai Medical University, Karachi, Pakistan. All the animals were acclimatized for housing condition before starting the experiment.

Experimental design: All the animals were randomly divided into three groups and each group comprised of 10 animals. Drugs were administered orally for 28 days as following schedule.

Group A: Control group received distilled water.

Group B: received RIF 100mg/kg as single daily dose [11].

Group C: received RIF 100mg/kg and propranolol 30mg/kg single daily dose [12].

Sacrifice of animals and collection of blood sample: After 24 hours of last dose , the thoracic cage was exposed, approximately 5ml of blood was collected from each rabbit by cardiac puncture technique[13]. Blood sample were then transferred into gel tube and sent to the laboratory, where serum was separated by centrifugation at 4000rpm for 8 min. Alkaline phosphatase (ALP), alanine transaminase (ALT/SGPT) and γ-glutamyl transaminase (γ GT) and total bilirubin were estimated within 2 hrs of serum separation on automatic analyzer. All the animals were sacrificed and tissue samples were collected.

Preparation of liver tissue for histological examination: The liver of the animals were also collected and flushed with saline and put into 10 % normal buffered formalin. After 24 hours, liver tissues were embedded in paraffin wax as standard protocol. Five micrometer thick section were carried out from these block and put into poly-1-lysine coated glass slide and stained with haemotoxylin and eosin as standard procedure [14]. The slides were observed under light microscope for histological changes induced by RIF alone and in combination with propranolol.

Statistical analysis of data: All the quantitative results were analyzed statistically using SPSS software version 21. Values were compared with control using ANOVA, considered $p<0.05$ was significant.

RESULTS

Gross toxicity estimation: The animals of group A were active, healthy and well-responsive to external stimuli. The animals of group B looked lethargic, less active and ill as compared to control animals. But all the animals were alive and responsive. The livers of group B animals had regular architecture with smooth surface. The color of livers also appeared normal but they are slightly bigger as compared to control. The animals of group C were active and responsive but not as healthy as the control animals. All the animals of this group are alive and alert for external stimuli. The livers of group C animals had the smooth surface and regular architecture with typical color. The livers were also not adhesive to any other tissues and not contractive in group B and C.

Biochemical assessment for liver functions: The serum analysis of bilirubin and liver enzymes SGPT (ALT), ALT, GGT between control and treated groups were used for assessment of hepatic injury.

Mean serum level of ALT (IU/L), ALP (IU/L), GT (IU/L) and bilirubin (µmol/L) of rabbits in group A, B and C and its comparison: Table 1and 2 showed the mean serum levels of ALT, ALP, GT and bilirubin and their comparison in group A, B and C.

The data showed the significant increase in the ALT level in RIF treated group when compared with control while there is insignificant ($P>0.05$) increase in ALT level was illustrated in RIF and propranolol treated group as compared to control. It was also indicated that there was significant ($P<0.05$) difference in the mean between RIF treated and RIF plus propranolol treated groups.

Table No.1: Mean serum level of ALT, ALP, γ GT and bilirubin in group A, D and E

Parameters	Group A n=10	Group B n=10	Group C n=10
ALT(IU/L)	40.80 ±1.14	87.10± 3.97	47.80± 1.81
ALP(IU/L)	42.60±2.79	70.40 ± 3.35	27.20 ± 1.43
γ GT (IU/L)	8.00 ± 0.93	20.70 ±1.28	12.60 ±0.60
Total Bilirubin (µmol/L)	8.52± 1.03	38.75 ± 2.50	19.10± 1.16

Data expressed as Mean±SEM

Table No.2: Comparison of serum level of ALT, ALP, γ GT and bilirubin in group A, D and E

Parameters	A vs B		A vs C		B vs C	
	Difference of mean	p-Value	Difference of mean	p-Value	Difference of mean	p-Value
SALT (IU/L)	-46.30*	0.000	-7.00	0.158	39.30*	0.000
ALP(IU/L)	-27.80*	0.000	-15.40*	0.001	43.20*	0.000
γ GT (IU/L)	-12.70*	0.000	-4.60*	0.007	8.10*	0.000
TOTAL BILIRUBIN(µmol/L)	-30.23*	0.000	-10.58*	0.000	19.65*	0.000

* $P<0.05$

When comparing the differences of means of ALP levels between these groups, it was showed that there is a significant ($p<0.05$) differences in mean between control group A verses group B, B verses C and A verses C.

The data of comparison of means showed the significant ($p<0.05$) increase in the γ GT levels in group D as compare to group A and group B as compare to group C. The difference of means of γ GT was insignificant ($p>0.05$) between group A and C.

The data showed that there was no significant ($p>0.05$) increase in the serum bilirubin level in group C verses A. But there was significant difference ($p<0.05$) in the level of bilirubin in group A verses B and group B verses C.

Microscopic examination

At 100X, liver sections of group A animals showed normal architecture of hepatic lobules. Each lobule is illustrated by a radial arrangement of hepatocytes around the central vein. The cell cords are separated by narrow blood sinusoid (a). At 400X magnification, the structure within the portal triad showed portal vein, hepatic artery and 1-2 bile ducts (b).

At 100X magnification, liver sections of RIF treated rabbit liver showed slightly disturbed hepatic architecture with moderate sinusoidal dilation especially in pericentral area. The central vein was observed moderately dilated and congested (c). The portal tract is moderately infiltrated with mononuclear cells and with moderate congestion. At 400X, mononuclear cell infiltrations observed with dilated and congested portal vein (d).

At 100X magnifications, liver sections of RIF and propranolol treated rabbit liver showed normal hepatic lobule radiating from the central vein with almost normal sinusoidal spaces. The central vein also appeared normal and congestion and inflammation is nearly absent (e). At 400X minimal inflammatory cells were spotted in portal tract. Portal tract consist of portal vein artery and bile duct also appeared normal and uncongested (f).

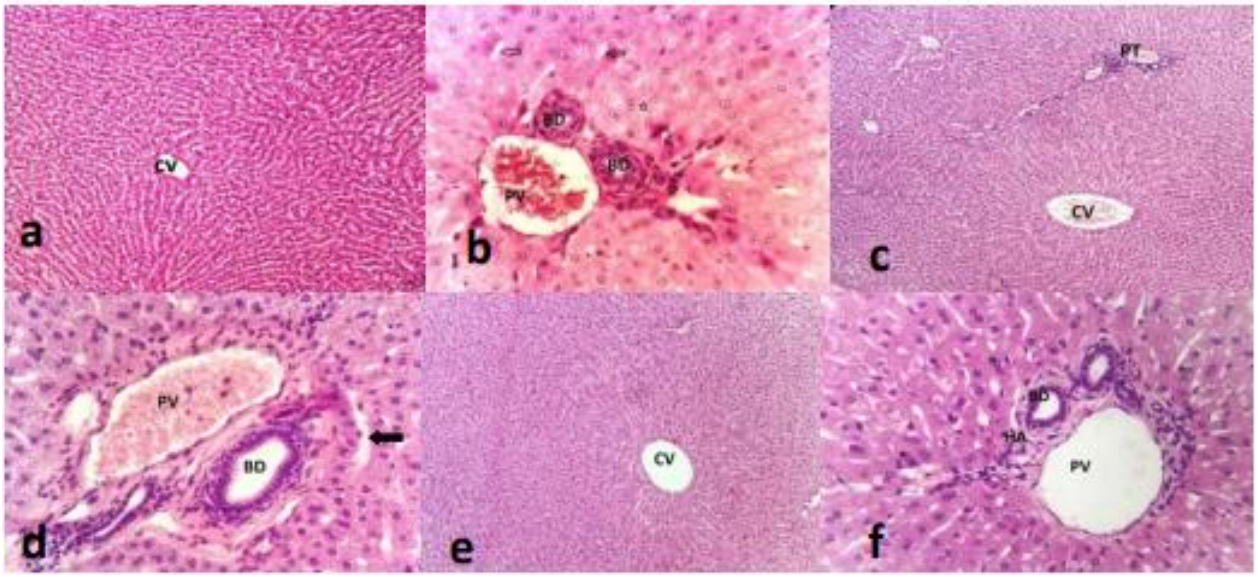

Figure No.1; Photomicrograph of 5 micron thick H & E stained paraffin section from liver of rabbits. (a) and (b) group A (control); (c) and (d) group B (RIF treated group); and (e) and (f) (RIF and propranolol treated group). Central vein (CV), portal vein (PV), duct (BD),

DISCUSSION

Hepatotoxicity of antitubercular drug is very important and unavoidable adverse effect. As RIF is the vital component of antitubercular therapy. Large body of literature discussed the hepatotoxicity of RIF in combination with other antitubercular drugs and alone.

In this research RIF alone and in combination with propranolol were administered to report the hepatotoxic effects of RIF and outcome of propranolol on RIF hepatotoxicity in rabbits. The animals of group B i.e. RIF treated group are lethargic and less active as compared to group A and group C. The liver of group B is slightly enlarged. But the liver of group C is nearly appeared as control.

Enzymatic and non enzymatic evaluation of hepatic functions considered as preliminary testing of hepatotoxicity [15]. In the present study the serum levels of ALT, ALP, γ GT and bilirubin were considerably high in group B as compared to group A and C (table 1). The comparison of mean values of ALT, ALP, γ GT and bilirubin (table 2) in group B and C was also statistically significant which revealed that RIF potentially raised the hepatic function which was also documented by many scientist [16, 17]. But coadministration of propranolol effectively reversed the raised levels of ALT, ALP, γ GT and bilirubin although these values are high in group C as compared to group A except mean value of serum ALT. It has been reported that propranolol alone and in combination with ginger can reduce the serum levels of ALP and ALT [18].

Histological evaluations of liver tissues of group B showed variable degree of damage presented as inflammation and dilation of central and portal vein with congestion. Minor fatty changes were also observed with swollen hepatocytes. Fatty changes and mononuclear cell infiltration within portal tract and pericentral area was mainly due to RIF itself and its toxic metabolites formed during biotransformation[19-21]. RIF and INH cause portal triditis, necrosis specially piecemeal necrosis and it was also concluded that 50 mg/Kg of both drugs are enough to produce hepatotoxic model [22]. It has been reported that esinophilic infiltrations in portal tracts, lobular inflammation, Kupffer cells hyperplasia apoptotic hepatocytes, sinusoidal dilations and central vein damage produced by combination of INH and RIF [23, 24]. In present study RIF alone also produced these kinds of hepatic changes especially sinusoidal dilation, portal triditis and apoptotic hepatocytes. In group C the rabbit liver showed mild degree of inflammation and slight sinusoidal dilation. The portal tract was also mildly congested with dilated portal vein. The comparison of group B and C disclosed that RIF induced toxicity was reduced by propranolol. The hepatotoxicity of RIF was reduced by cimitidine as it is the potent inhibitor of cytochrome P 450 [24]. Hepatotoxic effects of RIF and INH was also reduced by vitamin E and theses effects are highly comparable with cimitedine [25]. It has been recently reported that propranolol can reduced the hepatotoxicity of RIF showed by micrometric estimation of H and E stained liver tissue and scanning electron microscopy[26]. Similar results were observed in group C rabbits that RIF and propranolol was co administered and hepatotoxicity produced by RIF was reduced.

CONCLUSION

Thus it is concluded that propranolol is effective in reduction various hepatic complications. RIF is reported hepatotoxic drugs and produced classical signs of hepatotoxicity manifested as alteration in hepatic biochemistry and histology. These alterations were not exactly but to the significant level reversed by propranolol.

Author's Contribution:

Concept & Design of Study: Hina Abrar
Drafting: Muhammad Rashid Ahmed
Data Analysis: Asma Basharat Ali
Revisiting Critically: Hina Yasin, Sadaf Ibrahim
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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