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Research paper

Attenuation potential of rifampicin–phospholipid complex in murine hepatotoxicity model

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ABSTRACT

The study was aimed to investigate the potential of rifampicin–phospholipid complex (RMP–PC) in rifampicin (RMP) induced hepatic toxicity in rats. The protective effect of the RMP–PC was evaluated by biochemical analysis and histology examination of the liver in the test animals. Surface texture studies revealed the spherical vesicles in the particle size range of 500–600 nm. Moreover, RMP–PC exhibited enhanced *in-vitro* efficacy in MTB H37Rv strain. The biochemical analysis included aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin content, total protein, cholesterol and lipid peroxidation (MDA). RMP–PC administration resulted in a significant ($P < 0.001$) decrease in AST, ALT, ALP, MDA and cholesterol levels with elevation of total protein and albumin indicating its hepatoprotective activity. Histology of the liver sections further confirmed the reduction in hepatic injury. These findings suggest that the mechanism of protection elicited by RMP–PC, involves the hepatoprotective and antioxidant properties of phospholipid. Thus, the phospholipids have the potential to alleviate RMP-induced liver toxicity in tuberculosis treatment regimen.

1. Introduction

Phospholipids (PLs) are the principle components of biomembranes. Phosphatidylcholine also known as lecithin is the most abundant phospholipid in cell membrane, and also the principle phospholipid circulating in plasma. Further, phosphatidylcholine is a precursor of cytoprotective agents such as eicosanoids, prostaglandins and antioxidants [1]. The PLs are amphiphilic lipids arranged as lipid bilayers, in which the polar (hydrophilic) regions of the PL are directed towards the outer surface of the membranes and hydrophobic regions towards the inner compartment. In addition, they solubilize lipophillic drugs and enhance the oral bioavailability and antioxidant potential of phytopharmaceuticals [2–11]. Very few studies have reported hepatoprotective, antioxidant and cytoprotective effects of PLs via preservation of cell integrity, scavenging of free radicals, structural support and stabilization of hepatocyte membrane and increase in fluidity and prevented lipid peroxidation [12–16].

Tuberculosis (TB) is one of the widespread and fatal infectious disease, after acquired immunodeficiency syndrome (AIDS) [17]. Worldwide, it is reported that there were nine million people fell ill with TB in 2013, including 1.1 million cases among people living with HIV. In 2013, 1.5 million people died from TB, including 360,000 among people who were HIV-positive [18]. Although, it is more prevalent in the least-developed and the developing countries, it also remains a major concern for rest of the world as well, due to growing incidences of AIDS. Rifampicin (RMP) is front-line drug in the treatment of TB. However, it has been reported to cause severe hepatotoxicity, manifested by elevation in liver transaminase and other enzymes levels [19,20]. The exact mechanism of RMP hepatotoxicity is not well known [21,22] but several studies have shown that RMP causes hepatic injury of liver and its membrane. Consequently, liver plasma membrane gets damaged due to change in the permeability and fluidity. This leads to the leakage and release of aminotransferase enzymes present in the cytoplasm followed by in blood stream.

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In our previous studies, rifampicin–phospholipid complex (RMP–PC) showed improved solubility in distilled water and remarkable stability at pH 1.2. Moreover, it exhibited increase in AUC (area under curve) and MRT (mean residence time) which further suggested enhancement in the oral bioavailability of rifampicin in rats [17]. Therefore, aim of the present study was to investigate the potential of rifampicin–phospholipid complex on RMP-induced hepatotoxicity. Moreover, the activity of the RMP–PC was ascertained by *in-vitro* antimycobacterial activity in Mycobacterium *tuberculosis* (MTB) H37Rv strain. In addition, biochemical and histopathology evaluation was also carried out.

2. Materials and methods

2.1. Materials

Rifampicin was obtained by M/s. Sandoz Pharmaceuticals Ltd., Mumbai. The phospholipids (Lipoid S-75) were kindly provided by Lipoid Ludwigshafen, Germany, as a benevolent gift samples. Dichloromethane and other chemicals were obtained from Loba Chemie, Mumbai, India. 2-Thiobarbituric acid (TBA), trichloroacetic acid (TCA), Tris HCl and phosphate buffers were purchased from Hi-Media Laboratories (Mumbai, India). Kits for serum glutamate oxaloacetate transaminase (AST), serum glutamate pyruvate transaminase (ALT), alkaline phosphatase (ALP), albumin, total protein and cholesterol were purchased from Accurex Biomedical Pvt. Ltd. (Mumbai, India). All other chemicals used were of analytical grade.

2.2. Preparation of rifampicin–phospholipid complex (RMP–PC)

The phospholipid and RMP (as a molar ratio of 1:1) were placed in 100 mL round bottom flask and dissolved in dichloromethane (reaction medium). Subsequently, taken in a flask evaporator (IKA, India Private Limited) and refluxed at 60 °C for 24 h under constant stirring. After then, the dichloromethane evaporated off at 60 °C for 30 min. Thereafter, the dried product was collected and placed into an amber color glass vial at room temperature until further use [17].

2.3. TEM analysis

Surface morphology studies of RMP–PC were carried out employing transmission electron microscopy (TEM, Technai G2F20, Netherland). Briefly, a drop of RMP–PC dispersion was placed on carbon-coated copper grids and stained with phosphotungstic acid (2% w/v) and air dried and viewed under the TEM microscope and analyzed with an accelerating voltage of 200 kV at different magnifications.

2.4. Biological activity

2.4.1. Test strain

The antimycobacterial activity of the samples was tested on MTB H37Rv strain (Tuberculosis Research Centre, Chennai, India). Briefly, cultured the bacteria in Middlebrook 7H9 liquid medium (HiMedia, India) supplemented with 10% albumin, dextrose, and catalase (ADC; HiMedia, India) to mid-log phase and then frozen in aliquots at −70 °C until needed. The purity of the culture was checked by Ziehl–Neelsen staining (HiMedia, India). Before testing, the samples were dissolved in dimethyl sulphoxide (DMSO) and filter sterilized through 0.2 μm DMSO safe filters (PALL Life Sciences).

2.4.2. BACTEC 460 TB method

The BACTEC 460 TB system (Becton Dickinson, USA) was employed to determine a growth index (GI) of the MTB. GI is the quantitative measure of $^{14}CO_2$ liberated by metabolism of ^{14}C-labeled substrate in a medium and expressed in numbers on a scale from 0 to 999. In brief, 0.1 ml of ^{14}C-labeled substrate in the medium and expressed in numbers on a scale from 0 to 999. Briefly, 0.1 mL of the samples were transferred to 12B BACTEC vials, in duplicate for each sample/drug concentration unless mentioned otherwise, and incubated at 37 °C in 5% CO_2 atmosphere. Under aerobic condition the GI was calculated daily until a value greater than 30 was obtained in 1:100 controls. The undiluted control reading was employed to determine the percent inhibition. Appropriate positive and negative controls were also included in the calculation. The growth inhibition was expressed as a ratio of GI of drug to the respective control vial. The percent growth inhibition was calculated for each drug concentration [23,24].

2.5. Biochemical analysis and histology study

2.5.1. Animals

The effect of RMP–PC on RMP-induced hepatotoxicity was evaluated in Sprague Dawley rats (180–200 g) obtained from Central Animal House, National Institute of Pharmaceutical Education and Research, Mohali, India. The animals were housed in polypropylene cages with free access to food and water. They were fed a typical rat pellet diet and maintained under standard conditions of temperature (22 ± 2 °C) and humidity (50 ± 10%) with an alternating 12 h light/12 h dark cycle. Rats were acclimatized for two weeks before starting the experiments.

2.5.2. Experimental design

The study was carried out for two weeks. The animals were divided into three groups (n = 6). Group I (normal control) was administered with the standard diet. The Group II animals were orally administered RMP (50 mg/kg body weight/day). There exists a considerable variation in RMP dose in the literature for hepatotoxicity studies and the dose of 50 mg/kg body weight was selected based on a number of reports [19,25–29]. In Group III, rats were administered with RMP–PC (equivalent to 50 mg each/kg body weight/day of RMP) for a period of 14 days. The body weights of the animals were monitored throughout the study.

2.5.3. Effect on body weight and liver weight

At the end of two weeks study, the animals were sacrificed and the liver tissue collected. The % change in body weight and % relative liver weight were calculated:

$$\%\,Body\,weight\,change\,(g) = \frac{Final\,weight\,(g) - Initial\,weight\,(g)}{Initial\,weight\,(g)} * 100$$

$$\%\,Relative\,liver\,weight\,(g) = \frac{Absolute\,liver\,weight\,(g)}{Final\,body\,weight\,(g)} * 100$$

2.5.4. Biochemical parameters

Blood samples were left to clot at room temperature for 1 h and the serum collected by centrifugation of the samples at 10,000 rpm for 10 min at 4 °C. The AST, ALT and ALP serum levels were measured by commercial kits following the manufacturer's instructions.

2.5.5. Measurement of lipid peroxidation products

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) from liver tissue homogenate by the method as adopted by Wills, 1966. Liver tissues