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Ascorbic acid improves stability and pharmacokinetics of rifampicin in the presence of isoniazid

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ABSTRACT

Bioavailability of rifampicin (RIF) from fixed dose combination (FDC) products remains problematic for effective control of tuberculosis (TB) owing to its degradation in the presence of isoniazid (INH) in the stomach acid environment. Ascorbic acid (ASC) is being added to the dissolution medium as well as the plasma sample as anti-oxidant to prevent degradation of RIF and also daily intake of ascorbic acid is recommended to control TB infection. However the role of ASC on the interaction between dissolution stability and in vivo bioavailability of RIF in the presence of INH has not been explored and therefore examined in the present study. RIF and its degradation product 3-FRSV were measured by dual wavelength spectroscopy. ASC significantly reduced RIF degradation or formation of 3-FRSV in the presence of INH ($p < 0.001$) in the dissolution medium (pH 1.2) and showed increase in C_{max}, AUC_{0-24}, $AUC_{0-\infty}$ and $t_{1/2}$ of RIF ($p < 0.001$) as compared to that without ASC in rabbits. The study demonstrates that co-administration of ASC with RIF-INH combination can protect RIF from degradation in the acid environment and improve its bioavailability with effective control of TB.

1. Introduction

Tuberculosis (TB) is an infection caused by *Mycobacterium tuberculosis*. It is the world's second commonest cause of death from infectious disease after HIV/AIDS. WHO declares TB as a public health emergency. The prevalence of TB is higher in south East Asia and sub-Saharan Africa as compared to western countries [1]. TB remains a major cause of morbidity and mortality worldwide in the 21st century and prominent in international statistics of ill health mainly because it kills young adults. More than 80% of the burden of tuberculosis, as measured in terms of disability-adjusted life years (DALYs) lost, is due to premature death rather than illness. One third of the world's population is infected with TB. In 2011, nearly 9 million people around the world became sick with TB disease. There were around 1.4 million TB-related deaths worldwide. TB is a leading killer of people who are HIV infected [2].

Treatment of TB was initially complicated and challenging as it requires administration of drugs over a long period that resulted in poor patient compliance. WHO recommend a fixed dose combination (FDC) of rifampicin (RIF), isoniazid (INH), ethambutol (ETH) and pyrazinamide (PYR) to shorten the duration of treatment of TB besides overcoming resistant to individual anti-TB drugs. Although FDC products remain in use in the treatment of TB, the bioavailability of RIF has become unacceptable in a number of FDC anti-TB formulations owing to degradation of RIF in the acidic environment. RIF degrades in the stomach and the degradation varied from 8.5% to 50%, during the gastric emptying time for most dosage forms in humans [3] and decomposition of RIF is further influenced by the presence of INH in stomach after ingestion [4]. RIF is known to undergo hydrolysis in acidic medium to the insoluble 3-formyl rifamycin SV (3-FRSV). INH accelerates degradation of RIF into this poorly absorbed derivative (3-FRSV) in the acidic environment of the stomach via reversible formation of the isonicotinyl hydrazones of 3-FRSV with INH. Mechanism for this apparent degradation of RIF is that 3-FRSV and INH could possibly undergo Schiff's reaction to form a complex. The carbonyl groups and amine groups may rearrange to yield an ammonium ion. The C-4 hydroxyl group enhances the complex formation by possibly forming a hydrogen bond with the hydrogen atom attached to the nitrogen. This is a basic requirement of the Schiff's reaction. In addition, carboxylic acids and alcohols can also undergo carbonyl condensation reactions. INH could react with RIF in this manner, which could account for the instability of RIF when present together with INH.

Besides RIF is better absorbed at fasting condition of the stomach than in the presence of food and therefore the antituberculous FDC products are recommended to be administered on empty stomach; however this environment promotes degradation of RIF itself as well as in the presence of INH. There is a potential of failure

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of therapy in patients with FDC products owing to poor/variable bioavailability of RIF. Therefore there is an urgent need to develop stable formulations containing RIF-INH combination to withstand the acidic environment of the stomach.

The past several years have seen the development of a number of RIF controlled release formulations for the improvement of clinical efficacy of the drug and patient compliance. Liposomes and microspheres were developed for the sustained drug delivery of anti-TB drugs that have demonstrated better chemotherapeutic efficacy when investigated in animal models [5]. Nanoparticles [6], osmotic system [7], alveolar macrophages targeted nanoparticles [8], inhalable alginate nanoparticles [9], nebulized solid lipid nanoparticles [10], aerolized liposomes [11] and depot preparations [12] are other approaches attempted to improve the efficacy of anti-TB drugs. Alternately FDC products with improved RIF bioavailability can be designed by segregating delivery of RIF and INH by around 3–4 h as the two drugs show regional permeability; RIF being absorbed from the stomach and INH from all three segments of the intestine. Despite these developments there has not been much progress in controlling degradation of RIF in the presence of INH in the stomach environment as the FDC products remain the treatment option in clinical practice for tuberculosis because of its known patient compliance.

Previous study reveals that RIF collected in the plasma sample can be stabilized by using ASC as anti-oxidant. RIF degrades in plasma at ambient temperature, and a 54% loss was observed within 8 h and this degradation can be effectively prevented by adding ASC, thus prolonging stability for up to 12 h [13]. Furthermore, administration of ASC (1000 mg/day) is recommended in tuberculous patients [14] as ascorbate concentration of 1 mg/day, which is easily reached in blood, prevents the growth of cultures of *M. tuberculosis*. ASC is also added to the medium to prevent oxidative degradation of RIF during the in vitro diffusion [15] and dissolution [16] studies. While the above studies support the beneficial effects of ASC on in vitro stabilization of RIF or in the treatment of TB the role of ASC on the bioavailability of RIF in the presence of INH has not been explored. Therefore, the present study aimed to investigate the correlation between in vitro stability and in vivo bioavailability of RIF in the presence of INH under the influence of ASC.

2. Materials and methods

2.1. Materials

Rifampicin (RIF) and 3-formyl rifamycin SV (3-FRSV) were procured from Themis Lab, Mumbai (India) and isoniazid (INH) was obtained from Cadila Health Care Ltd, Maharashtra (India). Ascorbic acid (ASC) was purchased from Qualigens Fine Chemicals, Mumbai (India). Chloroform was procured from SRL Chem, Mumbai (India). Concentrated hydrochloric acid was purchased from Ranbaxy Fine Chemicals, Vijayawada (India). Anhydrous sodium sulphate was procured from Samir Tech Chem, Makarpura (Vadodara). Potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from S.D. Fine Chemicals, Bangalore (India). All the chemicals used were of analytical grade. HPLC grade acetonitrile (ACN), methanol and water were procured from Merck, Bangalore (India). All solutions were prepared using double distilled water.

2.2. Methods

2.2.1. Dissolution stability study

Dissolution stability study was performed on RIF alone, RIF-INH and RIF-INH-ASC combination in pH 1.2 medium. A solution of 0.1 N HCl (200 ml) was placed in the vessel of the USP dissolution apparatus 2 (USP XXIII, 1995) and the medium was equilibrated at 37 ± 0.2 °C with stirring at 100 rpm. 150 mg RIF, 100 mg INH and ASC in variable concentrations (125, 250, 500, 1000 mg) were selected for the study. Sample was accurately weighed, dissolved in and diluted to 100 ml with 0.1 N HCl (37 °C). The resulting solution was transferred immediately to the dissolution vessel at once and 5 ml of specimen was withdrawn immediately from a zone midway between the surface of the dissolution medium and bottom of the vessel (0 min sample). The aliquot withdrawn for analysis was replaced with equal volume of fresh dissolution medium at 37 ± 0.2 °C. Samples were withdrawn at 15 min intervals up to 60 min. The experiment was performed in triplicate.

2.2.2. Estimation of RIF

An aliquot, 1 ml was extracted immediately with 5 ml of chloroform using cyclomixer (3 min). The aqueous phase was discarded and anhydrous sodium sulphate was added to chloroform layer to remove traces of water. The sample was analyzed for RIF and 3-FRSV by DW-Spectrophotometric method at their characteristic wavelength ($\lambda_{475-507}$) [16] (Shimadzu 160A UV-Vis, Japan). The percent dissolution of RIF and percent formation of 3-FRSV were determined. From the percent dissolution of RIF percent degradation of RIF was calculated from the following equation:% degradation of RIF $= \frac{(\text{Initial concentration} - \text{Final concentration})}{\text{Initial concentration}} \times 100$.

2.2.3. Estimation of INH

An aliquot, 1 ml was made up to 10 ml with dissolution medium and drug content was determined by using UV–vis spectrophotometer at 263 nm [17] (Shimadzu UV-1800, Japan).

2.3. Statistics

The values are expressed in mean ± S.D. One way ANOVA followed by Tukey's multiple comparison test was used to analyze the data using Graph Pad Instat software, version 3.01. $p < 0.05$ was considered as significant.

2.4. In vivo studies

2.4.1. Pharmacokinetic evaluation in rabbits

New Zealand male white rabbits in the weight range of 1.5–2.5 kg were used in the study. The animals were maintained at standard laboratory conditions. The animals were overnight fasted before the experiment. They were administered samples orally. The animals were divided into the following 3 groups:

Group 1 administered RIF dispersed in 1% (w/v) tragacanth suspension.
Group 2 administered RIF-INH combination dispersed in 1% (w/v) tragacanth suspension.
Group 3 administered RIF-INH-ASC combination dispersed in 1% (w/v) tragacanth suspension (ASC was selected at a concentration which showed the least degradation of RIF in the dissolution medium).

(1% (w/v) tragacanth suspension–1 g of tragacanth is dissolved in 100 ml of purified water. The pH range of tragacanth suspension is 5–6.)

The animals were kept individually in cages. Blood samples (1 ml) were collected in heparinized tubes from the marginal ear vein at 0, 0.5, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12 and 24 h after drug administration and plasma was separated by using centrifugation and stored at −20 °C. Samples were analyzed by validated high performance liquid chromatography (HPLC) [18] (Shimadzu CLASS-VP, Japan) for estimation of RIF, 3-FRSV and INH. C_{max}, t_{max}, AUC_{0-24},