REVIEW ARTICLE

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Impaired Bioavailability of Rifampicin from Fixed Dose Combination (FDC) Formulations with Isoniazid

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Antitubercular Fixed Dose Combinations (FDCs) containing rifampicin (RIF) along with isoniazid (INH), and/or pyrazinamide (PZ) and/or ethambutol (ETB) ensure better patient compliance and help to minimize development of resistance to the drugs by *M. tuberculosis*. However, poor bioavailability of RIF in these FDC formulations is a subject of much concern for the last three decades. Poor bioavailability of RIF could lead to faulty treatment and to the drug resistance. WHO and IUATLD have now sounded a caution that antitubercular FDC formulations should be used only if the bioavailability of RIF has been demonstrated convincingly. It has been suggested that probable physical factors responsible for the variable bioavailability of RIF include particle size, crystalline nature, nature of excipients and lack of GMP. However, none of these explanations are found to be satisfactory. During the last few years, sufficient data has been generated, independently, in two Indian research laboratories, indicating that in the acidic medium of the stomach, RIF degrades to form an insoluble and inactive 3-formyl rifamycin SV, an aldehyde, involving a distinct catalytic role of INH. An elegant reaction mechanism has been proposed for the impaired bioavailability of RIF from FDC formulations containing INH. With a better understanding of the interaction of RIF and INH in the acidic medium, it should now be possible to formulate a stable FDC with acceptable and reproducible bioavailability of RIF.

Tuberculosis (TB) kills more people world over, than any other single infectious disease. It has made a comeback with vengeance especially in developing countries of Asia, Africa and South America. The disease is believed to claim about 3 million lives a year all over the world. Coinfection with AIDS has changed the organism into a challenging problem. More than 30% of Indians are believed to carry infection. The disease spreads rapidly because of urban migration, overcrowding and lack of sanitary facilities[1]. One Indian succumbs to the disease every minute.

Historical background:

In the late forties, two drugs, streptomycin and p-aminosalicylic acid were used together and the optimum period for the treatment was 2 years. While streptomycin, administered by intra-muscular route, is painful, PAS elixir was nauseating. Isoniazid (INH), the most effective drug, killing active and dividing-tubercle bacilli was introduced into clinical use in 1952. By combining all the three drugs the treatment length was brought down to 18 months[2]. Then in the late 1960's rifampicin (RIF), a new and perhaps the most important drug in the treatment of TB, was discovered. The drug was able to kill the slowly dividing bacteria and also the 'persisting' bacteria in a way that other drugs could not. It was found that RIF if combined with INH and pyrazinamide (PZ) the treatment could be dramatically reduced to 6 mo. World Health Organization (WHO) has now adopted the new treatment regimen, RIF+INH+PZ for 2 mo followed by RIF+INH for 4 mo which is the major offensive strategy of Direct Observed Therapy Short course (DOTS), for control and cure of TB[2]. DOTS programme has been recommended by WHO as a useful strategy to cure and control spread of TB.

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Variable bioavailability of RIF:

Fixed dose combinations (FDCs) containing RIF along with INH and/or PZ and/or ethambutol (ETB) are widely recommended by WHO and International Union against Tuberculosis and Lung Disease (IUATLD) for the treatment of TB, for better patient compliance and for avoiding development of resistance to drug by *M. tuberculosis*. However, poor bioavailability of RIF from a number of dosage forms of RIF and its combination with INH continues to be a subject of much concern[3-9].

In a symposium on quality control of anti-tubercular drugs, part of the scientific meeting of IUATLD in Dubrovnic, October 1989, Dr. Acocella (University of Pavia, Italy) presented studies on bioavailability of RIF in two- and three-drug FDC tablets[10]. His work confirmed, what was suspected for long by the medical community world over, that the bioavailability of RIF when given as FDC tablets, particularly the three-drug combination, could not be guaranteed. Furthermore, an apparently satisfactory *in vitro* dissolution test did not ensure acceptable RIF bioavailability[10]. In 1994, WHO and IUATLD cautioned that antitubercular FDC formulations should be used only if the bioavailability of RIF has been demonstrated convincingly[11].

It was observed that in normal adults the peak plasma concentration (C_{max}) after administration of 600 mg RIF alone is in the range of 6-13 µg/ml and AUC_{0-8} in the range of 55-60 µg.h/ml[12-15]; while on administration of RIF along with INH and/or PZ as "separate formulations" (administered at the same time) or as FDCs, the C_{max} values range from 3 to 6 µg/ml and AUC_{0-8} values in the range of 30-50 µg.h/ml[4,6,16-18].

However, simultaneously there were several contradictory reports suggesting that there is no statistical difference in the oral bioavailability of RIF after administration of RIF along with INH[5,12,16-23], thereby, adding to the confusion. Many of these reports, however, are based on nonspecific methods including microbiological methods[12,16,19].

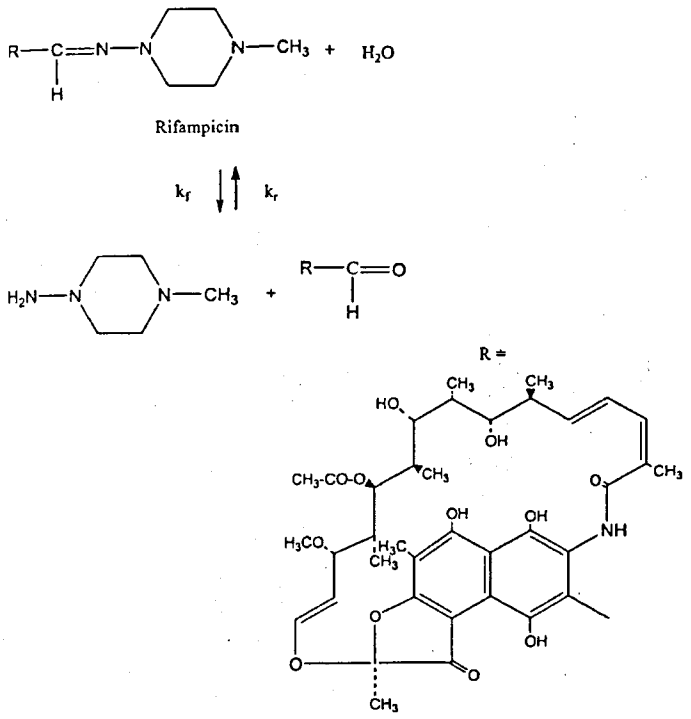
Manufacturers in US observed 18% reduction in the bioavailability of RIF from the three drug FDC formulations. In order to compensate for the reduced bioavailability, the dose of RIF was increased by 20%[24]. While RIF-INH FDC capsules made appearance in USP in 1980[25] and dissolution test for this formulation was introduced in 1995[26]. Interestingly, USP allows the RIF content of RIF-INH capsule to be in the range of 90 to 130%, probably to account for the overages added in FDCs in some parts of the world. On the other hand, the limits for RIF content for single component capsules range from 90 to 110%[27]. FDC formulations have yet not found a place in IP.

Interaction of INH with aldehydes and ketones in acidic medium:

Very early, it was known that INH is incompatible with aldehydes and ketones[28] and reacts with reducing sugars to form the corresponding hydrazones[29,30]. These hydrazones are insoluble and are poorly absorbed from the GI tract[31]. Hydrazone formation from INH and reducing sugars in 0.1 N HCl (stomach pH) at 37° was found to be a reversible reaction and follows second order kinetics, while the backward reaction is a pseudo-first-order reaction. The rate of forward reaction is maximum at pH 3.1. The rate of backward reaction, the hydrolysis of hydrazone, decreases with decrease in H^+ ion concentration[32]. Significantly, this study indicated that in the acidic environment of stomach the presence of excess aldehyde favors the forward reaction, the formation of hydrazone. But, because of the reversible nature of this reaction INH is regenerated, thus, there may not be any significant effect on its bioavailability.

Degradation of RIF in acidic medium:

Degradation of RIF is pH dependent[33]. In acidic medium RIF hydrolyzes to 3-formyl rifamycin SV (3-FRSV) and it undergoes air oxidation in alkaline medium to form inactive quinone derivative, rifampin quinone. 3-FRSV precipitates in acidic conditions[34]. The aldehyde shows high antimicrobial activity *in vitro*[35] but is poorly absorbed and is inactive *in vivo*[36]. Therefore, formation of 3-FRSV in the acidic environment of stomach leads to a decrease in the amount of RIF available for absorption and, thus, can be an important factor affecting bioavailability of RIF. In view of the exposure of this drug in oral dosage formulations to varying acidic conditions in the stomach and duodenum, Prankerd *et al.*[37], examined the stability of RIF over a wider range of acidic pH values (1.08 to 5.0). The kinetic study of the RIF degradation in acidic medium (pH 1 to 4) showed that the rate of degradation is maximum at pH 1.08. RIF, a hydrazone of N-methyl piperazine and 3-FRSV, undergoes fast reversible hydrolytic cleavage at its azomethine bond to give 1-amino-4-methylpiperazine (Scheme 1). The rate



Scheme 1: Degradation scheme of RIF in acidic medium where k_f is the rate of forward reaction and k_r is the rate of backward reaction

of hydrolysis of azomethine bond increases as the pH decreases. The decrease in the rate of hydrolysis is as much as 0.5 log unit over the pH range of 1 to 4.3[37]. The significant observations that RIF hydrolyses to an aldehyde, 3-FRSV, an inactive, less soluble product[31], which is not well absorbed and that isoniazid reacts with aldehydes/ketones[33] seems to have been overlooked while formulating FDC of RIF and INH.

Role of physical factors:

Though the reports had surfaced as early as 1976[12] that there is a serious problem with the bioavailability of RIF and the results were not reproducible, the bioavailability of RIF from FDC continued to be a jigsaw puzzle for the next 25 ye. RIF when combined with INH and/or PZ and/or ETB in 3 or 4 drug FDC results in poor bioavailability of only RIF, bioavailability of none other components - INH or PZ or ETB - from these FDCs gets affected. Significantly, the bioavailability of RIF from a single component formulation, tablets or capsules, also remains unaffected.

Because INH is the only one with a reactive group in a FDC formulation, while RIF and PZ do not have any reactive groups, it was argued that it is unlikely that there is any interaction between INH and RIF or PZ indicating nonparticipation of INH or PZ in the degradation reaction of RIF. This probably led to shifting the focus to the physical properties of RIF.

The probable physical factors responsible for the variation in bioavailability of RIF from different FDC formulations include particle size and crystalline form of the drug, excipients and even the manufacturing process[38-43]. However, none of these studies can explain, convincingly, the impairment of bioavailability of RIF from FDC formulations[44].

As far as crystalline form is concerned, it has been reported that different polymorphs of RIF show identical dissolution behavior in 0.1 N HCl[42] and whatever variability in bioavailability is expected due to difference in crystalline form it has to be reflected for both RIF alone formulations and FDCs containing RIF.

Adsorption of RIF on excipients in the FDCs cannot explain the variable or impaired bioavailability of RIF from FDCs, because these formulations contain high-proportion of active ingredients and only a small amount of excipients with any adsorbent property. Thus, possibility of unavailability of RIF due to adsorption is very less. According to WHO, if not carefully manufactured, bioavailability of RIF may be poor, which could lead to faulty treatment and eventually to drug resistance[10]. However, all these factors could have equally affected the bioavailability of single component RIF formulations, which does not seem to be the case. Thus, based on physical factors none of these studies explain, convincingly, the impairment of bioavailability of RIF from FDC formulations.

Evaluation of bioavailability studies of FDC formulations containing RIF and INH: WHO protocol:

Being concerned with the poor bioavailability of RIF formulations manufactured in the developing countries, WHO and IUATLD have developed a simplified and economical protocol for accurate assessment of bioavailability of RIF in FDC[45]. The samples of FDC are tested against a "loose mixture" of RIF and INH (and/or PZ and/or ETB). This protocol has been validated in India for all the types of FDCs containing 2, 3 or 4 drugs, available in the market[46].

Most of the bioequivalence studies of RIF from two or three drug FDCs compare various pharmacokinetic parameters of RIF obtained after administration of FDC against "loose mixtures" or "separate formulations" of individual components but administered at the same time[5,12,16-21,23]. However, both FDC and the "loose mixture" or "separate formulations" will face the similar acidic pH of stomach and RIF will suffer the same fate in both the cases (i. e. It will degrade to the same extent in presence of INH). Therefore, it is not surprising that no significant difference is observed in the bioavailability of RIF from various FDCs tested in these studies.

Bioavailability of RIF following multiple administration:

A potent enzyme inducer, RIF stimulates its own metabolism in liver[47]. On repeated administration of RIF, its plasma levels are found to decrease. Thus, the bioavailability of RIF decreased from 93% after the first single oral dose to 68% after 3 weeks of oral RIF therapy[48]. The plasma levels of RIF remain same whether administered as a FDC or as single component formulations of the respective drugs, administered simultaneously[5]. The blood kinetics of the three antitubercular drugs were determined on day 1, 15, 30 and 60 of the treatment with FDC containing 50 mg INH, 120 mg RIF and 300 mg PZ per tablet. The number of tablets ranging from 4 to 7 per day were administered according to the body weight of the patients. A fall in the plasma concentrations of RIF was observed on days 15, 30 and 60 when compared with plasma levels on day one. There was, however, no change in the INH concentration[49].

Defining the problem of impaired bioavailability:

Looking at all the data available, it becomes clear that any rational explanation for the impaired bioavailability of RIF from FDC must explain the following pieces in this jigsaw puzzle –

- RIF in single component formulation has no bioavailability problem.
- Bioavailability of RIF in presence of INH in 2-, 3- or 4-drug FDCs is impaired and not reproducible.
- If one is led to believe that INH interacts with RIF, then one has to explain why bioavailability of INH is not affected.
- What should be the reference formulation for comparative bioavailability and bioequivalence studies – a "loose mixture" or a "single component RIF formulation"?

***In vitro* dissolution studies:**

Earlier Prankerd *et al.*, had observed that the hydrolysis of RIF, being pseudo-first-order, increases if the piperazine is reacted with formaldehyde[37]. We, therefore, revisited our earlier study on INH-lactose hydrazone formation and thus, argued that INH could react with 3-FRSV. This prompted us to compare degradation rate of RIF, in the presence of INH and compare it with single component RIF formulation in the absence of INH in an acidic medium (0.1 N HCl). Therefore, with this view, two principally different methods, dual wavelength UV-Vis spectrophotometry (DW spectrophotometry) and HPTLC, were developed and confirmed to be specific, accurate and reproducible. It was found that degradation of RIF to 3-FRSV was almost two times faster in presence of INH than that of RIF alone. Similarly, RIF in market formulations of RIF and RIF-INH FDCs in dissolution medium (0.1 N HCl) was found to degrade by 12.4% to form 3-FRSV (single component RIF formulations) while in presence of INH the degradation is catalyzed to about 21.5% (RIF- INH formulations), in 45 min[9]. In 3 h the extent of RIF degradation in presence of INH is as high as 50%[50]. Singh *et al.*[51], have studied the extent of degradation of RIF, INH and PZ from prepared mixtures and market formulations containing RIF alone or in combination with two or three drugs (INH, PZ, ETB) under acidic conditions of stomach. While RIF was found to decompose by 17.8-24.4%, INH by 3.2-4.7%, PZ was found to be totally stable. The study confirmed that RIF degradation was essentially influenced by the presence of INH and not by PZ or ETB. At the same time wide variation was reported in the decomposition of RIF (7.5-33.3%) and INH (1.4-5.3%) in market formulations when compared to pure drugs or their prepared mixtures[51].

Although the USP dissolution test suggests comparison with the standard mixture of RIF and INH subjected to the similar dissolution conditions, the analysis method in USP dissolution test fails to differentiate the degradation product, 3-FRSV, formed in the dissolution medium from RIF because of the close absorption maxima values for RIF (475 nm) and 3-FRSV (492 nm). Thus, being a nonspecific method, it determines

RIF and 3-FRSV as total RIF. Therefore, USP dissolution test shows higher amount of RIF than actually released in the dissolution medium from the market formulations. Thus, correlation between *in vitro* dissolution and *in vivo* bioavailability is doubtful.

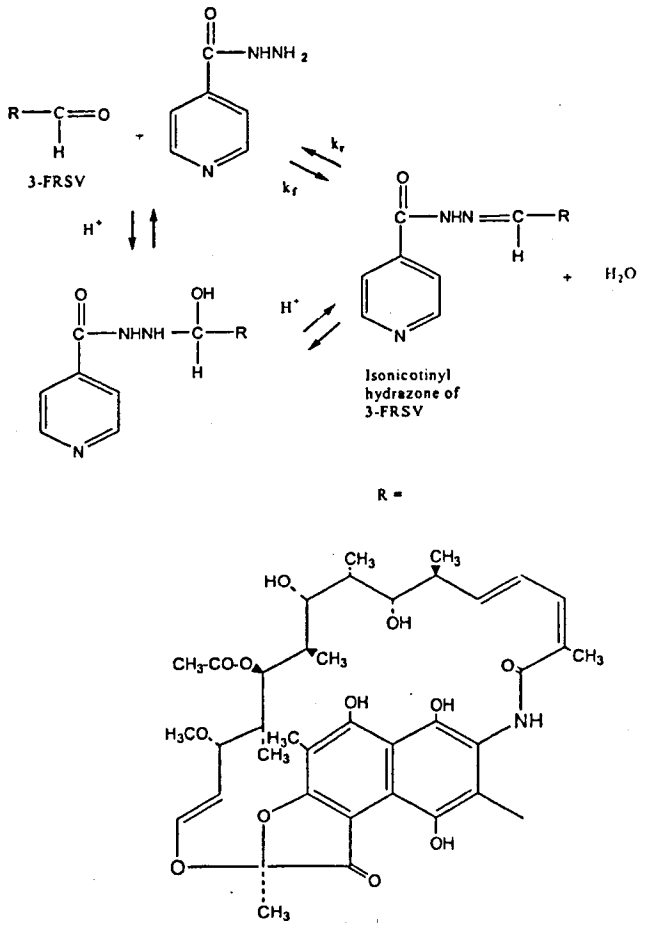
Further, formulations containing RIF in combination with INH showed more degradation (18.08% to 21.42%) as compared to those containing RIF alone (7.94%)[9]. Earlier Jindal *et al.*, also have reported degradation of RIF using 0.1 N HCl as dissolution medium to the extent of 10 - 23% in a RIF-INH combination formulation[52].

Interestingly, in case of formulations having faster release rate of RIF in dissolution medium, more amount of RIF is released in short time and gets exposed to the acidic medium for longer time. Thus, such formulations show higher RIF concentrations for initial short time interval in the dissolution medium followed by decrease in the concentrations. On the other hand, for the formulation showing slower initial release of RIF, fewer amount of RIF is available for degradation in the acidic dissolution medium and hence, shows higher concentrations of RIF as the time progresses. This may be an important factor for non-reproducible bioavailability of RIF from FDC formulations[9].

After oral administration of combined dosage form, appreciable amount of RIF is degraded in the stomach to 3-FRSV which is not absorbed and is inactive, which ultimately results into lower concentration of RIF available for absorption. This may, therefore, be responsible for poor bioavailability of RIF from combined dosage forms (RIF-INH) compared to formulations containing RIF alone.

Hydrazone formation of 3-FRSV with INH:

Independently, Singh and co-workers[50] published their study on the degradation of RIF in presence of INH using a specific HPLC method, which confirmed our results on the stability of RIF. They observed the presence of 3-FRSV-hydrazone peak in the HPLC analysis, which could be confirmed by comparing with a synthetic 3-FRSV-hydrazone. The hydrazone of 3-FRSV was found to be insoluble in aqueous medium. Proposing an elegant mechanism of interaction of INH and RIF, they postulated that there is a reversible formation of hydrazone of 3-FRSV with INH (Scheme 2). As a result, decomposition of RIF to 3-FRSV is pushed forward and an overall increase in the degradation of RIF is observed in the presence of INH. The mechanism explained all the missing



Scheme 2: Formation of hydrazone of 3-FRSV with INH where k_f is the rate of forward reaction and k_r is the rate of backward reaction

links in the jigsaw puzzle as all pieces fell into their right places. The mechanism also explained why the bioavailability of INH is not affected. The mechanism also raises doubts about the "loose mixture" used for comparison of bioavailability in WHO protocol.

In vivo bioavailability studies:

Using specific and sensitive HPTLC method the bioavailability of RIF from a single component RIF formulation has been compared with RIF-INH FDC on the basis of plasma levels[53] and urinary excretion data[54] of RIF and its major, active metabolite, 25-desacetylrifampicin (25-DAR).

Bioavailability of RIF was measured in terms of maximum concentrations of RIF and 25-DAR in plasma (C_{max}), AUC_{0-12} and $AUC_{0-\infty}$ for RIF and 25-DAR from RIF-INH FDC and from formulation containing RIF alone. There

was a reduction to the extent of 18.89% and 18.36% in C_{max} of RIF and 25-DAR, respectively, after administration of RIF-INH FDC capsule as compared to capsule containing RIF alone. Corresponding decrease in AUC_{0-12} values was to the extent of 24.27% and 16.72%[53].

Similarly bioavailability study based on the urinary excretion data has revealed reduction in cumulative amounts of RIF and 25-DAR excreted in 24 h to the extent of 32.35% and 27.90%, respectively, after administration of RIF-INH FDC capsule as compared to RIF alone capsule. Peak excretion rate for RIF and 25-DAR has shown a reduction of 21.18% and 24.02%, respectively and corresponding decrease in AUC_{0-24} values was to the extent of 34.24% and 29.26%. Statistical analysis (ANOVA, 90% confidence interval for ratio) of various pharmacokinetic parameters has indicated significant decrease in the bioavailability of RIF from FDC capsules as compared to that from capsules containing only RIF[54].

These results clearly indicate that there is a considerable reduction in bioavailability of RIF when it is administered along with INH in a FDC formulation, which correlates well with the results obtained from *in vitro* studies[9].

Both the *in vivo* studies[53,54] lend support to results obtained from *in vitro* studies and confirm our serious doubts about the stability of RIF in presence of INH in acidic environment of stomach, which probably is the main factor responsible for the reduced bioavailability of RIF from RIF-INH combination formulations.

Stable formulations of RIF and INH:

There is, therefore, a need to develop stable formulations containing RIF-INH combination to withstand the acidic environment of stomach, like enteric coated tablets or alternatively multilayered formulations so that INH is first released in stomach and RIF is released in the upper part of small intestine. Such a formulation may ensure good and reproducible bioavailability.

The considerable decomposition of RIF in presence of INH suggests that it might be more meaningful to conduct bioavailability and bioequivalence studies on FDCs by comparing the test FDC with a standard formulation containing RIF alone.

Administration of FDC:

Generally, it is recommended that RIF oral dosage form should be taken on empty stomach or at least 1 h before or 2 h after meals[36]. But, the pH of gastric fluid in an empty stomach remains more acidic (pH 1.4 to 2.1) as compared to fed stomach (pH 3.4 to 5.4)[55]. Therefore, there is a likelihood of higher decomposition of RIF after administration of FDC formulation on an empty stomach[9,53,54]. Recent studies have indicated that RIF degradation is relatively higher under simulated fasting conditions as compared to simulated fed conditions[56]. The acceleration of RIF degradation process due to presence of INH will further reduce the amount of RIF available for absorption, resulting in poor bioavailability of RIF from FDC. Therefore, it may be advisable to administer RIF FDC 1 h after meals and not on empty stomach. This requires further studies.

In conclusions, the world community needs to recognise that poor bioavilability of RIF from FDC formulations is a problem involving interaction between RIF and INH and needs to be addressed immediately.

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