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A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem☆

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Abstract

The problem of poor/variable bioavailability of rifampicin, which is shown in particular when the drugs are present in anti-tubercular fixed-dose combination (FDC) products, is a matter of serious concern. There is a potential of failure of therapy in patients with an active disease. It perhaps also is a contributory factor towards the increasing resistance to anti-tubercular drugs. Unfortunately, the origin and cause of the problem is not clearly understood, though GMP and crystalline changes in the drug are invariably cited as the principal reasons. In this write-up, various probable physical and/or chemical reasons are critically reviewed. The enhanced decomposition of rifampicin in the presence of isoniazid in stomach after ingestion is indicated to be the key factor behind the problem. Some simple solutions offered by the knowledge of the cause are discussed and it is concluded that there is a need to have a multifaceted approach to handle the problem.

Keywords: Rifampicin; Isoniazid; Pyrazinamide; Ethambutol; FDC products; Bioavailability problem; Reasons; Solutions

1. Introduction

The scourge of tuberculosis (TB) is spreading day-by-day. Almost a third of humanity is already afflicted. The dual affliction by human immuno deficiency virus (HIV) and the TB bacillus is a further-increasing danger that could prove even more serious. The disease has been righteously declared as a 'global emergency'.

The efforts to counter the disease through the use of anti-tubercular drugs have not been free from problems. There has been development of resistance to the first line drugs, rifampicin and isoniazid. The resistance to medications in TB is caused mainly due to inappropriate prescribing or taking of medications, effectively resulting in monotherapy. To reduce the possibility of the

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monotherapy, the drugs have been recommended to be taken in combination. Accordingly, a number of combinations of first-line drugs containing rifampicin, isoniazid, pyrazinamide, ethambutol, and streptomycin are in use today.

However, concern has been expressed on the poor bioavailability of rifampicin from fixed-dose combination (FDC) products containing isoniazid and/or pyrazinamide. The World Health Organisation (WHO) and International Union Against Tuberculosis and Lung Diseases (IUATLD) issued a joint statement in 1994 pointing out that anti-TB FDC products should only be used if the bioavailability of at least the rifampicin component has been demonstrated (IUATLD/WHO, 1994). Subsequently, a collaborative effort known as 'The Fixed Dose Combination Project' was launched. As a part of this exercise, a protocol has been established for bioequivalence testing of rifampicin from FDC products (Fourie et al., 1999). A network of bioavailability centres is being established in various parts of the world so that the manufacturers can get bioavailability testing of their products from laboratories with proven proficiency. Furthermore, strategies such as strengthening of procurement systems, development of planning activities and adherence to quality assurance policies by the manufacturers have been planned (Blomberg et al., 1999).

However, it seems that no serious effort has been made to know the basic cause for the falls in bioavailability of rifampicin, when it is administered along with isoniazid and/or pyrazinamide. In our opinion, a stage has come to pay attention to understand the root cause of the problem. This write-up is an effort in this direction. The probable physical and/or chemical reasons are reviewed and the possibilities, including those considered by international bodies, are critically assessed to pinpoint the real cause for the problem. The most fitting reason, which explains almost all the issues concerning the problem, is identified and the plausible solutions are discussed. The knowledge of the cause throws open a few solutions that are discussed in this review. There are several other points, which in view of the authors need immediate attention.

2. The reasons presently considered as the cause and whether they are really behind the occurrence of the problem

2.1. Changes in crystalline form of rifampicin

The existence of rifampicin in different crystal forms and the change from one form to another during processing and tabletting are repeatedly quoted as the reasons for the variable bioavailability of rifampicin from FDC products (WHO, 1999). The paper by Pelizza et al. (1977) is normally cited, which reports on the formation of different rifampicin polymorphs and the conditions for their inter-conversion. The said publication, however, gives no data to suggest that different polymorphs of rifampicin show different bioavailability. The assumption perhaps is based on a general premise that different polymorphs show different bioavailability behaviour.

Recently, an article has been published on the solubility and dissolution properties of five different rifampicin raw materials, in which three were polymorphic form II and two were the mixtures of form II and an amorphous form (Henwood et al., 2000). The data are presented for the dissolution behaviour in 0.1 M HCl, phosphate buffer pH 7.4 and water. While differences in the dissolution behaviour of the five samples were seen in water and in the phosphate buffer, it showed that 80–90% of rifampicin was dissolved in 0.1 M HCl within 10 min, and all samples showed an overlapping dissolution profile. It means that rifampicin, in the mostly used form II and in the amorphous form, rapidly goes into solution in the gastric fluid upon administration. So there is less chance of variability being seen from the formulations due to the presence of polymorphism. However, the differences in rate of dissolution of other polymorphic forms in gastric conditions still need to be investigated.

The polymorphic conversion is further unable to explain as to why a satisfactory dissolution test does not guarantee acceptable rifampicin bioavailability (WHO, 1999). As a matter of fact, different crystal forms ought to show either poor dissolution/poor bioavailability or good dissolution/good bioavailability. Also, if polymorphism

is the reason for variable bioavailability of rifampicin in FDC products, the same extent of problem should also exist in formulations containing rifampicin alone. Because the problem is more specific to FDC products containing rifampicin in the presence of isoniazid and pyrazinamide, then it means there is a role of isoniazid and/or pyrazinamide in polymorphic transformation. Though very unlikely, it still calls for in-depth comparative bioavailability studies involving different polymorphs in the presence and absence of isoniazid and/or pyrazinamide. Unfortunately, no such studies have been reported.

2.2. GMP

There is a report from WHO (1999), in which it is indicated that if produced according to GMP, FDC products show bioequivalence to the single preparations in all their active components, including rifampicin. From this it seems that the observed variable bioavailability of rifampicin in FDCs is closely linked to GMP. However, the same is true for production of any other drugs and their formulations. It would also be specifically true of single drug formulations of anti-tubercular drugs. It is unfortunate that there is no report that specifies the particular aspect(s) of GMP, which cause(s) variations to appear, typically in FDC products containing rifampicin and isoniazid and/or pyrazinamide. The lack of GMP practices in no way explains why bioavailability of rifampicin is more of a problem with FDC products and also why FDC products showing good dissolution show poor bioavailability and vice versa. This indicates that there is certainly a deeper scientific aspect to the problem, inadvertently overlooked till date.

3. Other probabilities

There are some other scattered studies in literature wherein the poor bioavailability of rifampicin was observed and the reasons were also reported. A discussion on the given reasons and the correlation, whether these are applicable to FDC products, will be worthwhile.

3.1. Drug adsorption by excipients

When rifampicin is administered along with *p*-amino salicylic acid, which is also an anti-tubercular drug, its absorption is delayed and the C_{max} and the AUC values are reduced to a half, than that obtained by the administration of rifampicin alone (Boman, 1974). This observation has been attributed to the adsorption of rifampicin on bentonite, which was used as an excipient in the manufacture of *p*-aminosalicylic acid granules (Boman et al., 1975).

The bioavailability of rifampicin is also reported to reduce significantly when it is administered along with antacids (Khalil et al., 1984). The effect of antacids on the bioavailability of rifampicin is shown to be in the order of magnesium trisilicate > aluminium hydroxide > sodium bicarbonate, and is ascribed to the combined effects of gastric pH elevation, chelation of drug by aluminium ions and binding of rifampicin with magnesium trisilicate. The drug is also bound to an extent of 16–20% by the partially neutralised magnesium silicate at pH 5.

It is unlikely that drug adsorption is the cause, in particular, for poor bioavailability of rifampicin from FDC products. First of all, the FDC formulations (tablets or capsules) contain excipients in amounts much lower than drugs present. Secondly, the solid formulations generally do not contain adsorbents as excipients in significant quantities. The only likelihood in that situation could be the inadvertent administration of antacids to patients along with the FDC products. But as this would happen even in formulations containing rifampicin alone, this reason even does not explain the typical poor bioavailability of rifampicin from FDC products.

3.2. Formulation factors

The formulation factors certainly can be a cause of variable bioavailability, as the performance of the formulation per se depends upon the quality of active ingredient(s), quality and combination of excipients, the process used in the manufacture, and the packaging. During development of formulations, several trials with different excip-

ients and/or process are made, before the formulation of desired characteristics and stability is obtained. It means that intermediate formulations exist that do not conform to desired specifications. In case the person involved in formulation is not fully experienced and trained, and is not in knowledge of the intricacies of the formulation development, the end product might be a half-baked poor-performing formulation, which might get into the market. Many pharmaceutical companies in several parts of the world do not have quality control measures available with them, and the performance of the formulations so produced cannot be judged before release. This problem is compounded by the absence of a simple in vitro test that can act as an effective substitute to in vivo bioavailability evaluation, which is a costly proposition. In such situations, formulation-to-formulation and batch-to-batch variations are bound to happen. The variations have been observed practically, for example, bioavailability of nine different single-drug rifampicin formulations of three pharmaceutical forms, syrups (2), tablets (4) and capsules (3), was evaluated by Mannisto (1976) and both inter- and intra-formulation differences were observed. Similarly, in a recent study, variations were reported in bioavailability of multiple marketed FDC products (Pillai et al., 1999).

Evidently, the variations due to formulation factors occur both in single rifampicin and the FDC products, suggesting that there is a still more specific reason that is responsible for the typical overall poor bioavailability of rifampicin from FDC products, as compared with the formulations containing rifampicin alone.

3.3. Drug decomposition in formulations

The decomposition behaviour of rifampicin, isoniazid and pyrazinamide has been well investigated under different reaction conditions. Table 1 summarises the information on each compound (Gallo and Radaelli, 1976). The order of sensitivity towards decomposition for the three drugs is rifampicin > isoniazid > pyrazinamide. Pyrazinamide, in true sense, is a known stable drug. There is very little information in literature on the stability of ethambutol.

As regards the correlation of drug decomposition to bioavailability, any fall in drug content in formulation due to decomposition is generally bound to influence its in vivo bioavailability. This is because the total drug available for absorption is decreased in such cases. The bioavailability may further vary from formulation-to-formulation, as the stability of a drug depends strongly upon formulation factors and the storage conditions. Hence on the face of it, the variable bioavailability from FDC products can be attributed to the drug decomposition in formulation environment. However, the critical points here again are, (i) whether the extent of drug decomposition in drug formulations differs in mono formulations versus multidrug FDC products, to explain the typical

Table 1
Stability of anti-tubercular drugs

Drug	Conditions	Decomposition products
Rifampicin	pH 2–3, 20–22 °C	3-Formyl rifamycin SV
	0.1 N HCl, 37 °C	Do
	pH 8.2, 20–22°C	Rifampicin quinone
	pH 8.2, 60-70°C	25-desacetyl rifampicin 25-desacetyl-21-acetyl rifampicin 25-desacetyl-23-acetyl rifampicin
	NaOH 5% in ethanol–water (1:1), 20–22 °C	25-Desacetyl rifampicin
Isoniazid	pH 3.1, anaerobic	Isonicotinic acid
	Alkaline, aerobic	Isonicotinic acid Isonicotinamide 1,2-diisonicotinoyl hydrazine
	Alkaline, anaerobic	Isonicotinic acid 1,2-diisonicotinoyl hydrazine
	Alkaline, anaerobic with EDTA	Isonicotinic acid
Pyrazinamide	Wet or dry atmosphere at solid state	Stable
	Natural day light	Stable
	Autoclaving of intravenous infusions	Stable

Table 2
Stability of rifampicin, isoniazid and pyrazinamide in suspension formulations

Drugs present	pH	Concentration (mg/ml)	Drug remaining after 28 days (%)		
			4 °C	24 °C	40 °C
Single drugs					
Rifampicin	4.05	5.88	91	96	91
Isoniazid	5.65	5.88	69	56	54
Pyrazinamide	6.10	11.76	89	80	56
Two-drug combinations					
Rifampicin	5.45	5.88	14	–	–
Isoniazid		5.88	54		
Rifampicin	4.22	5.88	100	–	–
Pyrazinamide		11.76	99		
Three-drug combination					
Rifampicin	5.23	5.88	3	–	2
Isoniazid		5.88	54		29
Pyrazinamide		11.76	95		95

poor bioavailability of rifampicin from FDC products; and (ii) does it explain the key observation that FDC products with stated drug content show poor bioavailability (WHO, 1999)?

On the first point, there exists a study on the suspensions of rifampicin, isoniazid and pyrazinamide by Seifart et al. (1991) in which the formulations of the drugs alone and their combinations were stored at 4, 24 and 40 °C for 28 days. The suspensions had a pH range between 4.05 and 6.10. Differential extents of decomposition were observed between suspensions of single drugs and mixtures of two and three drugs. The data are summarised in Table 2. Evidently, the degradation of rifampicin is insignificant when present alone or in combination with pyrazinamide. Conversely, the degradation is as high as 98% in the presence of isoniazid. This study strongly indicates that the probable cause for typical poor bioavailability of rifampicin from FDC products is the increased degradation of rifampicin in the presence of isoniazid.

The drug decomposition in formulation environment, however, does not explain the second point as to how the solid products with stated drug content show poor to variable bioavailability (WHO, 1999). Also it is unable to provide justification as to why the bioavailability problem is sometimes seen even in fresh formulations. Perhaps this is the reason that in vitro decomposition is not mentioned and considered as a causative factor.

3.4. Drug decomposition in situ in stomach

Another aspect that is closely correlated to a drug's bioavailability is the drug decomposition in situ in acidic conditions of the stomach. This is a change that happens on the administration of the drug. Several studies exist in literature where drugs are reported to show this kind of behaviour (Ertan et al., 1993; Yang et al., 1994). Recently, it was proposed by Shishoo et al. (1999) that the problem of poor absorption of rifampicin from combination products is also perhaps due to increased decomposition in stomach conditions.

There are a few reports in literature (Jindal et al., 1994; Shishoo et al., 1999) in which the extent of degradation of rifampicin, in the absence and presence of isoniazid, has been determined in 0.1 N HCl and simulated gastric fluid (SGF) at 37 °C in 45 min (USP dissolution test conditions). The data in Table 3 gives the range of the reported values. Apparently, rifampicin alone decomposes in the described conditions to an average extent of 6.33%, while the loss of rifampicin in the presence

of isoniazid increases on an average to 16.32%. This suggests a conclusion similar to which one makes from the study by Seifart et al. (1991), that the degradation of rifampicin is enhanced by the presence of isoniazid.

An important point here is whether the drug degradation in stomach explains the two points critical to the FDC products listed above under *Drug decomposition in formulations*. In this context, the first point is well explained, as an increased decomposition of rifampicin takes place in the presence of isoniazid that is always contained in FDC products. Further, the drug is expected to degrade in stomach even in fresh formulations and in this respect the behaviour differs from in vitro decomposition in a formulation environment, which does not explain this aspect. The observation that FDC formulations that show good dissolution show poor bioavailability or vice versa is also explained. Formulations with good dissolution ought to dissolve faster in the stomach and show significant decomposition within the period the drug remains in the stomach. On the other hand, formulations with poor dissolution ought to show less decomposition due to relative lower rate of dissolution, and hence a better bioavailability is seen.

Thus the in situ decomposition of rifampicin in the presence of isoniazid in the stomach seems to be the plausible reason for the problem of bioavailability of rifampicin in FDC products. The acceptance of the premise, however, raises several further questions. (i) What exactly is the mechanism of increased decomposition of rifampicin by isoniazid, if there are no reactive groups on rifampicin and pyrazinamide and only one on isoniazid? (ii) Do other co-drugs in FDC formulations, like pyrazinamide and ethambutol, also have any deleterious effect on rifampicin and whether they themselves are also lost to any extent? (iii) What is the extent of decomposition of rifampicin from marketed FDC products and how much does it influence the administered dose? (iv) Are any suggestions offered towards the solution of the problem, once the reason is known etc.?

Fortunately, answers to points i–iii have been put forth in a series of recent publications (Mariappan et al., 2000; Singh et al., 2000a,b). During

Table 3
Extent of decomposition of rifampicin in USP dissolution medium (0.1 M HCl)

Formulation/drugs	Drug strength (mg)	Decomposition of rifampicin at 45 min (%)	Method of analysis
Rifampicin capsules	450		HPTLC and dual-wavelength spectrophotometry
Formulation 1		4.58	
Formulation 2		6.61	
Formulation 3		7.79	
Rifampicin and isoniazid capsules	450 and 300		HPTLC and dual-wavelength spectrophotometry
Formulation 1		16.58	
Formulation 2		15.27	
Formulation 3		18.94	
Formulation 4		17.48	
Rifampicin and isoniazid tablets	450 and 300		HPTLC and dual-wavelength spectrophotometry
Formulation 1		16.31	
Formulation 2		13.10	
Formulation 3		15.07	
Formulation 4		16.59	
Rifampicin and isoniazid	450 and 300		HPLC
Formulation 1		12.73	
Formulation 2		31.66	

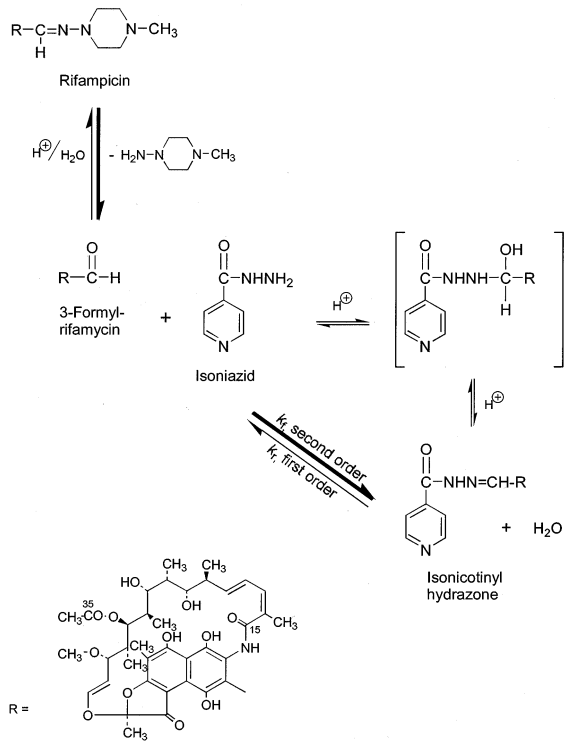

Fig. 1. Mechanism of enhancement of decomposition of rifampicin in the presence of isoniazid.

the development of a high-performance liquid chromatographic (HPLC) method of analysis, it was found by Mariappan et al. (2000) that a new peak emerged during decomposition of rifampicin in the presence of isoniazid, pyrazinamide and ethambutol. This peak was attributed to the formation of isonicotinyl hydrazone, based on the known reaction of isoniazid with reducing sugars (Devani et al., 1985). The hydrazone was eventually isolated as well as synthesised and its chemical structure was confirmed (Singh et al., 2000a). A reaction pathway explaining the mechanism for the increased decomposition of rifampicin in the presence of isoniazid and involving the formation of hydrazone was postulated (Fig. 1). It is proposed that once 3-formylrifamycin is formed under acidic conditions, it interacts with isoniazid to form the hydrazone, through a fast second-order reaction. The hydrazone, due to its instability in acidic conditions, regenerates isoniazid and 3-formylrifamycin by a pseudo first-order reaction. As the second-order forward reaction is faster than the preceding (rifampicin to 3-formylrifamycin) and the following (hydrazone to 3-formylrifamycin and isoniazid) first order reactions, the overall reaction is favoured towards the formation of hydrazone. As a result, the decomposition of rifampicin to 3-formylrifamycin is pushed forward and an overall enhancement of degradation of rifampicin is observed.

The fall in rifampicin was reported to be influenced only by the presence of isoniazid and not by pyrazinamide and ethambutol (Singh et al., 2000b). In both 0.1 M HCl and SGF, rifampicin was found to decompose from 17.8 to 24.4% at 37 °C in 50 min, while isoniazid was decomposed only between 3.2 and 4.7%. Pyrazinamide was found to be stable .In comparison to pure drugs and their mixtures, wide variations in decomposition of rifampicin (7.5–33.3%) and isoniazid (1.4–5.3%) were seen in the marketed FDC products, indicating a strong influence of the formulation and storage factors. The decomposition of rifampicin in the presence of isoniazid at 15 min was 8.5%, which increased to 50% after 3 h. The relative decrease in isoniazid in the same periods was 1.8 and 10.3%, respectively (Singh et al., 2000a).

A more recent and yet unpublished data from the author's laboratory indicate that the decomposition of rifampicin in the presence of isoniazid, after 50 min at 37 °C, increases from pH 1 to 2. So there is a likelihood of much higher decomposition of rifampicin after ingestion of the FDC formulation on an empty stomach, as the pH of stomach in fasted state ranges between 1.4 and 2.1 (Dressman et al., 1998). The decomposition might still be higher in patients with hyperthermia, which is normally associated with an active disease.

It means that varying rifampicin decomposition in situ, based on the stomach and pathological conditions, can be correlated directly to the variable bioavailability of rifampicin from FDC products. This strengthens the contention that in situ decomposition of rifampicin in the presence of isoniazid needs to be looked into critically.

4. The extent to which in situ decomposition of rifampicin explains other concerns that have been expressed for the therapy with FDC products

The question then arises whether this phenomenon of enhanced in situ decomposition of rifampicin in the presence of isoniazid answers other issues for which concern has been voiced from time-to-time, but no tangible reasons and solutions have been forthcoming. Several of these concerns were expressed during panel discussions at the IUATLD/WHO meet held at Bangkok in November 1998 (Anonymous, 1999). Some of the related questions, observations, and statements made were.

- Whether there are some physical parameters which are very important in FDC tablets which could also throw some light on the bioequivalence problem and eventually correct this problem of FDC in anti-TB therapy?
- There is no possible chemical reaction between the three drugs.
- The problem that faces us is knowledge about what dosage of rifampicin is really required. Are we right just to assume that the 600 mg dose for over 55 kg (450 under) is the correct dose?
- I want to ask whether it is not one beyond the dose issue, and whether it is not a blood level issue. We have seen a lot of evidence presented about the wide variability in drug concentrations that we see in patients given those standard doses. So is the issue a blood level issue rather than a dose issue?
- There are certainly very wide differences, if you just take 100 patients and do one blood level determination you will find a very wide variation, but if you take two or more on that patient they average out, In fact there is very little evidence for individual variation.
- We know that the manufacturer that licensed the three-drug FDC based in the US increased the dose by 20% because in their volunteers the absorption of rifampicin was reduced by 18%, and that is with a leading manufacturer, using the best available mechanisms, etc.

These statements clearly indicate that although hints were thrown during the discussions that the reason might be a physical one, the same was more or less ignored, under the assumption that all involved drugs were non-reactive to each other. It is also evident from the statements that a strong confusion prevails with respect to simple issues like dose, blood levels and drug absorption of rifampicin from FDC products. This is despite the fact that FDC products have been in existence for almost a decade, and a number of bioavailability studies have been carried out on them.

Fortunately, all the unresolved points are explained by the decomposition of rifampicin in situ in the presence of isoniazid. It justifies on one side that there is a physical/chemical aspect, and as the decomposition in reality takes place in situ, it clarifies why the problem remains undetected from the evaluation of the drug content and dissolution behaviour of the formulations. The decomposition in situ very well describes the decrease in the administered dose of rifampicin and the corresponding blood levels. It also directly explains the issue of reduction in the absorption of rifampicin, as the drug level falls due to decomposition in stomach before absorption. The in situ decomposition even, as discussed in the previous section, very well explains the reported variability in blood level data from subject-to-subject. The little evidence for variation in a single individual is explained on the basis that the in situ acidity, residence time in stomach and pathological conditions of one individual do not vary much, while the difference is well pronounced from one subject to another.

5. Suggestions that emerge from the knowledge of the cause

5.1. Conduct of bioavailability studies targeted to confirm the role of physical/chemical factors

Despite the recognition of the problem of bioavailability of rifampicin from FDC products several years ago, no actual study seems to have been done to correlate the assumed physical factors to in vivo bioavailability. For example, no practical study has ever been made to study the

influence of crystalline structure or polymorphism on the bioavailability of rifampicin. Hence, it is required that this ambiguity is removed and the studies are carried out to correlate the postulated factors to in vivo behaviour.

On similar lines, even the role of in situ decomposition of rifampicin from FDC products should be established by determining the extent of decomposition of several marketed formulations under in vitro acidic conditions, and the results should be correlated to those obtained by carrying out bioavailability studies. However, a modification, as discussed below, is suggested to be considered for incorporation in the test protocol, before taking up bioavailability studies.

5.2. The choice of reference preparations in bioequivalence studies on FDC products

The WHO has recently introduced a test protocol for bioequivalence studies on FDC formulations (Fourie et al., 1999). It proposes comparison of the test FDC formulation against a loose combination of single drug reference preparations. We suggest that in view of the rapid in situ decomposition of rifampicin in stomach, it might be more meaningful to carry out bioavailability and bioequivalence studies on FDC products by comparing the test FDC formulation against a standard formulation containing the drug of interest alone. Such studies are likely to provide more exact and complete picture on the drug's in vivo availability vis-a-vis the administered dose. In most cases, loose and FDC forms of drugs are expected to degrade to the same extent in the gastric environment, and hence no difference can be expected in terms of absolute bioavailability and levels of absorption. Here it might be significant to cite a very recent report (Rathod et al., 2000), where an almost 34% reduction in bioavailability was seen from combination formulation containing rifampicin and isoniazid, as compared with formulation containing rifampicin alone. A similar observation was made much earlier by Acocella (1989) who carried out a controlled human pharmacokinetics study using rifampicin alone, rifampicin and isoniazid, and three brands of triple fixed combinations of rifampicin, isoniazid and pyrazinamide. Some of the fixed-dose combinations were reported to be associated with rifampicin plasma levels significantly lower than those with the reference compounds.

An associated suggestion here is that the WHO protocol on bioequivalence studies on FDC products should include a requirement on comparison of the number of subjects in a study that show blood levels of rifampicin within the therapeutic window. Only those FDC products should be declared bioequivalent where this number is similar to that shown by the rifampicin alone. In a situation, where a failure of therapy even in one individual (due to delivery of sub dose) means spread of the disease to scores of others and also development of resistance, the comparison of averaged values of bioavailability parameters and the presently accepted 90% Cl range between 80 and 125% should not be the only bioequivalence determining criteria.

5.3. Another suggestion on bioavailability studies

The bioavailability studies should not be done only on fresh FDC products to assess and control their quality before release. Equally important is the regular bioavailability testing on aged samples in circulation. This is to assure that good bioavailability characteristics are retained till the defined date of the use of the formulation. In addition, such studies are expected to provide an insight into the role of formulation factors, if any.

5.4. In vitro quality control test as an alternate for the bioequivalence studies

A suggestion was made during the panel discussion of the WHO/IUATLD meet at Bangkok in 1998 (Anonymous, 1999) regarding the possibility of developing an in vitro dissolution test for the FDC products as a surrogate for bioavailability studies. In this regard, an in vitro dissolution test that duly accounts for in situ decomposition of rifampicin in the presence of isoniazid in acidic conditions has a better chance to give good correlations with the poor/variable bioavailability of the drug from FDC products. Our suggestion is the use of 250 ml 0.01 N HCl as the dissolution

medium. The reason for 250 ml dissolution medium against 900 ml is that, in general, a patient or volunteer takes the medicine with around 200 ml of water. The WHO model protocol also suggests administration of drugs to volunteers with 200 ml of tap water (Fourie et al., 1999). Once the volume of gastric fluid in the stomach in the fasted state ($\sim$ 30 ml; Dressman et al., 1998) is taken into account, the total volume comes to around 250 ml. As the pH in the fasted state varies from 1.4 to 2.1 (Dressman et al., 1998) and as our finding is that decomposition of rifampicin in the presence of isoniazid increases with the change in pH from 1 to 2, therefore, it might be useful to carry out the test at the pH of maximum decomposition, i.e. pH 2 (0.01 M HCl). We also suggest here that the samples should be analysed by a stability-indicating method, like the one recently reported by us (Mariappan et al., 2000). The use of a colorimetric method specified in USP23/NF18 (1995) is not expected to be suitable. This is because the two degradation products, 3-formylrifamyicn and hydrazone, show similar absorption spectra to rifampicin, but with different extinction values (unpublished findings). The results of colorimetric analysis, therefore, might not be very accurate for a decomposed solution.

Of course, this proposal needs to be validated by first carrying out bioavailability studies on a series of FDC products and correlating the in vivo results to in vitro drug decomposition data. Changes can be affected in the test conditions and if good correlation is developed, there is a strong likelihood that the test might obviate the need for currently advised human bioavailability and bioequivalence studies.

6. Plausible solutions to the problem of poor/variable bioavailability of rifampicin from FDC products

The identification of the in situ decomposition as the plausible cause for bioavailability problem of rifampicin from FDC products, the understanding of the mechanism involved, and the determination of the extent of decomposition of rifampicin from different marketed formulations (7–33%) suggest that there is rather a possibility of a permanent solution to the bioavailability problem typical to FDC products containing isoniazid. The following four approaches seem plausible. (i) Enteric coating of solid formulations or drug granules. (ii) Use of alkaliniser at the time of administration of FDC formulations. (iii) Exploitation of formulation factors, including addition of additives, etc. (iv) Segregation of delivery of rifampicin and isoniazid.

The enteric coating can provide sufficient protection against the acidic hydrolysis of rifampicin in situ, but here an important consideration is the site of absorption of both the drugs, which is not clearly defined. In case good absorption of rifampicin takes place from the intestine, then enteric coating can be a direct solution. There can be two approaches for enteric coating, one is the coating of the tablets/capsules and the second is coating of granules of either of the two drugs, among rifampicin and isoniazid. With respect to which of the two drugs ought to be preferred for coating of granules, the better choice would be rifampicin because that will prevent even the basic decomposition of rifampicin ($\sim$ 7%) that takes place in acidic conditions when the drug is present alone (Table 2). Isoniazid might be used uncoated, as it is stable in acidic condition. However, due trials need to be made to find out the better option. A problem that is envisaged in evaluation of the enteric-coated products is with respect to their dissolution testing. Acidic medium (0.01 M HCl) suggested in USP cannot be used because the formulations that are meant to release drug at duodenal intestinal pH ($>$ pH 5.5) would show poor release due to very poor solubility of rifampicin at pH $>$ 3. Dissolution medium containing sodium lauryl sulphate as a surfactant (Jindal et al., 1994) is the suggested alternative. The bioavailability testing of such products would also require standard formulations with enteric-coating of rifampicin or isoniazid.

The raising of gastric pH by simultaneous administration of an alkaliniser might act as a solution because lesser decomposition of rifampicin is expected to happen when the drug is in insoluble form. Here use of a soluble alkaliniser like sodium

bicarbonate is suggested. The use of antacids containing adsorbents, as discussed previously, decrease bioavailability of rifampicin (Khalil et al., 1984).

As regards the formulation approaches, they also seem to be the possibility. In our studies (Singh et al., 2000b) one of marketed FDC formulation was found to show decomposition of rifampicin to an extent of only 7%, almost the same level shown by rifampicin alone under the same conditions. There were a couple of other formulations that showed decomposition in a range of 11–13%. Although it could not be deciphered what exactly were the factors responsible for stabilisation, it might be the use of additives that knowingly or unknowingly reduce or stop the decomposition of rifampicin in acidic conditions in the presence of isoniazid.

The staggering of administration of rifampicin and isoniazid is an option that can be adopted if all other approaches fail in practical sense. Segregation of anti-tubercular drugs carries a disadvantage that it would tend to defeat the very purpose for which FDC products have come into being, i.e. to prevent missed doses that are a cause for monotherapy.

7. Isoniazid interaction with sugars and its implications

It is well known that in acidic medium, isoniazid forms hydrazones with reducing sugars such as galactose, lactose, glucose, maltose (Devani et al., 1985), etc. The isoniazid–sugar interaction can be a possibility even in FDC products that contain lactose or other sugars. A positive aspect of isoniazid–sugar interaction can be thought in terms of involvement of isoniazid with sugar and its non-availability for interaction with 3-formylrifamycin. That would spare rifampicin from rapid decomposition through 3-formylrifamyicn–isoniazid hydrazone mediated reaction (Fig. 1). However, as a matter of fact the addition of sugars, although it would spare rifampicin, can be in no way considered a solution to the problem of variable bioavailability of rifampicin from FDC products. The important point here is that the sugar–isonicotinyl hydrazones are poorly absorbed from the GIT, and any attempt to save rifampicin using this approach would mean sacrificing the total dose of isoniazid, which is not tenable. Hence, it becomes important that FDC products are formulated free from sugars. It may not be wrong to say that the FDC preparations in market should be tested for sugars, and wherever the test becomes positive, the preparations should be subjected to bioavailability studies to determine the levels of absorbed isoniazid. This might be important because there are reported instances of development of resistance even to isoniazid.

8. The present direction of thinking of the world bodies and whether a change is required in the same

As of today, the efforts of WHO/IUATLD combine are mostly oriented towards compulsory bioavailability/bioequivalence testing of all FDC products, laying down of technical requirements including protocols for these studies, and establishment of a network of approved laboratories. A lot of discussion is also being held on defining more exact registration requirements and on outlining the procedure for tendering for the purchase of FDC products. Talk is also revolving around on adoption of the WHO vaccine model for the distribution of FDC products, so that the formulations of only impeccable quality reach the end user.

Looking deeply into the direction of these activities, it becomes clear that the whole effort is seemingly being done in an absence of understanding of the root cause of the problem. The only reason, for this absence of the attention to the physical/chemical causes of the problem is perhaps the absence of representation from the pharmaceutical formulation and quality control scientists in the core anti-TB groups of both the organisations. The result of this is that ‘a simple pharmaceutics problem seems to have been blown out of all proportions’.

The data quoted above and the published reports (Shishoo et al., 1999; Rathod et al., 2000; Singh et al., 2000a,b) provide a strong evidence

that the enhanced decomposition of rifampicin in situ, which occurs post administration of a FDC product, is the reason for the problem of variable bioavailability of FDC products. The identified reason explains almost all the issues, including as to why FDC products even with apparently satisfactory assay and dissolution do not guarantee acceptable rifampicin bioavailability. Further, the fact that there exist formulations in market that are devoid of the problem, provide enough indication that solution is not out of hand, and is in very much reach of the formulation scientists.

Therefore, in our view, the world bodies should seriously consider shifting of its emphasis on pure biological solutions to the problem, to include investigation of the role played by physical and/or chemical factors besides looking at the mechanisms involved. The combine should direct its energies in development of a stable, simple and affordable FDC formula, which shows minimal decomposition both in vitro and in situ in stomach. Instead of pursuing the WHO's vaccine model, it would be much better if the organisations tread the path shown by the development of the standardised rehydration formula by the WHO. In addition, development of simple in vitro quality assurance tests should be given high priority. The world bodies must involve chemists, and formulation and analytical scientists in its core groups so that the root cause of the problem is addressed.

9. Conclusions

Among the various physical/chemical factors that can possibly be the reasons for the variable bioavailability of rifampicin from FDC anti-tubercular products, one that explains most of the issues related to this typical problem is the rapid decomposition of rifampicin in the presence of isoniazid in situ in stomach acidic conditions. The variable bioavailability is shown due to inter-individual variations in the gastric resident time and acidity. The failure of some marketed formulations in bioavailability studies is explained based on our practical observations in which different formulations were found to show different extent of acidic decomposition. The acidic decomposition of rifampicin in situ also explains as to why the products with satisfactory dissolution show poor bioavailability and vice versa. It is simply because stomach decomposition comes in-between drug dissolution and its absorption. Therefore, it is not wrong to say that the decomposition of rifampicin in stomach before absorption is a strong contributory factor for the treatment failure and emergence of resistance. It is expected that the drug decomposition, on administration in empty stomach, might range from 20 to 50%, which means a reduction in the dose from about 10 to 12 mg/kg (600 mg for patients above 60 kg and 450 mg for patients below 50 kg of body weight) to as low as 5–6 mg/kg of body weight. It was indicated by Long et al. (1979) that a decrease in the dose of rifampicin below 9 mg/kg of body weight results in loss of therapeutic efficacy.

Based on the reason, it seems the problem can be solved by using different approaches, like protecting rifampicin from exposure to acid by enteric coating, by simultaneous administration of soluble alkalinisers, through use of specific additives in formulation, and by segregation of delivery of rifampicin and isoniazid. However, further intense studies might be required to select the best among these approaches.

It is desired that WHO and IUATLD, the two world organisations, who are on the forefront in handling the issue of quality of FDC products should recognise the role of this factor in appearance of the problem. Research programmes should be initiated and funded to prove the role of this factor, towards development of a simple in vitro test that obviates the need of human bioavailability studies, and in the development of stable formulations.

Needless to say that this needs to be done immediately. After all it is a battle, against a dreaded disease. If we falter, the disease will take over us (as it tends to do presently), but if we take the 'right' steps, there is a good chance that we can effectively halt its ingress.

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