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Short communication

Study of the interaction between rifapentine and isoniazid under acid conditions

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Abstract

A well-known problem of anti-tuberculosis fixed-dose combination (FDC) products containing rifampicin (R) and isoniazid (H) is the fall in bioavailability, in particular of R, when two or more drugs are present together. The same has been ascribed to hydrolysis of R to 3-formylrifamycin (3-RIF) under stomach acid conditions and reaction of the latter with H to form isonicotinyl hydrazone (HYD). The objective of present study was to explore whether the same reaction occurred when H was present along with rifapentine (Rp), a newer long acting rifamycin, which is structurally similar to R. Clinical trials are currently undergoing for co-administration of Rp with H in patients who had completed 2 months of standard chemotherapy. For the purpose, first a validated HPLC method was developed for the separation of Rp and H, and the same was used for the study of interaction between the two drugs. Like R, Rp was also found to convert to 3-RIF in acid conditions, which reacted further with H to form HYD. The pH-rate profile was also similar in shape to that established with the combination of R and H; maximum decomposition occurred at pH 2, where Rp loss was to an extent of ∼30%, while corresponding decomposition of H was ∼9%. These values were similar to those reported for the combination of R (∼33%) and H (∼10%). Hence, the study suggests that co-administration of Rp and H should be avoided, like in case of R and H, and the two drugs should not be formulated directly into a single dosage form.

Keywords: Rifapentine; Isoniazid; Isonicotinyl hydrazone; pH-decomposition profile

1. Introduction

Despite availability of effective drugs and treatment, tuberculosis (TB) remains one of the most common causes of mortality worldwide. Complexity in the drug regimen and requirement of long duration of therapy are thought to be the main contributing factors for patient non-compliance [1]. Hence, there is an immediate need of drugs having long duration of action, which can decrease complexity in the treatment of this dreaded disease. Fortunately, US FDA granted accelerated approval to rifapentine (Rp) in June 1998 for the treatment of pulmonary TB [2]. The drug is a rifamycin derivative with an advantage of five times longer half-life than rifampicin (R) and it is recommended for use in intermittent therapy [3]. The administration of drugs at intermittent intervals tends to reduce the toxicity, improve patient compliance and of course reduce the cost of therapy [4]. Several trials have indicated that Rp and isoniazid (H) would be more effective than any other combination, if administered twice weekly during the intensive phase of TB treatment and once weekly during the continuation phase of treatment [5,6].

It has been shown that R and H interact with each other to form isonicotinyl hydrazone (HYD) in stomach acidic conditions, and the reaction is responsible, at least in part, for the fall in bioavailability of R from FDC products containing the two drugs [7,8]. Due to structural similarity, it was hypothesized that Rp would also react with H, in a similar manner to R. Hence studies were carried out to explore the occurrence of interaction between Rp and H in acid environment.

2. Experimental

2.1. Materials

Rp and H were gift samples from M/S Panacea Biotec Ltd., Lalru, India. HYD and 3-RIF were prepared and characterized by

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the methods already established in our laboratories [8]. All other materials were of analytical grade. Ultra pure water was obtained from a water purification unit (Elga Ltd., Bucks, England).

2.2. Instrumentation

The HPLC system consisted of a DGU-14A degasser module, FCV-10AL$_{VP}$ flow control valve, LC-10AT$_{VP}$ pump, SIL-10AD$_{VP}$ auto injector, CTO-10AS$_{VP}$ column oven, SPD-M10A$_{VP}$ photo-diode array (PDA) detector and an SCL-10A$_{VP}$ system controller. Data was acquired and processed by using CLASS-VP software Ver. 6.13 (all from Shimadzu, Kyoto, Japan). Zorbax XDB C-18 (250 mm × 4.6 mm, particle size 5 μm) column (Agilent Technologies, Wilmington, USA) was used for the chromatographic separations. Additional separation studies were also done using Discovery HS C18 (Supelco, PA, USA) column of the same dimensions and particle size. Other devices employed in the study were a pH meter (MA 235, Mettler Toledo GmbH, Schwerzenbach, Switzerland), sonicator (Branson, Ultra-Sonic Corporation, Danbury, CT, USA), analytical balance (AG 135, Mettler Toledo, Greifensee, Switzerland) and auto pipettes (Eppendorf, Hamburg, Germany).

2.3. Development and optimization of HPLC method

Solutions of Rp, H, 3-RIF and HYD were prepared in water–methanol mixture (50:50, v/v) and filtered through 2 μm nylon filters. The mobile phase was filtered through 0.45 μm nylon filter and degassed before use. It was pumped at a flow rate of 1 ml/min.

Initial trials for the separation of mixture of the drugs on HPLC were carried out using different ratios of water:methanol and water:acetonitrile. The peak shapes were better in water:methanol, hence further studies were carried out using this solvent combination. The method was optimized by varying both the pH and the buffer:organic modifier ratio.

2.4. Validation of the method

The developed HPLC method was validated for linearity, range, specificity, selectivity, accuracy and precision (intra-day, inter-day and inter-column). For linearity studies, different concentrations of Rp and H were prepared in triplicate from the primary stock solutions (1 mg/ml) of the two drugs. Standard plots within the selected concentration range were constructed by plotting concentration versus the mean area responses ($n = 3$) for the individual drugs. Specificity was determined by analysing acid degradation samples and observing the separation of drug peak from the degradation products. Overall selectivity was determined by checking peak purity of all the peaks including those of the degradation products using a PDA detector. For the determination of accuracy, synthetic mixtures of different ratio of the components were prepared and analysed. The intra-day precision was determined by repeating the studies three times on the same day. The experiment was repeated on three consecutive days to measure inter-day precision. The inter-column precision of the method was determined by repeating the experiment on C18 columns of two different brands.

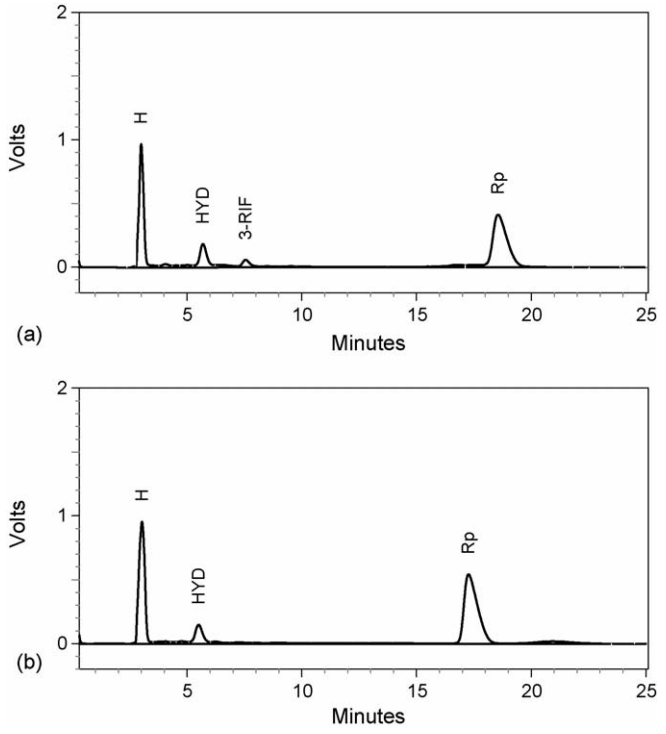

Fig. 1. Chromatograms showing resolution of the standard mixture of rifapentine (Rp), isoniazid (H), 3-formylrifamycin (3-RIF) and isonicotinyl hydrazone (HYD) (a) and formation of HYD as the main product on storage of the drug combination at pH 2 for 50 min (b).

2.5. pH-decomposition study

A stock solution of 1 M HCl was prepared by dilution of concentrated HCl. The same was standardized against 1 M NaOH, which was standardized previously against 1 M oxalic acid. The stock was diluted with water to achieve concentrations of 0.1, 0.03, 0.01, 0.003 and 0.001 M HCl (pH 1, 1.5, 2, 2.5 and 3, respectively).

Table 1
Linearity data for Rp and H ($n = 3$)

Drug	Concentration range (μg/ml)	Regression parameters	
		Equation of regression line	R^2 value
Rifapentine	50–1200	$y = 6380.6x + 31105$	0.9994
Isoniazid	50–500	$y = 8399.8x + 41283$	0.9985

Table 2
PDA peak purity parameters for Rp, H, HYD and 3-RIF

Drug	Peak purity index	Single point threshold
Rp	1.000000	0.999985
H	0.999999	0.999984
HYD	0.999904	0.997165
3-RIF	0.998590	0.962605

Table 3
Recovery studies on Rp and H

Drug	Added concentration (μg/ml)	Measured concentration (μg/ml)	% Recovery	Mean % recovery
Rp	50	50.39	100.79	100.15 ± 0.70
	100	100.25	100.25	
	200	198.80	99.40	
H	50	50.62	101.23	100.03 ± 1.37
	100	98.53	98.53	
	200	200.62	100.31	

Table 4
Intra- and inter-day precision studies ($n = 3$)

Drug	Added concentration (μg/ml)	Intra-day precision	Inter-day precision
		Measured concentration ± S.D. (μg/ml), R.S.D. (%)	Measured concentration ± S.D. (μg/ml), R.S.D. (%)
Rifapentine	100	99.80 ± 1.07, 1.07	101.82 ± 1.17, 1.15
	200	199.75 ± 2.11, 1.06	200.55 ± 2.89, 1.44
	400	401.70 ± 3.19, 0.79	403.83 ± 5.38, 1.33
Isoniazid	50	50.02 ± 0.59, 1.30	50.36 ± 0.98, 1.95
	100	99.86 ± 0.96, 0.96	98.97 ± 1.64, 1.66
	200	199.62 ± 1.98, 0.99	201.37 ± 3.70, 1.84

For decomposition at various pH, Rp (22.5 mg) and H (15 mg) were first weighed accurately and dissolved in 2 ml methanol. The mixture was then diluted with a solution of specific pH up to 25 ml. An aliquot (2 ml) of this solution was withdrawn, diluted to 10 ml with methanol and analysed by HPLC. The remaining solution was maintained in water bath at 37 °C for 50 min and then subjected to HPLC by the same method. The percentage degradation of Rp and H were calculated from difference in peak areas of initial and 50 min samples. The studies were conducted in triplicate.

3. Results and discussion

3.1. Chromatographic conditions for the optimized HPLC method

Good separations were achieved among the drugs and degradation products using a reversed-phase C18 column (Zorbax XDB C_{18}, 250 mm × 4.6 mm, particle size 5 μm) and employing a mobile phase consisting of 65% methanol and 35% 0.02 M phosphate buffer, pH 5.2 (Fig. 1a). The response was better at the detection wavelength of 238 nm.

3.2. Validation of the developed HPLC method

Data from linearity study are given in Table 1. Strictly linear relationships were obtained when mean area responses ($n = 3$) were plotted against the concentration for the individual drugs. The method was also found to be specific and selective as the peaks for Rp, H as well as 3-RIF and HYD were well resolved (Fig. 1a). All peaks were found to be pure through PDA purity studies. Peak purity parameters for all the peaks are given in Table 2. The percentage recovery in synthetic mixtures of different ratios of drugs are listed in Table 3, which shows that mean recovery (±S.D.) values were 100.15% (±0.70%) and 100.03 (±1.37%) for Rp and H, respectively. The data for intra- and inter-day precision studies are given in Table 4. As evident, percentage relative standard deviation (R.S.D.) values for intra- and inter-day precision were <2.0%. In inter-column studies, a small shift in retention time was observed (Table 5), while peak area values were similar.

Table 5
Inter-column studies

Column	Retention time (min)			
	Rp	H	HYD	3-RIF
Zorbax XDB-C18	18.55	3.10	5.80	7.75
Discovery HS-C18	17.83	2.83	5.30	7.58

3.3. pH-decomposition profile

The pH-decomposition profiles for the combination of Rp and H are shown in Fig. 2. Both the drugs showed bell-shaped pH-

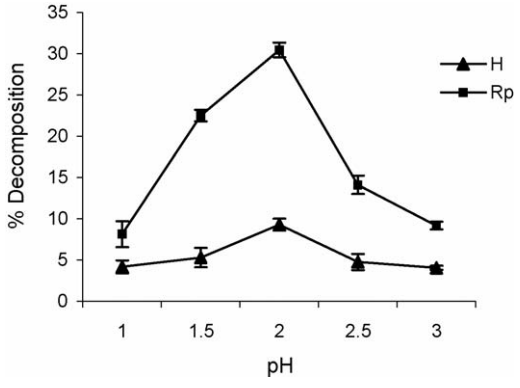

Fig. 2. Profile of pH-dependent decomposition of Rp and H.

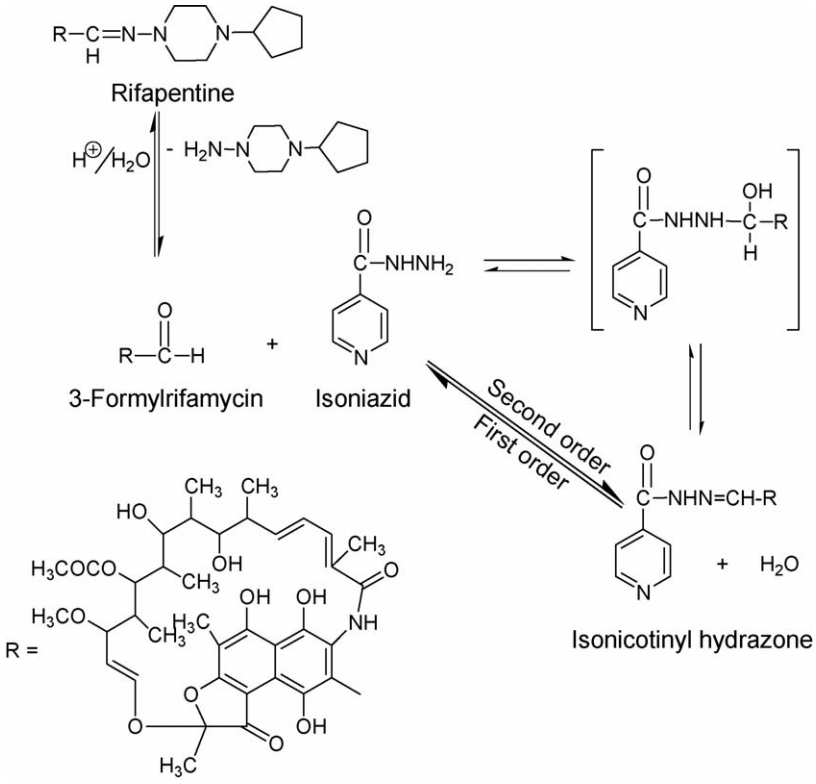

Fig. 3. Mechanistic scheme for the interaction of Rp with H in acidic conditions.

dependent decomposition, with maximum degradation occurring at pH 2, the loss of Rp and H being ∼30 and ∼9%, respectively. The profiles as well as the extent of decomposition were similar to that observed for the combination of R (∼33%) and H (∼10%) [9].

3.4. Mechanism of decomposition

The interaction of Rp and H is proposed to occur by the same mechanism, as outlined earlier for the reaction between R and H [8]. Fig. 2 shows that HYD was the main product, even in case of the Rp and H combination. It is hypothesized that Rp is initially converted to 3-RIF under acid conditions, and the latter reacts subsequently with H to form HYD. The mechanism is outlined in Fig. 3.

4. Conclusions

The present study clearly shows that Rp and H interact in a similar manner to R and H in acid environment. Hence co-administration of the two drugs should be avoided. The FDC products containing the two drugs need to be designed in a manner that chances of interaction between them are reduced to the minimum under stomach acid conditions.

References

[1] K. Duncan, C.E. Barry, Curr. Opin. Micro. 7 (2004) 460–465.
[2] http://www.pharmainfo.net/index2.php?option=com_content&do_pdf=1&id=2269 (accessed on 1.2.06).
[3] E. Miyazaki, R.E. Chaisson, W.R. Bishai, Antimicrob. Agents Chemother. 43 (1999) 2126–2130.
[4] M.E. Temple, M.C. Nahata, Ann. Pharmacother. 33 (1999) 1203–1210.
[5] N. Lounis, A. Bentoucha, C. Truffot-Pernot, R.J. O'Brien, A. Vernon, G. Roscigno, J. Grosset, Antimcrob. Agents Chemother. 45 (2001) 3482–3486.
[6] C.Y. Chan, C. Au-Yeang, W.W. Yew, C.C. Leung, A.F.B. Cheng, Antimicrob. Agents Chemother. 48 (2004) 340–343.
[7] S. Singh, T.T. Mariappan, N. Sharda, B. Singh, Pharm. Pharmacol. Commun. 6 (2000) 491–494.
[8] S. Singh, T.T. Mariappan, N. Sharda, S. Kumar, A.K. Chakraborti, Pharm. Pharmacol. Commun. 6 (2000) 405–410.
[9] R. Sankar, N. Sharda, S. Singh, Drug Dev. Ind. Pharm. 29 (2003) 733–738.