Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

THE EFFECT OF HYPERLIPIDEMIC STATE ON URINE EXPRESSION OF RIFAMPICIN AND ITS ASSOCIATION WITH PULMONARY TUBERCULOSIS-A PROSPECTIVE PILOT STUDY

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Received: 13 June 2014 Revised and Accepted: 15 Jul 2014

ABSTRACT

Objectives: Rifampicin is an antibiotic that imparts an orange-red colour to the urine for a few hours after a dose. About 7% of the administered drug is excreted through the urine. This study compared the urine colour expression of rifampicin in normal and hyperlipidaemic subjects.

Methods: A cohort pilot study was conducted at a tertiary care hospital in which eight subjects with tuberculosis with normal lipid profiles and twelve subjects with deranged lipid profiles were given one tablet of rifampicin (450 mg) in the morning on empty stomach over one month. After one month urine samples was collected at 1,3 and 5 hours. The quantity of rifampicin in urine was estimated using a spectrophotometer. The results were analysed using SPSS version 18.

Results: Twenty patients with pulmonary tuberculosis and on rifampicin 450mg were taken for this study. Their urine was analysed for concentration of rifampicin and correlated to their total cholesterol and triglycerides values. The median total cholesterol in the study group was 345.5 mg/dL whereas in control group was 145.5 mg/dL. The median triglycerides in study group was 436 mg/dL in comparison to control group 113.5 mg/dl. The mean urine concentration of rifampicin in study group at 1hr,3h and 5 h was (47.08 ±17.17),(102.5±134.57) and (63.75±38.027)mcg/dl respectively in comparison to control group (53.13±14.126),(199.38±146.2) and (215.63±164.825) mcg/dL respectively at 1,3 and 5 h.

Conclusion: The study shows that hyperlipidemia may be associated with decreased level of rifampicin, leading to therapeutic failure in tuberculosis patients.

Keywords: EGFR, Total cholesterol, Triglycerides, Urine concentration.

INTRODUCTION

Rifampicin is a bactericidal antibiotic drug belonging to rifamycin group. It is active against number of bacteria like Mycobacterium strains (*M. tuberculosis* and *M. leprae)*, *Listeria species Neisseria gonorrhoeae and Haemophilusinfluenzae.* It is commonly commonly used as a multidrug therapy in treatment of tuberculosis and leprosy. It is also used as monotherapyespecially for as prophylaxis against meningitis. due to quick development of resistance during long treatment of active infections, it is always used in combination with other antibiotics [1].

Rifampicin is an intensely red solid, and a small fraction reaches body fluids and imparts a harmless red-orange colour to the urine (also sweat and tears to a lesser extent) after a drug dose. Food decreases the maximum concentrations in the blood [2] [3] [4].

Rifampicin is easily absorbed from the gastrointestinal tract; its ester functional group is hydrolysed in the bile; and it is catalysed by a high pH and substrate-specific enzyme called esterase [5]. After about 6 hours, almost all of the drug is deacetylated. Even in this deacetylated form, rifampicin is still a potent antibiotic. Only about 7% of the administered drug will be excreted unchanged through the urine, though urinary elimination accounts for only about 30% of the dose of the drug that is excreted. About 60% to 65% is excreted through the faeces [2][3][4].

Rifampicin is a lipophilic drug and is easily absorbed in the gastrointestinal tract but hyperlipidaemia has been found to inhibit the absorption of lipohilic drugs. peak plasma concentrations are not reached as compared to non-hyperlipidaemics [6][7]. Studies have shown that insufficient exposure to unbound rifampicin may lead to the development of resistance. Both the duration and concentration of rifampicin in plasma may be affected due to the hyperlipidemia [8] [9].

The mechanisms by which hyperlipidemia affects pharmacokinetics of drugs are mainly undetermined[7].Hyperlipidemia may decrease the fraction of unbound drug in plasma and/or decrease intrinsic ability of the cytochrome P-450 systems due to excess membrane cholesterol[6][7].

According to a study, it was established that isoniazid, pyrazinamide, ethambutol did not interfere with the a peak plasma concentration of rifampicin; therefore, establishing that the co-administration of any of the antitubercular drugs does not hinder the urine concentration of rifampicin, making urine a reliable marker [10]

A study conducted on pharmacokinetics of rifampicin in diabetics, strongly suggest delayed rifampicin absorption in the diabetic subjects, compared with the non-diabetic subjects. As patients with diabetes are prone to dyslipidaemic conditions it could be extrapolated that hyperlipidaemia could have a confounding effect in the study [8].

Since the effect of hyperlipidemia on drug kinetics is relatively unstudied this research hopes to fulfil that need by evaluating the effect of hyperlipidemia on peak plasma concentration of rifampicin. The effect of the probable decrease in rifampicin concentration is significant as lowering of the peak concentration has an effect on antitubercular activity of rifampicin[9].

An animal study showed that hyperlipidemia significantly lowered the fraction unbound lipophilic drug in plasma by approximately 31%, reinstating the possible clinical significance of the effect of hyperlipidemia on the plasma concentration of rifampicin [7].

Except for cholesterol, all lipids tested (oleic acid, LDLs and HDLs) reduced lipophilic drug cell uptake in a concentration-dependent manner [6]. The antituberculosis activity of rifampicin is decreased by a mild dose reduction from 600 to 450mg [11]. It is well known

that sub-therapeutic plasma concentration of rifampicin leads to development of drug resistance[8][9].

This study will help us to find a non- invasive method of estimation of urine concentration of rifampicin,using its property of orange red discolouration of urine and help us in dosage adjustments of rifampicin in hyperlipidemic patients having decrease plasma concentrations of rifampicin.

Aims and objectives

1. To compare the urine levels of expression of rifampicin in normal and hyperlipidaemic subjects.

2. To correlate hyperlipidaemia and urine levels of rifampicin

MATERIALS AND METHODS

It was a pilot study done in collaboration with department of pharmacology, department of pulmonology and department of biochemistry, it was conducted on twelve patients with tuberculosis with deranged lipid profile All the patients were on rifampicin. and eight patients with tuberculosis, without deranged lipid profile and on rifampicin. The inclusion criteria comprised of patients of either sex,of age group between 18-70years, diagnosed with pulmonary tuberculosis, non-smokers and those who had normal liver function test (LFT) and renal function test (RFT). The exclusion criteria consisted of patients on oral contraceptives, on statins, on ART (Anti-Retroviral Therapy),patients diagnosed with intestinal tuberculosis and with known kidney disease. After taking the clearance from institutional ethical committee, the informed consent was taken from subjects. A total of 24 patients were enrolled for study. They were evaluated by taking a detailed medical history, Physical examination , RFT and LFT were done before they were recruited into the study. Twelve patients had hyperlipidemia and eight patients had no elevated lipid levels and four patients dropped out. The subjects took one tablet of rifampicin (450mg) in the morning on empty stomach for one month. Blood was collected in the morning on an empty stomach and analysed for serum triglycerides and total cholesterol and patients were classified as hyperlipidaemic (cases) and non hyperlipaedemic (controls) according to the latest ATP III classification [12].

Serum creatinine was also calculated for all the patients to check for any defective excretion of urine or drug.

The amount of water intake was standardised in each patient.After one month urine samples were collected at 1, 3 and 5 hours after oral administration of drug, in a sterile container for analysis of rifampicin concentration.

Chemical colour reaction test for urine

The basis of this test of antibiotic in the urine is upon the chemical property of free rifampicin and its desacetylated metabolite [13].Only free rifampicin passes through the kidney and into the urine. Semi-Quantitative and Qualitative Test: control-A set of 8 test tubes with 8ml distilled water and 2 ml chloroform was taken and increasing concentration of 50mcg of rifampicin matrix was added to the test tubes (50mcg to 500mcg). The rifampicin was extracted into the chloroform layer and concentration is measured spectrophotometrically at 540nm.

Test sample-8ml of each of the urine samples of the patients was taken in a test tube to which 2ml of chloroform was added. The rifampicin that gets extracted in the upper chloroform layer is measured spectrophotometrically at 540nm. The absorbance is directly proportional to the concentration of rifampicin in the urine. The absorbance was quantified using the standard values as described above and the concentration of rifampicin was determined [13]. The urine levels of rifampicin were taken as it directly correlated to the plasma levels of rifampicin[14].

RESULTS

Total of twenty four patients were recruited in the study. twelve cases and twelve controls. Four controls dropped out.So only 12 cases and eight controls completed the study.After analysing the urine and blood of the twelve cases and eight controls the following observations were made. The results were analysed using the Mann Whitney test using SPSS 18.0. P value <0.05 was considered significant.

The study was carried out on twenty subjects which included fifteen males and five females.

Table 1: Summary of Patient biochemical parameters (cases and control)

	Number	Minimum	Maximum	Mean± SD
Total cholesterol	20	66	762	277.8±177.371
Triglycerides	20	44	674	311.3±191.264
Serum creatinine	19	0.4	1	0.653 ±0.1679
Estimated glomerular filtration rate	19*	94	186	140.5±27.478

*eGFR was not calculated for one patient on account of old age. *Serum creatinine in* mg/dL.

Table 2: Comparison of lipid profile between both groups

Groups		TC	TG
Cases	N	12	12
	Median	345.5	436
	Interquartile range	231.50, 468.75	311.50, 548.00
	N	8	8
Control	Median	145.5	113.5
	Interquartile range	100.50, 178.25	63.00, 192.25

C= total cholesterol, TG= Triglycerides

Table 3: Urine concentration of rifampicin in cases and controls at 1 hour

	Number	Patients	Mean± S D
Urine concentration at 1 hour (in mcg/dL)	12	Cases	47.08±17.117
	8	Controls	53.13±14.126
	20	Total	49.5±15.886

The mean total cholesterol of the 20 patients was (277.75±177.371) mg/dl and mean serum triglycerides ADD was found to be (311.3±191.264)mg/dL. The mean estimated glomerular filtration rate (egfr) was found to be (140.47±27.478) mL/ min and the minimum filtration rate being 94 mL/ min showed add that none of the patients had a kidney disease. Table 1 The median value of the

serum total cholesterol values in hyper lipidaemics was 345.50mg/dL (231.5, 468.8), whereas for non hyperlipidaemics it was 145.5mg/dL (100.50, 178.25). It was statistically significant according to the Mann Whitney scale.

Table 2. The mean urine concentration of rifampicin of cases at 1 hour was (47.08±17.117)mcg/dl and that of controls was (53.13±14.126)mcg/dl. There was a difference of around (6.05±16.831) mcg/dl. The mean concentration of all the patients was (49.5±15.886) mcg/dl.

Table 4: Urine concentration of rifampicin in cases and controls at 3 hours

	Number	Patients	Mean± S D
Urine concentration at 3 hours(in mcg/dL)	12	Cases	102.5±134.578
	8	Controls	199.38±146.2
	20	Total	141.25±143.984

This table represents the urine concentration of rifampicin at 3 hours. It clearly shows that the cases (102.5±134.578 mcg/dL) had a decreased urine concentration of rifampicin as compared to controls (199.38±146.2 mcg/dL) amounting to a difference of about (96.88±146.881) mcg/dL.

Table 5: Urine concentration of rifampicin in cases and controls at 5 hours

	Number	Patients	Mean± S D
Urine concentration at 5 hours (in mcg/dl)	12	Cases	63.75±38.027
	8	Controls	215.63±164.825
	20	Total	124.5±129.126

At 5 hours the concentration of urine rifampicin was (63.75±38.027) mcg/dL in cases and 215.63±164.825 mcg/dL in controls showing a difference of (151.88±114.18) mcg/dL.

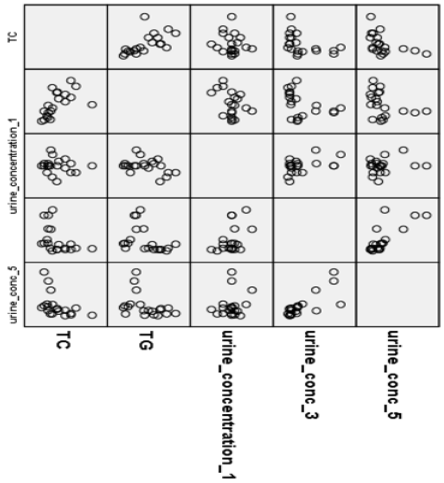

Fig. 1: Scatter diagram showing the lipid profile and urine concentrations of rifampicin.

TC= Total cholesterol (in mg/dL), TG= serum triglycerides (in mg/dL), Urine concentration at 1 hour, 3 hours and 5 hours (in mcg/dL)

The graph represents the urine concentration of rifampicin at 1, 3 and 5 hours and the corresponding serum triglyceride concentration of 20 patients (fig 1*)* shows a significant negative correlation (Evaluated using the Spearman's correlation coefficient(r)). At 1 hour there was a negative correlation between triglycerides and urine rifampicin concentration (r=-0.313, p<0.05).

At 3 hours, there was a significant negative correlation between serum triglyceride and urine rifampicin concentration at that time (r =-0.598, p<0.05)

Similarly, at 5 hours there was a negative correlation between serum triglyceride and urine rifampicin concentration (r=-0.499, p<0.05). This shows that with the increase in triglyceride concentration there is a corresponding decrease in urine concentration of rifampicin. Similarly the total serum cholesterol concentration was compared to the urine concentration of rifampicin at 1, 3 and 5 hours. There was a negative correlation with urine concentration of rifampicin and total cholesterol.

At 1 hour, negative correlation was established between the two variables (r=-0.085, p<0.05). Similarly at 3 hours(r=-0.588, p<0.05) and 5 hours(r=-0.639, p<0.05) it was found to have a statistically significant negative correlation. That is with the increase in serum cholesterol concentration there was a corresponding decrease in urine concentration of rifampicin.

In the time frame (5 hour) considered, peak concentration for controls was reached at 5 hours (215.63±164.825 mcg/dL) and cases at 3 hours (102.5±134.578 mcg/dL)

DISCUSSION

This study was done to detect a decrease in rifampicin absorption in hyperlipidaemics indicated by a decreased urine colour.Urine concentrations are directly proportional to plasma concentrations), which can be used as a marker for whether or not a dose of the drug has been effectively absorbed [14][15][10].Using the urine as a marker avoids the need for any invasive testing of the plasma concentration of rifampicin.

However, excretion of rifampicin in urine does not seem to be associated with any active process [15].This reinstates the reliability of use of urine concentration to analyse plasma levels of rifampicin.

The study was centred centred on the hypotheses that hyperlipidemia affects serum concentration of rifampicin and hence urine concentration (rifampicin follows first order kinetics in vivo). The study results agreed with our hypothesis and there was a negative correlation (spearman correlation coefficent at 1, 3 and 5 hours in relation to triglycerides = -0.313,-0.598 and -0.499 respectively) between the two variables.

With a single 600mg dose, peak serum concentrations of the order of 10µg/mL generally occur 2 hours after administration. The half-life of rifampicin for this dose level is of the order of 2.5 hours. Approximately 80% of rifampicin is transported in blood bound to plasma proteins, mainly albumin. Rifampicin is well distributed, although to a different degree, in the various tissues of the human body [3].

When lipid profile is deranged, the resulting changes may alter the drug transport and delivery system. Unbound drug in the plasma can also associate with lipoproteins, reducing the ability of drugs to freely enter tissues from the bloodstream. Interactions between the drug and the degradation products of the lipoprotein can lead to alterations in intracellular drug transport [6].

A study was undertaken to establish the degree of tissue penetrance and possible frequency of adverse drug reactions, as they reflect directly on the free drug concentration; this could arise from changes in plasma protein concentration which vary with age and diseases like hyperlipidaemia[16]. This further establishes the

clinical significance of determining how various pathologically altered body states might affect the concentration of serum rifampicin and hence the clinical outcome of disease treatment and possible adverse drug reactions.

A study conducted found that the bactericidal action of rifampicin depends on its peak concentration and duration of exposure to drug in vivo. In the results that we have obtained there is a decrease in the peak urine rifampicin concentration (which corresponds to the plasma concentration) of hyperlipidaemic patients as compared to non hyperlipidaemics. From these observations, it could be inferred that the adequate plasma concentration may not be attained leading to decrease in bactericidal activity which could further lead to a development of drug resistant M. tuberculosis strains in the community leading to therapeutic failure [9].

Our study eliminates the confounding factor of renal impairment, (which also reduces urine concentration of rifampicin) by the consideration of eGFR while selecting patients hence removing the bias that could have occurred [17].

The theoretical clinical significance is that a decreased plasma concentration of rifampicin has been shown in several studies to adversely affect cure rates as well as duration to obtain a clinical, microbiological and radiographical cure. This clinical significance of a decreased plasma concentration of rifampicin in a hyerlipidemic state needs to be evaluated further with a larger sample size and patient follow up to document cure rates and delays in cure. Confounding factors including coexistent diabetes and decreased albumin levels associated with a chronic disease process that is, in this case, pulmonary tuberculosis have to be eliminated.

Numerous studies have analysed the rifampicin concentration using mass spectrometry or liquid chromatographic tests which has high accuracy [10].This study aims to use a relatively less accurate but economical and cost effective method, more relevant to the socioeconomic situation present in India, to analyse urine concentration using a spectrophotometer. Though one of the limitations of our study is the enzyme inducing property of rifampicin.Hence blood samples were taken after one month usage of rifampicin.

The experimental design meets only the requirement for a pilot study, but the results show scope for further studies. Future studies should involve a larger sample size, include follow up of patients with tests for culture and sensitivity to evaluate the development of resistant strains and resolve the confounding factors identified by other studies as these confounding factors are very common among Indians and even more in patients with pulmonary tuberculosis.

Further studies need to be done to grade the effect of hyperlipidaemia on plasma rifampicin concentration. This could lead to a change in understanding the dosage of rifampicin in hyperlipidaemics (similar to the dose adjustment in patients with renal failure) and thereby achieve appropriate therapeutic concentration. We intend to further this study by increasing the sample size and microbiological culture and sensitivity of the patients after completion of the therapy and compare the possible delays in duration to obtain cure in cases and controls.

CONCLUSION

Further research is needed to elucidate the effect of hyperlipidemia on the urine concentration of rifampicin, because drug resistance in an emerging problem in India and it is important to identify the definitive cause of the sub-therapeutic concentration.

These researches should aim at accurately grade the effect of hyperlipidemia on plasma concentration of rifampicin, by increasing sample size, useing more accurate methods of analysis, and comparing the plasma and urine concentration of rifampicin, so that it can lead to appropriate dosage adjustment in hyperlipidaemics.

CONFLICT OF INTERESTS

Declared None

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