MICROBIOLOGICAL ASSAY VERSUS SPECTROPHOTOMETRY FOR DETERMINATION OF RIFAMPICIN IN URINE

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ABSTRACT : *A comparative study on the microbiological and spectrophotometric methods for estimation of rifampicin in urine was carried out in 15 individuals. The urinary levels of rifampicin were determined on 2nd, 8th and 15th days at 3 hour, 6 hour and 24 hour samples by the above methods after administration of 600mg rifampicin. The results suggest that the microbiological assay is more sensitive than spectrophotometric method. The difference was highly significant in all the cases by paired t-test. Incidentally it was also noticed that urinary excretion of rifampicin was comparatively more on 15th day.*

INTRODUCTION

Rifampicin (RFMP) is an antibiotic which exerts direct antibacterial activity against a wide variety of micro-organisms including *Mycobacterium leprae* (Rees, *et al* 1970, Binda, *et al* 1971; Shepard *et al* 1974: Levy *et al* 1976). Many techniques are available for the determination of the level of rifampicin in biological fluids. The colorimetric technique of Eidus and Harnanansingh (1969) and the spectrophotometric technique of Sunahara and Nakagawa (1972) are moderately sensitive ones. The microbiological assay technique of Mitchison *et al* (1970) and Dickinson *et al* (1974) using Staphylococcus aureus (NCTC 10702) are reasonably sensitive. The present report highlights the results of the comparative study of the spectrophotometric technique and microbiological assay for estimation of rifampicin in urine.

MATERIALS AND METHODS

Fifteen cases of lepromatous leprosy patients were included in this study. They were preferably untreated cases or cases receiving dapsone as monotherapy. The patients were admitted in the ward and kept under supervision during the period of investigation.

Each patient was given 600 mg Rifampicin along with 100 mg Dapsone daily for 15 consecutive days on empty stomach. The blood and urine

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specimens were collected on the 2nd, 8th and 15th days, at 3hr, 6 hr and 24 hr after the drug administration.

In all the cases, the blood specimens were collected in oxalated tubes and plasma was separated immediately. Drug assays were made either immediately or the plasma aliquots were frozen at —20°C till the assay was done. The urine samples were also kept in the refrigerator upto a maximum of two days, if assays were not carried out immediately.

Rifampicin levels in urine were determined by the spectrophotometric procedure of Sunahara and Nakagawa (1972). A Varian double beam spectrophotometer (Model 634S) was used throughout the experiment. Microbiological assay (Mitchison, *et al* 1971) of Rifampicin in urine and plasma were carried out with Staphylococcus aureus (NCTC 10702). For the microbiological assay, the urine samples were diluted appropriately with distilled water on the basis of the results obtained by spectrophotometry. Rifampicin concentrations of 2,4,6,8 and 10 μg/ml were used to plot the calibration curve. The standard Rifampicin powder was obtained from Lepetit, Milan, Italy. Statistical analysis were done by paired t-test.

RESULTS

The results of the comparative estimations of Rifampicin in urine by spectrophotometry and microbiological assay are presented in Table 1. All the results of microbiological assay were calculated from a calibration curve plotted, log Rifampicin concentration versus square of the zone of inhibition. Levels of Rifampicin in unknown which were too out of range of the calibration curve were calculated from the regression equation of the calibration curve. The results suggests that microbiological assay is more sensitive than spectrophotometric method which is highly significant in all cases by paired t-test.

Another interesting observation made during the course of study is that, there is a comparative increase in the Rifampicin level in urine on 15th day at 6 hr sample both by microbiological assay and spectrophotometric technique. A corresponding decrease was also noticed in the plasma Rifampicin level at 6 hr sample on 15th day (Table 2).

DISCUSSION

Comparisons of the spectrophotometry and the plate diffusion microbiological assay have shown that the two methods yield almost identical results both in standards and in urine samples containing rifampicin. How-

TABLE 1 : **Rifampicin in urine, μg/ml (n=15)**

DAY OF INVESTIGATION	TIME INTERVALS	MICROBIOLOGICAL ASSAY (MEAN±S.E.*)	SPECTROPHOTOMETRY (MEAN±S.E.)	LEVEL OF SIGNIFICANCE
2nd day	3 hour	265.77±41.33	206.96±35.64	P<0.005
	6 hour	251.96±32.08	211.64±26.57	P<0.05
	24 hour	57.05±14.02	38.77±10.65	P<0.001
8th day	3 hour	156.52±29.15	122.40±23.66	P<0.05
	6 hour	180.25±13.02	146.92±15.12	P<0.001
	24 hour	37.79± 7.41	28.73± 6.50	P<0.001
15th day	3 hour	154.31±26.59	117.78±17.84	P<0.001
	6 hour	256.41±58.02	215.04±43.18	P<0.005
	24 hour	35.85± 5.68	21.72± 4.56	P<0.001

*Standard error.

ever with regard to sensitivity, the microbiological assay is more sensitive than spectrophotometry which is evident from Table 1. The results are highly significant in all cases by paired t-test. At the same time it is interesting to note that the microbiological assay is significantly more sensitive for detecting lower concentrations of Rifampicin. This is clear from all 24th hour urine samples, in which the drug levels are very low as compared to 3rd hour and 6th hour samples. In all the cases of 24 hr. samples, the microbiological assay exhibit higher drug levels which are statistically highly significant (P<0.001).

TABLE 2: **Rifampicin in plasma, μg/ml (Mean±S.E.) n=15**

DAY OF INVESTIGATION	TIME INTERVALS		
	3 HRS.	6 HRS.	24 HRS.
2nd day	10.08±0.63	9.38±0.58	0.97±0.49
8th day	9.52±0.56	8.19±0.61	1.04±0.63
15th day	8.88±0.62	6.56±0.57	0.81±0.38

The spectrophotometric method employed in this study for the estimation of rifampicin in urine or plasma is sensitive upto 1μg/ml. It is very easy to perform, inexpensive and time saving when compared to the microbiological assay which is tedious and time consuming. The particular strain (NCTC 10702) involved in the microbiological assay is a highly potent one and resistant to most of the antibiotics so far invented. So extreme care should be taken while handling this dangerous strain. But the microbiological assay has got its own advantage over spectrophotometry as it requires only a very small amount of sample even less than 10 μl which is highly helpful for estimation of the drug in plasma and cerebrospinal fluid.

In microbiological assay, the zone of inhibition will be influenced by diffusibility and concentration of the drug. The nature and composition of the medium, the thickness of the medium, presence of inhibitory or stimulatory substances, pH value of the medium and time of incubation can also affect the zone size. While detecting Rifampicin in urine or plasma by microbiological assay, all the above factors should be taken into account.

During estimation of Rifampicin in plasma, the proteins as well as other nutrients present in plasma will serve as a stimulant to bacterial growth. As a part of the study conducted, this was observed in the 24th hour samples where in most of the cases the drug was totally absent. There was a very rich growth of bacteria surrounding the filter paper disc where there was no zone of inhibition.

Due to the reasons discussed above, the values obtained by microbiological assay for a particular sample may not be accurately reproduced necessitating a need for standard control for the purpose of comparison. Spectrophotometry gives consistent and reproducible results but less sensitive when compared to microbiological assay. Regarding the estimation of Rifampicin, the spectrophotometric technique has the advantage that the Rifampicin itself is a coloured compound and the absorption is measured directly after extracting the drug in a suitable organic solvent. The procedure is so simple that the whole work can be finished within 10 minutes.

It may be of interest to mention that a similar comparison between chemical (HPLC) and microbiological assay for the determination of Rifampicin in plasma was made by Peters *et al* (1977). The authors have reported almost identical results with the two methods. However the spectro-

photometric methods are inadequate to distinguish the Rifampicin metabolites, because the absorption spectra of Rifampicin and 25-desacetylrifampicin are nearly identical (475nm). The HPLC methods (Lecaillon, *et al* 1978, Gidoh, *et al* 1981) can distinguish Rifampicin from its metabolites specifically.

To conclude, a standardised microbiological assay will be the better one for estimation of Rifampicin for research purpose and the spectrophotometric method can be used for routine work.

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