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SIMPLE QUALITATIVE TESTS FOR RIFAMPICIN IN URINE

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Summary : Simple qualitative tests for rifampicin in urine, involving extraction with chloroform as well as with benzene were investigated on 50 patients. A positive test result was obtained in 98-100% of the specimens between 3 and 11 hours after drug administration by both methods. All specimens collected prior to administration of rifampicin as also specimens collected after ingestion of other anti-tuberculosis drugs were negative by both methods. However, the chloroform extraction method yielded positive reactions in 26% of 27 specimens collected after the administration of clofazimine.

Introduction

An important factor in the treatment of tuberculous patients is the regularity with which they take medicaments given to them for self-administration. A direct and reliable method of monitoring drug intake involves testing for the drugs or their metabolites in the urine. (Hobby, 1964; Rao et al, 1965, 1967; Venkataraman et al 1967). A simple chemical test for rifampicin and its desacetyl derivative in the urine has been described by Eidus and Harnanansingh (1969). A more sensitive microbiological assay procedure reported by Mitchison et al (1970) takes 24 hours and requires technical competence. This paper describes a controlled comparison of two extraction procedures, namely a simplification of the method of Eidus and Harnanansingh (1969) and another method involving benzene extraction on serial collections of urine after administration of rifampicin.

Material and Methods

Patients

A total of 785 specimens of urine was collected from the following three categories of patients attending the Centre :

Group 1 : Fifty patients receiving intermittent chemotherapy (once, twice or thrice weekly) which was administered under full supervision by the clinic staff with rifampicin (approximately 15 mg/kg) *plus* isoniazid and, in some cases, streptomycin and pyrazinamide also. Each patient was asked to empty the the bladder (O-hour) and a specimen of urine was collected. He was then administered his scheduled chemotherapy (with rifampicin) on an empty stomach. Specimens of urine were collected at hourly intervals from 1 to 12 hours after drug administration. A specimen was also collected in the clinic the next day at 24 hours. The mean body-weight of the patients was 42.6 kg (range 24.3-54.8 kg) and the mean dosage 15.4 mg/kg (range 12.9-18.5 mg/kg).

Group 2: Twenty-nine patients receiving supervised chemotherapy with two or more drugs other than rifampicin were included as control subjects. The drugs included streptomycin, isoniazid, pyrazinamide, cycloserine, ethionamide and ethambutol. From each of these patients two specimens of urine were collected, one prior to the administration of drugs (O-hour) and the other three hours after drug administration.

Group 3 : As an additional control, 27 patients receiving chemotherapy for leprosy with clofazimine (100 mg) and dapsone were studied. From each of these patients, one urine specimen was collected three hours after the administration of the drugs.

The specimens were stored in the laboratory at 5-10°C for not more than 72 hours prior to testing.

Test Procedures

(a) Chloroform extraction : Five ml of urine was taken in a test tube and an equal volume of chloroform was added. The mixture was shaken by hand vigorously for 10-15 seconds and allowed to separate. By this process, rifampicin, if present, was extracted into the organic layer and depending on the concentration, produced a faint pink to deep orange-red colour. Tubes showing no coloration of the solvent layer were reported as negative.

(b) Benzene extraction : The procedure and interpretation were the same as above, except that the chloroform was substituted by benzene.

All specimens were randomized and coded; thus the readers were unaware of the source

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or other details of any individual specimen. Ail the tests were read independently by three readers and the majority opinion was considered for analysis.

Result

O-hour specimens : All of 79 specimens of urine collected prior to the administration of drugs (Groups I and II) were reported as negative by all three readers by both chloroform and benzene extraction methods. Thus there was no incidence of false positive reactions.

Urine specimens after rifampicin administration : The test results, by both methods, for 50 patients who had received rifampicin are set out in Table 1.

Fourteen patients (28%) yielded a positive test result at I hour after the administration of rifampicin by both methods. The proportion of positive results at 2 hours was 88% by the chloroform method and 90% by the benzene method. A positive result was obtained in 98-100% of the specimens between 3 and 11 hours after drug administration by both methods, during which period only 2 specimens out of the 450 tested by each method were negative. The proportions at 12 hours were 94% by the chloroform and 96% by the benzene method. At 24 hours, only 8% of the specimens yielded positive reactions by each method. Of these, 3 specimens were positive by both methods while 1 each was positive by one method only.

A total of 6 specimens (one each at 2, 3, 11 and 12 hours and 2 at 24 hours) yielded discrepant results Of these, 4 were positive by the benzene but negative by the chloroform method, while 2 specimens yielded converse results ($P > 0.2$).

Urine specimens after the administration of drugs other than rifampicin : All 29 specimens collected from tuberculous patients 3 hours after the administration of drugs other than rifampicin were reported as negative by all three readers by both the chloroform and the benzene method. Thus, the tests were specific for rifampicin.

Urine specimens after administration of clofazimine : Out of 27 urine specimens collec-

Table 1

Percentage of patients with positive test results by the chloroform and benzene methods after administration of rifampicin (15 mg/kg)

Hour of collection	Chloroform method	Benzene method
0	0	0
1	28	28
2	88	90
3	98	100
4	100	100
5	100	100
6	100	100
7	100	100
8	100	100
9	100	100
10	98	98
11	100	98
12	94	96
24	8*	8*
No. of Patients	50	50

* Three specimens were positive by both methods and one each by one method only.

ted from leprosy patients three hours after the administration of clofazimine (and dapsone), 7 specimens (26%) gave a positive reaction by the chloroform method. No specimen was reported positive by the benzene method by any of the three readers, a significant difference (P=0.01).

Reader variation : Of 700 tests (by each method) from patients receiving rifampicin, one reader disagreed with the other two on five occasions by each of the two methods. In the remaining 695 tests, identical results were reported by all three readers.

In the case of the 27 urine specimens containing clofazimine, on 11 occasions one reader disagreed with the other two by the chloroform method, while there was no disagreement on any specimen by the benzene method.

Discussion

Tests for the detection of rifampicin in urine have been described using simple extraction of the urine with either chloroform or benzene. Both tests gave 98-100% positive reactions between 3 and 11 hours after the administration of approximately 15 mg/kg body-weight of rifampicin in this study.

The tests described by Eidus and Harnanansingh (1969) yielded 100% positive reactions from two to eight hours after 300 mg dose using urine and chloroform in the ratio of 5 : 1. The microbiological method of Mitchison et al (1970) yielded positive results upto 12 hours after the conventional dose of 600 mg. This test, though simple, can be performed only in laboratories equipped to carry out microbiological work, and further, it requires at least 24 hours before the results are available.

The present method using chloroform is a simplification of the Eidus and Harnanansingh (1969) procedure and can be performed even under field conditions. The tests are simple, specific and sensitive, and no positive reactions were obtained among the O-hour specimens or among specimens from patients receiving other anti-tuberculous drugs with either test. Clofazimine, which also yields a reddish coloration of the urine similar to rifampicin, gave positive reactions only with the chloroform method. Further, chloroform has a tendency to form an emulsion with the aqueous layer, necessitating centrifugation on some occasions (Eidus and Harnanansingh, 1969). Also, chloroform being heavy, forms the lower layer, and hence, there is a reflection of the upper aqueous layer, especially in dark-coloured urines, which might interfere with the reading. Finally, chloroform is twice as expensive as benzene. Thus, the benzene method appears to be superior to the chloroform extraction procedure. The benzene extraction procedure in this study yielded 98-100% positive results for rifampicin in urine between 3 and 11 hours after oral administration of the drug. In clinical practice, this means that if the drug is self-administered in the morning, on rising, or a supervised dose is given on attendance at a clinic, then positive results could be expected at any time between 3 hours after the administration of drug to midday or late in the evening.

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