Indian J Med Res 63, 2, February 1975. pp. 323-328

Classification of Subjects as Slow or Rapid Inactivators of Isoniazid Oral Administration of a Slow-release. Preparation of Isoniazid and Determination of the Ratio of Acetyisoniazid to Isoniazid in Urine

S. Kailasam, Chandra Immanuel, N.G.K. Nair, S. Radhakrishna and S.P. Tripathy

Tuberculosis Chemotherapy Centre, Madras-600031.

Received for publication, April 24, 1974.

A simple method for classifying subjects as slow or rapid inactivators of isoniazid has beenevaluated on large numbers of patients. The method consists of determining the ratio of acetylisoniazid to isoniazid in a 24-26 h. urine collection following the oral administration of a slow-release preparation of isoniazid 30 mg./kg. body-weight. In a group of 101 patients, there was 100 per cent agreement between the classification based on this method and that based on a standard method, consisting of estimation of the serum isoniazid concentration, 4½ h. after an intramuscular dose of ordinary isoniazid 3 mg./kg. body-weight. Subsequent studies in other patients have confirmed that the method is efficient, and demonstrated that the classification is highly reproducible.

Introduction

Methods for classifying subjects as slow or rapid inactivators of isoniazid on the basis of the ratio of acetylisoniazid to isoniazid in urine following an intramuscular or intravenous dose of isoniazid have been described by Eidus **et al.** (1971), Venkataraman *et al.* (1972) and Ellard *et al.* (1973b). Obviously, it would be far more convenient, especially under field conditions, to administer the drug orally rather than intramuscularly or intravenously. Recently, Ellard *et al* (1973a) reported, from their investigations on 20 patient-volunteers, that subjects could be conveniently classified as slow or rapid inactivators by determining this ratio in a 24-25 h. urine collection following the oral administration of a slow-release preparation of isoniazid (matrix isoniazid, Smith and Nephew HS 82) in a dose of 30 mg./kg. body-weight. This paper reports, on considerably larger numbers of patients, the findings of similar investigations with two doses of matrix isoniazid (30 mg./kg. and 37.5 mg./kg. body-weight).

Material and Methods

Patients: Adult tuberculous patients under treatment at the local Government Tuberculosis Sanatorium, or under follow-up at the Tuberculosis Chemotherapy

Centre, were included in this investigation. The mean weight was 42 kg. for the former and 43 kg. for the latter patients, the ranges being 25-60 kg. and 27-67 kg. respectively.

Test procedure : On the day of the test, a specimen of urine was collected from the patient and tested for acetylisoniazid by the method of Eidus and Hamilton (1964) (for patients under treatment, all antituberculosis drugs were withheld for at least 24 h. before the test). After verifying that the result was negative, the dose of matrix isoniazid was orally administered in the form of 300 mg. tablets (together with pyridoxine 20 mg.), usually before breakfast. Next day, at 24 h., after the dose, the patient was instructed to empty his bladder. At 26 h., a urine specimen was collected from him, and the ratio of acetylisoniazid to isoniazid was determined.

Estimation of acetylisoniazid and isoniazid : Acetylisoniazid was estimated by the direct method of Venkataraman **et al.** (1968), without prior oxidation using potassium permanganate and without the reverse blank procedure*. Isoniazid was estimated by the method of Rao **et al.** (1971).

Investigations : The first investigation was in 49 known slow inactivators and 52 known rapid inactivators, who were under follow-up at this Centre. The isoniazid inactivation status of these patients had been determined previously by a standard method, consisting of the estimation of the serum isoniazid concentration 4 h. after an intramuscular dose of ordinary isoniazid 3 mg./kg. body-weight (Tuberculosis Chemotherapy Centre, Madras, 1973)]. A dose of matrix isoniazid 30 mg./kg. body-weight was administered to these patients, and. the 24-26 h. urine collections randomized before they were processed.

Subsequent investigations were undertaken in patients under treatment at the sanatorium, matrix isoniazid doses of 30 mg./kg. and 37.5 mg./kg. body-weight being administered to 97 and 193 patients, respectively.

Results

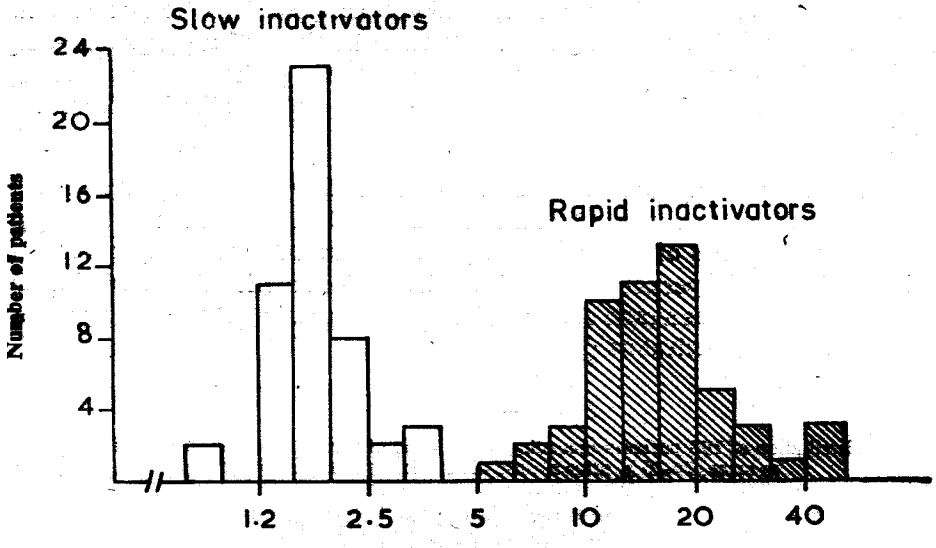
Findings with the 30 mg/kg. dose : The findings of the investigation in known slow and known rapid inactivators are illustrated in Graph 1. There was complete agreement between the 'known' classification and that based on the ratio. Thus, all the slow inactivators had a ratio of less than 4 (highest value 3.4), whereas all the rapid inactivators had a ratio of more than 5 (lowest value 5.5). The mean ratio was 1.8 for the slow inactivators and 15.7 for the rapid inactivators ($P<10^{-5}$).

Graph 2 illustrates the findings in another series of 97 patients, whose isoniazid inactivation status had not been 'previously determined. The distribution clearly demonstrates the presence of two groups of patients, and suggests that the criterion for a rapid inactivator could be a ratio of 5 or more. Accordingly, 45 patients were

* Our subsequent experiencc indicates that it would be better to employ the extraction method of Sarma **et al.** (1974), as the direct method fails if sugar (> 25 mg./ml.) is present in the urine; fresh criteria would, however, have to be derived for classification purposes.

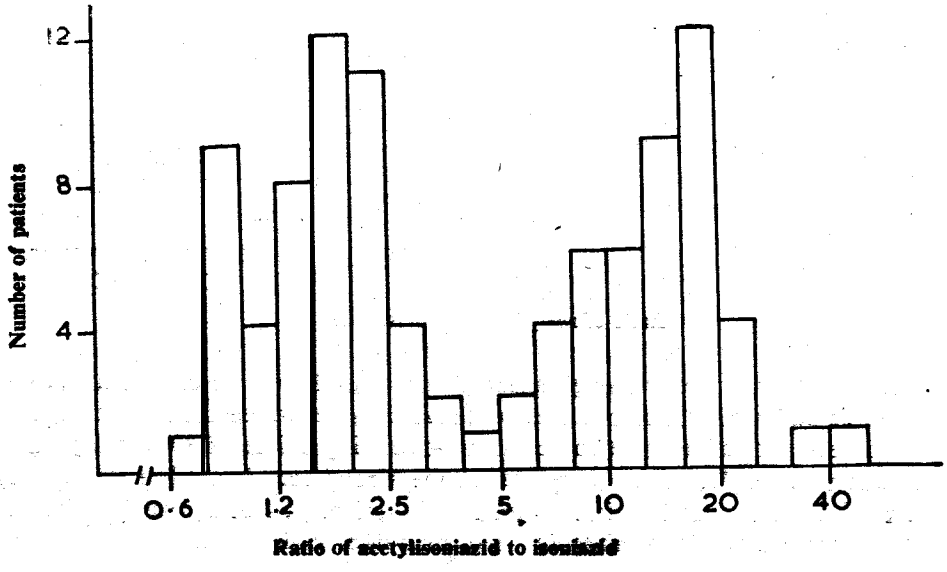
Graph 1

Distribution of 49 known slow inactivators and 52 known rapid inactivators according to the ratio of acetylisoniazid to isoniazid in urine, 24-26 h. after a dose of matrix isonfazid 30 mg./kg.



Graph 2

Distribution of 97 patients according to the ratio of acetylisoniazid to isoniazid in urine, 24-26 h. after a dose of matrix isoniazid 30 mg./kg.



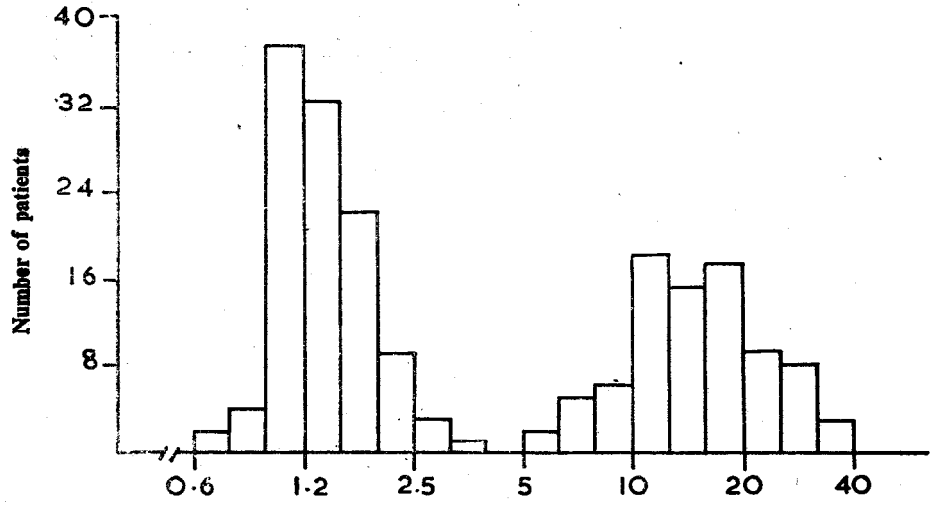
classified as rapid and 52 as slow inactivators. As in the first investigation, the mean ratio for the rapid inactivators (13.2) was considerably higher than that for the slow inactivators (1.6), the difference being highly significant ($P < 10^{-5}$).

These findings clearly indicate that the ratio of acetylisoniazid to isoniazid in a 24-26 h. urine collection following a dose of matrix isoniazid 30 mg./kg. body-weight would be a highly satisfactory measure for classifying patients as slow or rapid inactivators, and that a ratio of 5 or more could be taken as the criterion for a rapid inactivator.

Findings with the 37.5 mg./kg. dose : The distribution of 193 patients according to the ratio of acetylisoniazid to isoniazid is illustrated in Graph 3. As with the 30 mg./kg. dose, two distinct groups of patients, namely 110 slow inactivators (57 per cent) and 83 rapid inactivators (43 per cent), can be identified. The criterion for a rapid inactivator could again be taken as a ratio of 5 or more. The mean ratio was 14.7 for the rapid and 1.4 for the slow inactivators ($P<10^{-5}$).

Graph 3

Distribution of 193 patients according to the ratio of acetylisoniazid to isoniazid in urine, 24-26 h. after a dose of matrix isoniazid 37.5 mg./kg.



Consistency of the test in classifying patients as slow inactivators or rapid inactivators : There were 55 patients in the second and third investigations whose isoniazid inactivation status had been determined on two occasions-following a dose of 30 mg./kg. on the first occasion and a dose of 37.5 mg./kg. in the second occasion. The classification on the two occasions was identical on *all* the 55 patients, 34 being classified as slow inactivators and 21 as rapid inactivators. [An analysis of variance (not tabulated here)

of the ratios obtained on the 2 occasions showed that there were consistent differences among the 34 slow inactivators, and among the 21 rapid inactivators].

Association between serum isoniazid concentration and ratio of acetylisoniazid to isoniazid : There was no association between the serum isoniazid concentration and the ratio of acetylisoniazid to isoniazid in the 49 slow inactivators in the first investigation. with the 30 mg./kg. dose. However, among the 52 rapid inactivators, the mean ratio was 20.8 for 9 patients with a serum isoniazid concentration of less than 0.20 µg./ml. compared with 14.8 for 43 patients with a concentration of 0.20-0.89 µg./ml. This difference is significant (P=0.02), and indicates that there is heterogeneity among the rapid inactivators.

Very rapid inactivators : In the second and third investigations, a total of 235 patients-was investigated, and 128 (54 per cent) classified as slow inactivators (ratio <5) and 107 (46 per cent) as rapid inactivators (ratio > 5). Among the latter, 14 had very -high ratios (> 25) and were probably homozygous rapid inactivators. According to the Hardy-Weinberg law, the expected number of homozygous rapid inactivators in this population is 16.

Concluding remarks

The method described in this paper for classifying subjects as slow or rapid inactivators of isoniazid is efficient and yields highly consistent findings. The method is also convenient, for *(a)* the drug is administered orally, rather than intramuscularly or intravenously, and *(b)* no collection of blood is necessary, unlike the case with several of the conventional methods for determining isoniazid inactivation status. As both the doses investigated yielded efficient classification, the lower dose (30 mg./kg.) is to be preferred.

Acknowledgment

We are grateful to Dr. G.A. Ellard for advice in the planning stages, to Dr. K.V. Krishnaswami (Medical Superintendent, Government Tuberculosis Sanatorium) for his co-operation, to Mrs. Jeyalakshmi Monga for valuable assistance in the organisation of the studies, to Mrs. S. Joseph Mathew for the serum isoniazid assays, and to Dr. G.R. Sarma for valuable comments.

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