Inactivation of isoniazid by Canadian Eskimos and Indians

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Summary: Phenotyping for isoniazid inactivation in Canadian Eskimos and Indians showed that the former are all fast acetylators, while only 63.4% of the Indians examined belonged to the same group. Further studies are suggested to confirm this initial finding.

During the investigation metabolic studies were carried out to devise a reliable urine test for phenotyping of isoniazid inactivators, to replace the fall-off technique which required venipunctures. The simplicity of the new urine test makes it suitable for mass examinations.

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Isoniazid (INH), the most potent antituberculous drug, is acetylated and inactivated in the liver by acetyltransferase enzyme. The rate of acetylation is genetically controlled; it exhibits a bimodal pattern. Based on the elimination rate of free INH from the blood, individuals may be divided into slow and fast inactivators. Slow inactivation is inherited as a homozygous autosomal recessive trait, while fast inactivation is due to either a homo- or heterozygous dominant gene. Japanese Thais, Koreans,[1] Saame Lapps[2] and Eskimos[3] are mainly fast acetylators, while the majority of Caucasians are slow inactivators.[4-6]

Fast inactivation is of little consequence in daily treatment, particularly with triple regimen;[7] however, in intermittent chemotherapy or irregular self administration of the drugs, it may contribute to treatment failures. Therefore, investigation of INH metabolism as well as the introduction of a simple urine test for phenotyping of INH inactivators on a large scale are of great importance in the planning of an effective domiciliary treatment program particularly for the population of the Canadian Far North and for fast inactivators in general.

A study was carried out in co-operation with the Chronic Disease Control and the Northern Medical Research Unit at the Charles Camsell Hospital, Edmonton, with the following objectives:—

a) To determine the INH inactivation rate in Canadian Eskimos and Indians.

b) To establish correlation between INH inactivation (estimated in plasma specimens by the fall-off technique) and excretion of INH and its metabolite in urine in order to devise a simple urine test for mass phenotyping, avoiding thereby repeated venipunctures.

c) To assess the need for changes in INH dosage and frequency of administration or for different drug preparation (with protracted absorption rate) in order to compensate for fast acetylation.

Procedure

Twenty-six Eskimos and 46 Indian patients received 8 mg./kg. INH intramuscularly. Ten ml. blood was collected in heparinized vacutainers before, and two and four hours after drug administration. The plasma was separated by centrifugation, frozen immediately, and stored at −20°C until examination. The free INH concentrations of the two- and four-hour plasma samples were estimated in μg./ml. The values obtained

were plotted on a logarithmic scale against the time in minutes registered on a linear scale and the half-life of the INH determined graphically.[8]

In 60 patients, urine specimens were also collected before drug administration, and two, four, six, eight and 10 hours thereafter. On each occasion, the patients emptied the bladder completely. The total volume of all samples voided was measured and a portion of each was stored in deep freeze at −20°C. In these specimens the concentrations (μg./ml.) of free INH, acetylisoniazid (Ac-INH) and isonicotinic acid (INA) were estimated and the two-hourly excretion of these compounds determined in mg. The results were then expressed as free INH, and the recovery rate of INH and its metabolite calculated in proportion to the dose administered.

Methods

For estimation of free INH in plasma, the method of Eidus and Harnanansingh[9] was used with a minor modification for urine specimens.[10] Determination of acetylisoniazid is based on colour reaction, described by Eidus and Hamilton,[11] and modified to a quantitative method by Venkataraman, Eidus and Tripathy.[12] Isonicotinic acid was estimated by the method of Nielsch.[13] During the entire study, all chemical determinations were carried out in duplicate.

Results

Patients producing half-life values (t ½) of 160 minutes or over may be regarded as slow inactivators, while the upper limit of fast acetylators is set at 110 minutes. Out of 72 patients examined in this study, only 20 were slow inactivators, with an average half-life of 288.4 minutes. The larger group (52) were fast acetylators, exhibiting a t ½ of 81.2 minutes. Three patients of the latter group, with a half-life of 111, 128 and 134 minutes, could perhaps be classified as intermediate. Such a distinction was, however, not made in this study, and for practical reasons they were included in the group of fast acetylators.

Among the 26 Eskimos examined, six were of Delta

Table I

INH inactivation rates in various Eskimo and Indian groups

Ethnic origin	Tribe	Total	Fast	av. t ½	Slow	av. t ½
a) ESKIMO						
a) Delta and Alaskan		6	6	70.2	—	—
b) Central and Eastern		20	20	80.6	—	—
Total:		26	26	78.2	—	—
%			100			
b) INDIAN						
a) Algonquin-speaking	Northern Cree	11	5	79.0	6	306.2
	Prairie Cree	3	2	112.0	1	456.0
b) Athabascan-speaking	Loucheux Indian	4	1	95.0	3	231.0
	Slave Indian	6	2	94.0	4	251.3
	Southern Yukon Indian (Little Stick Indian)	8	4	95.5	4	291.8
	Tlingit	2	1	111.0	1	375.0
	Yellowknife Band Indian	2	2	83.5	—	—
	Dogrib Indian	2	2	74.0	—	—
	Chippewyan Indian	8	7	68.9	1	234.0
Total:		46	26	84.3	20	288.4
%			56.5%		43.5%	

Table II

Average excretion rate of INH and its metabolite during 10-hour period in urine specimens collected every two hours following drug administration.

Phenotype	Compound	Collection time in Hours 2	4	6	8	10	Total excretion rate in 10 hours
Fast	INH	6.86	3.36	1.36	0.59	0.24	12.41
	Ac-INH	10.03	11.61	9.34	6.52	3.88	41.38
	INA	3.95	5.83	4.83	3.35	2.18	20.14
Total 2-hourly excretion rate:		20.84	20.80	15.53	10.46	6.30	73.93
Slow	INH	7.10	7.66	5.55	4.28	2.52	27.11
	Ac-INH	3.28	4.58	4.05	4.08	2.64	18.63
	INA	1.32	2.39	2.02	2.40	1.73	9.86
Total 2-hourly excretion rate:		11.70	14.63	11.62	10.76	6.89	55.60

origin and the remainder belonged to the Central and Eastern Eskimos (Table Ia). Except for one patient with a half-life of 134 minutes, all Eskimos produced values under 110 minutes, exhibiting a t ½ average of 78.2 minutes. The patient producing the high half-life value (134') had, according to an unconfirmed source, a Caucasian father. One of the patient's siblings had Caucasian features but the patient himself did not show such features.

Out of 46 Indians, 26 (56.5%) were fast, and 20 (43.5%) were slow inactivators (Table Ib). Ib). If we exclude from these calculations five Metis, all of them slow inactivators, the ratio is changed to 63.4% fast and 36.6% slow inactivators. The patients investigated can probably be divided into two major groups: Indians living along the Mackenzie fur trader's route, with a considerable white admixture. To this group belong the Algonquin-speaking Northern Cree and the Athabascan, and the Tlingit-speaking Southern Yukon Indians and Slave Indians from the Mackenzie. The second group, the Yellowknife Band, Dogrib and Chippewyan Indians, were relatively untouched until about 1930, living away from the main traffic and trade routes. In 12 patients belonging to the latter tribes, only one slow acetylator was found, while the rate of slow activators was considerably higher among the rest of the Indians. After deduction of the five Metis and 12 members of the second group, the proportion becomes 15 (51.7%) fast versus 14 (48.3%) slow inactivators. It seems that Caucasian mixture plays an important role in the outcome of the phenotyping. The number of patients is far too small and the investigation is lacking in detailed genetic markers and kinship studies, therefore no final conclusions can be drawn. Further investigations should follow this pilot study to confirm the present observations.

The results of the metabolic studies are shown in Table II. They demonstrate the two-hourly average excretion rate for INH and its metabolite in fast and slow inactivators. It may be noted that free INH rapidly decreases in urine specimens of fast acetylators, and only 12.41% of the dose administered is excreted unaltered during a 10-hour collection period. The excretion rate of free INH is two and one-half times higher in slow inactivators, namely, 27.11%. On the other hand, the average recovery rates of Ac-INH and INA in the same period are twice as high in fast as in slow inactivators. These differences are statistically significant at the P>.001 level.

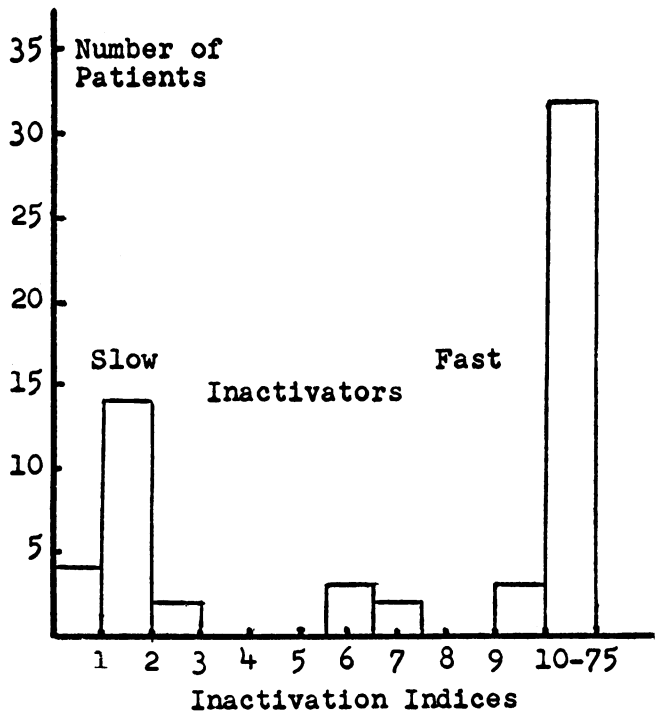

FIG. 1—Inactivation indices of urine specimens collected from 40 fast and 20 slow inactivators in the six- to eight-hour period following drug administration.

In fast inactivators INH is rapidly metabolized and the excretion rate of the derivatives, particularly Ac-INH, is greater than that of the original compound. Therefore, six hours following drug administration 57.17% of the dose given is already excreted in the urine, while only 37.95% could be recovered in slow inactivators. During the 10-hour period the excretion of INH increases in fast acetylators to 73.93% in comparison with the 55.60% recovery rate in slow metabolizers. In the latter group approximately 50% of the recovered drug is free INH. In slow inactivators the metabolic transformation of INH occurs gradually, resulting in even excretion of the derivatives in the four-, six- and eight-hour collection periods.

In both groups of inactivators the rate of isonicotinic acid excretion closely follows that of Ac-INH, and is approximately half of it in the four-, six- and eight-hour collection periods. According to Peters, Miller and Brown,[15] the primary metabolic change, depending on the inactivator status, is acetylation of isoniazid. INA is a product of Ac-INH as a secondary reaction. The high excretion of INA in fast inactivators is due to the greater availability of the acetylated compound for conversion.

Earlier, an attempt was made to utilize the recovery rate of free INH in pooled overnight urine specimens for phenotyping of inactivators.[16-18] A repetition of the method in this laboratory showed that the recommended urine test is not suitable for phenotyping INH acetylators, as it does not produce a sharp division between the two groups of inactivators and overlapping values frequently occur.

Isoniazid and Ac-INH are present in reverse proportion in urine specimens of slow and fast acetylators, therefore it is more practical to use the fraction of Ac-INH versus INH concentration for phenotyping of inactivators. The proportion between the concentrations (μg./ml.) of Ac-INH and free INH in urine is termed by us "Inactivation Index". An average of these indices, calculated from the urine specimens of 40 fast and 20 slow inactivators, collected in two-hourly intervals, is shown in Table III. It may be noted that the indices of slow inactivators remained fairly even, increasing from 0.63 at 2-hour, to 1.60 at 10-hour collection times. The indices of fast inactivators increased rapidly with every collection period, from 2.14 at 2 hours to over 50 at 10 hours. In urine specimens collected between six and eight hours the average indices of fast acetylators were 19.24 times higher than those of slow inactivators.

Fig. 1 demonstrates inactivation indices of 60 patients determined in urine specimens collected in the six-hour to eight-hour period after intramuscular administration of 8

Table III

Average inactivation indices of 40 fast and 20 slow acetylators at two, four, six, eight and 10 hours after intramuscular administration of 8 mg./kg. isoniazid

	Collection time in hours				
Phenotype	2	4	6	8	10
Fast	2.14	5.45	13.10	25.98	>50
Slow	0.63	0.86	1.05	1.35	1.60

mg./kg. INH. The slow inactivators produced indices of 3 and under, while fast acetylators exhibited high indices. However, five patients in the latter group produced somewhat lower indices than the majority (5.5 to 7.5). Three of the above patients, with indices from 5.5 to 6.5, also exhibited longer plasma half-life values than the rest of the fast acetylators, namely 111, 128 and 134 min. The remaining two patients with indices between 6.5 and 7.5 showed plasma half-lives of 96 and 98 min. The rest of the fast acetylators (35), with indices between nine and 75, showed an average INH half-life of 75.6 min. The indices of the urine specimens seem to follow closely the half-life values obtained by the fall-off technique. A comparison of the t ½ values with the inactivation indices of the same patient shows that, with a decrease of the half-life values, the indices are increasing in reverse proportion (Fig. 2). This means that there is an association between t ½ and indices and that rapid elimination from the blood corresponds with high indices. The lowest indices in the group labelled as fast inactivators were produced by three patients with t ½ values between 111 and 134 min. while the highest indices are exhibited by 15 patients with a half-life between 60 and 69 min. This remarkable agreement between index and half-life values confirms that urine tests can be employed for phenotyping of INH inactivators.

While inactivation indices provide a distinct division between the two groups of inactivators, overlapping values were obtained when the recovery rate of free INH had been calculated in the same urine samples. In the 60 urine specimens discussed above, four slow inactivators exhibited a recovery rate which would place them into the group of fast acetylators, while one fast acetylator produced a recovery rate identical to that of slow acetylators. Thus, five erroneous groupings would be received if only the excretion rate of free INH were taken into consideration.

Discussion

The inactivation of INH was studied in Canadian Eskimos and Indians. It was found that all 26 Eskimos investigated inactivated isoniazid rapidly. Out of 41 Indians, 26 (63.4%) belonged to the fast acetylators, while five Metis, also included in this study, were slow acetylators. It seems that the white admixture plays an important role in the outcome of the phenotyping. In 216 Eskimos, Armstrong and Peart[3] found 95% rapid inactivators. In 14 American Indians, Mitchell, Bell and Riemensneider[19] observed 79% fast inactivators. Scott, Wright and Weaver[20] reported from Alaska that 79% of the Eskimos and 62% of Athabascan-speaking Indians were fast inactivators. According to Sunahara, Urano and Ogawa,[1] 89% of Koreans, 85% of Ryukyuans, 72% of Thais and 88% of Japanese are rapid acetylators. The rate of fast inactivators is considerably lower in Caucasians. Only 32% of Swedes[6] and 41% of Finns[21] are fast inactivators. In North America, Harris, Knight and Selin[4] examined whites of European descent and found that 45% were fast acetylators. Jessamine, Hamilton and Eidus[22] reported in Canada that of a small group of white patients seven out of 24 (approximately 30%) were fast acetylators.

In contrast to previous reports,[17, 23-25] it is generally accepted today that rapid acetylation of INH has no effect on the outcome of chemotherapy with daily double or triple regimens of primary antituberculous drugs. It may, however, influence unfavourably intermittent INH treatment.[26, 27] According to Tiitinen,[21] the reason for not responding to treatment and thus developing chronic tuberculosis depends either on the therapeutic mismanagement or on the patient himself. Alcoholism, psychopathic behaviour and poor social environment often play a more important role in development of drug resistance than physiological conditions. In the past, during the initiation of chemotherapy, inadequate treatment (prescription of a single drug or administration for too short a period) was one of the causes for developing chronic tuberculosis. Today, we are again at the crossroads in the treatment of tuberculosis by rationalizing it and changing over from sanatorium to domiciliary and probably to intermittent therapy. If we want to avoid the mistakes of the past, efficient and realistic principles have to be established for domiciliary treatment and the latter has to be controlled by reliable supervision. For planning and monitoring intermittent chemotherapy, it is important to possess a method for phenotyping which is relatively simple, dependable and does not require repeated venipunctures. It produces results comparable to those of the fall-off technique and can be performed on a large scale. Methods employed in the past for estimation of INH in serum often suffered from great inaccuracy; furthermore, in a number of studies the criteria for distinguishing the two groups of acetylators were arbitrarily set. Tests based on the recovery rate of free INH in urine specimens[16-18] proved to be unsatisfactory, owing to overlapping values. Tiitinen[28] determined the excretion of free INH in three-hour urine samples of 335 subjects, as a percentage of total hydrazides, following an intravenous injection of 5 mg./kg. isoniazid. The results of the urine test did not correlate with the INH half-life values estimated by the fall-off technique. Out of 192 slow inactivators, 31 fell in the group of fast acetylators, according to the urine test. Furthermore, out of 143 fast acetylators in the same study, 15 emerged as slow inactivators when phenotyped by urine specimens.

Recently, Russell[29] introduced a method for determination of acetylator phenotypes. A repetition of his test did not give satisfactory results. The method employs small INH doses (100 mg. INH taken orally three times per day), and therefore produces too low drug concentrations in urine. Furthermore it utilizes semi-quantitative proce-

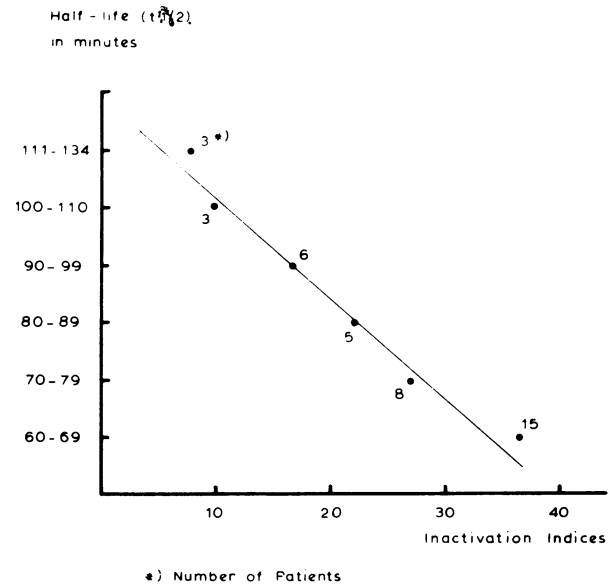

FIG. 2—Relation between half-life values (t ½) and averages of corresponding inactivation indices of fast acetylators in the six- to eight-hour collection time of urine specimens.

dures not suitable for this purpose. The introduction of a urine test for phenotyping should be based on detailed metabolic studies, aimed to devise optimal conditions for this procedure. The Inactivation Index recommended by us ensures a distinct division between the two groups of acetylators and is in agreement with the results of the fall-off technique. An additional simplification could be achieved by adaptation of the method to a colorimeter often available even in poorly equipped laboratories.

The introduction of the urine test for phenotyping of INH acetylators is not only useful for intermittent chemotherapy; it may also assist in studying INH-Matrix preparations with attenuated absorption, in order to compensate the fast acetylation. A double or triple dose, as mixture of isoniazid and INH-Matrix preparation, would probably ensure a similar effect in fast acetylators as that of plain isoniazid in slow inactivators. With the increased dosage of an attenuated preparation, the addition of Vitamin B_6 is essential. But even so, an excessively high dose may cause side effects if administered to slow inactivators. A distinct division of patients according to their inactivation status seems therefore an important prerequisite for such trials.

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Résumé

L'inactivation de l'isoniazide chez les Esquimaux et les Indiens du Canada

Il résulte de cette étude que l'inactivation de l'isoniazide, chez ce groupe phénotypique, est attribuable à une acétylation rapide. Par contre chez les Indiens examinés, 63.4% seulement appartiennent à ce phénotype. Il faudra procéder à des études plus poussées pour confirmer cette première constatation.

Au cours de l'enquête, les auteurs ont tenté de mettre au point une nouvelle épreuve fiable de dépistage de l'anomalie, à partir de l'urine, en vue de remplacer l'ancienne technique qui exige de multiples ponctions veineuses. La simplicité du nouveau test urinaire est telle qu'il peut convenir au dépistage systématique en masse.

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