LEVELS OF ACETYL HYDRAZINES AND RATE OF ACETYLATION OF ISONIAZID IN ADULT TUBERCULOSIS PATIENTS

E. B. BHALERAO AND S. V. BHIDE

Carcinogenesis Division,
Cancer Research Institute,
Tata Memorial Centre, Parel, Bombay – 400 012

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Summary: Patients suffering from pulmonary tuberculosis were investigated for the levels of isoniazid (INH) and its metabolites viz. acetyl-INH, mono and diacetyl hydrazines and ammonia. It was observed that 50% of the patients are slow inactivators of INH and almost all show an increase in circulating levels of NH₃ at 6 hrs. Mono and diacetyl hydrazine levels in blood and urine were detectable in all the patients up to 24 hrs though the maximum levels were observed at different intervals after the intake of INH.

Key words: slow and rapid acetylators isoniazid mono and diacetyl hydrazines

INTRODUCTION

A large variation in the metabolism of Isoniazid (INH) is reported by Hughes (12) which was related to variable rate of disappearance of the drug from the blood or to the proportion of acetyl INH/INH that are excreted in urine. The rate of inactivation of INH is genetically determined (8) and humans could be divided into two distinct groups of rapid and slow inactivators. Other metabolites of INH, detected in human urine are isonicotinic acid, mono and diacetyl hydrazines, ammonia and also hydrazine of pyruvic acid. Hepatotoxicity of INH is attributed to increased ammonia levels. Hepatitis in rapid inactivators of INH was suggested to be due to presence of acetyl hydrazine, a metabolite of INH (17). Various studies have been reported in literature pertaining to the rate of inactivation of INH in Western countries (2, 8, 10, 6) as well as in Japan (5,22) but few reports are available on Indian population.

Present paper reports major findings of such a study.

MATERIAL AND METHODS

Subjects: Indoor male patients between the age group of 25 to 40 years, suffering
from pulmonary tuberculosis and admitted to T.B. Hospital, Bombay, G.T. Hospital, Bombay
and Cardio Thoracic Centre of Millitary Hospital, Pune constituted the study subjects.

INH treatment was withdrawn for 24 hr and 300 mg of INH was administered
orally at 8.00 a.m. (5-6 mg/kg) after blood and urine samples were collected. Subse-
quently, blood samples were collected at 2 and 6 hr for ammonia estimation and at 3 and
6 hrs for the estimation of mono and diacetyl hydrazines. Urine samples were collected
6 and 24 hr after the treatment for the estimation of INH, acetyl INH, mono and diacetyl
hydrazine levels. Circulating levels of ammonia were determined in oxalated blood sam-
ple (16), and expressed in $\mu g/100 \, ml$ of blood. Changes in ammonia levels were ex-
pressed as % change over control (zero hour value=100%).

Urine samples were analyzed at 0 and 6 hrs for INH and acetyl-INH whereas levels
of monoacetyl hydrazines (MAH) and diacetyl hydrazines (DAH) were measured at 0.6
and 24 hr after the intake of drug (6). INH (AR, BDH). MAH (AR, Ega Chemie, West
Germany) and other AR grade reagent were used as standards.

RESULTS

Those patients who showed the ratio of acetyl INH to INH below 2.5 in urine at 6
hrs after the drug administration were considered as slow inactivators (2). The proportion
was 55% (43 out of 77 patients).

Fig. 1 shows the percent change in blood ammonia levels in adult patients at 2
and 6 hr respectively. It may be noted that 10 out of 18 i.e. (60%) adult patients
showed increases in ammonia in 2 hrs while 7 show persistent increase at 6 hr after the
treatment. However, no correlation between slow or rapid inactivators and increase in
ammonia levels, was observed.

Fig. 2 gives the values of monoacetyl hydrazine in urine of patients treated with
INH. Even at 0 hr, i.e. 24 hr after previous INH administration, 10 patients have values
between 2 and 8 $\mu g/100 \, ml$ of urine. While for 19 others, values were below 1 $\mu g/100 \, ml$.
At 6 hr all patients show detectable levels and 2 to 5 $\mu g/100 \, ml$ are observed in 20 out of
29 patients. At 24 hr only 2 are above 7 $\mu g/100 \, ml$ range. 6 return to zero values
and rest lie between 2 to 5 $\mu g/100 \, ml$ range.

Fig. 3 shows the levels of diacetyl hydrazine in the urine of T.B. patients treated
with isoniazid. It is interesting to note that even at zero hour i.e. 24 hr after the previous
INH administration, 17 patients out of 29 have DAH values between 2 and 10 $\mu g$ ; while
12 show values below 2 $\mu g$. At 6 hr only 3 show values below 2 $\mu g$ and 26 have
values ranging between 2 and 60 $\mu g$ level. At 24 hr, 8 cases have values below 2 $\mu g$ and
21 have values between 2 and 18 $\mu g/100 \, ml$ of urine.
% CHANGE IN CIRCULATING LEVELS OF AMMONIA IN ADULT T. B. PATIENTS TREATED WITH ISONIAZID

Fig. 1

URINARY LEVELS OF MONO-ACETYL HYDRAZINE IN ADULT T. B. PATIENTS

Fig. 2

URINARY LEVELS OF DI-ACETYL HYDRAZINE IN ADULT T. B. PATIENTS

Fig. 3
CIRCULATING LEVELS OF MONO & DI-ACETYL HYDRAZINE IN ADULT T. B. PATIENTS

Fig. 4

Fig. 4 depicts the levels of serum MAH and DAH at 0.3 and 6 hr after INH administration. Out of 10, 9 cases show detectable level of MAH. Even at 0 hr 4 patients show DAH value from 0.25±0.05 μg/100 ml. These subjects apparently metabolise INH at much slower rate, DAH being due to earlier INH treatment.

DISCUSSION

That 55% of these patients were slow inactivators of INH, agrees with previous reports (20, 24). It is also interesting to note that all the patients show an increase in circulating levels after the intake of the drug in accordance with Zipporin et al. (25). Incidentally, the present data qualitatively resemble our earlier finding that Swiss mice showed an increase in blood ammonia levels at 24 hr after INH administration (13).
Our finding of MAH and DAH in blood as well as in urine accords with the well documented evidence (19, 23, 21). Further, the rapid inactivators of INH are reported to show larger amount of urinary MAH and they also suffer from hepatotoxicity due to presence of MAH (17). On the other hand slow inactivators have presence of MAH for longer duration and hence the hepatotoxicity (15). In our present study, we observed some circulating MAH even at 0 hr, in case of 4 out of 10 patients and 3 out of 24 patients are slow inactivators. However, no correlation could be observed in case of urinary MAH levels recorded at zero hr. According to Ellard et al. (7) rapid acetylators of INH acetylate MAH more rapidly than do show acetylators and as a consequence they eliminate it more rapidly than do slow acetylators. According to present study it appears that the circulating levels of MAH are more indicative of the rate of acetylation.

Since MAH has been shown to be mutagenic in Ames test (14) as well as hepatotoxic (18), it is interesting to note that patients treated with INH show detectable levels of MAH and DAH upto 6 hrs and some even at 24 hr. We have earlier reported that mice are susceptible to INH-tumorigenicity (12). They are slow inactivators of INH and have detectable levels of MAH, while rats are refractory to INH-tumorigenicity (14) and are rapid inactivators of INH. Further, we have observed that MAH is tumorigenic in mice and it acts as a potent carcinogen than INH (1). It is difficult to extrapolate inferences of animal studies to humans but above data seems to call for prospective studies from this viewpoint in patients who show detectable levels of urinary MAH.

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REFERENCES


