EFFECT OF ISONIAZID ON FOLIC ACID STATUS IN SWISS MICE AND RATS

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(Received on October 8, 1984)

Summary : Effect of insoniazid (INH) and its metabolites e.g. mono and diacetyl hydrazines (MAH and DAH respectively) was studied on circulating and tissue folates in mice (a species susceptible to INH tumorigenicity) and rats (a species resistant to INH carcinogenicity). It was observed that ip injection of INH, MAH and mydrazine sulfate (HS, 0.18 *mg/g*) decreased blood folates in mice while only HS and MAH decreased blood folates in rats. DAH had no effect on blood folates of mice or rats. Long term feeding of MAH and HS to mice decreased blood folates in treated mice at the age of 17 and 22 months respectively.

Key words : isoniazid blood folates tissue folates

INTRODUCTION

Tumorigenicity of isoniazid (INH) in Swiss mice under different experimental conditions has been reported by several workers (6, 17, 4). Tumorigenic effect of INH is completely prevented if folic acid is co-administered in mice (12). Further, simultaneous co-administration of INH with folic acid (or other antioxidants) prevented INH induced inhibition of nucleic acid biosynthesis in lung tissue, the target organ of INH action (5). Folic acid also prevents teratogenic effects of hydrazine, a metabolite of isoniazid (18)

As a natural corollary of these observations, it was of interest to see if INH treatment decreased blood or tissue folic acid levels and whether folic acid alters the useful properties of INH.

MATERIAL AND METHODS

Inbred Swiss mice (strain, Virus Research Centre, Pune) were given standard laboratory diet (20) and water *ad libitum*. Inbred Wistar rats (strain, Memorial Hospital, New York) were also used.

Drugs used were ^{14}C labelled acetate (specific activity, 49.3 mCi/mM). Bhabha Atomic Research Centre, Bombay, India, Isoniazid (Fluka, Switzerland), Mono and diacetyl hydrazines (Ega-Chemie, West Germany) and folic acid (AR, BDH).

Blood or tissue (liver or lung) folates were assayed microbiologically using S. faccalis (ATCC 8043) as test organism and DIFCO folic acid assay medium (1).

INH, MAH, DAH or HS were given orally (1.1 *mg/animal*) or ip (0.09 or 0.18 *mg/gm*). Mice and rats of same age were used as controls. At the given interval, the animals were sacrificed and blood or tissues (liver or lung) were collected for analysis.

Influence of folic acid on 'bactericidal' effect of INH on micobacteria of H37 RV strain was tested radiometrically. Measurement of bacterial metabolism was done by biphasic liquid-scintillation vial system for radiometry as described by Ganatra *et al.* (9).

RESULTS

Effect of folic acid on activity of INH :

Micobacterium (H 37 RV) cultures were incubated with folic acid (10, 50 or 100 *ng/nm*) alone, isoniazid (1 *µg/ml*) alone or isoniazid + Folic acid (in concentrations given above).

TABLE I : Growth of mycobacteria (H 37 RV) expressed in terms of CPM of $14CO_2$ liberated in presence of isoniazid and folic acid
(Values are means of 4 observations ±SEM)

	5 days	7 days
Blank	9384±160	4627±920
Untreated culture	94149±1826	102012±9451
Isoniazid 1 *µg/ml*	48876±1926*	45517±2963*
Isoniazid (1 *µg/ml*) + 10 *ng* FA	49710±7820*	45346±980*
Isoniazid (1 *µg/ml*) + 50 *ng* FA	47827±1002*	47205±4200*
Isoniazid (1 *µg/ml*) + 100 *ng* FA	57960±970*	46536±1623*
100 *ng* FA	98848±9112	116244±7020
50 *ng* FA	98848±9112	116244±7020
100 *ng*	98600±1892	129809±19162

*Value differs significantly ($P<0.05$) from corresponding control.

Significant decrease was observed in CO_2 production by 5 days in presence of isoniazid alone. This decrease was persistent even on the day 7. Folic acid itself had no effect by itself or on activity of INH (Table I).

TABLE II : Mean folate levels in blood (*ng/ml*)±SEM in mice treated with isoniazid.

	Injection ip		*Oral feeding*
	0.09 *mg/gm*	0.18 *mg/gm*	
Control	44.76±3.16 (21)	44.76±3.19 (21)	44.76±3.19 (21)
Treated 2 hrs	40.33±0.47 (6)	44.30±5.85 (13)	
Treated 24 hrs	39.20±1.92 (5)	33.76±3.32* (13)	43.28±6.83 (7)
Treated 7 days	23.75±2.59* (4)		35.12±3.10 (4)

*Value differs significantly ($P<0.05$) from corresponding controls (number of animals given in parenthesis)

TABLE III : Effect of INH, DAH, MAH and HS on blood (*ng/ml*) and tissue (*ng/mg.* dry weight) folates.

	Rats	*Mice*		
	Blood	*Blood*	*Lung*	*Liver*
Control	46.5±3.4 (4)	44.76±3.19 (21)	0.32±0.008 (3)	3.5±0.1 (4)
INH	44.23±1.76 (4)	33.76±3.32* (13)	0.31±0.01 (4)	3.6±0.21 (4)
DAH	40.3±2.9	44.00±5.55 (5)	—	—
MAH	39.0±3.4* (4)	28.00±4.08* (3)	0.25±0.08 (3)	3.4±0.24 (4)
HS	28.5±3.8* (4)	20.00±1.63* (3)	0.21±0.003* (3)	2.51±0.28* (3)

Each inj (0.18 *mg/g*) was injected ip 24 hrs before.
*Value differs significantly ($P<0.05$) from corresponding controls (parentheses show number of anima's in a group).

Effect of folic acid on folate levels : Effect of INH administration on blood folate levels in mice treated with INH (po or ip) was studied at 2 and 24 hr after the treatment.

It is evident (Table II) that INH (0.09 *mg/g, ip)* had no effect at 2 and 24 hr, but a decrease was observed in mice injected with this dose daily, for 7 days. Reduction at 24 hr was observed only when dose was higher (Table II). Oral administration of a dose 1.1 *mg/mouse* (usually considered to be tumorigenic), for 7 days also produced no effect on folate levels.

Comparative effect of INH and its metabolites in mice and rats : Short term and long term study : Effect of INH, MAH, DAH and HS injected ip (0.18 *mg/g*) on folate levels in blood, lung and liver at 24 hours is shown in Table III. INH treatment decreased circulating levels of folates in mice but not in rats DAH did not show any effect in mice or rats. MAH and HS show significant decrease in circulating folates levels in rats and mice. In HS treated mice lung and liver folate levels were found to decrease significantly. INH and MAH did not cause significant alterations in tissue folate levels even though significant decrease in circulating folate levels was observed in the same group of mice.

TABLE IV : Effect of long term oral feeding of INH, MAH, DAH and HS on blood (*ng/ml*) and tissue (*ng/mg*, dry weight) folates.

	17 months			*22 months*
	Lung	*Liver*	*Blood*	*Blood*
Control	0.36±0.03 (9)	3.55±0.38 (10)	20.20±1.48 (9)	13.00±1.00 (3)
INH	—	—	—	6.8*±1.30*
MAH	0.21±0.03* (3)	2.56±0.26* (8)	18.16±3.19* (4)	13.80±2.45 (3)
DAH	0.13±0.01* (3)	3.57±0.65 (3)	21.24±2.10 (3)	—
HS	0.10±0.01* (7)	1.76±0.34* (9)	10.12±1.20* (7)	

Each compound was fed every day (1.1 *mg/animal*) MAH and DAH treatment was stopped after 8 months.
*Value differ significantly ($P<0.05$) from corresponding control (parentheses show the number of animals in a group)

Daily oral dose of 1.1 *mg/mouse* of INH, or MAH and HS is tumorigenic in mice in long term studies. INH and HS treatment was well tolerated and hence the two compounds were continued till animals were sacrificed at the age of 22 months ; whereas MAH and DAH caused toxicity and hence the treatment was discontinued after 8 months though animals were to be sacrificed at 22 months. Blood folates were measured in untreated controls and treated groups.

It was seen (Table IV) that with increasing age circulating folate decreased. MAH and DAH caused no significant change in folate levels at any age and that HS caused a decrease from the age of 12 months while INH caused significant decrease only at the age of 22 months.

DISCUSSION

Foregoing data show that ip injection of INH (0.18 *mg/day/*mouse) caused a significant decrease in 24 hr in mice but not in rats. This is pertinent since mice are reported to be susceptible and rats refractory to tumorigenic action of INH (13). Further equivalent doses of MAH and HS cause a significant decrease in both the species when injected ip. Again HS induced tumors both in rats (12) and mice (13). MAH also produces lung tumors in mice (7).

Above data also show that oral feeding of HS and INH do not affect blood folate levels at earlier time periods but cause significant decrease at the age periods when tumors have been reported to develop. In case of HS (3,19,7) tumors begin to appear from the age of 12 months and in case of INH at the age of 20-22 months (4). It is not possible at this stage to determine if decrease in folates is due to mere association with the growing tumor or if it plays any causative role in tumor development. In view of the fact that concomitant treatment of folic acid prevented INH tumorigenicity and other adverse effect, we assume that folic acid may play some role in INH toxicity and carcinogenesis. There are no reports on folate depressing effect of INH in experimental animals though a few clinical reports support this contention. Klipstein *et al.* have observed that 15 out of 29 patients treated with combination therapy of INH + Cycloserine reported decrease in circulating folate levels (11). It is also observed that the two drugs when used together cause hyperchromatin anemia (15,16). Since these effects are observed as a result of combination therapy, it is difficult to attribute these to INH alone. However, there are reports describing sideroblastic anemia in patients treated with INH alone (8,14).

We observed that folic acid does not interfere with an equivalent of therapeutic action of INH and it seems worthwhile to use folic acid (12) as an adjuvant to INH therapy, to prevent possible toxicity and tumorigenicity, along with pyridoxine, though INH is not yet equivocally established as a human carcinogen (10).

ACKNOWLEDGEMENTS

Authors are grateful to Dr. D. Shan, Radiation Medicine Centre, BARC, for help in the radiometric assay.

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