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TRANSPLACENTAL PASSAGE OF ISONIAZID (INH) AND ITS INTERACTION WITH FETAL TISSUES OF MICE

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Abstract—When INH was administered orally or intraperitoneally to pregnant female mice, the concentrations of acetyl INH and acetyl hydrazines did not vary significantly in circulating blood and in amniotic fluid. However, the concentration of INH in the amniotic fluid was significantly higher than that observed in the serum. Autoradiographic studies using ^{14}C-labelled INH revealed that INH crosses the placental barrier and grains can be observed in lung and liver tissues of the fetus. Studies on interaction of ^{14}C-labelled INH with nucleic acids clearly demonstrated that significant amounts of radioactivity were present in all the macromolecules of the whole embryo as well as in those isolated from fetal lung and liver tissues.

Mutagenic and carcinogenic activities of isoniazid (INH)† have been reported by this laboratory and by several other workers [1–4]. We have also observed an increase in lung tumor incidence in F_1 Swiss mice when they were treated with INH pre- and postnatally [5]. Passage of INH across the placental barrier has been reported earlier [6, 7]. It therefore seemed interesting to quantitate the concentrations of INH and its metabolites in the circulating blood of the mother as well as in amniotic fluid of pregnant females treated with INH. In a further effort to understand the mechanism of transplacental carcinogenesis of INH, it seemed worthwhile to study the interaction of ^{14}C-labelled INH with macromolecules from whole embryos as well as from those of fetal tissues. The present paper reports salient observations on these studies.

MATERIALS AND METHODS

For studying the rate of acetylation, 6–8 week-old Swiss mice, female and pregnant mice of day 15 gestation were either injected intraperitoneally (0.09 mg/g body wt) or fed orally (1.1 mg) with INH. Two and three hours after the administration, the animals were killed, blood was collected and serum separated. INH and its metabolites, acetyl INH, MAH and DAH, were measured colorimetrically from serum and amniotic fluid.

Interaction studies. Pregnant Swiss mice (days 10 and 19 of gestation) were injected i.p. with 0.5 μCi/g body wt of [^{14}C]INH [carbonyl ^{14}C, sp. act. 22 mCi/mmole (Radiochemical Centre, Amersham, U.K.)].

Fifteen minutes after the injection, animals were killed by cervical dislocation and maternal lung, liver and kidney tissues were dissected out. Embryonic lung and liver were dissected from day 19 fetuses. The tissues were homogenised in a Potter–Elvehjem homogeniser using a homogenising solution consisting of *para*-aminosalicylic acid (6%), sodium dodecyl sulfate (1%), sodium chloride (1%) and *n*-butyl alcohol (6%). DNA, RNA and protein were extracted from the homogenate by Diamond *et al.*s [8] method. Radioactivity was measured with a Beckman LS 100 scintillation counter in a dioxan–PPO–POPOP–naphthalene–toluene–ethylene–glycol cocktail. Results were expressed as dpm/mg RNA, DNA or protein.

To the ^{14}C-labelled DNA isolated by the aforementioned procedure, a few crystals of DNase were added and incubated at 37° for 3–5 hr. The pH of this mixture was raised to 8.5 by addition of Tris buffer. Alkaline phosphatase (100 μl) and snake venom phosphodiesterase (10 μl) were added and incubation was continued for 18 hr. At the end of this period, the DNA was loaded on a Dowex–formate column (45 × 1 cm), equilibrated with NH_4OH (pH 9.9). A constant flow of buffer was achieved from a gradient [300 ml of NH_4OH (pH 9.9) and 300 ml of ammonium formate (pH 4.2)]. Five-millilitre fractions were collected and readings at 260 and 340 nm were recorded. After eluting the deoxyguanylic acid, the column was washed with ammonium formate (pH 4.2). The fractions were collected and absorbance at 260 and 340 nm were recorded.

Uptake of [^{14}C]INH by maternal and fetal lung (day 19 of gestation) was studied by autoradiography using AR 10 Kodak Films. The label was administered intraperitoneally (0.5 μCi/g body wt) 15 min prior to killing to day 19 pregnant mothers. Tissues were fixed in formalin and sections were taken on subbed slides. The procedure followed was that of Pelq [9].

RESULTS

Table 1 gives the circulating levels of intact INH and its metabolites after oral and intraperitoneal

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† Abbreviations: INH, isoniazid; HS, hydrazine sulfate; MAH, monoacetyl hydrazine; DAN, diacetyl hydrazine.

Table 1. Circulating levels of INH, MAH, DAH and acetyl INH in the serum of normal males, virgin females and pregnant females, and in amniotic fluid, after INH was administered along different routes

Compound	Time (hr)	Serum			Amniotic fluid
		Males	Virgin females	Pregnant females	
Oral route					
INH	2	7.0	7.5	8	12
	3	5.4	4.5	5.4	12
Acetyl INH	2	25	22.5	25	27.5
Intraperitoneal injection					
INH	3	9	7	12.12	16
MAH	3	0.0025	0.00	0.006	0.00
DAH	3	0.0013	0.014	0.01	0.0073

INH was administered orally at a dosage of 2.2 mg/animal, 2 and 3 hr before killing.

INH was administered intraperitoneally at a dosage of 0.1 mg/g body wt. In both routes, pregnant mice were killed on day 15 of gestation.

Observations are means of three values from pooled samples of four animals each and expressed in μg/ml of serum.

Estimations are done as described in the text.

administrations. Levels of INH in the serum did not vary considerably among the males, females and breeders. In both cases, amniotic fluid retained the maximum amount of intact INH. Amounts of other metabolites also did not differ significantly in the serum or amniotic fluid.

Interaction studies

Considerable amounts of radioactivity could be observed in the nucleic acids as well as in the proteins. Table 2 gives the data on the interaction of [^{14}C]INH with whole embryos and maternal tissues on day 10 of gestation, 15 min after its administration. Specific activities of the DNA and RNA of all tissues are comparable, except in the maternal liver, where the specific activity of RNA was significantly higher than that of DNA. Table 3 gives the interaction data on day 19 of gestation. DNA from embryos had a significantly higher amount of radioactivity than RNA from embryos. As in the case of day 10, liver RNA had significantly higher radioactivity than liver DNA. Interaction of INH with embryonic lung and liver nucleic acids is given in Table 4. The specific activity of lung DNA was higher than that of lung RNA whereas the specific activity of liver RNA was higher than that of liver DNA.

Attempts to degrade the labelled DNA to nucleotides gave some interesting results. Previous reports [10, 11] have shown that [^{3}H]INH interacts with nucleic acids and the modified nucleotides have a characteristic absorption maxima at 340 nm. In the present studies using ^{14}C-labelled DNA, the deoxycytidylic acid fraction gave 65 dpm/mg nucleotide. The second fraction containing deoxyadenylic acid gave 270 dpm while deoxythymidilic and deoxyguanylic acid gave negligible counts. The fraction eluted after deoxyguanylic acid gave significant

Table 2. Interaction of [^{14}C]INH with nucleic acids and protein from embryo and maternal tissues on day 10 of gestation.

Tussue	DNA	RNA	Protein
Embryo	2750 ± 1120 (4)	2662 ± 817 (4)	93 ± 14 (4)
Lung	2723 ± 681 (3)	2547 ± 177 (3)	47 ± 12 (3)
Liver	677 ± 67 (3)	2370 ± 288* (3)	460 ± 87** (4)
Kidney	1430 ± 400 (3)	1590 (1)	213 ± 52** (4)

Pregnant mice on day 10 gestation were injected with [^{14}C]INH (0.5 μCi/g body wt). Animals were killed 15 min after the injection.

Values are expressed as dpm/mg DNA, RNA and proteins. Figures in parentheses indicate the numbers of observations.

Values are means ± S.E.

* Statistical significance when specific activity of RNA is compared with that of DNA and P value is < 0.05.

** Statistical significance when specific activity of embryo protein is compared with protein from maternal tissues.

Table 3. Interaction of [^{14}C]INH with nucleic acids and protein from embryo and maternal tissues on day 19 of gestation

Tissue	DNA	RNA	Protein
Embryo	3138 ± 325* (4)	2148 ± 71 (4)	661 ± 7 (4)
Lung	1809 ± 225 (4)	2464 ± 871 (3)	330 ± 83 (4)
Liver	1005 ± 79 (4)	1649 ± 197 (4)	1747 ± 461 (4)
Kidney	1729 ± 338 (4)	2076 ± 248 (4)	523 ± 202 (4)

Pregnant animals on day 19 of gestation were injected intraperitoneally with [^{14}C]INH (0.5 μCi/g body wt) for 15 min.

Specific activity is expressed as dpm/mg DNA, RNA or protein.

Values are means ± S.E.

* Statistical significance when specific activities are compared between RNA and DNA of each tissue and when P value is < 0.05.

Table 4. Interaction of [^{14}C]INH with embryo tissues on day 19 of gestation

Tissue	DNA	RNA	Protein
Lung	6322 ± 1316* (4)	3009 ± 699 (4)	195 ± 26 (4)
Liver	2664 ± 612 (4)	5059 ± 445* (4)	316 ± 114 (4)

Pregnant mice of day 19 were injected with [^{14}C]INH (0.05 μCi/g body wt) and killed 15 min later.

Specific activity is expressed in dpm/mg DNA, RNA or protein. Figures in parentheses indicate the numbers of observations.

Values are means ± S.E.

* Statistical significance when P value is < 0.05, and compared between different tissues.

radioactivity (207 dpm). This fraction, after necessary concentration, gave an absorbance at 340 nm (0.34) while none of the previously eluted fractions gave any absorbance at 340 nm.

Autoradiographic studies on day 19 of gestation of pregnant females treated with ^{14}C-labelled INH show the uptake of the drug into maternal and fetal lung respectively 15 min after its administration.

DISCUSSION

Autoradiographic studies in the present experiment showed that [^{14}C]INH could be detected in both maternal and fetal lung as early as 15 min after its administration. Quantitative studies on intact drug in serum and amniotic fluid further substantiate the passage of INH across the placental barrier along with the previous reports. That the amniotic fluid contained high levels of the intact drug is of great concern in the light of the observation that many pregnant women in India manifest TB during pregnancy and are put under INH therapy.

The present study also reports the interaction of [^{14}C]INH with nucleic acids and proteins. Bartmann and Massmann [10] obtained the half-life value of INH in mice as 50 min. The radioactivity observed at 15 min in the present study might therefore be ascribed at least partly to intact INH. Further, degradation of the DNA to nucleotides showed that a considerable amount of radioactivity was present in both deoxyadenylic acid as well as the fraction eluting after deoxyguanylic acid. The radioactivity in the adenylic acid may be attributed to incorporation of the label whereas that in the fifth fraction, because of its absorbance at 340 nm, may be because of interaction.

Maru and Bhide [11, 12] observed interaction of [^{3}H]INH with nucleic acids *in vivo* as well as *in vitro*. The interaction was more with RNA than with DNA. In the present experiments also, the specific activity of RNA of maternal tissues was comparatively higher than that of DNA, even though this difference was not statistically significant.

However, interaction of INH with DNA from whole embryos was significantly higher than that of RNA. Since DNA is responsible for carrying the genetic information, this observation is of great importance. It is pertinent to mention here that in the F_2 generation of mothers (F_1) which received pre- and postnatal INH treatment, we observed 83% tumor incidence (unpublished data). Among the fetal tissues also, INH had more interaction with DNA from the lung than with RNA. This observation is interesting in view of the fact that 55% of F_1 mice treated with INH during gestation and subsequently from the adult stage developed lung tumors [5]. Since a greater interaction of INH is observed with DNA from fetal lung and RNA from maternal lung, it is probable that a two-stage mechanism is operating in the transplacental carcinogenesis of INH. Lung tumors observed in the F_1 generation may be the result of interaction of INH with the genetic material at the fetal stage; this genetic alteration is then perpetuated by an epigenetic mechanism operating at the cellular level in the adult stage.

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