THE METABOLISM AND PLACENTAL TRANSFER
OF ISOCARBOXAZID IN PREGNANT RATS

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Isocarboxyazid has been widely used in the treatment of depressive diseases, however, there is no pertinent information on metabolism and placental transfer of the drug although a few reports have appeared concerning metabolism of this drug in male rats (1) and man (2). Preceding papers (3-6) demonstrated that the metabolism of isocarboxazid was initiated by enzymatic hydrolysis of amide bond of the drug to form benzylhydrazine, and this enzyme was found to localize mainly in the microsomal fraction of liver. Moreover, there was a significant species difference in the enzyme activity, that is, the guinea-pig has an approximate ten-fold higher enzyme activity than rats.

The present paper is concerned with the metabolism of isocarboxazid in pregnant rats and determinations to elucidate the placental transfer of this drug.

MATERIALS AND METHODS

1. Animals
Female, Wistar strain rats were used throughout the experiments. Isocarboxazid was suspended in 0.5% carboxymethylcellulose solution before use and the drug was administered orally (0.2 ml/100 g body weight). Controls were administered an equivalent volume of the vehicle. Female rats in the proestrus stage of the cycle were used as controls.

2. Enzyme preparation
The animals were sacrificed by decapitation, and tissues except for uterus, were removed, washed in cold 1.15% KCl, then homogenized in a Potter type homogenizer with a Teflon pestle. The uterus was scraped with a blunt knife and then homogenized in a glass homogenizer. In all cases a 20% homogenate in 1.15% KCl was prepared. Foetuses were detached from the placentas, and livers harvested from each litter were pooled and frozen until required.

Concentrations of benzylhydrazine in each tissue were measured according to the method previously described (3) and were reported in terms of microgram of benzylhydrazine formed/g of wet weight tissue/hr.

3. Enzyme assays
Monoamine oxidase (MAO) activity was determined by employing 20% tissue homogenate with serotonin creatinine sulfate as substrate. The reaction mixture contained 3 μmoles serotonin as free base, 1 ml of tissue homogenate, 1 ml of 0.2 M phosphate buffer (pH 7.4) and added distilled water up to 4 ml. After incubation for 30 min at 37°C, the
reaction mixture was inactivated with 1 ml of 10% trichloracetic acid followed by centrifugation. Aliquots of supernatant were used for determination of residual serotonin content according to the method of Udenfriend et al. (7). MAO activity was expressed as μmoles serotonin metabolized g of wet weight tissue/hr. Assay of isocarboxazid hydrolase activity was carried out according to the method as described previously (5).

RESULTS

1. Tissue MAO activities during gestation

MAO activities of several tissues were determined in pregnant rats during gestation (Fig. 1). Activities of plasma, liver and brain did not vary during the course of pregnancy, that is, the levels of these activities in pregnant rats were similar to those of the pregnant controls, i.e., 36.5 ± 2.4, 32.9 ± 2.1 and 9.4 ± 1.0 μmoles serotonin metabolized/ml of plasma or gram of liver and brain in one hr, respectively.

On the other hand, the placental MAO level increased during 14th and 16th day of gestation and reached a maximum during the 17th and 20th day. In contrast, significant decrease in the enzyme level of uterus occurred during 8th and 10th day then gradually declined until delivery.

FIG. 1.

2. Effects of isocarboxazid on tissue MAO activities in non pregnant rats

The following experiments were designed to investigate the ability of isocarboxazid to

| Time (min) | No | Liver | Uterus | Brain |
|------------|----|-------|--------|-------|
|             |    | Benzylhydrazine | MAO* | Benzylhydrazine | MAO | Benzylhydrazine | MAO |
| 15          | 5  | 38.2±4.50 | 40±5.4 | 22.0±4.11 | 45±5.6 | 7.1±0.82 | 84±9.1 |
| 30          | 5  | 49.4±5.94 | 50±6.8 | 20.5±3.92 | 60±7.4 | 6.3±0.74 | 91±9.8 |
| 45          | 6  | 46.3±6.12 | 52±6.6 | 17.6±2.91 | 57±7.8 | 3.5±0.44 | 80±11.2 |
| 60          | 5  | 32.4±4.83 | 60±7.4 | 15.4±3.00 | 55±6.5 | 3.7±0.53 | 89±10.6 |
| 120         | 4  | 20.5±3.60 | 61±7.8 | 8.6±0.90 | 59±7.0 | 2.7±0.47 | 86±11.4 |
| 240         | 4  | 8.9±1.00  | 59±6.9 | 4.5±0.64 | 62±7.2 | 1.3±0.25 | 90±12.5 |
| 360         | 4  | 1.4±0.32  | 60±8.0 | N.D.  | 60±7.6 | N.D.  | 90±16.0 |
| 24(hr)      | 5  | N.D.    | 58±7.1 | N.D.  | 57±6.6 | N.D.  | 91±15.3 |

Each values is the mean of at least four animals .S.E.
N.D.: not detectable, * Per cent inhibition of control. Benzylhydrazine levels represent μg/g wet weight tissue.
inhibit MAO in non pregnant rats and to determine the duration of effect. Isocarboxazid was administered to intact female rats in a dose of 30 mg/kg, and animals were sacrificed at stated intervals. The brains, livers and uteri were homogenized and assayed for both MAO activity and tissue benzylhydrazine level.

As shown in Table 1, MAO inhibition was initiated at 15 min and persisted for at least 24 hr after administration. Tissue levels of benzylhydrazine show a maximum at 30 min and then gradually decline to undetectable levels within 24 hr after dosing. Remarkable inhibition of tissue MAO activities was recognized despite disappearance of the drug from the tissues at 24 hr.

3. Distribution of benzylhydrazine and MAO inhibition after isocarboxazid administration in pregnant rats

Tissue distribution of benzylhydrazine and MAO inhibition were determined in pregnant rats after a single administration of isocarboxazid (30 mg/kg) on 17th day of gestation. As shown in Table 2, tissue benzylhydrazine levels elevated significantly within 1-2 hr after treatment, then decreased gradually to lower or undetectable levels at 24 hr. Subsequently, changes in MAO activities of several tissues were studied in pregnant rats, and resulted in

| Time (min) | No | Liver (µg/g) | Uterus (µg/g) | Brain (µg/g) | Placenta (µg/g) | Amniotic fluid (% inhibition) | Foetal liver (% inhibition) |
|------------|----|--------------|---------------|--------------|-----------------|-------------------------------|-----------------------------|
| 15         | 5  | 45.2 ± 5.74  | 8.7 ± 0.98    | 7.5 ± 1.00   | 6.5 ± 0.88      | 2.5 ± 0.34                   | 2.8 ± 0.38                  |
| 30         | 3  | 58.3 ± 8.23  | 10.1 ± 2.06   | 10.8 ± 2.45  | 7.4 ± 0.80      | 2.7 ± 0.38                   | 2.2 ± 0.34                  |
| 45         | 5  | 59.6 ± 6.55  | 15.4 ± 3.21   | 9.6 ± 0.91   | 11.7 ± 1.98     | 4.0 ± 0.51                   | 3.1 ± 0.46                  |
| 60         | 5  | 60.1 ± 6.90  | 21.5 ± 4.30   | 6.3 ± 0.80   | 16.4 ± 3.20     | 4.8 ± 0.56                   | 3.3 ± 0.50                  |
| 120        | 4  | 64.5 ± 7.86  | 29.7 ± 4.82   | 4.4 ± 0.57   | 20.4 ± 3.29     | 5.2 ± 0.66                   | 4.0 ± 0.53                  |
| 240        | 5  | 47.1 ± 6.23  | 26.0 ± 4.21   | 4.6 ± 0.51   | 17.0 ± 2.18     | 4.5 ± 0.53                   | 3.4 ± 0.51                  |
| 360        | 4  | 31.4 ± 5.40  | 18.5 ± 2.11   | 2.7 ± 0.30   | 5.6 ± 0.66      | 3.7 ± 0.41                   | 2.7 ± 0.39                  |
| 24 (hr)    | 4  | 15.6 ± 3.00  | 9.8 ± 1.40    | N.D.         | N.D.            | 2.0 ± 0.34                   | N.D.                        |

Pregnant animals were used on 17th day of gestation.
Legends see Table 1.

| Time (min) | No | Liver (µg/g) | Uterus (µg/g) | Brain (µg/g) | Placenta (µg/g) |
|------------|----|--------------|---------------|--------------|-----------------|
| 15         | 5  | 40 ± 7.4     | 54 ± 7.0      | 83 ± 9.1     | 30 ± 4.6        |
| 30         | 4  | 50 ± 8.0     | 64 ± 6.8      | 85 ± 9.2     | 68 ± 7.6        |
| 45         | 4  | 50 ± 7.9     | 65 ± 8.4      | 86 ± 9.9     | 67 ± 9.1        |
| 60         | 3  | 58 ± 7.7     | 64 ± 7.9      | 89 ± 10.4    | 70 ± 8.8        |
| 120        | 4  | 54 ± 8.1     | 65 ± 8.6      | 88 ± 9.6     | 68 ± 8.5        |
| 240        | 4  | 53 ± 6.9     | 67 ± 8.0      | 89 ± 11.0    | 67 ± 7.6        |
| 360        | 4  | 56 ± 8.4     | 62 ± 8.5      | 87 ± 12.0    | 66 ± 7.8        |
| 24 (hr)    | 4  | 59 ± 7.8     | 64 ± 9.1      | 90 ± 12.5    | 70 ± 9.4        |

MAO activities represent per cent inhibition.
Legends see Table 1.
a remarkable inhibition in MAO activities of all tissues tested (Table 3). Tissue MAO activities of non-treated rats referred to as controls were recognized from values at 17th day of gestation (Fig. 1).

This inhibitory effect of benzylhydrazine formed from isocarboxazid on tissue MAO activity was initiated within 15 min after drug administration.

In these experiments the activity of brain MAO seemed to be particularly sensitive to the effect of the inhibitor, as brain MAO was more vigorously inhibited than other tissues in spite of the lower level of benzylhydrazine, and reached a value of approx 20% of that of control within 15 min after isocarboxazid.

4. The isocarboxazid hydrolase activity in pregnant rats

The enzyme activity responsible for hydrolysis of isocarboxazid to benzylhydrazine in pregnant rats (17th day of gestation) was compared with that in non-pregnant rats. Mean enzyme activity in non-pregnant rats was found to be 6.5 ± 0.12 and pregnant rats had a mean value of 5.4 ± 0.24 benzylhydrazine formed/g of wet weight tissue/hr. There was no significant difference in values from non-pregnant and pregnant rats.

DISCUSSION

In accordance with other papers (8, 9), MAO activity in blood of normal pregnant rats did not differ from enzyme activities found in the non-pregnant. It is also recognized from the results given in Fig. 1 that the MAO activity of uterus tends to decrease to a low level by the 10th day of gestation and then remains constant until delivery. In contrast, placental MAO activity, expressed per gram of placenta, appears to increase as the placental ages until 17th day of gestation and continues at this level to delivery.

Following a single administration of isocarboxazid, metabolism of benzylhydrazine in pregnant rats appears to be at a much slower rate than in non-pregnant controls. Benzylhydrazine was cleared from brain, liver and uterus of non-pregnant rats as well as brain and placenta of pregnant rats and foetal liver within 24 hr (Tables 1 and 2), while detectable amounts of benzylhydrazine in liver, uterus and amniotic fluid remain in pregnant animals at 24 hr (Table 2).

With regard to the placental transfer of benzylhydrazine, results in Table 2 imply that at least orally administered isocarboxazid is hydrolyzed to benzylhydrazine in maternal liver, then it crosses the placenta and is distributed throughout the foetus. Little or no hydrolase activity was found in foetal liver (6).

The results shown in Tables 2 and 3 suggest that the MAO activity of brain seems to be particularly sensitive to the effect of benzylhydrazine, compared with other tissues tested. This is the same phenomenon as other MAO inhibitors such as iproniazid (10) and pargyline (11). These facts may be associated with the deleterious effect of isocarboxazid on the foetus (12). In fact, Botros et al. (13) and Poulson et al. (14) suggested that the deleterious effect of iproniazid acting at the drug level of brain in relation to MAO inhibition, interfered with the mechanism responsible for the maintenance of the corpus luteum.

It can be speculated that inhibition of placental MAO by benzylhydrazine causes
alterations in blood flow, the transport of nutrients or waste products accounting for the deleterious effect of isocarboxazid. The lethal effect of MAO inhibitors, therefore, may be due to serotonin accumulation in the brain rather than direct action of drugs on the foetus, as a number of studies having been reported on the deleterious effect of serotonin (16–18).

Details remain for further investigations.

SUMMARY

Plasma, liver and brain MAO activities do not vary during normal pregnancy, however, the enzyme activity of uterus remarkably decreases. In contrast, placental MAO level increases progressively from the 14th day of gestation until delivery. Following oral administration of isocarboxazid to rats, metabolism of benzylhydrazine in pregnant rats occurs at a much slower rate than in non-pregnant controls, with the formed benzylhydrazine crossing the placental barrier and entering into the foetal liver. Benzylhydrazine clears from tissues of non-pregnant rats treated with isocarboxazid within 24 hr, while, detectable amounts of the compound are retained in liver, uterus and amniotic fluid of pregnant animals 24 hr after dosing.

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