Synthetic flavonoids cross the placenta in the rat and are found in fetal brain

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Schröder-van der Elst, J. P., D. van der Heide, H. Rokos, G. Morreale de Escobar, and J. Köhrle. Synthetic flavonoids cross the placenta in the rat and are found in fetal brain. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E253–E256, 1998.—The synthetic flavonoid EMD-49209 is a potent inhibitor of the in vivo and in vitro binding of thyroxine (T4) to transthyretin (TTR). We studied the distribution of 125I-labeled EMD-49209 in maternal tissues, intestinal contents, and fetal tissues in rats that were 20 days pregnant (from 1 to 24 h after intravenous injection). The percent dose of EMD decreased quickly with time. In maternal brain no radioactive flavonoid could be detected. EMD was excreted very rapidly from the intestines. In the fetal compartment the percent dose of EMD increased with time; after 24 h it contained 17% of the EMD. The flavonoid was found in all fetal tissues investigated and also in the fetal brain. Because TTR concentrations are high in the fetal rat, especially in the brain, the transfer of flavonoid to the fetal brain might be linked to TTR expression. The presence of flavonoid in the fetal brain raises the possibility of an essential interference of flavonoids with the availability of T4 in the fetal compartment.

thyroid hormones; goitrogen

NATURAL FLAVONOIDs, which are plant pigments found in edible plants, contribute to our normal diet. Earlier studies have indicated that flavonoids are able to induce goiter (13, 14). Together with this increase in the weight of the thyroid, a decrease in iodide organification was found. Recently it was shown that flavonoids, as constituents of one of the widely consumed African millet species, are the cause of goiter (11, 22). Next to the high intake of millet there is also iodine deficiency in these areas, but no correlations have been found among goiter incidence, the individual iodine intake, and urinary iodine excretion. A connection among iodine, thyroid hormone availability for tissues, and natural (11, 13, 14, 22) as well as synthetic flavonoids (5, 15, 20, 24, 25) has been discussed. Synthetic flavonoids have proven to be a valuable tool for studying thyroid hormone metabolism in vitro and in vivo. These flavonoids were developed by molecular drug design as thyroxine (T4) analogs due to their capacity to inhibit T4 binding to transthyretin (TTR) and to block T4 deiodination by the type I 5′-deiodinase enzyme in vitro (6, 7, 12, 28). These flavonoids do not bind to albumin or thyroid hormone-binding globulin (8, 16, 27). The synthetic flavonoid 3-methyl-4′,6-dihydroxy-3′,5′-dihydrophilavone (EMD-49209) has been shown to enter tissues and is eliminated and excreted very quickly (26).

As in humans (2, 10), thyroid hormones of maternal origin are found in fetal rat tissues before the onset of fetal thyroid function (9, 17–19) and are at present believed to play a role in both early development (3, 21) and protection against impaired fetal thyroid function (4). TTR might be involved in the transfer of maternal T4 to the fetal compartment. Because TTR is the principal carrier protein in the fetus (23, 30), it is important to know whether the transport of T4 especially to the fetal brain is influenced by TTR.

Recent data (20) showed an increase in free T4 in the maternal and fetal circulations after administration of EMD-21388 to rats, which is considered evidence that the flavonoid crosses the placenta. To confirm this and to investigate whether flavonoids also enter fetal tissues and interfere with thyroid hormone metabolism, we administered synthetic flavonoids to rats that were 20 days pregnant. In this study the 125I-labeled flavonoid EMD-49209 was given as an intravenous bolus injection. At different time points, rats were bled via the vena cava inferior and perfused with saline via the cannula under ether anesthesia. The distribution and elimination of the labeled flavonoid in the maternal and fetal compartments were studied.

MATERIALS AND METHODS

Animals. The experiments were approved by the local committee of animal care. One group of eight Wistar rats (Central Roefdier Bedrijf/Wageningen Universiteit, Iffa Credo, Brussels, Belgium) was used. The rats were individually housed at 22°C, with a 14:10-h light-dark cycle. They were fed the American Institute of Nutrition Diet (1). To prevent reutilization of free 125I from 125I-EMD-49209, the rats received potassium iodide in their drinking water (10 mg/l) for 4–5 days. The addition of potassium iodide to the drinking water does not affect thyroid function (29).

After two regular cycles the rats were mated. The day that sperm appeared in the vaginal smear was taken as day 0 of gestation. One week before the experiment all rats underwent a surgical procedure; a cannula was inserted into the right jugular vein and extended to the right atrium (24).

Materials. 125I-EMD-49209, containing ~20 µCi 125I/µg of EMD-49209, was provided by Henning Berlin (Henning, Germany) (26). Just before use the purity of the labeled compound was checked by high-performance liquid chromatography (HPLC) (26). There was <0.1% iodide present, and no other labeled metabolites could be detected.

The 400-µl bolus injection consisted of a tracer amount of 125I-EMD-49209 (7 µCi, equivalent to 350 ng of the flavonoid) in saline, containing 5% normal rat serum, 0.3 U/ml heparin (Organon, Tilburg, The Netherlands), and 0.4 mg/ml ticarcil-
lin (Ticarpen; Beecham, Heppignies, Belgium). Ticarcillin is used to prevent eventual bacterial infection and diminishes nonenzymatic deiodination of the labeled compound (29); heparin is used to keep the cannula patent.

Experimental design. At 1, 2, 3, 4, 8, 14.5, 18.5, and 24 h after the bolus injection one pregnant rat was bled via the vena cava inferior under ether anesthesia; the rats were then perfused with saline via the cannula. Maternal tissues (blood, liver, kidney, brain, and thyroid); six fetal units (fetus and placenta in amnion sac), three from each uterine horn; and several fetal tissues (blood, liver, brain, placenta, and carcass) from all remaining fetuses \( n = 5–8 \) were removed, placed on ice, homogenized, counted, extracted, and submitted to HPLC to separate iodide, \(^{125}\text{I}-\text{EMD-49209}\), and its metabolites as described before in detail (24). In short, whole small tissues or weighed samples were minced and homogenized at 0°C in methanol-ammonia. The dried extracts were dissolved in 0.1 ml 0.2 M ammonia and put through HPLC to separate the flavonoid, iodide, and its metabolites. Analyses were performed with HPLC using a reverse phase C8 10 × 0.4 cm column (Chrompack, Middelburg, The Netherlands); the mobile phase was 0.625 M ammonium acetate, pH 3.8, and methanol (58:42, vol/vol). The fractions were collected and counted in a gamma counter, and their radioactivity was calculated as a percentage of the dose of \(^{125}\text{I}-\text{EMD-49209}\) [corrected for trapped plasma (29)] and expressed per whole organ. The maternal and fetal sides of the placentas were analyzed separately.

The maternal intestines were divided into five segments: three equal lengths of small intestine (I, II, and III), the cecum, and colon; and the contents was taken out. Because the animals were housed in metabolic cages, feces and urine could be collected separately. All of these radioactivity measurements were calculated as percent dose of \(^{125}\text{I} \).

To check that the injected \(^{125}\text{I}-\text{EMD-49209}\) was present in the intact form and no unknown metabolites were present, the samples were extracted with methanol-ammonia. The dried extracts were dissolved in 0.1 ml 0.2 M ammonia and put through HPLC to separate the flavonoid, iodide, and its metabolites. Analyses were performed with HPLC using a reverse phase C8 10 × 0.4 cm column (Chrompack, Middelburg, The Netherlands); the mobile phase was 0.625 M ammonium acetate, pH 3.8, and methanol (58:42, vol/vol). The fractions were collected and counted in a gamma counter, and their radioactivity was calculated as a percentage of the dose of \(^{125}\text{I}-\text{EMD-49209}\) [corrected for trapped plasma (29)] and expressed per whole organ. The maternal and fetal sides of the placentas were analyzed separately. The maternal intestines were divided into five segments: three equal lengths of small intestine (I, II, and III), the cecum, and colon; and the contents was taken out. Because the animals were housed in metabolic cages, feces and urine could be collected separately. All of these radioactivity measurements were calculated as percent dose of \(^{125}\text{I} \).

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Table 1. Distribution of \(^{125}\text{I}-\text{EMD-49209}\) radioactivity as percentage of dose of \(^{125}\text{I}-\text{EMD-49209}\) in maternal tissues (per whole organ) and fetal compartment 1–24 h after bolus injection

<table>
<thead>
<tr>
<th>Hours</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>8.5</th>
<th>14.5</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>54</td>
<td>45</td>
<td>33</td>
<td>28</td>
<td>13.8</td>
<td>6.5</td>
<td>3.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Liver</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>1.3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Brain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>The rest</td>
<td>18</td>
<td>24</td>
<td>24</td>
<td>22</td>
<td>15</td>
<td>15</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Fetal comp</td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>No. fetuses</td>
<td>14</td>
<td>13</td>
<td>14</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>

For blood, liver, brain, and residual tissues (the rest), and fetal compartment (fetal comp) percent dose is given as \(^{125}\text{I}-\text{EMD-49209}\). Residual tissues are all organs together except blood, liver, brain, fetal compartment, and intestines plus content. Fetal compartment consists of total number of fetal units per dam.

Fig. 1. Percent dose of \(^{125}\text{I}-\text{EMD-49209}\) in several fetal tissues (blood, brain, liver, carcass, total placenta), calculated as mean tissue values multiplied by number of fetuses per dam per time period. Data were analyzed by regression analysis, with time as independent parameter and represented as best fit, \( R^2 \), and \( P \) values. Blood and tissues were extracted; extracts were submitted to high-performance liquid chromatography to separate \( \text{EMD-49209} \), iodide, and its eventual metabolites. Amount of iodide found in samples ranged between 2 and 8%; metabolites were not detected. Fetal and maternal placenta are presented as whole placenta.
the label was added as a spike to the fetal plasma and submitted to HPLC. With the exception of free iodide (<0.1%) no other radioactive metabolites were detected. The extraction efficiency for 125I activity from all tissues was higher than 95%.

The computer program Kaleidograph for MacIntosh was used for least squares nonlinear curve-fit analysis; it was developed by Abelbeck Software and published by Synergy Software.

RESULTS

In maternal blood there is a fast decrease in percent dose 125I-EMD-49209 with time; in contrast, after 1 h delay there is a gradual increase in the percent dose flavonoid in fetal blood.

In Table 1 the percent dose 125I-EMD-49209 in maternal blood, liver, brain, and the residual tissues is given according to time. The residual tissues include all organs and tissues together except blood, liver, brain, and intestinal contents; muscle and skin accounted for most of the radioactivity (about 10% each) of the residual tissues. There is a decrease in percent dose 125I-EMD-49209 in maternal blood, liver, and the residual tissues, whereas there is an increase in percent dose 125I-EMD-49209 in the total fetal compartment with time. No flavonoid could be detected in the maternal brain. Thyroidal uptake of the flavonoid was below the detection limit. At 24 h most of the flavonoid (60–70%) had been elimated from tissues and plasma and excreted via feces and urine (data not shown).

Figure 1 shows the increase in the percent dose 125I-EMD-49209 in fetal tissues, blood, brain, liver, and carass with time, presented together with the best fit, R2, and P values. For the total placenta a decrease in the percent dose of 125I-EMD-49209 was found.

DISCUSSION

The disappearance of the flavonoid from maternal blood was comparable to the disappearance from the blood of nonpregnant rats; the decrease in radioactivity in maternal tissues and increase in the radioactivity in intestinal content were also comparable (26). As shown previously, the intact flavonoid or its metabolite does not cross the blood-brain barrier in the adult rat (26). This finding indicates that flavonoids might affect thyroid hormone transfer to the brain via either the blood-brain or the blood-chorioid plexus-cerebrospinal fluid barrier without being transported themselves by TTR or other carriers (5, 27).

Our data indicate that the flavonoid crosses the placenta and enters fetal tissues. The increase in the flavonoid concentration in fetal tissues is striking. Twenty-four hours after injection of the labeled compound, 60–70% of it had already left the maternal body, whereas about 17% of the initial dose of 125I-EMD-49209 was still present in the fetal compartment, i.e., 1.2–1.5% per fetus, indicating a relative accumulation in the fetal rat compared with the mother. We have shown that this synthetic flavonoid can reach the fetal brain, in contrast to the maternal brain. In some fetal tissues the flavonoid concentration was even higher than in maternal tissues. Thus it seems that there is a transfer of the flavonoid to the fetal compartment. The question of whether this is caused by the large amount of TTR in the fetal compartment has yet to be answered (23, 30).

Synthetic flavonoids have been shown to displace T4 from TTR (5, 15, 25). TTR is the principal high-affinity thyroid hormone carrier protein in the fetal rat. After birth the TTR level decreases to nearly zero, but later it increases again to adult levels (23, 30). Because the flavonoid is present in the fetus, it is of importance to realize that these flavonoids might interfere with T4 binding to TTR and thus might influence T4 availability in tissues during a period when the hormone is very important for fetal development (20). An essential question still remains, namely, are these effects of a synthetic flavonoid representative of those of natural flavonoids that are common in our diet?

In conclusion, synthetic flavonoids cross the placenta and enter fetal tissues, where they reach higher concentrations than in the tissues of the mother. It is tempting to speculate that the fact that flavonoids reach the fetal compartments, including the brain, implies that the availability of thyroid hormones could be restricted in fetal tissues. If transfer for natural flavonoids that act as goitrogenics (11, 22) also occurs, it is important to realize that fetal development could be impaired by the intake of these flavonoids that are part of the daily diet; this is particularly true if there is also severe or moderate iodine deficiency, which unfortunately is still true in a large part of the world.

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