In-vivo study of diazepam transfer across the first trimester human placenta

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Diazepam transfer by the first trimester human placenta was investigated at pregnancy termination between 6 and 12 weeks of gestation. Fetal fluid samples were obtained from the exocoelomic and amniotic cavities of 65 pregnancies between 8 and 25 min following the i.v. administration of 0.1 mg/kg diazepam to the mother. Diazepam was detected in one-third of coelomic fluid samples and two-thirds of amniotic fluid samples. Maternal serum and urine diazepam concentrations correlated negatively and positively respectively, with time from drug injection to sampling. Individual diazepam concentrations were low on the fetal side, and the corresponding concentrations were independent of maternal serum concentrations and the time from drug injection to sampling. Amniotic fluid diazepam content increased significantly with advancing gestational age. A multiple regression analysis showed that the diazepam content of the coelomic fluid was not influenced by maternal serum diazepam concentration, the time from drug injection to sampling or gestational age, whereas only gestational age contributed to the diazepam content of amniotic fluid. These data demonstrate that the placental transfer of diazepam occurs from week 6 of gestation, indicate a preferential transfer of this drug to the amniotic cavity and suggest that diazepam may accumulate in fetal circulation and tissues during organogenesis.

Key words: diazepam/first trimester/placenta/pregnancy/transfer

Introduction

The placental transfer of drugs has been studied mainly in experimental animals such as guinea pigs and monkeys because they have haemochorial placentas similar to those of humans (Burton, 1992). Considerable embryological differences exist between humans and these animal species, which limit the value of data concerning the mechanisms of transfer of most drugs and their interference with organ development in the human fetus. Materno-fetal drug transfer in humans has been investigated more satisfactorily in vitro by using isolated, dually perfused placenta (Sibley and Boyd, 1992). However, because of technical limitations, similar studies are not feasible during the first half of pregnancy, and there is very little information on the placental transfer and metabolism of drugs during this particular period of gestation.

Previous studies of diazepam pharmacokinetics and pharmacodynamics in human pregnancies have shown that placental transfer during the third trimester is rapid and fetal uptake appears significant (Bakke and Haram, 1982). The possible relationship between the maternal use of diazepam during the first trimester and fetal cleft lip with or without cleft palate has generated numerous debates (Safra and Oakley, 1975; Rosenberg et al., 1983; Bergman et al., 1992). However, there are no data on the transfer of benzodiazepines at the time of fetal organogenesis. Recently it has become possible to selectively aspirate coelomic and amniotic fluids inside the human gestational sac from the beginning of the second month of pregnancy. We have examined the concentration of diazepam in the fetal fluid compartment, following the maternal administration of this drug during the first trimester of pregnancy.

Materials and methods

Patients and sample collection

Fetal fluids and peripheral venous blood samples were obtained from 65 healthy women between 6 and 12 weeks of gestation before the elective termination of pregnancy for psychological indications. This study was approved by the Research Ethics Committee (King's College Hospital, London, UK), and written consent was obtained from each patient after receiving complete information on the procedure. Only women with uncomplicated pregnancies presenting with a fetal heart rate within the normal range for gestation were included in the study group. The 65 patients in this study consented to the sampling of peripheral venous blood and embryonic fluids during the surgical procedure. Only 40 patients consented to bladder catheterization. Gestational age was determined from the date of the last menstrual period and confirmed by ultrasound. All patients had normal electrolytes and haematological values. Their ages varied between 18 and 32 years (mean 27.3 years) and weights between 55 and 92 kg (mean 67.3 kg).

Pregnancy termination surgical procedures were performed following the protocol used routinely in our departments for uterine evacuation under local anaesthesia; this includes a pre-operative i.v. injection (0.1 mg/kg) of diazepam (Diazemul®, Dumex, Herts, UK) in 15 s via a catheter inserted in the left forearm and a paracervical block using 1% lignocaine. All patients were asked to empty their bladder 20 min before the procedure. Coelomic and amniotic fluid samples were obtained from the corresponding cavities as described previously (Jauniaux et al., 1991) between 8 and 25 min after the end of the bolus of diazepam. In brief, coelomic fluid was first
aspirated using a 20 gauge needle introduced transvaginally into the coelomic cavity under continuous ultrasound guidance. Within 15 s, another needle of the same size was inserted into the amniotic cavity and the corresponding fluid aspirated. Both cavities were emptied to evaluate their corresponding volume. A puncture of an antecubital vein of the right arm and bladder catheterization were performed simultaneously to obtain maternal blood and urine at the time of fetal fluid aspiration (between 10 and 13 min in 17 cases, 14 and 17 min in 17 cases, 18 and 21 min in 16 cases, and 22 and 25 min in 15 cases).

On seven occasions the needle was left inside the exocoelomic cavity and fluid was aspirated at 1, 2 and 3 min intervals after the beginning of the procedure, which started respectively 10, 14, 16, 18, 20, 22 and 23 min after the diazepam i.v. injection. In these cases, no amniotic fluid was aspirated and maternal serum and urine were collected simultaneously with the first coelomic fluid aspiration. Samples of maternal serum and urine and fetal fluids were stored at -20°C.

**Diazepam assay**

Diazepam and its main metabolite (N-desmethyldiazepam and N-methyloxazepam) concentrations were determined by the fluorescence polarization immunoassay technique (Abbott Laboratories, Chicago, IL, USA). The limit of sensitivity for this assay was 5 ng/ml. Commercially available benzodiazepine serum controls at different concentrations were used to determine the intra-assay coefficients of variation, which were 12.0, 2.7, 3.2 and 3.1% at diazepam concentrations of 15, 75, 300 and 700 ng/ml respectively. Between 5 and 10 ng/ml, the intra-assay coefficient of variation was 16% on eight paired series of amniotic and coelomic fluid samples. Five control series of samples from women not taking benzodiazepines were used to obtain blank values for the assay.

**Statistical analysis**

Linear regression equations were calculated by the least-square methods and their slopes tested for significance by the F ratio test. Multiple linear regressions including gestational age, maternal serum diazepam concentration and time from drug injection to sampling were performed to examine the relationships between these variables and the diazepam content in coelomic or amniotic fluid, which was calculated as the product of diazepam concentration and volume of the corresponding fluids. A biomedical data processing statistical package (Statgraphics; Mercia Software Ltd, Birmingham, UK) was used for the analysis. Results were considered statistically significant at $P < 0.05$.

**Results**

Coelomic fluid was aspirated in 55 pregnancies and amniotic fluid was obtained in 57 pregnancies, including in total 47 series of matched samples of maternal serum and coelomic and amniotic fluids. The gestational ages at coelocentesis and amniocentesis ranged from 6.3 to 11.5 and from 8.1 to 12.1 weeks respectively. Because of insufficient space, no amniotic fluid could be aspirated before 8 weeks of gestation and no coelomic fluid could be aspirated after 11 weeks of gestation. Diazepam concentrations were measured on all 65 serum samples and on the 40 urine samples available. Diazepam concentrations above the lower limit of the assay were detected in 20 out of the 55 (36.4%) coelomic fluid samples, including some samples collected during the 6th and 7th weeks of gestation and in 38 out of the 57 (66.7%) amniotic fluid samples.

The mean total diazepam concentration was $444 \pm 202$ ng/ml in maternal serum, $21.8 \pm 8.6$ in maternal urine, $7.3 \pm 1.8$ in coelomic fluid and $7.1 \pm 1.5$ in amniotic fluid (Table I). The diazepam concentration correlated with the time from drug injection to sampling on the maternal side (serum, $r = 0.47$, $n = 65$, $P = 0.0001$; urine, $r = 0.32$, $n = 40$, $P = 0.045$) but not on the fetal side (coelomic fluid, $r = 0.30$, $n = 20$, $P = 0.051$; amniotic fluid, $r = 0.27$, $n = 38$, $P = 0.121$). No significant correlation was found between the diazepam concentrations measured in the different maternal and fetal compartments. Amniotic fluid diazepam content increased significantly ($r = 0.80$, $n = 38$, $P < 0.0001$) with gestational age (Figure 1). No significant ($r = 0.32$, $n = 20$, $P = 0.16$) change was found in the amount of diazepam in coelomic fluid with advancing gestational age (Figure 2). The multiple regression analysis showed that gestational age was the only significant contributor to the diazepam content of amniotic fluid (Table II).
Time intervals
Table L
Comparison between maternal and fetal diazepam concentrations with advancing time

<table>
<thead>
<tr>
<th>Time intervals (min)</th>
<th>Maternal serum (ng/ml)</th>
<th>Maternal urine (ng/ml)</th>
<th>Coelomic fluid (ng/ml)</th>
<th>Amniotic fluid (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 17</td>
<td>n = 6</td>
<td>n = 4</td>
<td>n = 4</td>
</tr>
<tr>
<td>10-13</td>
<td>568.2 ± 227.6</td>
<td>12.3 ± 4.9</td>
<td>6.3 ± 0.6</td>
<td>6.6 ± 1.7</td>
</tr>
<tr>
<td>14-17</td>
<td>507.4 ± 118.3</td>
<td>16.9 ± 13.4</td>
<td>6.9 ± 1.1</td>
<td>6.8 ± 1.6</td>
</tr>
<tr>
<td>18-21</td>
<td>343.1 ± 172.8</td>
<td>28.0 ± 26.3</td>
<td>6.7 ± 1.6</td>
<td>7.7 ± 1.6</td>
</tr>
<tr>
<td>22-25</td>
<td>287.7 ± 94.6</td>
<td>30.1 ± 26.5</td>
<td>9.2 ± 2.4</td>
<td>6.8 ± 1.1</td>
</tr>
<tr>
<td>Total</td>
<td>444.1 ± 202.3</td>
<td>21.8 ± 8.6</td>
<td>7.3 ± 1.8</td>
<td>7.1 ± 1.5</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD.

Table II. Multiple linear regression analysis between the total amount of diazepam in fetal fluid and the main independent variables

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Diazepam content</th>
<th>Coelomic fluid</th>
<th>Diazepam content</th>
<th>Amniotic fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>t value</td>
<td>p value</td>
<td>Coefficient</td>
</tr>
<tr>
<td>Gestational age</td>
<td>1.49 ± 0.157</td>
<td>8.28</td>
<td>&lt;0.0001</td>
<td>0.365</td>
</tr>
<tr>
<td>Time</td>
<td>1.27 ± 0.223</td>
<td>-1.85</td>
<td>0.072</td>
<td>0.977</td>
</tr>
<tr>
<td>Maternal serum diazepam</td>
<td>-0.03 ± 0.977</td>
<td>-0.92</td>
<td>0.365</td>
<td>0.365</td>
</tr>
</tbody>
</table>

Diazepam was detected in two out of the seven cases for which sequenced samples of coelomic fluid were available. In these cases, puncture of the exocoelomic cavity started 10 and 14 min respectively following the i.v. injection, and the corresponding very low (5.6 and 6.2 ng/ml) coelomic diazepam concentrations were found in the third sample.

Discussion

There are few pharmacokinetic studies of the transfer of diazepam or other drugs across the placenta during early human pregnancy. Most studies are carried out in the third trimester, while most damage from drugs occurs early in the first trimester. The results of the our study indicate that diazepam molecules are transferred in small amounts by the human placenta between weeks 6 and 10 of gestation when the fetal face forms (Moore, 1982). These findings concur with its putative teratogenic potential and suggest that sampling the exocoelomic cavity can be of relevance to the study and quantification of drug transfer from the maternal to the fetal compartments in early pregnancy. However, causes of left lip and cleft palate include genetic and non-genetic factors which seem to operate by influencing the amount of neural crest mesenchyme that migrates into the embryonic facial primordia (Ross and Johnston, 1972). Bergman et al. (1992) have shown in their survey of 104 000 women delivered during 1980-1983 that the high rate of teratogenicity after heavy maternal benzodiazepine intake occurs with multiple alcohol and substance exposure, and thus may not be caused by benzodiazepine exposure alone.

Diazepam is a lipophilic and undissociated drug with a low molecular weight of ~280 Da which easily crosses biological membranes via water-filled extracellular channels or directly through the cells, as is the case for the trophoblastic barrier (Kanto, 1982). Diazepam is mainly metabolized to N-desmethyl-diazepam and N-methyloxazepam by the liver. In humans, this metabolism has been observed during fetal life from 13 weeks of gestation onwards (Ackermann and Richter, 1977). Idanpaan-Heikkila et al. (1971) and Erkkola et al. (1974) have compared the concentrations of diazepam in fetal blood and tissues and maternal plasma between 12 and 16 weeks of gestation. The patients received a single i.m. injection of 5-10 mg diazepam before pregnancy termination, and fetal samples were obtained at hysterotomy. In these cases, similar concentrations were found in fetal and maternal plasma 40 and 120 min after injection of the drug. Diazepam was also found in fetal liver, brain and placenta, but interestingly never in amniotic fluid samples.

Using a sensitive assay, we have demonstrated that diazepam is found in two-thirds of amniotic fluid samples collected during the second and third months of pregnancy, and that the total amount of diazepam in amniotic fluid increases with advancing gestation. The placental transfer of most drugs is greater in late pregnancy than in early pregnancy, mainly because uterine circulation increases and the trophoblastic layer becomes thinner as pregnancy advances. Lipophilic drugs including diazepam rapidly cross the placenta, the rate of transfer being limited only by blood flow rates (Sibley and Boyd, 1992). After i.v. injection of the mother at term, the materno-fetal distribution equilibrium of diazepam is attained rapidly within 5–10 min (Bakke and Haram, 1982; Kanto, 1982). Thereafter, the feto-maternal ratio increases with time, indicating accumulation in the fetus, in particular in its liver tissue (Erkkola et al., 1974). The mean diazepam concentration in this study was low on the fetal side, corresponding to a small fraction of the total mean diazepam concentration in maternal serum. Furthermore, amniotic and coelomic fluid diazepam concentrations were not correlated with either maternal serum concentrations or the time from drug injection to sampling within the 25 min limit of the study. These findings could be explained by the absence of continuous maternal intervillous circulation before the end of the first trimester (Hustin and Schaaps, 1987).

Diazepam concentration was more frequently measurable in the amniotic fluid, suggesting a preferential transfer which may be due to binding of this drug to fetal serum albumin. There is an increase in the concentration gradient between
the exocoelomic and amniotic cavities for molecules with molecular weights \( >100 \) Da (Gulbis et al., 1992; Jauniaux et al., 1993, 1994, 1995), suggesting that the coelomic fluid protein turnover is slow and/or that the transfer of molecules through the amniotic membrane is limited. Thus, it is likely that, over a short period of time, diazepam molecules crossing the trophoblast are transferred mainly to the fetus via the chorionallantoic and/or the yolk sac circulations (Jones and Jauniaux, 1995), and do not accumulate in the exocoelomic cavity. In the first trimester, fetal urine is probably not the source of amniotic fluid diazepam because the definitive kidney produces urine only from ~10 weeks of gestation (Moore, 1982). Thus, the transfer of these drugs from the fetal circulation into the amniotic cavity probably occurs through the fetal skin, which becomes keratinized only during the second trimester.

Because of ethical considerations in the experimental design of this study, fetal fluids could be aspirated only up to 25 min following maternal injection of the drugs. The pharmacodynamics and distribution of diazepam metabolites on the fetal side during the first trimester, after a longer period of time or after chronic maternal intoxication, requires further investigation.

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References


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