Liver Blood Perfusion as a Possible Instrument for Fetal Growth Regulation


Universitätsklinikum Hamburg-Eppendorf, a Klinik für Frauenheilkunde und Geburtshilfe, and b Neurologische Klinik, Abteilung für Experimentelle Medizin, Martinistraße 52, 20246 Hamburg, Germany

Placental and fetal liver blood perfusions are reduced in intrauterine growth-restricted human fetuses. We hypothesized that changes in fetal liver blood supply can alter fetal growth. In nine ewes with twin pregnancies at a gestational age of 119 ± 2 days, a stent (4 mm) was placed into the ductus venosus of one twin (DVstent group). Alternatively, in 17 near term sheep with twin (n=11) or singleton (n=6) pregnancies, a DV was blocked with an embolization coil (DVcoil group) for about one week. The cell proliferation rate (pKi-67) was determined in the liver, heart, skeletal muscle, kidneys and placenta. The dilatation or occlusion of the DV did not change placental perfusion on the first day or later after surgery. The liver blood supply was decreased in the DVstent group by more than half from 499 ± 371 to 278 ± 219 ml min⁻¹ (mean ± s.d., n=4), and increased two-fold in the DVcoil group (P<0.05). The percentage of liver/body weight was decreased from 3.9 ± 0.6 per cent in control twin to 3.0 ± 0.2 per cent (n=3) in the DVstent group. Occlusion of the DV lead to an increase in the percentage of liver/body weight from 3.4 ± 0.8 per cent to 4.3 ± 0.8 per cent (n=11, P<0.05). Reduced liver blood supply in the DVstent group was associated with a decrease of cell proliferation in the liver from 12.43 ± 2.31 to 6.5 ± 0.62 (nuclei μm⁻² 10⁻⁴, n=3, P=0.058), in heart from 1.14 ± 0.03 to 0.93 ± 0.02 (nuclei μm⁻² 10⁻⁴, P<0.05), and in skeletal muscle from 0.82 ± 0.05 to 0.54 ± 0.01 (nuclei μm⁻² 10⁻⁴, P<0.05). The increased liver blood perfusion following occlusion of the DV increased cell proliferation sixfold in the liver, (n=9, P<0.005) and twofold in heart muscle, skeletal muscle and the kidneys (P<0.05), whereas no significant difference was seen in the placenta. The expression of mRNA for IGF-I and IGF-II in the liver was increased in the DVcoil group. In conclusion, these results suggest that liver blood perfusion can regulate fetal growth.

INTRODUCTION

Intrauterine growth retardation (IUGR) is one of the leading causes of perinatal morbidity and mortality in developed countries (Brar and Rutherford, 1988). There is no successful procedure for treatment of IUGR fetuses, except for premature termination of the pregnancy. Premature delivery could itself increase the risk of perinatal death (Allen et al., 1993) and of neurological, behavioural and cognitive function later in life (Dammann et al., 1996; Stewart et al., 1999). The effort to increase placental blood perfusion and/or 'normalize placental functions' in order to treat growth-retarded fetuses could not be efficient, because the placenta of growth-retarded fetuses as a rule is damaged in early gestation (Caniggia et al., 2000).

Due to the anatomical position of the liver, its role in controlling the distribution of nutrients from the placenta is obvious. The liver is the first fetal organ to receive well-oxygenated blood from the placenta, which also is the richest in amino acids and glucose. In fetal sheep, one-third of the umbilical blood flow bypasses the liver tissue via the ductus venosus (DV), and two-thirds of the umbilical blood flow supplies the liver (Rudolph, 1983). The ductus venosus/umbilical vein (DV/UV) flow ratio is quite variable. Under hypoxic stress in fetal sheep, placental blood perfusion is reduced, and the DV/UV flow ratio is increased from 36 ± 5 per cent to about 53 ± 6 per cent (mean ± s.d.) (Tchirikov et al., 1998a). In human intrauterine growth-restricted fetuses, we observed a decrease in umbilical blood flow and an increase of DV/UV flow ratio to 62 ± 8 per cent (Tchirikov et al., 1998b). Blood flow through the DV is maintained to ensure survival of the fetus in stress situations by supplying important fetal organs such as the brain and heart with oxygen (Rudolph, 1983; Reuss and Rudolph, 1980). However, the liver blood perfusion in IUGR fetuses is reduced markedly from 76 ± 35 to about 43 ± 23 (ml/min/kg), because the intrahepatic branches of the portal vein react significantly stronger to catecholamines, compared to the isthmic portion of the DV (Tchirikov and Schröder, 2001). Additionally, the diameter of the DV in IUGR fetuses may be increased (Bellotti et al., 2001).
The liver produces proteins, lipids and carbohydrates, and is involved in their metabolism (Seifer and Englard, 1994). In addition, the liver synthesizes IGF-I, IGF-II, IGF binding proteins and other growth factors (Sjögren et al., 1999). In the present study, we propose that the reduction of the liver blood supply in IUGR fetuses can influence fetal growth and could itself lead to growth retardation.

Our previous studies have shown a significant increase of cell proliferation in the fetal organs after an increase of the liver blood perfusion via the obstruction of the ductus venosus. (Tchirikov et al., 2001). The increase of the proliferation rate in the organs may be associated with an increase of mRNA expression for IGF-I and IGF-II in the fetal liver. The influence of reduced liver blood supply on fetal growth has not been investigated.

To test the influence of liver blood flow on fetal growth, we inserted a stent into the DV in fetal sheep in order to decrease fetal liver blood perfusion. Additionally, the expression of mRNA for IGF-I and IGF-II in the fetal liver and the plasma concentration of amino acids, glucose, lipids, IGF-I, IGF-II and norepinephrine in fetuses with a closed ductus venosus were investigated.

MATERIALS AND METHODS

Procedures

The experimental procedures were approved by the board on animal studies of the State Authority for Labor, Health and Social Issues of the State of Hamburg, Germany. The experiments were carried out in two groups: in a DVstent group and in a DVcoil group. The DVstent group included nine ewes, which had twin pregnancies at a gestational age of 119 ± 2 days. The DVcoil group included 17 near term sheep with twin (n=11) or singleton (n=6) pregnancies. While a portion of the data from the DVcoil group has been previously published (Tchirikov et al., 2001), this paper reports results on an increased number of animals.

DVstent group experiments

The ewes were sedated with xylazine i.m. (Rompun®, 0.25 mg/kg). Then the animals were anaesthetized with thiopental-sodium i.v. (Trapanan®, 1.0 g), followed by ventilation with 1.0–1.5 per cent isoflurane in O₂/N₂O (2 : 1).

The maternal abdomen was opened under aseptic conditions. An incision was made in the uterus and the head and neck of one fetus was delivered. A catheter (5F HNB-5.0–38–45-PW–NS–NPA, Cook®) and guide wire were passed through a catheter port (RCFW, Cook®), inserted into the right external jugular vein of the fetus, and advanced through the right atrium and the ductus venosus into the intrahepatic part of the umbilical vein under ultrasound control (Acuson Aspen®, USA) (Tchirikov and Schröder, 1998). A coronary stent (10 or 15 mm length and 4 mm diameter in inflated state, Biotronik Tenax®, Berlin, Germany) with a balloon catheter was placed in the isthmic portion of the ductus venosus. Then the balloon was inflated for 1 min and the stent was fixed in the isthmic portion of the ductus venosus (Figure 1).

The inserted catheter was removed and replaced by a short, chronic venous catheter. Additional catheters were placed in the right carotid artery and in the amniotic cavity in six experiments. The fetal head was returned into the uterine cavity and the uterus was closed. The head and neck of the twin fetus were exposed through the second uterotomy. The sham catheterization of the ductus venosus was performed (see above). Then catheters were inserted in the right external jugular vein and into the carotid artery as described above. The fetal incisions were closed, the fetal head was returned and the uterine wall was sutured. This fetus served as a control. All catheters were led to the left flank of the ewe and stored in a pouch attached to the maternal skin.

The catheters were flushed daily with heparinized saline (1000 U/ml). Antibiotic (cefalozin-sodium, 1 g/day i.v. and 1 g/day into the amniotic fluid) was used to protect fetuses from infection. Blood gases were determined daily from carotid arterial blood samples (Radiometer Copenhagen ABL 710). Blood pressure, heart rate, and blood flow rate in the umbilical vein and the ductus venosus were measured at days 1 and 4, and later if possible.

Doppler blood flow measurements

Blood flow rates in the umbilical vein and ductus venosus of both fetuses were measured using Doppler ultrasound (Acuson Aspen®, USA) with a 7 MHz convex ultrasound transducer (Tchirikov et al., 1998a). The blood flow profiles in the hepatic veins were analysed.

Termination of experiments

The experiments were terminated by injection of a proprietary euthanasia solution T61® (Hoechst). Autopsies were performed on both fetuses in parallel, and the weights of the livers, kidneys and hearts were determined (Tchirikov et al., 2001). Tissue samples from the right and left liver lobes, hearts, kidneys, quadriiceps femoris and from randomly selected cotyledons of the placenta were immediately placed in liquid nitrogen. Additional tissue samples were placed in a 3.5 per cent formaldehyde solution for 24 h and then embedded in paraffin.

Detection of cell proliferation

All paraffin blocks were prepared for Mib-1 immunohistochemistry to detect proliferating cells (Tchirikov et al., 2001). The Mib-1 antibodies are directed against the nuclear antigen pKi-67 (Cattoretti et al., 1992) which is expressed in all phases of the cell cycle during proliferation, except the G₀ phase. The number of Mib-1 positive cell nuclei per square micron was determined automatically on a C.A.S.T.-Grid system (Olympus, Denmark).
DVcoil group experiments

In 17 near term sheep (122 ± 4 days) with twin (n=11) or singleton (n=6) pregnancies, the ductus venosus was blocked with a Jackson embolization coil (MWCE 38–4–4, Cook®) in one fetus and left open in the twin (control). In six sheep with singleton pregnancies, the DV was occluded. Cell proliferation was analysed after 5 ± 2 days post operation in tissue samples from the right and left liver lobes, hearts, kidneys, quadriceps femoris, and from randomly selected cotyledons of the placenta.

Biochemical investigation of fetal and maternal plasma

Plasma samples were obtained from 17 fetuses with closed ductus venosus, from control twin fetuses and ewes on the 1st, the 4th and in seven cases on the 8th postoperational days.

Table 1. Cell proliferation index of fetal liver, heart, skeletal muscle and kidney in three fetuses with ductus venosus dilated by a stent and in control twins expressed as the density of nuclei (number of nuclei μm² 10⁻⁴). Mib-1 antibodies were used to detect the nuclear proliferation antigen pKi-67. Mean ± s.d.

<table>
<thead>
<tr>
<th>Fetal organ</th>
<th>Control twin (number of nuclei μm² 10⁻⁴)</th>
<th>DVstent fetus (number of nuclei μm² 10⁻⁴)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>12.43 ± 2.31</td>
<td>6.5 ± 0.62</td>
<td>0.058</td>
</tr>
<tr>
<td>Heart</td>
<td>1.14 ± 0.03</td>
<td>0.93 ± 0.02</td>
<td>0.007</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.82 ± 0.05</td>
<td>0.54 ± 0.01</td>
<td>0.007</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.45 ± 0.05</td>
<td>1.34 ± 0.12</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Figure 1. Correct position of a stent (4 mm diameter and 15 mm length) in the ductus venosus at autopsy. The small section of the stent (3 mm) is placed in the Rex recessus. A swab marks the umbilical vein. The ligature is tied on the inferior caval vein.
Measurements of essential amino acids (threonine, valine, methionone, isoleucine, leucine, phenylalanine and lysine) and non-essential amino acids (taurine, serine, asparagine, glutamate, glycine and alanine), glucose, lipids, IGF-I, IGF-II and norepinephrine in fetal and maternal plasma samples were performed in the central clinical laboratory. Because there were no significant changes in the concentration of most plasma constituents with time in experiments which lasted 7 days or longer ($n=7$), all data were pooled for experimental fetuses with closed DV, for control twin fetuses and for measurements of maternal plasma. Samples were deproteinized, neutralized and injected into a Biochrom 20 HPLC amino acid analyser (Pharmacia) and concentrations were derived from peak areas. Catecholamines were analysed with an HPLC system with Millipore Waters 460 column as an electrochemical detector. Substances were identified by the retention times and standards, and concentrations were calculated based on peak areas. The coefficient of intra-assay variation was 4.0 per cent for norepinephrine and 4.3 per cent for epinephrine.

Free fatty acids were measured with a commercial kit (Roche Diagnostics, 13831/001), following the instructions of the manufacturer.

Plasma samples were extracted to remove IGF binding proteins using the acid-ethanol cryoprecipitation method (Owens et al., 1991). Immunoradiometric assay kits (Diagnostic Systems Laboratories, Texas, USA) were used to measure IGF-I (DSL-5600) and IGF-II (DSL-9100).

Determination of mRNA for IGF-I and IGF-II in fetal livers with closed ductus venosus

Paraffin sections of liver tissues from five twin animals were analysed for IGF-I and IGF-II mRNA expression by in situ hybridization using $^{35}$S-labelled IGF-I and IGF-II antisense riboprobes (Courtesy of V. K. M. Han, London, Ontario, Canada). The levels of IGF-I and IGF-II mRNA were quantified by computer-controlled photometry and, after development of the hybridized tissue sections, also by counting the number of autoradiograph signals per cell.

Statistics

Data are presented as mean ± s.d. The non-parametric Mann–Whitney $U$-test was used to detect the significance between mRNA expression levels for IGF-I and IGF-II, and between biochemical parameters. Calculations were carried out using Statistica$^\text{®}$ software (Statsoft, Tulsa, USA). $P$ values <0.05 were considered to be statistically significant.

RESULTS

In six fetuses, the stent was found in the correct position during the autopsy (Figure 1). However, in two fetuses the stent deformed the outlet of the ductus venosus, and the reduction of resistance in the DV was less than expected. In two fetuses, the stent was placed incorrectly in the Rex recessus for the isthmic portion of the ductus venosus.
Fetuses died before autopsy. The stent implantation in the DV chronically instrumented with vessel catheters. One of these fetuses survived 4 (n=2), 8 and 10 days (two fetuses were not chronically instrumented with vessel catheters). One of these fetuses died before autopsy. The stent implantation in the DV reduced liver perfusion by more than half from 499 ± 371 to 278 ± 219 ml min⁻¹ on the 1st day after the operation (P<0.05, n=4). The umbilical blood flow rate was not affected. However, the DV blood flow rate increased from 110 ± 37 to 362 ± 50 ml min⁻¹ (P<0.001). A reverse of blood flow in the liver vein was observed in fetuses with successful stent implantation (n=4, data not shown).

The percentage of liver/body weight was decreased from 3.9 ± 0.6 per cent in control twin to 3.0 ± 0.2 per cent (n=3) in the DVstent group (N.S.). Table 1 presents data on the cell proliferation index in fetal organs. The reduction of liver blood perfusion led to decreased cell proliferation in the liver, heart and skeletal muscle.

The increase of liver blood perfusion following occlusion of the DV with an embolization coil significantly increased liver weight and cell proliferation in the liver, heart, kidney and skeletal muscle [Figures 2 (a and b)]. We did not observe any difference in the placenta. Occlusion of the DV slightly decreased fetal arterial Po₂ from 17.3 ± 6.6 to 14.6 ± 6.9 mmHg (n=6, P>0.05).

Figure 3 shows the IGF mRNA expression in the liver with a closed DV. We could not determine any significant change in the biochemical values between fetuses with closed DV and control fetuses (Table 2).

**DISCUSSION**

The liver plays a central role in supplying sources of energy from placenta to peripheral tissues. It is also a primary fetal organ of hematopoiesis (Seifer and Englard, 1994). The liver is the major site of oxidation of essential amino acids and lipids, as well as the source of growth factors (Hellerstein and Munro, 1994; Seifer and Englard, 1994). Because the liver blood perfusion is dramatically reduced in IUGR (Tchirikov et al., 1998b), we hypothesized that reduced liver blood supply in IUGR fetuses can decrease fetal growth.

The results of this study demonstrate that decreased liver blood supply may reduce cell proliferation in fetal liver, heart and skeletal muscle. On the other hand, increased liver blood perfusion in fetal sheep significantly stimulated cell proliferation in fetal liver, heart, kidney and skeletal muscle, but did not alter cell proliferation in placenta. The results of the present study did not support our previous observation that increased liver blood flow could decrease cell proliferation in the placenta (Tchirikov et al., 2001). The increased rate of cell proliferation in fetal liver with closed DV was related to increased expression of IGF-I and IGF-II mRNAs in fetal liver. We did not detect any significant biochemical deviations in the levels of essential (threonine, valine, methionone, iso-leucine, leucine, phenylalanine and lysine) and non-essential amino acids (taurine, serine, asparagine, glutamate, glutamine, glycine and alanine), glucose, lipids, IGF-I, IGF-II and norepinephrine in fetal plasma from animals with a closed DV. In this regard, the demand for and consumption of amino acids and fatty acids in the fetuses with increased liver supply are likely well-balanced (Hellerstein and Munro, 1994), in that placental blood perfusion and the rate of cell proliferation were not altered. On the other hand, a number of other growth factors and parameters (i.e., IGF binding proteins, insulin, leptin, epidermal and hepatocyte growth factors, interleukins) could be involved in the regulation of fetal growth by the liver (Somerset et al., 1997; Clemmons, 1998). Occlusion of the DV slightly reduced the Po₂ value in the carotid artery from 17.3–14.6 mm Hg (N.S.). Alterations in Po₂ could influence cell proliferation in fetal organs, as reported by Ahmed et al. (2000) that reduced Po₂ can increase cell proliferation in placenta. However, chronic hypoxia in fetal sheep has also

<table>
<thead>
<tr>
<th>Plasma component</th>
<th>Fetus</th>
<th>Control</th>
<th>Ewe</th>
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<tbody>
<tr>
<td>IGF-I (ng/ml)</td>
<td>70 ± 56</td>
<td>70 ± 58</td>
<td>290 ± 172</td>
</tr>
<tr>
<td>IGF-II (ng/ml)</td>
<td>588 ± 310</td>
<td>733 ± 344</td>
<td>202 ± 177</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>28.7 ± 8.3</td>
<td>31.6 ± 11.6</td>
<td>66.5 ± 12.4</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>32.3 ± 4.8</td>
<td>31.7 ± 3.4</td>
<td>55.6 ± 4.7</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>13.8 ± 4.4</td>
<td>14.9 ± 1.9</td>
<td>23.8 ± 3.0</td>
</tr>
<tr>
<td>Total AA (mmol/l)</td>
<td>4.4 ± 1.1</td>
<td>4.2 ± 1.0</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>Essential AA (mmol/l)</td>
<td>0.83 ± 0.27</td>
<td>0.76 ± 0.2</td>
<td>0.49 ± 0.19</td>
</tr>
<tr>
<td>Non-essential AA (mmol/l)</td>
<td>1.3 ± 0.45</td>
<td>1.2 ± 0.43</td>
<td>0.69 ± 0.29</td>
</tr>
<tr>
<td>FFA (μmol/l)</td>
<td>32 ± 21</td>
<td>35 ± 18</td>
<td>403 ± 206</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>277 ± 257</td>
<td>222 ± 110</td>
<td>92 ± 28</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>1206 ± 1285</td>
<td>1050 ± 505</td>
<td>1168 ± 562</td>
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</tbody>
</table>
been reported to reduce DNA synthesis (Hooper et al., 1991; Gagnon et al., 1995; Asano et al., 1997).

The liver blood supply can be regulated by a change in the diameter of the ductus venosus, because of parallel arrangement of branches of the portal vein and the DV in fetal liver. Occlusion of the DV usually results in an increase in diastolic blood flow. In contrast, the successful implantation of the stent in the isthmic portion of the DV can lead to reverse blood flow in hepatic veins and can induce pulsation in the umbilical vein. These observations were very helpful in the correct implantation of the coil or stent into the DV. The incorrect implantation of the stent, or deformation/reduction of outlet of the ductus venosus by the stent could be predicted because the diastolic blood flow in the hepatic veins was not reversed. Thus, these findings may be helpful in understanding the blood flow velocity profiles in the ductus venosus under acute hypoxic situations in fetuses.

In conclusion, the increase of liver blood supply following occlusion of the ductus venosus stimulated cell proliferation in fetal organs. In contrast, the decrease of liver blood perfusion in hepatic veins and can induce pulsation in the umbilical vein. These observations were very helpful in the correct implantation of the coil or stent into the DV. The incorrect implantation of the stent, or deformation/reduction of outlet of the ductus venosus by the stent could be predicted because the diastolic blood flow in the hepatic veins was not reversed. Thus, these findings may be helpful in understanding the blood flow velocity profiles in the ductus venosus under acute hypoxic situations in fetuses.

The pharmacological regulation of liver blood supply may offer therapeutic opportunity for the treatment of growth-re retarded fetuses.

REFERENCES


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