Endothelin concentrations in monochorionic twins with severe twin–twin transfusion syndrome

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The objective of this study was to determine endothelin (ET-1) concentrations in monochorionic twin fetuses with and without twin–twin transfusion syndrome (TTTS). Fourteen monochorionic twin pregnancies complicated by TTTS and six without TTTS were studied. Matched maternal and fetal blood samples were also collected from twin pairs. ET-1 concentrations were measured by radio-immunoassay. ET-1 concentrations in recipient fetuses were higher than in the donors both in utero (P < 0.001) and at birth (P < 0.01). Fetal concentrations of ET-1 in donors were similar to non-TTTS twins. Plasma ET-1 concentrations were significantly higher (P < 0.01) in recipient fetuses with severe hydrops than those with mild/no hydrops. Maternal concentrations of ET-1 were comparable in the two groups. Endothelin concentrations in recipient twins were 2² times higher than in their co-twins and this was related to the severity of hydrops.

Key words: endothelin-1/fetal cardiac dysfunction/twin–twin transfusion syndrome

Introduction

Twin–twin transfusion syndrome (TTTS) occurs in 4–25% of monochorionic multiple pregnancies and accounts for 17% of perinatal mortality in twins (Steinberg et al., 1990). For cases presenting in the second trimester, perinatal survival has recently improved from <10% to 65% with serial aggressive amnio-ecdysin treatment (Elliott et al., 1994; Kilby et al., 1997; Trespide et al., 1997). As severe cases now progress to the third trimester, the late sequelae of haemodynamic instability between the two fetuses are becoming increasingly apparent. The recipient twin frequently develops cardiac dilatation and hypertrophy and in some cases tricuspid regurgitation and right ventricular outflow tract (RVOT) obstruction (Tolosa et al., 1993). These may manifest in utero with cardiac failure, or postnatally as pulmonary stenosis (Zosmer et al., 1994). We recently reported a series of recipient twins with cardiac dysfunction in utero who developed RVOT obstruction requiring surgical correction in infancy (Zosmer et al., 1994).

The mechanism of recipient cardiac dysfunction is not clear. Historically, this has been attributed to transfer of blood from one twin to the other along placental vascular anastomoses. This increase in circulatory volume in the recipient twin may cause an increase in right ventricular output with subsequent cardiac dilatation and hypertrophy. Consistent with this theory, Doppler studies have also shown that severe cases are often associated with pulsatile umbilical venous waveforms. Although hydrops in the recipient has been assumed to be secondary to volume overload, recent evidence suggests that umbilical venous pressure is not always raised in hydropic fetuses. This along with the observation of neonatal hypertension in the recipient twin (Tolosa et al., 1993) suggests that increased peripheral resistance may contribute to cardiac dysfunction in the recipient twin.

Animal experiments suggest that intravenous infusion with vasoconstrictive peptides such as endothelin (ET-1) and angiotensin (Ag-II) through a common pathway can induce cardiac hypertrophy secondary to pressure overload (Yorikane et al., 1993; Ito et al., 1994). In humans, increased concentrations of ET-1 and Ag-II have been implicated in the pathogenesis of chronic cardiac failure (Rosenberg et al., 1993; Wei et al., 1994). Increased feto–placental vascular resistance seen in fetuses suffering from intrauterine growth retardation may be related to elevated concentrations of endothelin (McQueen et al., 1993; Schiff et al., 1994). To investigate the association between recipient cardiac dysfunction, and circulating vasoactive peptides, we measured ET-1 concentrations in utero and at birth in twin pairs with and without TTTS.

Materials and methods

Patients

This observational study of twin pregnancies undergoing fetal blood sampling was conducted in a tertiary referral centre between 1992 and 1994. Fourteen cases of monochorionic (MC) twins with TTTS were compared with six MC twin pregnancies without TTTS as the control group. All fetuses had normal cardiac anatomy on detailed scan at 18 weeks with normal chromosome and toxoplasmosis, rubella, cytomegalovirus and herpes (TORCH) studies.

Monochorionicity was established on ultrasound by demonstration of all three of the following criteria: (i) concordant genitalia; (ii) interfetal membrane thickness <2.0 mm; and (iii) single placental mass, and was confirmed by placental histology after birth. Cases where both fetuses were alive at the time of fetal blood sampling (FBS) were included in this study.

As no gold standard exists for the diagnosis of TTTS, the inclusion criteria were as follows: (i) monochorionic placentation; (ii) growth discordance of ≥15%; and (iii) discordance in amniotic fluid volume, i.e. polyhydramnios in one sac (amniotic fluid index of ≥40 cm) and...
anhydramnios or oligohydramnios (single deepest pool of ≈2 cm) in the other (Reisner et al., 1993). All TTTS pregnancies were monitored by serial scans and haemodynamic changes sought using echocardiography and colour flow Doppler as described previously (Zosmer et al., 1994). Hydrops was graded as severe if the fetus had ascites with pleural or pericardial effusion or skin oedema, and mild when only ascites was present. The echocardiographic and Doppler data in this study were obtained within 1 week of FBS. All babies born alive had cardiac assessment within the first week of life. Clinical details of some of these pregnancies have been reported elsewhere (Zosmer et al., 1994).

Inclusion criteria for control pregnancies without TTTS were: (i) monochorionic twin pregnancies with normal amniotic fluid volume in both sacs and (ii) an amniotic fluid index of ≈24 cm on fortnightly ultrasound scans from 18 weeks gestation.

**Fetal blood sampling**

In the TTTS group, fetal blood sampling was only performed during the study period as per unit policy, in women with severe disease to establish fetal well being and to exclude major haematomatological discordance. In the non-TTTS group, the indications for FBS were suspected aneuploidy due to fetal growth discordance with normal liquor volume (n = 3), the presence of chromosomal markers on ultrasound (n = 2), and maternal age (n = 1). All women gave written informed consent to collection of additional research samples as approved by the hospital ethics committee. Immediately prior to FBS, maternal peripheral venous blood was collected. FBS was performed at the intrahepatic vein or the umbilical vein at its placental cord insertion, and 1 ml additional fetal blood was obtained for ET-1 estimation. The fetal source of blood was confirmed by separate cord insertion, and 1 ml additional fetal blood was obtained for ET-1 estimation. The fetal source of blood was confirmed by separate mean cell volume peaks on a Coulter Channelizer (Coulter Electronics, Luton, UK) and the standard test known as the Kleihauer–Betke method, where the blood film is stacked to detect red cells containing adult haemoglobin in a blood sample obtained by fetal blood sampling in utero.

**Amniotic fluid samples**

Amniotic fluid was collected from both sacs in seven pairs of TTTS fetuses and four pairs without TTTS at the time of fetal blood sampling.

**Endothelin-1 assay**

Blood was collected into chilled tubes containing ethylenediamine tetra-acetic acid, centrifuged at 3000 g for 15 min, and plasma stored at –20°C until batch assay. Radioimmunoassay was performed using a commercial kit (Amersham International, Amersham, Buckinghamshire, UK) after extraction of the acidified plasma using Amprep C2 minicolumns (Amersham International). The antibody was 100% specific for ET-1 with cross reactivity of <0.01% with ET-2 and ET-3 and 1% with human big ET-1 (formed when the precursor proteins are post-translationally processed into biologically inactive intermediate forms of ET-1 which are eventually processed into mature ET-1 peptide), on a molar basis. The intra- and inter-assay coefficients of variation were <12%.

**Statistical analysis**

Clinical data were expressed as medians and ranges, while endothelin concentrations were expressed as mean and 95% confidence interval (CI). Delta values (Δ) of ET-1 in the study group were calculated as difference between recipient and donor twin, while those in the control group by subtracting values of twin 1 from twin 2, with the presenting fetus labelled as twin 1. For parametric data, the paired t-test was used to compare values within twin pairs and Student’s t-test was used for comparisons between groups. Fisher’s exact test was used for blocked comparisons. For non-parametric data, correlations were sought using the Spearman coefficient, and comparison between groups by the Mann–Whitney test. Percentage growth discordance was defined as the difference in growth as a proportion of the birth weight of the larger twin.

**Results**

Clinical features of the MC twins with or without TTTS are shown in Table I. All patients in the TTTS group underwent amnioreduction.

Out of 14 TTTS pregnancies, hydrops was severe in eight recipients, mild in four and absent in two. Four recipient fetuses had absent end-diastolic flow in the umbilical artery (one progressed to reverse end-diastolic flow) in the presence of normal pulsatility indices in their co-twins. Seven recipient twins had pulsatile umbilical venous flow. There was no evidence of cardiomegaly in the donor twin or in the non-TTTS fetuses in utero, at birth or on autopsy.

The perinatal loss rate in the TTTS group was significantly higher than in the control group. Out of 14 sets of twins, six pairs were born alive, of which one twin pair died in the early neonatal period. In one case, both twins died in utero. Two patients opted for termination of pregnancy. In the remaining five pairs, the donor twin died in utero in all cases and three of the five recipients died neonatally either from respiratory distress syndrome with renal failure or cardiac dysfunction. Autopsy findings on the seven recipient fetuses showed varying degrees of cardiac enlargement with myocardial hypertrophy. Out of 11 recipient twins born alive, six showed cardiac dysfunction postnataally. Five babies subsequently went on to develop structural valvular or infundibular pulmonary stenosis which required valvoplasty within 6 months of birth. Furthermore, in the non-TTTS group there was no intrauterine fetal loss, but one patient had termination of pregnancy for cloacal malformation and one baby died in the early neonatal period following surgery for tracheo–oesophageal fistulae.

### Table I. Clinical features of pregnancies with mid-trimester twin–twin transfusion syndrome (TTTS) in comparison to the control group without TTTS

<table>
<thead>
<tr>
<th></th>
<th>TTTS (n = 14)</th>
<th>Control (n = 6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at diagnosis (weeks)</td>
<td>21 (16–28)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gestational age at fetal blood sampling (weeks)</td>
<td>23 (19–30)</td>
<td>30 (21–34)</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum amniotic fluid index (cm)</td>
<td>58 (35–79)</td>
<td>16 (25–37)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Maximal discordance in estimated fetal weight (%)</td>
<td>38 (19–55)</td>
<td>29 (3–56)</td>
<td>NS</td>
</tr>
<tr>
<td>No. amnioreductions</td>
<td>2 (1–7)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total volume removed (l)</td>
<td>5.7 (0.2–19)</td>
<td>0.8 (0.1–2.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin discordance at fetal blood sampling (g/dl)</td>
<td>3 (0.1–4)</td>
<td>0.8 (0.1–2.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>30 (23–37)</td>
<td>34 (25–37)</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight discordance (%)</td>
<td>24 (12–73)</td>
<td>35 (5–61)</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 1. Endothelin (ET-1) concentrations in monochorionic twins with or without twin–twin transfusion syndrome (TTTS) (a) at the time of fetal blood sampling in utero; (b) in amniotic fluid, and (c) in umbilical vein (V) and artery (A) at the time of delivery. NS = non-significant.

Endothelin concentrations

Fetal blood in utero

Fetal concentrations of ET-1 were significantly higher in the recipient than the donor twin (mean Δ118; CI 88–148 fmol/l; \( P < 0.001 \)) (Figure 1a). There was no significant difference in the ET-1 concentration between the twin pairs in the control group (mean Δ11; CI –6–29 fmol/l). In the recipient, ET-1 concentrations were higher than in twin 1 (196; CI 167–225 fmol/l versus 95; CI 79–112 fmol/l; \( P < 0.001 \)) and twin 2 (84; CI 63–105 fmol/l; \( P < 0.001 \)). In contrast, ET-1 concentrations in the donor twin were comparable with those of the non-TTTS fetuses as a group (78; CI 70–88 fmol/l versus 81; CI 67–95 fmol/l).

Amniotic fluid

ET-1 concentrations in amniotic fluid (Figure 1b) were significantly higher in recipient than donor sacs (mean Δ84; CI 54–113 fmol/l; \( P < 0.01 \)) but similar in the two sacs of the control group (mean Δ16; CI –8–24 fmol/l). The concentration of ET-1 in the donor twin was comparable with those of control twin sacs.

Umbilical cord blood at delivery

As in utero, ET-1 concentrations in venous cord blood in the recipient were higher than in the donor (mean Δ 66; CI 37–96 fmol/l; \( P < 0.01 \)) (Figure 1c), whereas in non-TTTS pregnancies concentrations were similar to twin pairs (mean Δ 7; CI –13–27 fmol/l). Cord ET-1 concentrations in the donor were comparable to those of non-TTTS twin pairs as a group (55; CI 40–69 fmol/l; versus 66; CI 54–78 fmol/l). The highest cord ET-1 concentration (192 fmol/l) was found in a recipient fetus with severe hydrops in utero who failed to respond to aggressive amnioreduction, and transplacental digoxin therapy and progressed to develop reverse flow in the ductus arteriosus. In two cases with co-twin death in utero, cord blood was obtained only from the recipient twin but concentrations were still higher (107 and 119 fmol/l) than in the control group. ET-1 concentrations were significantly higher in the umbilical vein than the artery in both TTTS (mean Δ 8; CI 7–8 fmol/l; \( P < 0.001 \)) and non-TTTS groups (mean Δ6 CI 5.5–6.6; \( P < 0.001 \)), but venous to arterial differences were similar in the two groups (8; CI 6.8–9.1 fmol/l versus 6; CI 4.8–7.8 fmol/l) respectively.

Fetal endothelin concentrations in relation to hydrops

ET-1 concentrations in recipient fetuses with severe hydrops were higher than those with nil/mild hydrops (228; CI 201–254 fmol/l versus 155; CI 117–193 fmol/l; \( P < 0.01 \)) (Figure 2). Out of seven recipient twins who survived the neonatal period, five developed right ventricular obstruction and required balloon valvotomy or open heart surgery. The intrauterine ET-1 concentrations in these five fetuses (204; CI 165–243 fmol/l) were 2.5-fold higher than those of their co-twins with normal cardiac function. There was no relationship between ET-1 concentrations and gestational age, estimated fetal weight or percentage discordance in estimated fetal weight at the time of FBS.
placenta. Although the cause for different ET-1 concentrations in donor and recipient is unclear, we speculate that it could be due to differential rate of secretion of ET-1 from each twin’s half of the placenta. As hypoxia is a potent stimulus for production of ET-1 (Isozaki-Fukuda et al., 1991; Huaibin et al., 1994), it is possible that discordant levels in TTTS twins could be attributed to poor feto–placental gaseous exchange in the recipient twin’s half of the placenta. High blood viscosity, hydrostatic–osmotic, and amniotic pressures may all contribute towards placental bed hypoxia in the recipient twin (Talbert et al., 1996). However, further studies are necessary to confirm this hypothesis, as fetal acid–base status may not be a true reflection of the tissue oxygenation, particularly in the presence of a hydropic recipient twin.

Furthermore, our finding of less marked differences in umbilical cord compared to in-utero ET-1 concentrations in the TTTS group raises the possibility of improved feto–placental circulation as a result of various therapies. In this light, Bower et al. have reported a substantial improvement in uteroplacental blood flow as a consequence of amnioreduction (Bower et al., 1995). The change in ET-1 concentrations seems unlikely to be a gestational age effect as we, like others, did not find an association between ET-1 concentrations and gestational age (Radunovic et al., 1995). However, further studies are necessary to elucidate the effect of serial amnio-reduction on fetal ET-1 concentrations.

The biological function of the 2- to 3-fold higher endothelin concentrations in the recipient twin remains unclear. As endothelin is a potent vasoconstrictor, raised values provide indirect evidence of higher systemic resistance in the recipient twin as compared to the donor. However, the significance of this in the pathogenesis of TTTS remains speculative. It is conceivable that increased peripheral resistance in the recipient twin may play a role in the maintenance of haemodynamic stability between the two twins’ circulations; and thus have a beneficial effect on the disease. On the other hand, increased pressure might have a deleterious effect by increasing cardiac load. Anecdotal reports of higher blood pressure in the recipient twin during the first 24 h of age (Tolosa et al., 1993), and observation of reverse flow during diastole in the ductus arteriosus in one of the recipient fetuses studied here, support this proposition. However, cardiac dysfunction of similar magnitude is not found in the other naturally occurring models of raised after load, e.g. increased placental bed resistance in intrauterine growth retardation (IUGR). One reason could be that pregnancy complicated by IUGR is short-lived, often being terminated on maternal or fetal grounds. Nevertheless, lesser degrees of right ventricular dysfunction have been reported in IUGR fetuses in utero (Rizzo and Arduini, 1991). Tricuspid regurgitation is commonly seen in another clinical model of raised ventricular afterload, i.e. constriction of the ductus arteriosus in fetuses exposed to indomethacin in utero (Moise et al., 1988). In this study, no attempt was made to relate ET-1 concentrations to the echocardiographic features.

We appreciate that the underlying mechanism of chronic fetal cardiac failure is complex and the simple demonstration that ET-1 concentrations are raised cannot explain the pathophysiology. Nevertheless, the severity of hydrops in the recipient twin does relate to the degree of elevation in ET-1 concentrations.

**Maternal concentrations**

Maternal endothelin concentrations were similar in TTTS (65; CI 57–74 fmol/l), and non-TTTS (67; CI 55–79 fmol/l) and were lower than in fetal blood (P < 0.05).

**Discussion**

This study demonstrates elevated ET-1 concentrations both in utero and at birth in recipient fetuses of severe TTTS of second trimester origin, especially in those with hydrops. Our findings are consistent with recent reports of a 2- to 3-fold increase in plasma endothelin concentration in adult patients with chronic cardiac failure (McMurray et al., 1992).

The source of high ET-1 concentrations in recipient twins is not clear. Transplacental passage of maternal endothelin seems unlikely because ET-1 concentrations in the fetus were higher than in the mother. Furthermore, fetal concentrations of ET-1 were different in the two groups despite similar maternal concentrations. This is suggestive of a fetal source of ET-1. It is unlikely that high concentrations in the recipient twin are due to reduced clearance of ET-1 by the kidney or the placenta (Gasic et al., 1992; Myatt et al., 1992), because cord venous–arterial differences in ET-1 concentrations were similar in TTTS and non-TTTS pairs. Furthermore, higher endothelin concentrations in the amniotic fluid of recipient compared with donor twin sac suggest increased renal clearance.

Increased production of ET-1 in the recipient twin’s compartment thus seems the more likely explanation. Although endothelin might be produced by the fetal heart (Wharton et al., 1991), the cord arterio–venous gradient suggests instead that higher concentrations are due to increased production by the placenta. Although the cause for different ET-1 concentrations...
concentrations. It is possible that as in adults (Kiowski et al., 1995), increasing concentrations of ET-1 in the recipient twin might lead to increased placental bed resistance due to vasoconstriction resulting in higher ventricular filling pressures (Yorikane et al., 1993), ventricular hypertrophy, falling right ventricular stroke volume, tricuspid regurgitation, and development of hydrops. Alternatively, ET-1 could caus cardiac dysfunction by its direct positive inotropic and chronotropic effects and mitogenic activity (Garcia et al., 1990) or indirectly by an angiotensin II-mediated pathway (Kanno et al., 1993). This assumption is also substantiated by our observation that intrauterine ET-1 concentrations in the five recipient twins who required cardiac surgery for RVOT was higher than in those with normal heart function. Similarly, ET-1 has been shown in adult subjects with chronic cardiac failure, to be a better prognostic marker than haemodynamic variables and atrial natriuretic peptide in predicting mortality (Krum et al., 1993). Taken together, these findings suggest that endothelin may be a useful marker of cardiovascular morbidity and mortality in recipient fetuses.

In summary, this study implicates ET-1 activity in the pathophysiology of cardiac dysfunction in the recipient twin in TTTS and for the first time provides the biochemical support for a pressure overload mechanism.

References


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