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Twin Fetuses: Intravascular Microbubble US Contrast Agent Administration—Early Experience¹

PURPOSE: To explore the feasibility of administering SH U 508A by using a single-needle procedure at ultrasonography (US) in twin pregnancies to confirm interfetal transfusion in monochorionic twins and delineate placental angioarchitecture in pregnancies with twin-twin transfusion syndrome.

MATERIALS AND METHODS: Fourteen twin pregnancies were studied over 12 months: seven with monochorionic twins, including six with twin-twin transfusion syndrome; two of unknown chorionicity; and five with known dichorionic twins discordant for fetal karyotype or anomaly and undergoing selective feticide in the third trimester. Bolus injection of 100 μ L/kg of estimated fetoplacental weight of 400 mg/mL of SH U 508A was performed in the intrahepatic vein of one twin, and evidence of interfetal transfusion was sought by means of digital analysis of power Doppler signals in the contralateral twin.

RESULTS: Contralateral twin echo enhancement was seen in four of the nine ultimately histopathologically proved monochorionic twins. As expected, no evidence of echo enhancement in the contralateral twin was seen in any of the five dichorionic twin pregnancies. There was no evidence of fetal compromise associated with the procedure.

CONCLUSION: These pilot results suggest that microbubbles can be used to demonstrate interfetal transfusion but not to delineate placental vascular anatomy.

Microbubble contrast agents have been used extensively in internal medicine to enhance vascular imaging. SH U 508A (Levovist; Schering, Berlin, Germany) produces enhancement of 15–25 dB with power Doppler imaging and was the only licensed agent producing good systemic enhancement during the study period (1). It has been used widely in human and animal studies and has an excellent safety profile. Results from pilot work in perfused human placentas (2) demonstrated that power Doppler imaging enhanced with SH U 508A delineated villous architecture at dose-concentration ratios appropriate to in utero application. We recently reported the use of a microbubble contrast agent to confirm interfetal transfusion in a monochorionic twin pregnancy (3).

Twenty percent of twins share a single monochorionic placenta in which vascular anastomoses are virtually omnipresent, which allows interfetal transfusion as a normal event (4). These anastomoses either lie on the surface of the chorionic plate (bidirectional arterioarterial or venovenous anastomoses) or deep within the placenta (arteriovenous anastomoses).

Twin-twin transfusion syndrome results from an imbalance in interfetal transfusion and affects 15% of monochorionic twin pregnancies (5). The net “donor” fetus becomes growth restricted, oliguric, and oligohydramniotic, while the net “recipient” becomes overloaded, with polyuric polyhydramnios. In 25% of monochorionic twin pregnancies with twin-twin transfusion syndrome, there is a substantial interfetal hematocrit discordance (6). If twin-twin transfusion syndrome is left untreated, mortality exceeds 80%, although this is halved with current treatments (7). In addition, neurologic (8) and cardiac (9) sequelae occur in survivors.

The most rational therapy for twin-twin transfusion syndrome would involve interruption of interfetal transfusion through vascular anastomoses. At present, either color or power Doppler ultrasonography (US) can be used to identify the superficial arterioarterial anastomoses, but these are often absent in twin-twin transfusion syndrome (10); unfortunately, the deep unidirectional arteriovenous anastomoses that set up the dysequilibrium in interfetal transfusion cannot be readily identified, even by means of direct fetoscopic visualization.

Monochorionic twins with placental anastomoses are at risk of acute interfetal transfusion after death in utero of one of a monochorionic pair. After the demise of the first, the second twin may exsanguinate into the first via placental anastomoses. This results in the death of the second fetus in approximately 25% of cases (Fusi L, unpublished data, 1998) or the development of hypoxic-ischemic cerebral or renal damage in a further 25% (11). This has implications for selective feticide for discordant anomaly in monochorionic twins and contraindicates injection of lethal doses of potassium chloride (12). Hence, so-called occlusive methods such as ligation of the umbilical cord or laser coagulation are used.

The remaining 80% of twin pregnancies have dichorionic placentas, although these often lie adjacent to one another in utero and may appear fused. These were previously considered to be functionally separate, but this has been challenged by demonstration of interfetal transfusion in occasional dichorionic twin pairs (13,14). Because of this, one group of authors (13) recommended that evidence of interfetal transfusion be sought in dichorionic twins before feticide and that, if evident, occlusive rather than cardiotoxic feticide be performed. Demonstration of interfetal transfusion in the absence of an identifiable arterioarterial anastomosis relies on injection of a marker substance, such as maternal red cells (15), into the first twin followed by a further procedure to confirm its presence in the second twin.

The purpose of this study was to explore the feasibility of administering SH U 508A by using a single-needle procedure at US in twin pregnancies to confirm interfetal transfusion in monochorionic twins and delineate placental angioarchitecture in pregnancies with twin-twin transfusion syndrome. The ultimate objective lay in delineating areas of placental perfusion to allow targeted therapeu-

tic ablation in twin-twin transfusion syndrome.

MATERIALS AND METHODS

Patients

All patients were examined at the Centre for Fetal Care (London, England), a tertiary referral center, during 12 months (June 1997 to May 1998). Three separate groups are reported on: (a) monochorionic twins with or without twin-twin transfusion syndrome, (b) twins of unknown chorionicity, and (c) dichorionic twins. We studied 14 twin pregnancies undergoing clinically indicated fetal blood sampling. These comprised seven monochorionic twins, including six with twin-twin transfusion syndrome; two of unknown chorionicity; and five known dichorionic twins undergoing selective feticide in the third trimester for discordant fetal karyotype or gross anomaly.

Monochorionicity was diagnosed following the US demonstration of a single extraembryonic celom in the first trimester or, in the second trimester, a single placental mass, absent twin peak sign, thin interfetal septum, and concordant external genitalia (7).

After consenting to the clinical procedure, parents were also counseled before consideration of SH U 508A administration; they were aware that the injection of contrast agent was part of a research project, with both local ethical committee and UK Medicines Licensing Authority approval, that aimed to develop a rational therapy for twin-twin transfusion syndrome by depicting the shared areas of the placenta. In all cases written consent was obtained.

Monochorionic twins (n = 7).—Six of the pregnancies in this group were diagnosed as having twin-twin transfusion syndrome. Patients were treated with aggressive serial amnioreduction with excess amniotic fluid drained through a needle with US guidance. Fetal blood sampling was indicated, once amnioreduction had ensured viability, to determine fetal hematologic and acid-base status to facilitate obstetric decision making. For example, a major difference in hematocrit between the fetuses is taken as an indication for early delivery because of poor prognosis (6). The remaining monochorionic pregnancy had discordant fetal growth as detected with serial US assessment. In this case, fetal blood sampling was clinically indicated to establish the acid-base status and to perform rapid karyotype analysis. SH U 508A in this group was

administered for research purposes as approved by the ethics committee.

Unknown chorionicity (n = 2).—The two pairs of twins of unknown chorionicity were referred from other institutions because of major differences in fetal size and fetal well-being indices (eg, umbilical arterial Doppler waveforms). Fetal blood sampling for analysis of acid-base status and karyotype was indicated in both cases. SH U 508A was administered on the clinical grounds that demonstration of interfetal transfusion in pregnancies with discordant fetal well-being would necessitate delivery to prevent the sequelae of acute transfusion.

Dichorionic twins (n = 5).—The indication for selective feticide in the dichorionic twins was aneuploidy in three cases, schizencephaly in one, and anencephaly with polyhydramnios in one. The fetal karyotype had been determined previously in both twins in all except the anencephalic case. A detailed description of fetal and placental position, cord insertion, and, where appropriate, fetal sex or anomaly, had been meticulously recorded to avert any possibility of confusion between the affected and nonaffected fetus. The placentas appeared to be fused in all cases. Terminations were performed in accordance with the 1990 Human Fertilization and Embryology Act, which in the UK allows for feticide beyond 24 weeks for severely handicapping abnormalities. SH U 508A was administered on the clinical grounds that demonstration of interfetal transfusion would necessitate an occlusive method of feticide.

Technique

Fetal blood sampling was performed in all cases via the intrahepatic portion of the umbilical vein with continuous US guidance. In cases of twin-twin transfusion syndrome or discordant karyotype, the donor, or affected twin, always underwent sampling first. An XP10 US unit (Acuson, Mountain View, Calif) was used for the first two cases; thereafter, a Sequoia 512 US unit (Acuson) was used for the remaining studies. A 5C2 probe (Acuson) at 5 MHz was used for all studies. Scanner settings were adapted to minimize bubble destruction, and a low power output of -6 dB was used. In addition, to optimize image quality, we used a high-spatial-weighting low-edge function for maximum resolution and high persistence to increase smoothing over time. A wide gate and low filter value were used to enhance detection of low volume. Anticipating a 10–25-dB sig-

Summary Data for Study Population

Case No.	Gestational Age*	Chorionicity [†]	Diagnosis [‡]	Volume of SH U 508A Administered [§] (mL)	Enhancement		Placental Enhancement	Postnatally Identified Vascular Anastomoses [#]
					Twin 1	Twin 2		
1	30 + 5	NK	Discordant growth	0.15	Yes ($P < .004$)	Yes ($P < .01$)	None	2 AVA, 1 AAA
2	24 + 6	MC	TTTS	0.05	Yes ($P < .01$)	Yes ($P < .004$)	Patchy	2 AVA
3	30 + 1	NK	Discordant growth	0.12, 0.19	Yes ($P < .01$)	No	None	1 AVA, 1 AAA
4	27 + 0	MC	TTTS	0.05	Yes ($P < .001$)	Yes ($P < .009$)	None	1 AVA, 1 AAA
5	28 + 4	MC	Discordant growth	0.15, 0.22	Yes ($P < .007$)	No	None	NP
6	22 + 1	MC	TTTS	0.04	Yes ($P < .02$)	No	None	1 AVA
7	28 + 3	MC	TTTS	0.13	Yes ($P < .005$)	No	None	7 AVA, 1 VVA
8	27 + 2	MC	TTTS	0.07	Yes ($P < .001$)	Yes ($P < .02$)	Patchy	1 AVA
9	28 + 5	MC	TTTS	0.16	Yes ($P < .004$)	No	None	2 AVA
10	31 + 2	DC	Discordant aneuploidy	0.15, 0.22	Yes ($P < .007$)	No	None	NP
11	18 + 6	DC	Discordant aneuploidy	0.07	Yes ($P < .004$)	No	None	NP
12	33 + 1	DC	Discordant anomaly	0.43	Yes ($P < .01$)	No	None	NP
13	20 + 0	DC	Discordant aneuploidy	0.06	Yes ($P < .004$)	No	None	NP
14	32 + 0	DC	Discordant anomaly	0.12	Yes ($P < .006$)	No	None	NP

Note.—Total monochorionic (MC) twin enhancement was nine (100%) of nine for twin 1 and four (44%) of nine for twin 2. Overall twin enhancement was 14 (100%) of 14 for twin 1 and four (29%) of 14 for twin 2.

* Data are the number of weeks plus days.

[†] DC = dichorionic, NK = not known.

[‡] TTTS = twin-twin transfusion syndrome.

[§] A second dose was administered in cases 3, 5, and 10.

^{||} Patchy enhancement was not significant.

[#] AAA = arterioarterial anastomosis, AVA = arteriovenous anastomosis, NP = not performed, VVA = venovenous anastomosis.

nal enhancement from SH U 508A, we adjusted the power Doppler gain to be just below threshold (ie, no signal in the absence of microbubbles) for the region of interest (ie, the cardiac chambers for the first case and the intrahepatic or umbilical vein thereafter).

These settings were not changed for the remainder of the procedure. The primary goal was to detect intertwin transfusion; however, evidence of placental enhancement within a convenient region of placenta close (approximately 2 cm) to the cord insertion of the twin who underwent sampling was also sought.

For fetal blood sampling, we used a 20-gauge 14-cm-long needle (Cook Ob/Gyn, Spencer, Ind) with a dead space of 0.12 mL. After aspiration of fetal blood for hematologic and acid-base analysis, a saline solution flush of 1–2 mL was administered to confirm correct positioning of the needle. We administered 100 μ L/kg of estimated fetoplacental weight (estimated fetal weight \times 1.25 [16]) of 400 mg/mL of SH U 508A by means of bolus injection, again followed by a saline solution flush of 2 mL. The entire procedure, including the subthreshold settings, was recorded

on videotape (Super Video Home System; TDK Recording Media Europe, Bascharage, Luxembourg) for subsequent analysis.

After administration of SH U 508A (time = 0 second), increased power Doppler signals confirmed the presence of the agent within the fetus receiving the injection (time = 0–5 seconds). The transducer was then positioned over the placental territory of the first twin, which was identified as adjacent to its umbilical cord insertion, including surface chorionic vessels and evidence of echo enhancement sought (time = 5–30 seconds). This was repeated over either the umbilical vein, intrahepatic vein, or cardiac chambers of the contralateral twin (time = 30–120 seconds). Thereafter, all sites were revisited to check for echo enhancement; the total scanning time was limited to 5 minutes.

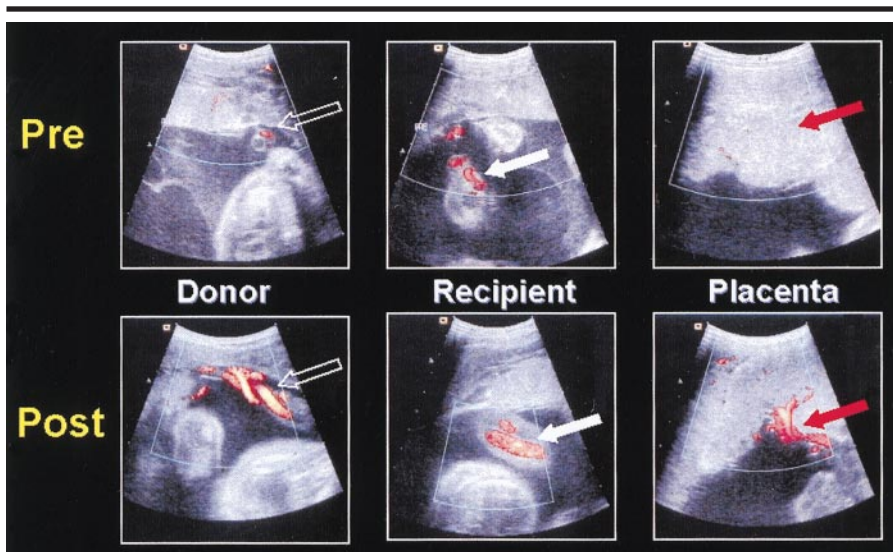
If no signal enhancement was noted in the contralateral twin, a second dose of 150 μ L/kg of estimated fetoplacental weight of 400 mg/mL of SH U 508A was considered, and intertwin transfusion of contrast agent was sought in a similar manner. Fetal well-being was checked before and after the procedure by using

analysis of a single measurement of umbilical arterial pulsatility indices.

Analysis

Analysis was performed subjectively by means of consensus on image quality by two authors (M.L.D., N.M.F.) and objectively undertaken by means of digital comparison of views obtained before and after contrast agent injection as previously described (17). This quantification system measures absolute Doppler signal intensities and corrects for logarithmic compression and nonlinear machine processing to give the integrated intensity in the region of interest from captured video frames (18). The integrated intensity is 0–1, with zero representing no energy signal and one representing the maximum, with use of arbitrary units. As the preprocedural settings were set below threshold (ie, integrated intensity = 0), any integrated intensity greater than zero was taken to indicate the presence of contrast agent echo enhancement.

Analysis was performed at the following sites: (a) intrahepatic or umbilical



Power Doppler US images show the in utero appearance of SH U 508A in a monozygotic twin pair. In each case, the images show the enhancement of signal attributable to the contrast agent during maintenance of constant US parameters. Left: cross section through the first (*Donor*) twin's cord (arrow) before (top) and after (bottom) the administration of contrast agent. Middle: cross section through the contralateral (*Recipient*) twin's cord (arrow) before (top) and after (bottom) intertwin transfusion of contrast agent. Right: placental appearance (arrow) before (top) and after (bottom) injection of contrast agent.

vein of the first (rejected) twin, (b) placental territory of the first twin, and (c) intrahepatic or umbilical vein of second twin. The right ventricle of the contralateral twin was used as an analysis site in the first case, but concerns that movement artifact might affect the power Doppler signal meant that the nonpulsatile venous sites were chosen in subsequent cases.

After delivery, the placenta was examined and chorionicity confirmed. In addition, placental injection studies, as described previously (10), were performed by a perinatal pathologist blinded to the US findings. This was not possible in the dichorionic twins owing to autolysis of the terminated fetus's placenta because of the long interval between feticide and delivery.

Statistical analyses of pre- versus post-procedural pulsatility indices and integrated intensities were performed by using a paired Student *t* test, with a *P* value less than .05 considered significant.

RESULTS

The median gestational age at the time of study was 28 weeks (range, 20–33 weeks). The findings are summarized in the Table. All twins classified as monozygotic or dichorionic antenatally had their chorionicity correctly confirmed histopatho-

logically after delivery. The two cases in which chorionicity was equivocal at the antenatal examination were shown at delivery to be monozygotic.

Both subjective visual and objective digital analysis showed clear echo enhancement in the contralateral twin in four of the nine ultimately proved monozygotic twins (Figure), but no clear enhancement was seen in the remaining five cases, either in the placenta or the intrahepatic or umbilical vein. In two cases of monozygotic twins in which no contralateral twin enhancement was seen with the first dose, a second injection of SH U 508A (150 μ L/kg of estimated fetoplacental weight of 400 mg/mL) also failed to show contralateral twin enhancement. In the remaining three monozygotic twins, a second dose was not given owing to a shift in fetal position that dislodged the needle from the fetal intrahepatic vein. There was similarly no evidence of echo enhancement in the contralateral twin in any of the five dichorionic twin pregnancies, including after a second injection in one case. In two cases, qualitative patchy enhancement was visible at subjective analysis, but this did not reach significance at digital analysis.

There was evidence of contralateral twin echo enhancement in three of the six cases of twin-twin transfusion syndrome, compared with evidence of contra-

lateral twin echo enhancement in one of the three unaffected monozygotic pregnancies. The small numbers involved in the series precluded formal statistical analysis of correlation between either gestational age at the time of the procedure or the number or type of anastomosis with evidence of contralateral twin echo enhancement.

Postnatal injection studies could be performed in all monozygotic twins except in one case in which the placenta was disrupted at delivery. There was no significant difference in the number or type of anastomoses seen in placentas between pregnancies in which contralateral echo enhancement was evident and pregnancies in which contralateral echo enhancement was not evident.

There was no evidence of fetal compromise associated with the procedure, with all fetuses except those undergoing feticide remaining hemodynamically stable throughout the procedure, as indicated by maintenance of preprocedural umbilical arterial Doppler pulsatility indices (mean difference, 0.181; *P* = .28). Postnatally, all surviving monozygotic twins were admitted to the neonatal intensive care unit for the management of prematurity. In none was there evidence of excess morbidity or abnormalities at cranial or cardiac US that were attributable to the injection of the microbubble contrast agent. Furthermore, in the twins that underwent feticide or died owing to other complications of pregnancy and subsequently underwent postmortem examination (*n* = 2), no evidence of organ damage attributable to SH U 508A injection was noted.

DISCUSSION

The results of this series document early experience with use of a microbubble contrast agent in human fetuses to demonstrate intertwin transfusion. There was evidence of contralateral twin echo enhancement in 50% (three of six) of cases of twin-twin transfusion syndrome, compared with 33% (one of three) of unaffected monozygotic pregnancies (Table). In all cases in which contralateral twin echo enhancement was noted, placental vascular anastomoses were present. Although evidence of contralateral twin echo enhancement was seen in half the total monozygotic twin pregnancies, SH U 508A yielded poor delineation within the placental substance, with subjectively patchy enhancement at best in two of nine cases. The numbers involved in the study were too

small to identify differences in anastomotic patterns between monochorionic twins that did and those that did not demonstrate contralateral enhancement. Exact understanding of the rates and directions of interfetal transfusion awaits further study.

Flow rates down deep anastomoses are uncertain and are likely to vary at different times of the pregnancy and also for different fetuses. It seems likely therefore that demonstration of contralateral twin enhancement will depend on interfetal transfusion rates at the time of the procedure. Furthermore, rates might also be affected by changes in fetal physiology (eg, changes in blood pressure) in response to the fetal blood sampling procedure.

After encouraging results in placental perfusion experiments (2), the comparative failure of placental echo enhancement in vivo was surprising. There may be several explanations. First, the in vitro work was performed in optimal conditions in a water bath, which averted the clinical problems associated with loss of US information owing to tissue attenuation.

Second, SH U 508A has a short half-life of 3–5 minutes. It seems likely that during this short period, only tiny volumes of SH U 508A, too small to be detected, may have crossed from one fetal circulation to the other in some of the monochorionic twins. In other cases in which there was clear enhancement in the contralateral twin, the maximum signal was achieved at 1–3 minutes, which is consistent with SH U 508A's known half-life. Agents with longer durations of action may improve visualization of interfetal transfusion, although recirculation through the contralateral twin and subsequent retransfusion back to the first twin may hamper interpretation of initial volumes transfused interfetally.

Third, the dose-concentration ratio used in this study was based on clinical experience in adult medicine and also on in vitro placental perfusion studies (2). This led to the use of very small injection volumes, often less than 0.05 mL. The dead space of the needle was 0.12 mL, and although contrast agent injection was followed by a saline solution flush, it remained possible that some of the injected volume did not reach the fetal circulation, instead being retained in the needle or syringe. Placental perfusion study results indicate that the concentration of 400 mg/mL of SH U 508A provides optimal villous enhancement (2).

Finally, flash artifact secondary to fetal movements and changes in maternal position rendered comparison of before and after patterns difficult. We used a technique of quantitative digital analysis on video recordings, the accuracy of which has been validated previously by our group in human and animal models (19). Although this confirmed contrast agent-derived echo enhancement, it did not allow for accurate quantification because of the effect of fetal movements during the course of the procedure, an issue that will be addressed in future studies.

SH U 508A was chosen because of its excellent safety profile and licensing considerations and because its constituent agents are all naturally occurring and nontoxic substances. In adults, no serious complications have been noted, with minor local reactions occurring at the site of injection in fewer than 10% of cases (Carter EC, verbal communication, 1997). In this study, no evidence of adverse reaction was noted, albeit within the limits of the relatively crude methods used. Better echo enhancement might result from newer generation microbubbles once their safety profiles have been demonstrated.

In conclusion, the results of this study demonstrate that microbubble contrast agents can be used to demonstrate interfetal transfusion in some monochorionic twin fetuses. The agent used in this series, however, was not able to help demonstrate reliably either monochorionicity or placental vascular anatomy. Future work is indicated to evaluate newer generation microbubble contrast agents in the delineation of placental vascular perfusion.

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