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Placental Perfusion In Uterine Ischemia Model as Evaluated by Dynamic Contrast Enhanced MRI

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Abstract

Background—To validate DCE MRI method of placental perfusion estimation and to demonstrate application of the method in a rabbit model of fetal antenatal hypoxia-ischemia.

Methods—Placental perfusion was estimated by dynamic contrast imaging with bolus injection of Gd-DTPA in 3 Tesla GE magnet in a rabbit model of placental ischemia–reperfusion in rabbit dams at embryonic day 25 gestation age. Placental perfusion was measured using steepest slope method on DCE MRI before and after intermittent 40 min uterine ischemia. Antioxidants (n = 2 dams, 9 placentas imaged) or vehicle (n = 5 dams, 23 placenta imaged) were given systemically in a separate group of dams during reperfusion–reoxygenation. Placental perfusion was also measured in two dams from the antioxidant group (10 placentas) and two dams from the control group (12 placentas) by fluorescent microspheres method.

Results—While placental perfusion estimates between fluorescent microspheres and DCE MRI were significantly correlated ($R^2 = 0.85$; $P < 0.01$), there was approximately 33% systematic underestimation by the latter technique. DCE MRI showed a significant decrease in maternal placental perfusion in reperfusion–reoxygenation phase in the saline, 0.44 ± 0.06 mL/min/g ($P = 0.012$, t-test), but not in the antioxidant group, 0.62 ± 0.06 mL/min/g, relative to preocclusion values (0.77 ± 0.07 and 0.84 ± 0.12 mL/min/g, correspondingly).

Conclusion—Underestimation of true perfusion in placenta by steepest slope DCE MRI is significant and the error appears to be systematic.

Measurements of placental perfusion have important applications to assess capacity for maternal–fetal gas and nutrient exchange. Compromised uteroplacental perfusion is associated with chronic and acute gestational pathologies, such as fetal growth restriction, preeclampsia^{1,2} and abruptio placentae. Direct perfusion quantification using dynamic contrast-enhanced magnetic resonance imaging (DCE MRI) has distinct advantage over indirect estimation of placenta perfusion by Doppler ultrasound routinely used in clinical settings. The disadvantages of Doppler are that it requires standardization to compare results between sessions and patients, as well as poor reproducibility⁽³⁾.

Most previous knowledge on placental function originates from animal studies.^{4–6} While contrast use is currently limited in humans due to the safety concerns to fetus, DCE MRI

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with contrast injection is currently a method of choice in small and medium size animal studies due to the relatively high signal to noise and high spatial resolution of the measurements by this method relative to noncontrast MRI techniques. Estimation of rodent placental perfusion and permeability has been successfully performed using pharmacokinetic compartment models⁷⁻⁹ but uses the steepest slope method.¹⁰ The pharmacokinetic compartment model theoretically provides estimates of placental permeability and maternal–fetal transport rates, but it requires fitting multiple parameters that may not be accurate, given noisy imaging data in small animal models and the complexity of composition of placental tissue.¹¹ While the steepest slope method has the distinct advantage over the compartmental models due to its robustness, it has been suggested on simulated data¹² that the method underestimates perfusion, because it does not account for the contrast outflow.

The primary objective of the study was to evaluate the accuracy of the steepest slope method of placental perfusion estimation by DCE MRI against estimation using microspheres. The secondary objective was to apply DCE MRI to monitor changes in placental perfusion induced by drug interventions. Using a rabbit model of fetal hypoxiaischemia (H-I) injury after global uterine ischemia, resulting in postnatal cerebral palsy phenotype,¹³ we examined changes in placental perfusion occurring after episodes of ischemia in the uterus and placenta. We tested whether maternal administration of antioxidants has any influence on placental perfusion after a period of uterine ischemia.

MATERIALS AND METHODS

Animal Instrumentation

The Institutional Animal Care and Use Committee approved all experimental procedures with animals. We studied placental perfusion in timed pregnant New Zealand White rabbits (Myrtle's Rabbits, Thompson Station, TN) at 25 days gestation (79% term, embryonic day [E] 25). The setup was used in a model that mimics acute placental insufficiency at a premature gestation. This is induced by sustained uterine ischemia resulting in global H-I of fetuses.¹³ Surviving kits exhibited a spectrum of sensory and motor deficits, including hypertonia and characteristic posture, resembling human CP, as well as impaired locomotion, suck, swallow and righting reflexes.^{14,15}

The surgical procedure has been described previously.¹⁴ Briefly, dams were anesthetized with intravenous fentanyl (75 µg/kg/h) and droperidol (3.75 mg/kg/h), followed by spinal anesthesia using 0.75% bupivacaine. A balloon catheter was introduced into the left femoral artery and advanced into the descending aorta to above the uterine and below the renal arteries. The catheterized animal was placed inside the MR scanner. Body core temperature was maintained at 37 °C with a water blanket wrapped around the dam's abdomen and connected to a temperature-controlled heating pump. Respiration and heart rates, blood oxygen saturation, and blood pressure from air cuff, wrapped around rabbit leg, were monitored throughout the experiment. After the dam was positioned in the magnet, the balloon was inflated for 40 min causing uterine ischemia and subsequent fetal H-I. At the end of H-I, the balloon was deflated, resulting in uterine reperfusion–reoxygenation. The imaging session lasted approximately 1.5 h.

MR Imaging

MR imaging was performed in clinical 3.0 Tesla (T) Twin Speed scanner with Excite technology (General Electric Medical Systems, Milwaukee, WI) using a knee phased array coil. Single shot fast spin echo (SSFSE) T2-weighted images were taken for detailed anatomical reference in axial, coronal and sagittal planes of the trunk of the rabbit dam, with 50–76 axial slices covering all fetuses inside the dam. Slice thickness was 2 mm, matrix 256×192 , and field of view was 16 cm.

DCE perfusion weighted imaging with contrast injection was performed twice for each dam, once before aortal occlusion and again 5 min after occluder opening during the reperfusion–reoxygenation phase. The perfusion sequence consisted of nine 6-mm slices with 5-mm gap, acquired in the transverse plane of the dam's trunk using a fast T1-weighted SPGR sequence. Imaging parameters were: T1-weighted SPGR sequence, echo time 1.1 ms, repetition time 3.3 ms, flip angle 25, bandwidth 83.3, 256×64 matrix, 18 cm field of view, 2 s per time point, 50 time points, no electrocardiograph triggering. The acquisition started 20 sec before and continued during and after intravenous infusion of 0.6 mL of Magnevist (Gadopentetae dimeglumine, Berlex, NJ) contrast, dissolved in saline to 0.30 mmol/kg concentration, at the rate 0.15 mL/s followed by 3 mL saline flush through a catheter placed in ear vein. Perfusion scans took approximately 1.5 min each. A separate SSFSE scan was taken with the same slice geometry for in-plane anatomical reference to visualize placentas (Fig. 1). The F1 slab was placed in the center of abdominal volume, covering several fetuses and placentas. Three to six individual fetal placental units could be visualized in each rabbit dam. To minimize effect of the slice crosstalk in the multi-slice perfusion sequence, order of slices advanced from caudal to rostral. Blood velocity in abdominal aorta and caudal vena cava was measured using a phase contrast sequence before each contrast injection.

Perfusion Quantification From MR Imaging

A set of 9 reference phantom tubes with known T1-values, ranging from 0.2 to 2 s, were imaged with each rabbit. T1 values for the phantoms were obtained using fitting signal from inversion recovery sequence versus inversion time. Signal intensity of the phantom tubes was fitted with a third degree polynomial function that was used to convert signal intensity across the image into R1 values (Fig. 2). The phantom tubes were placed next to the dams' abdomen and the regression equation was updated for each experiment.

Flow-corrected calibration were not performed because the blood velocity in aorta was found not to be significantly different between pre- and post H-I and was relatively low (32 ± 8 cm/s) due to the relatively low heart rate in deeply anesthetized rabbit dams (145 ± 22 bpm). Perfusion imaging with T1-weighted sequence with matrix 256×64 used in our study was shown to be least sensitive to the inflow artifact.¹⁶

Polygonal ROIs were manually placed on cross-section of aorta and individual fetus placentas, as shown on Figure 1B, and automatically propagated across all time points of the perfusion sequence to obtain arterial and placental transit curves (Fig. 3). Perfusion was estimated in ROIs placed on the maternal parts of placentas, deciduas,¹⁷ identified by large signal enhancement with contrast infusion. Perfusion in deciduas was also determined by

fluorescent microspheres method, described below. To minimize a possibility of inclusion voxels outside indented structures of interest because of ROI displacement due to maternal respiration and fetal motion, values of voxels with intensities above 25 percentile were averaged for each ROI to calculate arterial input function and the time course of the contrast uptake in placentas. Renal perfusion was estimated in the same manner as for placenta by placing ROI on kidneys cortex.

The absolute value of placental perfusion per unit of volume (PP/Volume) was determined using the steepest slope method^{18,19} as a ratio of the maximum slope of contrast media uptake by placental tissue divided by the maximum change of 1/T1 in the aorta according to the formula:

$$\frac{PP}{Volume} = \frac{max\ slope_{placenta}}{max\ \Delta R_{1aorta}} \quad (1)$$

To obtain the more commonly used unit of mL/min/g, this quantity divided by the placenta tissue density 0.95 g/mL.²⁰

Validation of Perfusion Quantification From MR Imaging by Fluorescent-Microspheres Method

To evaluate the accuracy of perfusion estimated by DCE MRI, an injection of fluorescent microspheres²¹ was performed on a subset of the animals used for DCE MRI. After the second perfusion measurement with contrast injection on MRI, animals were rapidly transferred to the adjacent surgical facility. The microsphere injection was performed by means of the second channel of the dual lumen Fogarty balloon catheter, the balloon of which was used for the aortal occlusion to induce H-I. To ensure homogeneous mixing of microspheres, the catheter was advanced from occlusion site (just below renal arteries) to 5 cm rostrally toward to the aortic arc. Approximately 2 million of 15 μ m orange-fluorescent microspheres (Invitrogen, cat# F8841) were first sonicated in water bath for 30 min, and then manually injected into the descending aorta over a 30-s time span, 15 min after the MRI perfusion measurement. This was followed by an injection of 5-mL saline flush. Reference blood sample was withdrawn from a catheter placed in left femoral artery using Harvard syringe pump at rate 2 mL/min in a heparinized syringe, starting 30 s before microspheres injection and continued 2 min thereafter. After the injection of microspheres, animals were euthanized with anesthetic overdose and all placentas were obtained using laparotomy. Based on our imaging data and published angiographic data,¹⁷ the majority of maternal blood circulates in the maternal part of placental unit in E25 rabbit placentas. So, the decidua was separated from fetal part of placenta and both samples were weighed and processed separately.

Determination of the absolute perfusion values by fluorescent microspheres method was based on Tan et al.²¹ Placental tissue was dissolved in 4 M ethanolic KOH with 0.5% with Tween 80 (at least 3 mL/g tissue) during 5 days protected from light. The samples were then centrifuged at 1000 g at 25 °C for 20 min and the supernatant was carefully discarded by suction until <1 mL remained above the sediment. The sediment was then re-suspended in

50 mL deionized water and centrifuged again. After the final centrifugation, the supernatant was removed carefully by hand using a Pasteur pipette, taking care not to either disturb or dry the pellet (~100 μ L of supernatant left). Three milliliters of 2-ethoxyethyl acetate (Cellosolve acetate, Sigma Aldrich, MO) was added to the pellet, and the tubes were stirred using a Vortex mixer and then allowed to stand for at least 4 h to extract the fluorescent dye from the microspheres. They were then stirred again and centrifuged, leaving a clear dye-containing solution from which 200- μ L aliquots were pipetted in triplicate into individual wells of a 96-microwell plate. Fluorescence was measured on Gemini EM microplate spectrofluorometer (Molecular Devices, CA) with 540/560 nm excitation/emission. Perfusion rate was calculated as reference sample withdrawal rate (mL/min) multiplied by ratio $I_{\text{placenta}}/I_{\text{reference}}$, where I denotes fluorescent intensity.

Effect of Antioxidant Treatments

Effects of antioxidants on maternal placental circulation in this model were studied using DCE MRI. The experimental group included two rabbit dams (9 placentas imaged) and the vehicle control group included five dams (23 placentas imaged). In addition to DCE MRI, placental perfusion was measured in two dams from the antioxidant group (10 placentas) and two dams from the control group (12 placentas) by microspheres method. The experimental group was administered antioxidants during H-I (antioxidant group). After aortal occlusion and the onset of H-I, the dams were administered by means of ear vein a 10 mL/kg volume bolus consisting of 100 mg/kg Trolox (Sigma Aldrich) (dissolved in 5 mL/kg 0.9% saline) and 1600 mg/kg ascorbic acid (dissolved in 5 mL/kg 0.9% saline) during 15 min, followed by a 10.2 mL/kg/h continuous infusion of 50 mg/kg/h Trolox (concentration, 5 mg/mL) and 60 mg/kg/h ascorbic acid (concentration, 300 mg/mL) for 45 min.²² In this manner, antioxidants could reach placentas and fetuses only during reperfusion–reoxygenation phase, because uterine circulation was blocked during occlusion of aorta. The control group of rabbits were administered saline at a matched infusion rate and total volume of infused liquid.

Statistical Analysis

Differences in mean values of placental perfusion before and after uterine ischemia, and between saline and antioxidant groups were tested using Student's t-test. Although most of the data can be presented as paired measurement obtained from the same placentas before and after ischemia, substantial fetal motion occurred between measurements during H-I period, causing change in orientation of placentas and sometimes movement out of the imaging slice. Therefore unpaired two-sided t-test was applied. Data presented as means $\pm$ standard error of means.

RESULTS

Placental Perfusion Quantification From MR Imaging and by Fluorescent Microspheres

Individual maternal–fetal placental units (thick black arrow on Fig. 1A) can be identified on T2-weighted images as dark bi-lobular structures. Corresponding fetal structures can be also identified, including fetal brain (thin black arrow on Fig. 1A. Arrival of contrast produces signal enhancement in band-shaped outer part of placenta (Fig. 1B, thick white arrow),

corresponding to maternal decidua,²³ where majority of maternal circulation occur in E25 rabbit placenta.¹⁷ Characteristic lacunar structures, formed by maternal circulation, are also visible as bright blobs (thin white arrow on B). Because the rate and magnitude of enhancement on MR images with contrast administration was visibly larger in decidua than the rest of maternal–fetal placental unit, we chose to estimate perfusion in those anatomical parts separately. In E25 rabbits the decidua could be readily separated from the rest of placenta on dissection. In our study, the average weight of decidua was 1.2 g and the average weight of the whole placenta was 5.6 g. Comparing microspheres data from four placentas, obtained from the same sham control dam, perfusion values were 0.99 ± 0.19 mL/min/g in decidua and 0.33 ± 0.03 mL/min/g in the rest of placenta. The maternal perfusion values are depicted from decidua tissue by MRI and by microspheres methods. Placental perfusion, estimated using aortic injection of fluorescent microspheres between 15 and 20 min of reperfusion–reoxygenation time yielded decidual perfusion values of 0.72 ± 0.02 mL/min/g in saline and 0.94 ± 0.07 mL/min/g in antioxidant groups, which were not significantly different (t-test; $P = 0.07$).

Positions of fetuses in uterus were identified on structural MR images¹⁵ and correspondence was established when the tissue samples were obtained by laparotomy. Twelve placentas were reliably identified, having both MRI and microspheres perfusion measurement. Perfusion values, estimated in the same placentas by the two methods and both obtained during reperfusion phase, significantly correlated with each other ($P < 0.01$) as shown in Figure 4. Estimation of perfusion by DCE MRI (average 0.56 ± 0.05 mL/min/g) was systematically lower than by microspheres method (average 0.82 ± 0.08 mL/min/g), or by 33.3%. Typical signal-to-noise ratios were 22–28 in aorta and 9–12 in placentas; absolute change in image intensity was 30–35% and 21–25% relative to baseline in aorta and placentas correspondingly.

Placental Perfusion During Reperfusion–Reoxygenation Phase After Acute H-I

Placental perfusion was significantly lower during reperfusion–reoxygenation phase (0.44 ± 0.06 mL/min/g) compared to the baseline values (0.77 ± 0.07 mL/min/g) in the saline group (Fig. 5), as estimated by DCE MRI. On the other hand, in the antioxidant group there was no significant difference between baseline and during reperfusion–reoxygenation phase (0.84 ± 0.12 mL/min/g before and 0.62 ± 0.06 mL/min/g after occlusion). There was no significant difference in placental perfusion between antioxidant and control groups before occlusion. Blood velocity in descending aorta (31.7 cm/s) and caudal vena cava (11.2 cm/s) were not significantly different before and after uterine ischemia. Maternal heart rate dramatically increased above 150 bpm immediately after cessation of ischemia, but returned to 125 ± 12 bpm at 5 min of reperfusion–reoxygenation when perfusion measurement was done, not significantly different from preocclusion values.

Reduction of placental perfusion during reperfusion–reoxygenation is illustrated by decreased slope of contrast accumulation on Figure 6, showing perfusion transit curves obtained from the same placenta in saline group before and after occlusion. This reduction in maternal placental blood flow could not be attributed to the decreased cardiac output

because there was no similar reduction in the renal cortical perfusion (Fig. 6). The renal arteries branch off from aorta above the occlusion site and there was no ischemia in kidneys.

DISCUSSION

Even though the administration of contrast agent is not permitted in human pregnancy, DCE MRI of placenta in animal models offers highly valuable information for basic understanding of disease mechanisms. Small and medium size animal models are amenable to mechanistic manipulations to answer specific research questions, but signal to noise ratio (SNR) of perfusion measurement is typically low. The first major finding of the study was significant linear relationship between perfusion measured by the steepest slope MRI method and by fluorescent microspheres, validating the steepest slope MRI method as a simple, reliable and robust method of perfusion estimation. The advantage of method is relatively higher SNR of measurements, compared to perfusion estimation methods without exogenous contrast infusion, and numerical robustness, because it does not require fitting numerous parameters, as in pharmacokinetic perfusion models. Those two advantages are especially beneficial in small animal imaging of internal organs with substantial motion that may throw off estimations by more complex models. The numerical robustness of the steepest slope technique is seen in the lower standard deviation of the measurement 0.44 mL/min/g in the present study, compared with 0.54 mL/min/g in mouse placenta using pharmacokinetic compartment model.⁸ In the current study, this allowed detecting statistically significant difference in means with effect size 0.73 standard deviations in comparisons of placental perfusion with drug interventions.

It is well known that the steepest slope method to analyze DCE MRI data systematically underestimates tissue perfusion due to the underlying assumption of the negligible amount of outflow during measurement.¹² This study represents an effort to assess the extent of the bias by means of comparative measurements performed by an established reference technique using fluorescent microspheres. Our results demonstrate that placental perfusion measured by the steepest slope method was underestimated approximately 33% relative to the perfusion measurements by fluorescent microspheres.

One of the reasons of the inaccuracy is that the steepest slope model does not account for the outflow of the contrast, with errors becoming larger in highly perfused tissues. According to simulations in Brix et al¹² underestimation of perfusion values obtained by the steepest slope method were under 16% or 23%, depending on the noise level and sampling frequency and a true perfusion being less than 0.5 mL/mL/min. Above this limit, the bias increases markedly to more than 30% at true perfusion of 1 mL/mL/min. In our data true perfusion in rabbit placenta (as determined by microspheres method) was in the range between 0.72 and 0.94 mL/min/g thus placing our result in the theoretically predicted range of accuracy. Average placenta perfusion value obtained in this study, 0.82 ± 0.08 mL/min/g, was comparable with previously reported measurements of 1.1 mL/min/g in whole placenta of E28 rabbit² using fluorescent microspheres, 0.56 mL/min/g in whole placenta of E27 rabbit using radioactive microspheres,²⁴ 1.26 ± 0.54 mL/min/g in mouse using compartment model MRI⁸ and 1.12 ± 0.32 mL/min/g using steepest slope method MRI,¹⁰ 1.15 mL/min/mL in rats⁷ using compartment model MRI. Placenta is an organ with relatively

high perfusion (compared with other organs) and the outflow effect became significant, introducing error in the MRI perfusion estimation by steepest slope. Perfusion in rabbit kidneys in our study was even higher at 2.26 mL/min/g. Systematic underestimation of renal perfusion by the steepest slope method has been previously reported in rabbits' kidney cortex, although the data were correlated well with the ultrasound measurement.¹⁹

Free radical injury is one of the leading mechanisms of the fetal brain injury caused by H-I.²⁵ It has been demonstrated that maternal administration of antioxidants protects fetal brains from H-I injury.^{22,26} It is unclear, however, that with maternal systemic administration, whether neuroprotection can be attributed exclusively to the free radical scavenging in fetal brains. We speculated that improvement of placental circulation may also produce beneficial effect on fetuses by accelerating their recovery in reperfusion–reoxygenation phase after H-I insult. We found that the placental perfusion was significantly reduced in first 5 min of reperfusion–reoxygenation relative to the baseline. The decrease was less in the group with antioxidants and not significantly different from the baseline, suggesting that antioxidants accelerated re-perfusion recovery. Decrease of placental perfusion during reperfusion stage after ischemia could not be explained by decrease in systemic maternal circulation because the maternal heart rate was actually increased during reperfusion phase before returning to the baseline values. The mechanisms of the perfusion decrease may involve oxygen preserving blood flow redistribution, as observed during an episode of acute maternal hypoxia⁵ that persists after restoration of blood flow in aorta, explaining the delay before placental perfusion recovery. It is also possible that slow recovery of the blood flow in placenta after a period of ischemia involves oxidative stress, similar to no-reflow phenomenon in myocardium.²⁷ Whether acceleration of placental reperfusion is beneficial to fetus is controversial because, while improving re-oxygenation, it theoretically may cause more damage by providing more free radicals and toxins to fetal brain. If the latter factor is found to be negligible, then the increase of maternal placental blood flow could be a promising strategy to protect fetal brain at risk of H-I injury caused by acute or episodic repetitive placental insufficiency.

There were several limitations in the study that could introduce error in the measurements. First, MRI and microspheres measurements were performed at different times. In future such limitation could be overcome if the reference blood withdrawal for microsphere infusion can be performed in the scanner during MRI contrast injection. The second limitation is that arterial input function was derived from ROI in maternal aorta because umbilical arteries were too small in rabbits to make measurement feasible on a clinical magnet. This may explain in part the variation observed between measurements in different fetuses because in rabbits, the uterine blood supply originates from uterine arteries with collaterals from ovarian arteries. Small number of animals is also a limitation of the study.

We conclude that the steepest slope method to analyze DCE MRI provided a robust measurement of perfusion in rabbit placentas with significant but systematic underestimation of the values against those obtained by an invasive method of microspheres infusion. Therefore comparison of perfusion values between experimental groups obtained by the steepest slope method could be performed if the arterial input function is maintained approximately equivalent.

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FIGURE 1.

A: T2-weighted SSFSE images were obtained as an in-plane anatomic reference. Thick black arrow points to maternal–fetal unit; thin black arrow, fetal brain. **B:** A series of fast T1-weighted images acquired during contrast administration for perfusion measurement. Typical ROI placed on decidua is shown with dotted line. Thick white arrow indicates maternal deciduas part of placenta. Thin white arrow point to lacunar structures, formed by maternal circulation in placenta

FIGURE 2.

Signal intensity on SPGR perfusion sequence of the phantom tubes, filled with solutions with known R1, was fitted with a polynomial function that was used to convert signal intensity across the image into R1 values. The phantom tubes were placed next to the dams' abdomen and the regression equation was updated for each experiment.

FIGURE 3.

Arterial and placental transit curves obtained following injection of Gd-DTPA bolus prior to initiating placental ischemia. The blood flow (0.66 mL/min/g in the figure) was measured from the slope of placental perfusion curve and the peak enhancement in the aorta. The signal intensity, measured on time series of T1-weighted images, was converted to $R1\ s^{-1}$ using calibration curve obtained from a set of phantoms with known T1. The phantoms were placed next to the dam's body in each imaging session.

FIGURE 4.

Values of absolute maternal placental perfusion measured on the same rabbit placentas by DCE MRI method are plotted against perfusion measurements by fluorescent microspheres. Fitted linear regression is plotted as dashed line. The identity line is given for reference and is the dotted line.

FIGURE 5.

Absolute maternal placental perfusion was quantified by DCE MRI in rabbit dams at E25 gestation age. The perfusion was measured repeatedly before and after placental ischemia caused by aortic occlusion. Antioxidants were given systemically in a separate group of dams during reperfusion–reoxygenation. Maternal placental perfusion decreased in reperfusion–reoxygenation phase in both groups, but the difference reached statistical significance only in the saline group. There was no difference in perfusion between saline and antioxidant groups either before or after ischemia. * - $P = 0.012$, t-test

FIGURE 6.

Transit curves, obtained from the same placenta before aortic occlusion and 5 min after beginning of reperfusion–reoxygenation. Note the decreased slope of the signal enhancement in the reperfusion curve indicating lesser placental perfusion after ischemia (0.69 mL/min/g) than before (1.21 mL/min/g). In comparison, perfusion in kidney cortex, where the blood flow were not interrupted, did not change significantly before (2.26 mL/min/g) and after occlusion (2.00 mL/min/g).