Nitric oxide (NO) donors and haemodynamic changes in fetal growth restriction

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Abstract

Objective: To evaluate maternal cardiovascular effects of nitric oxide (NO) donors in pregnancies complicated by fetal growth restriction (FGR)

Methods: 26 women with a diagnosis of FGR were treated with transdermal patches of NO donors and plasma volume expansion. We compared the treated group to a control historical FGR untreated group of patients evaluated longitudinally. We obtained haemodynamic indices using UltraSonic Cardiac Output Monitor system.

Results: At enrolment, the two groups were similar in terms of maternal and haemodynamic characteristics. In the treated group, we found a significant increase in cardiac output, stroke volume and a decrease of systemic vascular resistance after therapy. No significant differences were found after two

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weeks in the untreated group. At birth the treated group also gave birth to babies with higher birth weight centile.

Conclusions: Despite the observation nature of the data, the combined therapeutic approach of NO donor administration and plasma volume expansion in FGR significantly improves maternal hemodynamic indices.

Keywords: fetal growth restriction (FGR), UltraSonic Cardiac Output Monitor, nitric oxide (NO) donors, maternal hemodynamic, total vascular resistance (TVR)

Introduction

Fetal Growth Restriction (FGR) is a condition in which a fetus is unable to achieve its genetically determined potential size. It affects about 8% of pregnancies and is associated with an increased perinatal mortality and morbidity (1).

Several studies in the literature have investigated the cardiovascular adaptation in pregnancies complicated by FGR demonstrating that they are characterized by a reduced expansion of the maternal intravascular space, a lack of increase in stroke volume (SV) and cardiac output (CO) in

the very early phase of pregnancy (2,3) and a decreased preload compared with normal pregnancies.

Nitric oxide (NO) is an autocrine and paracrine signalling molecule that is synthesized from L-arginine by a family of calcium–calmodulin-dependent enzymes called nitric oxide synthases (NOS). It is released by endothelial cells with a physiological vasodilating effect that increases blood flow. Moreover, it induces a decreased responsiveness to vasopressors and inhibition of platelet function (4).

NO has an important effect in regulating placental vascular tone and development. (5) In fact, during normal pregnancy it appears to play an important role in normal placental development; its production occurs in the uteroplacental tissues and is associated with a reduction in vascular resistance of the fetoplacental and uterine circulations (6). These important features of NO donors in improving uteroplacental perfusion could be a therapeutic strategy in the management of pregnancies complicated by FGR.

Objective

The aim of the study was to evaluate the maternal cardiovascular effects of NO donors therapy in pregnancies complicated by FGR. The remarkable vasodilatory function of NO donors, associated with plasma volume expansion, derived by co-administration of oral fluids, may improve pregnancy outcome in FGR acting on the maternal systemic vascular resistance (SVR).

Materials and Methods

This was a prospective cohort study conducted at the Department of Obstetrics and Gynaecology, Policlinico Casilino Hospital, Rome with the collaboration of the Fetal Medicine Unit, St George's Hospital, University of London.

We enrolled 26 pregnancies complicated by FGR, defined as an estimated fetal weight below the 10th percentile and abnormal umbilical artery (UA) Doppler, over a continuous period of six months. The exclusion criteria were: multiple pregnancy; chromosomal abnormalities; preterm rupture of membranes; intrauterine infection; undetermined gestational age; history of maternal heart disease; tobacco use; pre-existing chronic medical problems. The 26 patients of Group A were treated with NO donors (transdermal patches of glycerine trinitrate at a dosage of 5 mg/24 h, which were utilized for 12 h a day to avoid tolerance) and oral fluid intake (2.5 L of fluid per day). For each case enrolled in the 'standard treatment' group (Group A), one control, matched for maternal age (± 1 year), body mass index (BMI) (± 1 kg/m2), gestational age and severity of disease from a historical group of untreated FGR patients followed longitudinally, was included in the Group B.

Approval of the local ethics committee was obtained based on a submitted protocol, and informed consent was obtained from all patients prior to enrolment.

All patients were subjected to UltraSonic Cardiac Output Monitor (USCOM®, USCOM Ltd., Coffs Harbour, Australia) evaluation at the enrolment and after two weeks.

Fetal ultrasound examination

For the ultrasound examinations, a 3.5-MHz sector ultrasound transducer was used. Fetal biometry and estimated fetal weight were assessed according to local reference values. Doppler measurements were obtained from the UA, middle cerebral artery (MCA) and uterine artery.

Fetal biometry and Doppler measurements were obtained by a single specially trained examiner, using a Voluson E6 ®, GE Healthcare.

Maternal hemodynamic assessment

All haemodynamic measurements were obtained with USCOM®, a non-invasive Doppler ultrasonic technology for determination of haemodynamic variables. The USCOM® has been validated against invasive gold standards (7,8,9) USCOM® uses a non-imaging, continuous-wave Doppler transducer placed at the suprasternal notch to measure transaortic or transpulmonary blood flow. The acoustic beam directed in line with the valvular flow and a time–velocity spectral display is generated showing variations of the blood flow velocity with time. Once the optimal flow profile is obtained, the trace is frozen on the screen and the flow profiles automatically traced allowing the SV to be calculated as the product of the velocity-time integral and the cross-sectional area (CSA) of the chosen valve. The CSA of the aortic valve is determined from the proprietary height-indexed regression equations. The CO is automatically calculated as the product of the heart rate (HR) and SV. SVR is calculated following the entry of systolic blood pressure (SBP) and the diastolic blood pressure (DBP) or the mean arterial pressure (MAP) (10).

Only two trained operators were responsible for taking measurements.

Statistical Analysis

Values are expressed as mean ± SD. Clinical data were compared by means of independent samples Student's t-test. Differences were considered as significant with $p < 0.05$.

Results

Table 1 reports the demographic and pregnancy characteristics at the enrolment and the comparison of the outcome between the two groups. The two study populations were similar and

comparable at the enrolment in terms of parity, maternal age, height, weight and BMI; no significant differences were found in estimated fetal weight (EFW) at diagnosis of FGR, in fetal Doppler (UA pulsatility index [PI] and MCA PI), in gestational age at the enrolment and in the gestational age at delivery. There was a significant difference in terms of estimated fetal weight centile at the enrolment that was higher in group A (9.2±1.2 vs 6.5±1.7, p<00001). The uterine artery PI was significantly higher in the pregnancies in Group A (treated) than those in group B. The birth weight in grams and birth weight centile were significantly higher in Group A than Group B (2538.88±490 vs 2303±447 grams, $p<0.05$; 11±0.5 vs 3.66±1.5, $p<0.05$).

In table 2 there is the comparison between the EFW at the enrolment and birth weight centile for each group evaluated longitudinally. In the group A there is a statistically significant increase of centile value after two weeks; on the contrary, group B shows a significant longitudinal reduction of the same.

There were no statistically significant differences between the two groups in any of the cardiovascular parameters at the enrolment (Table 3).

Table 4 shows the longitudinal evaluation of the hemodynamic parameters in the treated group (group A) and the untreated group (group B). As reported in table 3 and figures 1-3, in the treated group there were higher values of SV, SV index (SVI), CO and cardiac index (CI) at the second measurement ($p<0.05$). Moreover, the SVR values were significantly lower after two weeks of therapy ($p<0.05$). No significant differences were found after two weeks in the untreated group followed longitudinally, despite a slightly decrease of SVR values.

Discussion

Summary of findings

The normal cardiovascular adaptation of the mother to pregnancy includes a number of haemodynamic changes such as the increase in CO and the SV associated with the reduction of

SVR and systemic arterial pressure values. These changes take place from the earliest weeks of pregnancy, reach their peak during the second trimester and then remain relatively constant until delivery.

In our prospective cohort study we investigated the therapeutic effect of NO donors in pregnancies complicated by FGR and, in particular, their effect on maternal cardiovascular assessment.

For this purpose, we compared maternal cardiovascular parameters in two groups of patients subjected to a different management of FGR: a treated group enrolled at the diagnosis of FGR and evaluated after two weeks therapy and a second group of untreated patients followed longitudinally.

As shown in table 1, the two study populations were comparable in terms of maternal characteristics. Instead, in the treated group we noticed higher uterine artery Doppler PI values compared with Group B (untreated), suggesting these cases might present a worst impaired placentation process.

Our results showed an overall improvement, after two weeks' therapy, in cardiac function indices, particularly in CO, SV and SVR in the group treated with NO donors and oral fluids. On the contrary, the cardiovascular assessment in the untreated group, evaluated after two weeks, reflects a stable worst condition with SVR values persistently high.

Another important aspect of NO donors treatment is the increase in heart rate, which is typically lower in pregnancy complicated by FGR. In our study there was a slight increase in HR two weeks after therapy although it does not reach statistical significance. The enrolment of a second historical control group of untreated FGR patients allows us to demonstrate that the finding of elevated SVR values in these kind of patients in not only an accidental result but might be replicated in different clinical setting, investigated with the same method. The availability of a

control-untreated group permitted to testify that the therapy regimen adopted gives a significant reduction of SVR in the treated group.

Although it wasn't our first endpoint and we especially focused on cardiovascular effect of NO donors therapy, our data showed a statistically significant improvement of fetal growth. The estimated fetal weight percentile for the treated group at study enrolment was on the 9th percentile, higher than the control group (6th percentile). Even if the treated group "starting point" could be considered better, the analysis of birthweight centile showed a significant difference (11^{th} vs third centile, respectively) demonstrating that therapy may be also effectiveness in improving fetal growth.

Comparison with existing literature

Several studies in literature have shown that abnormalities of these adaptive mechanisms in the placental vascular bed are related to several disorders of pregnancy, in particular, pregnancy-induced hypertension (PIH) and FGR. Transdermal GTN patches have been widely studied for both prevention and management of preeclampsia and related disorders (11) and it seems an attractive tool in improving uterine and umbilical blood flow. Moreover, it protects syncytiotrophoblast from apoptosis, lipid peroxidation, and superoxide formation following hypoxia-reperfusion insults.

A prospective case-control study conducted by Melchiorre demonstrated that growth-restricted pregnancies are characterized by a lower CI and higher SVRI than expected for gestation (12). As regard therapy with NO donors, several studies in the past have demonstrated their effectiveness in improving outcome of FGR pregnancies. Some authors have, also, demonstrated the reappearance of end-diastolic flow in the UA associated with an improvement in fetal outcome during NO donors treatment (13,14).

Research and clinical implications

On the basis of our results, we can speculate the beneficial effect of NO donors and fluid management. The most interesting aspect is the double and synergistic action on both afterload and preload of our therapy. In fact, oral fluid management induces an increase in preload, that is typically reduced in pregnancies complicated by FGR. At the same time, NO donors administration reduces afterload acting on SVR.

We can speculate that CO and SVR could therefore represent a good method to evaluate the efficacy of our combined therapy, confirming that improvement in maternal cardiovascular function could potentially have a positive effect on fetal growth. On the basis of the results of our study, the maternal "cardiac pump" would play a central and decisive role in the evolution of the pregnancy and the study of maternal cardiovascular function could be useful in understanding the maternal response to impaired placentation, and therefore, improving our prognostic ability.

Strenght and weakness

Most of the current existing studies in literature investigated both the maternal hemodynamic changes and effectiveness of therapy using transthoracic echocardiography. This is a widely accepted methodology for CO estimation in pregnancy, considered safe and acceptable due to its non-invasive nature. However, this method requires both costly equipment and clinical expertise, thereby limiting availability. In the current study we use a non invasive method, the USCOM® system, simple to use with measurements obtained in minutes, with minimal training required to achieve operative proficiency.

The accuracy of the system has been proven in a number of studies, when compared with other methods and in various clinical applications. USCOM® comparisons with flow probes in computer controlled flow simulator demonstrated a variation of less than 5% and, as shown in a previous study (15), the mean difference of CI was 0.16 ± 0.59 l/min/m, and the correlation coefficient was

0.87. Several studies demonstrated a good reproducibility of USCOM in pregnant women. A study conducted in 2016 by Khalil et al demonstrate that USCOM® has a good agreement with echocardiography in the third trimester. The level of agreement between USCOM® and echocardiography in the third trimester meets the recommended level of clinical acceptability. The intra-class correlation coefficient for CO estimation was 0.969 (95% CI 0.953-0.980). The interobserver reproducibility varied from 0.899 (95% CI 0.790-0.952) in the first trimester, 0.969 (95% CI 0.88-0.992) in the second trimester, and 0.965 (95% CI 0.185- 0.990) in the third trimester. The intra-class correlation coefficient for CO was 0.896 (95% CI 0.812-0.944) (16).

A limitation of this study could be the small sample size and the observational nature of the study. However, despite the small sample size, it was adequate to demonstrate the effectiveness of therapy.

Conclusions

In conclusion, despite the observation nature of data and several limits of the study, our results might open new perspectives in the treatment of fetal growth restriction, focusing on main maternal cardiovascular anomalies. This view would place the focus of the evaluation of TVR and CO, becoming a central target for the therapies.

Although these initial results are encouraging, they must be confirmed in a properly controlled RCT, with an adequate study population**.**

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Table 1. Characteristics of the study population

	Group A (treated)	Group B (untreated)	p value A-B
	(n=26)	(n=26)	
Nulliparous	18 (69.23)	20 (76.92)	0.39
Maternal age (years)	32.27±4.9	33.33±3.8	0.38
Maternal height (metres)	1.62±0.1	1.58±0.07	0.10
Maternal weight (kilograms)	65.13±15.8	60.16±9.6	0.17
Maternal BMI (kg/m2)	24.8±1.8	23.82±2.14	0.08
Estimated fetal weight in grams at recruitment	1437±465.4	1405±291.39	0.76
Estimated fetal weight centile at recruiment	9.2±1.2	6.5±1.7	**p<0.0001**
Umbilical artery pulsatility index(PI)	0.99±0.3	1±0.2	0.88
Middle cerebral artery PI	1.85±0.3	1.83±0.41	0.84
Uterine artery PI	1.2±0.2	0.90±0.16	**p<0.05**
Gestational age at enrollement	30.77±0.33	30.33±1.21	0.07
Birth weight (grams)	2538.88±490	2303±447	**p<0.05**
Birth weight centile	11±0.5	3.66±1.5	**p<0.05**
Gestational age at delivery	37.19±2.5	37 ±2.6	0.78

Table 2. Difference in percentile pre and post in each group longitudinally

	EFW centile at recruiment	Birth weight centile	p value
Group A (treated)	9.2±1.2	11±0.5	p<0,01
Group B (untreated)	6.5±1.7	3.66±1.5	p<0,01

Table 3. Maternal hemodynamic parameters at enrolment

	Group A (treated)	Group B (untreated)	p value A-B
Heart Rate (HR)	78.33±12.7	77.83±11.8	0.88
Ejection Time % (ET%)	40.95±5.7	41.48±6.97	0.76
Stroke Volume (SV)	73.76±11.5	71.9±17.11	0.64
Stroke Volume Index (SVI)	42.98±7.9	42.8±0.6	0.9
Cardiac Output (CO)	5.81±1.1	5.48±0.9	0.24
Cardiac Index (CI)	3.46±0.7	3.29±0.37	0.27
Sistolic Arterial Pressure (SAP)	112.58±13.3	115±12.24	0.49
Diastolic Arterial Pressure (DAP)	71.92±10.8	73.66±7.73	0.50
Mean Arterial Pressure (MAP)	84.15±11.3	87.16±8.7	0.28
Systemic Vascular Resistence (SVR)	1215.39±230.2	1314.83±219	0.11
SVR Index (SVRI)	2100.51±447.5	2140±257.40	0.69

Table 4. Maternal hemodynamic in group A (treated) before and after therapy and in group B (untreated)

	Group A (before therapy)	Group A (after therapy)	Δ (%)	p value	Group B untreated	Group B untreated (after 2 wks)	Δ (%)	p value
Heart Rate (HR)	78.33±12.7	80.57±10.48	2.86	0.48	77.83±11,8	79.33±8.53	1.89	0.60
Ejection Time % (ET%)	40.95±5.7	42.86±6.50	4.66	0.25	4.,48±6.97	44.33±7.11	6.87	0.15
Stroke Volume (SV)	73.76±11.5	83.11±16.78	12.68	**p<0.05**	71.9±17.11	70.96±16.4	-1.31	0.84
Stroke Volume Index (SVI)	42.98±7.9	48.13±10.22	11.98	**p<0.05**	42.8±0.6	42.5±0.74	-0.70	0.11
Cardiac Output (CO)	5.81±1.1	6.60±1.03	13.60	**p<0.05**	5.48±0.9	5.74±0.72	4.74	0.25
Cardiac Index (CI)	3.46±0.7	3.88±0.66	12.14	**p<0.05**	3.29±0.37	3.6±0.98	9.42	0.13
Sistolic Arterial Pressure (SAP)	112.58±13.3	108.81±12.98	-3.35	0.30	115±12.24	109±14.16	-5.22	0.10
Diastolic Arterial Pressure (DAP)	71.92±10.8	69.42±11.12	-3.48	0.41	73.66±7.73	69.83±10.34	-5.20	0.13
Mean Arterial Pressure (MAP)	84.15±11.3	82.77±11.08	-1.64	0.65	87.16±8.7	82.83±10.8	-4.97	0.11
Systemic Vascular Resistence (SVR)	1215.39±230.2	1022.43±195.35	-15.88	**p<0.05**	1314.83±219	1290±135	-1.89	0.62
SVR Index (SVRI)	2100.51±447.5	1786.04±387.56	-14.97	**p<0.05**	2140±257.40	2112±249.28	-1.31	0.69

Figure Legends:

Fig. 1: SV variation after two weeks therapy

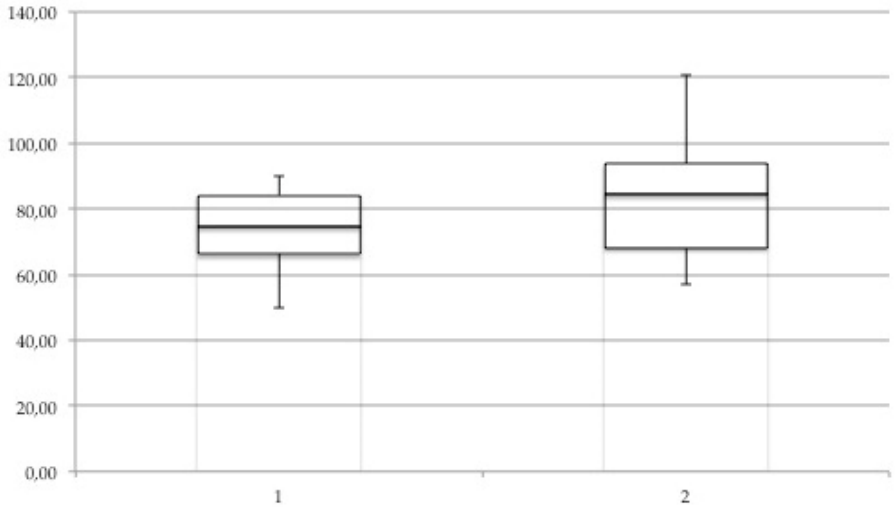

Fig. 2: CO variation after two weeks therapy

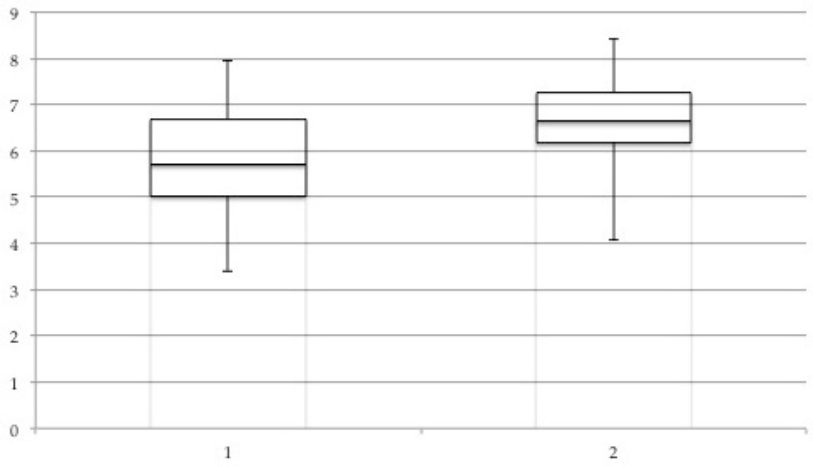

Fig. 3: SVR reduction after two weeks therapy

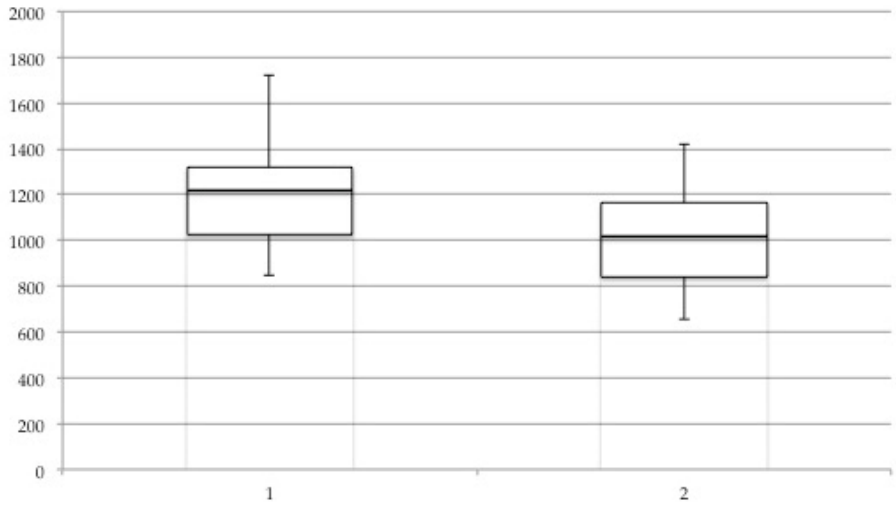

Fig 4: Strobe flowchart