

Effect of intravenous hydralazine infusion on maternal plasma nitric oxide levels in gestations complicated with severe preeclampsia: a pilot study

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Abstract

Aim To investigate the effect of intravenous hydralazine infusion on maternal nitric oxide (NO) levels.

Methods This pilot study comprised 40 ($n = 40$) gestations complicated with severe preeclampsia to whom maternal plasma NO levels were determined by chemiluminescence before and after hydralazine administration. Blood pressure values were concomitantly assessed.

Results After 20 min of intravenous hydralazine infusion blood pressure values decrease significantly in term and preterm gestations. This was accompanied by an overall significant decrease in mean plasma NO values

(38.7 ± 12.9 to 35.4 ± 13.9 $\mu\text{mol/L}$, $p < 0.05$). Despite this, NO values decreased in 67.5% of cases (a 17.6% from baseline) and increased in 32.5% (a 14.8% from baseline) ($p < 0.05$ for both). Blood pressure decrease (%) was lower (systolic and diastolic) among those displaying a NO decrement than in the increment group. Interestingly, gestational age was higher in the group displaying decreased NO; however, this did not reach statistical significance (37.5 ± 2.7 vs. 35.9 ± 2.8 weeks, $p = 0.08$).

Conclusion The results of this study fail to demonstrate a similar NO secretion after hydralazine infusion in women with severe preeclampsia.

Keywords Hydralazine · Nitric oxide · Preeclampsia · Pregnancy · Hypertension

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Introduction

In normal and abnormal situations the vascular system is subject to many endocrine, inflammatory and metabolic mediators with significant gender differences [1]. Pregnancy induces significant vascular remodeling in the uterine and systemic circulation in order to fulfill functional and metabolic requirements of the developing fetus. Preeclampsia is a frequent disorder of the human pregnancy (5–10% of all pregnancies) precipitated by genetic, nutritional and environmental factors [2]. From a clinical point of view, it remains a leading cause of maternal and fetal morbidity and mortality [3]. Altered normal hemodynamic response and endothelial dysfunction are major pathophysiological manifestations of this entity [3, 4]. Reports related to nitric oxide (NO) secretion and preeclampsia are contradictory, some studies indicating altered production and/or activity [5–11], others not [12–14]. These

contradictions are most likely due to differences in the research approach, the analyzed NO metabolites and/or the applied measuring methods.

Vasodilating antihypertensive drugs used to treat preeclampsia may increase endothelial cell NO production or improve NO bioactivity [15]. Hydralazine, a direct-acting smooth muscle relaxant, is a frequently used antihypertensive drug in gestations complicated with preeclampsia [16, 17]. Despite this, cellular mechanisms relating to the vasodilating properties of hydralazine are still not completely understood. Similar to organic nitrates, hydralazine mediates counter regulatory neurohormonal activation as demonstrated by increases in plasma catecholamines and renin activity. Theoretically, this would be expected to aggravate counter regulatory adjustments to nitrate and worsen nitrate tolerance resulting from frequent administration of isosorbide dinitrate [18, 19]. Despite this, hydralazine has shown to prevent glyceryl trinitrate tolerance in experimental models [20–22]. Information regarding its effect over serum NO among gravids complicated with preeclampsia is lacking. The present pilot study aimed at assessing short term plasma NO and blood pressure responses after intravenous hydralazine infusion in women complicated with severe preeclampsia.

Methods

Participants and study design

This study was approved by the Bioethics Committee of the Biomedical Center of the Central University of Ecuador. Women with severe preeclampsia attended at the “Hospital Gineco–Obstétrico Isidro Ayora” of Quito, Ecuador (2,800 m altitude) were included. Severe preeclampsia was defined as a blood pressure $>160/110$ mmHg and proteinuria >300 mg/dL. Based on institutional protocol, all women with severe preeclampsia must receive intravenous magnesium sulfate 4 g as a seizure prophylaxis. A blood sample (5 ml) was taken from the antecubital vein using a 21 g heparinized catheter before intravenous hydralazine administration (5 mg) and then after 20 min post-infusion. Drawn blood was immediately transferred into a polypropylene vial containing sodium citrate 3.15% (v/v) and centrifuged for 10 min at 1,500 rpm and 4°C. Plasma was removed and aliquoted into 500 μ l samples which were then frozen at -40°C until analysis. In patients whose blood pressure did not decrease after 20 min (first dose), additional hydralazine (10 mg) was administered. Blood pressure readings were recorded at baseline and at 20, 40 and 60 min after the initial intravenous hydralazine administration.

Nitric oxide (NO) assay

Plasma NO content was determined by chemiluminescence (Sievers NOA-280, Boulder, USA) as previously described [23]. Samples were measured in duplicates and mean values used for statistical analysis.

Statistical analysis

Statistical analysis was performed with SPSS statistical package (Version 10.0 for Windows, SPSS, Chicago, IL, USA). Data are presented as mean \pm standard deviations and percentages. The Kolmogorov–Smirnov test was used to determine the normality of data distribution. According to this, non-parametric paired continuous data were compared with the Wilcoxon signed-rank test. Percentages were compared with the χ^2 test. A p value of <0.05 was considered as statistically significant.

Results

A total of 40 gestations complicated with severe preeclampsia entered the study. Maternal and neonatal data are depicted on Table 1. Mean maternal age was 23.6 ± 5.7 years (range 14–36 years). An 80% of women were nulliparous and 45% delivered by cesarean section. Gestational age at birth was 36.9 ± 2.8 weeks. In this series, 57.5% of neonates had low birth weight ($<2,500$ g), 42.5% were preterm and 13.5% had low Apgar scorings at 5 min.

Depicted on Table 2 is the effect of hydralazine over maternal blood pressure and plasma NO levels. Blood

Table 1 Maternal and neonatal data

Variables	$n = 40$
Maternal and delivery data	
Age (years)	23.6 ± 5.7
Nulliparous (%)	32 (80)
Cohabiting with partner (%)	20 (50)
Magnesium sulfate (%)	40 (100)
Dipstick protein (range)	2 (1–3)
Cesarean delivery (%)	18 (45)
Neonatal characteristics	
Gestational age (weeks)	36.9 ± 2.8
Birth weight (g)	$2,333 \pm 648.9$
Birthweight $<2,500$ g (%)	23 (57.5)
Preterm rate (%)	17 (42.5)
Apgar score <7 at 5 min	5/37 (13.5)
Stillbirths (%)	3 (7.5)

Data are presented as mean (\pm standard deviation), median [interquartile range] and percentages

Table 2 Effect of hydralazine over maternal blood pressure and plasma NO levels

Variables	Systolic BP (mmHg)	Diastolic BP (mmHg)	NO ($\mu\text{mol/L}$)
All ($n = 40$)			
Baseline	160.7 \pm 15.2	117.7 \pm 5.0	38.7 \pm 12.9
20 min	149.9 \pm 16.8*	102.6 \pm 17.1*	35.4 \pm 13.9*
40 min	145.7 \pm 14.6*	96.0 \pm 7.2*	
60 min	146.8 \pm 12.4*	93.0 \pm 5.3*	
Term gestations ($n = 23$)			
Baseline	160.9 \pm 18.4	112.1 \pm 6.4	38.5 \pm 12.7
20 min	151.4 \pm 19.4*	99.6 \pm 8.9*	34.7 \pm 14.9*
40 min	145.4 \pm 17.5*	95.3 \pm 7.4*	
60 min	149.2 \pm 14.7*	91.9 \pm 5.6*	
Preterm gestations ($n = 17$)			
Baseline	160.3 \pm 9.7	111.0 \pm 1.8	38.9 \pm 13.5
20 min	147.9 \pm 12.9*	106.7 \pm 23.9	36.3 \pm 13.1
40 min	146.1 \pm 9.6*	96.9 \pm 7.1*	
60 min	143.5 \pm 7.6*	94.4 \pm 4.7*	

Data are presented as mean \pm standard deviation

BP blood pressure, NO nitric oxide

* $p < 0.05$ as compared to baseline

pressure values (systolic and diastolic) significantly decreased 20, 40 and 60 min after intravenous hydralazine infusion as compared to baseline. This effect was similar among the term and preterm gestations. Overall, as compared to baseline, NO plasma levels significantly decreased at 20 min of hydralazine infusion (38.7 ± 12.9 to $35.4 \pm 13.9 \mu\text{mol/L}$, $p < 0.05$). Although the trend was observed for preterm and term gestations, only the latter was found to be significant.

Blood pressure and NO plasma values before and after 20 min of hydralazine infusion in accordance to NO response are presented on Table 3. After 20 min of hydralazine infusion NO values decreased in 67.5% (a 17.6% from baseline) and increased in 32.5% (a 14.8% from baseline) ($p < 0.05$ for both). Blood pressure values (systolic and diastolic) decreased in both NO responding groups; however, this was significant in both groups for systolic while significant only for diastolic readings in those displaying a NO increment after 20 min. Blood pressure decrease (%) was lower (systolic and diastolic) among those displaying a NO decrement than in the increment group. Interesting was finding that gestational age was higher in the group displaying decreased NO; however, this did not reach statistical significance (37.5 ± 2.7 vs. 35.9 ± 2.8 weeks, $p = 0.08$).

Discussion

Hydralazine is a potent arterial vasodilator exerted through arterial smooth muscle relaxation. It has been widely used

Table 3 Blood pressure and NO plasma values before and after 20 min of hydralazine infusion in accordance to NO response

NO secretion after hydralazine infusion	Systolic BP (mmHg)	Diastolic BP (mmHg)	NO ($\mu\text{mol/L}$)
Decreased ($n = 27$)			
Baseline	160.4 \pm 17.3	111.6 \pm 5.8	41.0 \pm 12.9
20 min	151.5 \pm 16.6*	105.1 \pm 19.1	33.8 \pm 14.5*
Decrease (%)	5.5	5.9	17.6
No change in BP (%)	7 (25.9)	6 (22.2)	
Increased ($n = 13$)			
Baseline	161.2 \pm 10.1	111.8 \pm 2.9	33.8 \pm 11.6
20 min	146.6 \pm 17.5*	97.3 \pm 10.8*	38.8 \pm 12.7*
Decrease (%)	9.0	12.2	14.8
No change in BP (%)	2 (15.4)	2 (15.4)	

Data are presented as mean \pm standard deviation

BP blood pressure, NO nitric oxide

* $p < 0.05$ as compared to baseline

to manage preeclampsia hypertension [24]. This drug lowers both peripheral and pulmonary vascular resistance and increases cardiac output [25]. Peripheral vasodilatation results in decreased arterial blood pressure. Hydralazine has several other effects including increase in plasma renin activity and secondary increase in angiotensin II which stimulates aldosterone secretion. Hydralazine also increases renal and cerebral blood flow. Despite the aforementioned, it is less effective than other anti-hypertensive drugs for the treatment of severe preeclampsia [26]. Nevertheless, an intravenous hydralazine bolus is safe and effective in the management of hypertensive emergencies [7, 24–27]. More than 10 years ago, Lopez-Jaramillo et al. [28] reported that hydralazine infusion in preeclamptic women was related to an increase in plasma and urinary cGMP levels, suggesting that the drug might improve endothelial cell NO production. However, our group found that although plasma NO levels were similar among normal and preeclamptic women, intraplatelet cGMP levels were lower in those with preeclampsia [13]. This suggests that during preeclampsia NO action rather than its production is abnormal. In addition to several of the previously reported mechanisms of action of hydralazine, its novel antioxidant effect has been recently shown [29].

The present study found that 20 min after intravenous hydralazine infusion in women with severe preeclampsia, plasma NO were found to increase or decrease. This situation seemed to be correlated to the gestational age. Indeed, those displaying NO decrement were basically term and blood pressure decrease in this group was lower than those displaying NO increase in which blood pressure decrease was more pronounced. In other words preterm

preeclamptic women seem to have responded better to hydralazine, situation evidenced by higher plasma NO levels. Given that preeclampsia is characterized by free radical production [30], it could be possible that hydralazine might reduce oxidative stress and favor that equal amounts of NO be enough to stimulate soluble guanylate cyclase (GC) [13]. The final consequence of this action would be vascular dilatation and blood pressure decrease, however with different secretion patterns depending on gestational age.

Unfortunately due to small sample size of this pilot study and the limited period of observation (20 min) we cannot determine if the different NO responses apparently related to gestational age are in fact ethnically or genetically related (i.e. polymorphisms). Ethnic differences in NO-dependent vascular relaxation have been reported [31]. Some reports indicate that eNOS polymorphisms relate to differences in endothelium-dependent dilatation implicated in normal vascular adaptation to pregnancy [9, 32, 33]. Some of these polymorphisms may be protective against severe forms of preeclampsia. Significant eNOS polymorphisms have been described in Colombian and Brazilian women and related to increased preeclampsia risk [34, 35]. Although Colombia, Brazil and Ecuador are geographically closely related, ethnical characteristics are not presumably similar. Despite this, genetic aspects should be investigated in our population, moreover if gene polymorphisms and other mutations have been identified and correlated to preeclampsia prevalence and severity [36–38]. Current reports, on the other hand, show that preeclampsia increases the risk of developing chronic hypertension, metabolic syndrome and cardiovascular morbidity and mortality later in adult life. Thus, the negative impact of preeclampsia is not only confined to pregnancy [39].

Although this pilot study presents a very provocative hypothesis, unfortunately due to the limited sample size further work is needed to confirm our preliminary findings. NO has a major role in adapting circulation to pregnancy. Hydralazine exerted some beneficial effects on small vessels which could be linked to systemic hypertension prevention and/or local effects which are not uniformly detectable with our approach. More research is warranted to determine other involved biochemical factors or NO related polymorphisms that could explain the different NO response reported here at different gestational ages.

Conflict of interest The authors declare no conflict of interest.

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