Effects of transdermal nitroglicerin patches on inflammation and vascular lesion biomarkers in preeclamptic patients

Efectos de los parches de nitroglicerina sobre bio-marcadores de inflamación y lesión vascular en preeclámpticas

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Short title: nitroglycerine patches in preeclamptic patients

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Resumen

El objetivo de la investigación fue establecer los efectos de tratamiento con parches de nitroglicerina sobre las concentraciones de los bio-marcadores de inflamación y lesión vascular en preeclámpticas. La muestra fue de preeclámpticas nulíparas con embarazos de más de 25 semanas que acudieron a la emergencia obstétrica del Hospital Central "Dr. Urquinaona", Maracaibo, Venezuela. Se midieron las concentraciones interleucinas 6 y 10, factor de necrosis tumoral alfa, interferón gamma, proteína C reactiva, selectinas (P, E y L) y dimetilarginina asimétrica. El tratamiento fue con parches transdérmicos de nitroglicerina de 5 mg, aplicado en la región torácica anterior o lumbar de las pacientes por un período de 12 horas, por un tiempo total de 48 horas. La edad materna promedio fue de 21,77 ± 3,07 años y la edad gestacional fue de 34,23 ± 1,85 semanas. Se observó una disminución significativa en la presión arterial sistólica y diastólica al comparar los valores antes y después del tratamiento ($p < 0,05$). Sin embargo, no se encontraron modificaciones en las concentraciones plasmáticas de interleucina 6, interleucina 10, factor de necrosis tumoral alfa, interferón gamma, proteína C reactiva, selectinas y dimetilarginina asimétrica luego del uso de los parches de nitroglicerina al compararlo con los valores iniciales (p = ns). Se concluye que el tratamiento con parches de nitroglicerina no produce modificaciones en las concentraciones séricas de bio-marcadores de inflamación y lesión vascular en pre-eclámpticas.

Palabras Clave: Pre-eclampsia; Nitroglicerina; Inflamación; Disfunción endotelial; Bio-marcadores, TNF-α.

Abstract

Introduction and Objective: Pre-eclampsia is a multisystem disorder that complicates 3%–8% of pregnancies in Western countries and constitutes a major source of morbidity and mortality worldwide. The aim of this study was to examine the effects of transdermal nitroglycerin patches on biomarkers of inflammation and vascular lesion in pre-eclamptic patients.

Materials and methods: a prospective study was done in 40 nulliparous preeclamptic patients over 25 weeks of gestation who attended the Obstetric Emergency at Hospital Central "Dr. Urquinaona", Maracaibo, Venezuela. Interleukin 6 and 10, tumor necrosis factor alpha, interferon gamma, C-reactive protein, selectins (p, E and L) and asymmetric dimethylarginine concentrations were measured before and after nitroglycerin transdermal patches treatment (5 mg/twice a day) for 48 hours. Results: The mean maternal and gestational age was 21.77 ± 3.07 years and 34.23 ± 1.85 weeks respectively. A significant reduction was observed in systolic and diastolic blood pressure after treatment ($p<0.05$). However, no modifications were observed in Interleukin 6, Interleukin 10, tumor necrosis factor alpha, interferon gamma, C-reactive protein, selectins and asymmetric dimethylarginine concentrations. We concluded that treatment with nitroglycerin patches did not produce modifications in serum concentrations of inflammation and vascular lesion biomarkers in preeclamptic patients.

Keywords: Pre-eclampsia; Nitroglycerin; Inflammation; Endothelial dysfunction; TNF-α.

Introduction

Preeclampsia is a multisystem disorder that involves liver, kidneys, brain and placenta. It affects 2 to 8 percent of pregnancies[1] and is associated with a substantial increase maternal and fetal morbidity and mortality[2]. Maternal complications may include eclampsia, cerebrovascular disease, renal or hepatic failure and alterations on the coagulation. Severe preeclampsia is associated with different degrees of fetal injury, especially, undernutrition as a result of uteroplacental vascular insufficiencies, which leads to intrauterine growth restriction or even causes fetal death. Long-term follow-up studies have demonstrated that babies who suffered intrauterine growth retardation are more likely to develop hypertension, coronary artery disease, and diabetes in adult life[2].

The etiology of preeclampsia still unknown, but there is strong scientific evidence supporting that the reduction of the blood flow with placental hypoxia and/or ischemia, excessive oxidative stress, in association with endothelial dysfunction. The release of soluble factors from the ischemic placenta into maternal plasma plays a central role in the ensuing endothelial dysfunction that is the most prominent feature of this disease[3]. Endothelial dysfunction causes generalized vasoconstriction, platelet activation, thrombosis and decrease in plasma volume with later multi-organic blood flow reduction[4]. The main pathophysiologic finding of the preeclampsia is the alteration of the vasodilation of maternal vessels, due to different compounds produced in the endothelium[5].

Nitric oxide (NO) is a potent vasodilator and inhibitor of platelet aggregation produced by endothelial cells from L-arginine by the catalytic action of constitutive nitric oxide synthase (cNOS)[6]. Once synthetized, NO Immediately diffuses through adjacent smooth cells increasing the intracellular concentration of cyclic guanosine monophosphate (cGMP), yielding vascular smooth muscle relaxation. A placental reduction in both, L-arginine concentration and nitric oxide synthase activity have been found in women with preeclampsia[7]. Data derived from experimental models show that chronic inhibition of cNOS results in high blood pressure, intrauterine growth retardation, and thrombocytopenia[8]; thus, NO deficiency may be an important factor in preeclampsia development[2].

Some data indicate that the administration of nitroglycerin, a nitric oxide donor, conduces to vasodilation in the maternal circulation but not in the fetal vasculature[9]. Drugs that can be converted in NO (known as NO donors) are widely available and have been used for years as therapeutic agents in CVD such as angor pectoralis and hypertension[10]. NO donors have been previously studied in several obstetric complications, including premature labor, prevention of pre-eclampsia, intrauterine growth restriction, and hypertensive crisis. In this context, the decrease in morbidity and mortality is the main reason for the use of NO donors in obstetrics[9,11].

It has been shown that transdermal nitroglycerin (TDN) exposure modifies the vasomotor response to a number of endothelium-dependent stimuli in both, healthy subjects[12,13] and coronary artery disease carriers[14]. The mechanism of this vasomotor endothelial function modification induced by nitrates is unknown, but there is evidence, at least in part- related to an increased superoxide anion concentration in relation to an inadequate NO synthesis, suggesting abnormalities in eNOS function as part of this pathophysiologic conundrum[15]. In this regards, it has been hypothesized that TDN administration attenuates the vascular injury and inflammation more than functional or biochemical changes in endothelial cells. Also, the effects of TDN on inflammation and vascular lesion markers are unknown in pregnant women with preeclampsia. Because of the latter, the aim of this study was to assess TDN patches treatment on inflammation and vascular lesion biomarkers in pre-eclamptic women.

Materials and methods

Study design and sample features

The present prospective study was performed in a sample of 40 nulliparous women with more than 25 weeks of pregnancy and confirmed a diagnosis of preeclampsia, who attended the Obstetric Emergency department at Central Hospital "Dr. Urquinaona", Maracaibo, Venezuela. The research was approved by the Committee on Ethics and Research of the hospital and a written consent was obtained from all participants.

Exclusion criteria

The following conditions were considered exclusion cause to participate in this study: Polyhydramnios pregnant women, third trimester bleedings (abruptio placentae, placenta praevia), suspected intrauterine fetal growth restriction (top of the pubic symphysis–fundal height measurements, abdominal circumference and femur length under 10 percent of reference values with postnatal confirmation), HELLP syndrome, disorders of fetal heart rate, multiple gestation pregnancy, active maternal or intrauterine infection, chronic hypertensive disease (before 20 weeks of pregnancy), antihypertensive drugs treatment, cardiac, liver, and renal diseases or chronic systemic, pre or gestational diabetes mellitus and smoking habit. The patients who refused to participate in the research were also excluded.

Operative definitions, treatment protocol and patients´ evaluation

Preeclampsia was defined as systolic blood pressure of 140 mmHg or more, or diastolic blood pressure of 90 mmHg or more, confirmed by 6 h or more of difference, while proteinuria was defined as 300 mg or more in a 24-hour sample or 1-2 proteinuria crosses in a qualitative test after 20 weeks of gestation[4]. The blood pressure was measured in seated position after 15 minutes of rest before the determination. During the procedure, the arm was at the same level of the heart, being the systolic pressure the first sound that is heard (phase 1) and diastolic pressure the point where the sound fades (phase 5).

Treatment protocol consisted in 5 mg nitroglycerin transdermal patches applied in the anterior or lumbar thoracic region of the patients for 12 hours for a total period of 48 hours (a total of 4 patches). The patch was placed on the skin, on the lateral side of the thorax[9].

Blood samples from the antecubital vein were collected before and after 48 hours of the treatment and were let clot at room temperature. All the samples were centrifuged at 6.500 R.P.M for 10 minutes and then stored at -70°C until their processing. For determining interleukin-6, interleukin-10, TNF-α, and interferon-γ, an enzyme-linked immunosorbent assay was used and all samples were tested in duplicate, using the arithmetic mean of the 2 measures as the final value. The ELISA assays sensitivity were 10 pg/ml for interleukin-6, 1 pg/ml for interleukin-10, 3 pg/ml for interferon-γ and 3.5 pg/ml for TNF-α. The Inter and Intra variation coefficient were less of 7%.

Asymmetric dimethylarginine (ADMA) plasma concentration was measured using the enzyme-linked immunosorbent assay. Inter and intra-assay variation coefficient did not exceed 10% and 5%, respectively. C-reactive protein was assessed by a chemiluminescence essay with inter and intra-assay coefficient of variation of 8.7% and 7%, respectively. The sensitivity detection was 0.01 mg/dl. The soluble selectins concentrations were measured using ELISA test. The lower limit of detecting P-selectin quantification was 1.30 ng/ml, 0.25 ng/ml for L-selectin and 0.33 ng/ml for E-selectin detection. Intra and inter-assay coefficients of variation were lower than 5%.

The values obtained were presented as arithmetic means ±standard deviation. The t Student test for related samples was used for analyzing the values of high blood pressure and marker concentrations studied before and after the treatment. $p<0.05$ was considered statistically significant.

Results

The characteristics of the selected patients are shown in table 1. The average age of mothers was 21.77 ± 3.07 years and the gestational age was 34.23 ±1.85 weeks.

TABLE 1. General characteristics of women with preeclampsia treated and untreated with nitroglycerin patches

(n = 40)	
Age of the mother, years	21.77 ± 3.07
Gestational age, weeks	34.23 ± 1.85
Systolic blood pressure, mm of Hg	147.06 ± 11.49
Diastolic blood pressure, mm de Hg	110.10 ± 7.00
Proteinuria, g/24 hours	3.1 ± 1.9

The treatment effects with nitroglycerin patches on the mean blood pressure value are shown in table 2. A significant reduction in the systolic and diastolic blood pressure was observed when comparing the values before and after the treatment ($p<0.05$).

TABLE 2. Systolic and diastolic blood pressure on women with preemclampsia before and after using nitroglycerin patches

(n = 40)	
Systolic blood pressure, mm of Hg	
Initial	147.06 ± 11.49
48 hours	142.61 ± 15.71 ¥
Diastolic blood pressure, mm of Hg	
Initial	110.10 ± 7.00
48 hours	105.13 ± 8.65 ¥

¥ $p < 0.05$ compared to the initial value.

Regarding interleukin-6, interleukin-10, TNF-α, interferon-α, C-reactive protein, P-, E- and L- selectins and ADMA concentration no changes in plasma concentrations were found after using nitroglycerin patches compared to the initial values (p = ns; table 3).

Table 3. Biomarker concentrations in women with preeclampsia before and after the application of nitroglycerin patches

(n = 40)	Before the treatment	After the treatment	p
Interleukin 10, pg/ml	15.9 ± 3.1	17.5 ± 6.2	ns
Tumor necrosis factor alpha, pg/ml	9.7 ± 4.6	9.0 ± 5.1	ns
Interferon gamma, pg/ml)	75.5 ± 27.7	74.2 ± 29.6	ns
Interleukin 6, pg/ml	31.4 ± 2.5	29.5 ± 6.4	ns
C-reactive protein, mg/L	6.1 ± 2.2	6.3 ± 2.8	ns
P-Selectin, ng/ml	104.8 ± 9.4	102.6 ± 13.5	ns
E-Selectin, ng/ml	59.3 ± 9.9	58.1 ± 10.2	ns
L-Selectin, ng/ml	550.0 ± 74.2	561.4 ± 81.5	ns
Asymmetric dimethylarginine, pmol/L	0.547 ± 0.034	0.552 ± 0.027	ns

Discusion

Several studies in the last two decades have documented that treatment with nitroglycerin has deleterious effects on endothelial function, especially, when intermediate to long-term therapy is indicated, in which a significant reduction in smooth muscle responses have been observed in both, healthy individuals or CAD carriers treated with TDN with a similar protocol proposed in this trial[15,16]. These changes would be attributed to eNOS decoupling in the context of increased oxygen free radicals (OFR) production induced by nitroglycerin or ischemia-reperfusion process.

The secondary increase in vascular oxidative stress lead by nitroglycerin exposure is well documented from a variety of possible metabolic sources, including xanthine oxidase, NADH oxidase, mitochondrial electron chain, P450-cytochrome oxidase as well as NOS[17-19]. These free radicals increase has implications beyond nitrates resistance development, thus OFR oxidizes the carbohydrates, lipids, proteins and DNA and also altering the biochemical homeostasis inducing biological damage[20]. The evidence of vasomotor and endothelial dysfunction drive by nitroglycerin has been associated with aldehydes production and isoprostanes cytotoxicity, both products of lipid peroxidation[21], as well as deflective endothelial progenitor cells, function[14]. Because of the latter, the objective of this study was to investigate whether the treatment with TDN would be associated with an increase in vascular inflammation and endothelial lesion markers concentrations in women with preeclampsia. The results of this investigation show no changes in these bio-markers, an effect derived from an interplay between reactive oxygen species toxicity and the protective effect of NO released by nitroglycerin. In a research carried out by Berrazueta and col.[22], nitroglycerin treatment decreased C-reactive protein and E-selectin concentration, with no changes in other inflammation markers of vascular lesions in patients with the severe peripheral vascular disease. The explanation for this inconsistency is not clear, but it is possible that the protector effects of NO may prevail in patients with cardiovascular diseases, or in the case of our work, the absence of these effects may be due to the short lapse administration.

The particular relationship between the nitrate therapy and ADMA has been proposed via an increase in OFR bioavailability with subsequent functional alteration of ADMA hydrolase, the enzyme responsible for the metabolism of ADMA[23-26]. It should be noted in this context two observation from In vitro studies[25]: 1) endothelial cells exposed to nitroglycerin exhibit a decrease in ADMA hydrolase activity, which produces an increase in ADMA concentration. 2) Endothelial cells treatment with ADMA also cause aldehyde dehydrogenase-2 activity reduction, one of the key enzymes involved in the biotransformation of nitroglycerin. These studies concluded that the exposure to the nitroglycerin reduces the dimethylarginine aminohydrolase, ADMA increase and decreases in the activity of aldehyde dehydrogenase[25,27].

Even though crucial pharmacology and therapeutic facts about nitrates have been elucidated in the last years[13,28], there are still a lot of questions to be solved, especially in the practice of obstetric. One of these problems is the impact of nitrates on the prognosis in pregnant women[15]. Based on our results, it is concluded that the treatment with TDN does not produce changes in the serum concentrations of inflammation and vascular lesion biomarkers in women with preeclampsia.

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