

# Hydrocortisone reduces serum concentrations of inflammatory biomarkers in patients subjected to carotid endarterectomy

## *Hidrocortisona reduz as concentrações séricas dos biomarcadores inflamatórios séricos em pacientes submetidos a endarterectomia de carótida*

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### Abstract

**Background:** Hydrocortisone may reduce serum and tissue concentrations of inflammatory biomarkers. **Objective:** To analyze the inflammatory activity of serum and tissue high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor (TNF)- $\alpha$  and vascular endothelial growth factor (VEGF) after intraoperative administration of hydrocortisone, after carotid endarterectomy (CEA). **Method:** Twenty-two patients were allocated to a Control Group (5 asymptomatic and 6 symptomatic patients) and were not administered hydrocortisone or to Group 1 (4 asymptomatic and 7 symptomatic patients) and were administered 500 mg intravenous hydrocortisone. Serum levels of hsCRP, TNF- $\alpha$  and VEGF were tested for the preoperative period and at 1 hour, 6 hours and 24 hours after CEA. Levels of TNF- $\alpha$  and VEGF were also measured in carotid plaques. **Results:** Group 1 exhibited lower concentrations of serum TNF- $\alpha$  at 1 hour ( $p=0.031$ ), 6 hours ( $p=0.015$ ) and 24 hours ( $p=0.017$ ) after CEA and lower concentrations of serum VEGF at 1 hour ( $p=0.006$ ) and 6 hours ( $p=0.005$ ) after CEA, relative to controls. Symptomatic patients in group 1 exhibited lower concentrations than controls for serum TNF- $\alpha$  at 1 hour and 6 hours after CEA and lower concentrations than controls for serum VEGF at 1 hour after CEA. There were no statistical differences in tissue concentrations of TNF- $\alpha$  or VEGF between the control group and group 1. **Conclusion:** Hydrocortisone reduces postoperative concentrations of serum TNF- $\alpha$  and VEGF, especially in symptomatic patients; but does not reduce tissue levels of these biomarkers.

**Keywords:** hydrocortisone; inflammation; carotid stenosis.

### Resumo

**Contexto:** A hidrocortisona pode reduzir a concentração dos biomarcadores inflamatórios séricos e teciduais. **Objetivo:** Analisar a atividade inflamatória da proteína C-reativa ultrasensível (PCR-US), do fator de necrose tumoral (FNT)-alfa e do fator de crescimento do endotélio vascular (FCEV) séricos e teciduais, mediante administração intraoperatória de hidrocortisona, após endarterectomia de artéria carótida (EAC). **Método:** Vinte e dois pacientes foram divididos em Grupo Controle (5 assintomáticos e 6 sintomáticos) – não foi administrada hidrocortisona – e Grupo 1 (4 assintomáticos e 7 sintomáticos) – foram administrados 500 mg intravenoso de hidrocortisona. O PCR-US, o FNT-alfa e o FCEV séricos foram dosados no pré-operatório e em 1 hora, 6 horas e 24 horas após a EAC. Na placa carotídea, mensuramos os níveis de FNT-alfa e FCEV. **Resultados:** O grupo 1 exibiu menor concentração sérica de FNT-alfa em 1 hora ( $p=0,031$ ), 6 horas ( $p=0,015$ ) e 24 horas ( $p=0,017$ ) após a EAC, e menor concentração de FCEV em 1 hora ( $p=0,006$ ) e 6 horas ( $p=0,005$ ) após a EAC, em relação ao grupo controle. Os pacientes sintomáticos do grupo 1 exibiram menor concentração de FNT-alfa em 1 hora e 6 horas após a EAC, e menor concentração de FCEV em 1 hora após a EAC, em relação ao grupo controle. Não houve diferença estatística entre as concentrações teciduais de FNT-alfa e FCEV entre o grupo controle e o grupo 1. **Conclusão:** A hidrocortisona reduz as concentrações séricas pós-operatórias de FNT-alfa e FCEV, em especial nos sintomáticos; porém, não reduz os níveis teciduais destes biomarcadores.

**Palavras-chave:** hidrocortisona; inflamação; estenose das carótidas.

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Financial support: None.

Conflicts of interest: No conflicts of interest declared concerning the publication of this article.

Submitted: March 18, 2015. Accepted: June 11, 2015.

The study was carried out at Pontifícia Universidade Católica de Campinas (PUC-Campinas) and Faculdade de Ciências Médicas da Santa Casa de Misericórdia de São Paulo, São Paulo, SP, Brazil.

## ■ INTRODUCTION

Inflammatory biomarkers, which are produced by immunocompetent cells in atheromatous plaques, can orchestrate both systemic and tissue inflammatory responses, contributing to formation of complex atherosclerotic plaques, which are responsible for manifestation of ischemic neurological events, such as transitory ischemic attacks (TIA) and cerebral vascular accidents (CVA).<sup>1-4</sup>

Elevated expression of inflammatory biomarkers in patients with unstable carotid plaques suggests that immunomodulatory treatments focused on the inflammatory process within the carotid plaque should be tested with a view to reducing progression of the disease and the risk of TIA and CVA.<sup>5-7</sup>

Glucocorticoids have anti-inflammatory and immunosuppressive properties, reducing secretion of inflammatory biomarkers by monocytes and macrophages.<sup>8,9</sup> Elenkov<sup>10</sup> points out that glucocorticoids are immunomodulatory medications capable of modulating the systemic inflammatory response. The objective of this study was to analyze the inflammatory activity of hsCRP, of TNF-alpha and of VEGF in serum and tissue in response to intraoperative administration of hydrocortisone.

## ■ METHODS

### Population

A total of 22 patients were treated with EAC from October of 2012 to September of 2013. Their ages ranged from 50 to 84, with a mean of  $69.50 \pm 9.09$  years. In terms of sex distribution, 68.18% (15) were male and 31.82% (7) were female. With regard to neurological symptoms, 50% (11) were symptomatic and 50% (11) asymptomatic. Contralateral carotid stenosis percentages were as follows: 54.54% (12) of the patients had < 50% stenosis; 36.36% (8) had from 50% to 69% stenosis, and 9.1% (2) had stenosis  $\geq$  70%.

Symptomatic and asymptomatic patients were enrolled on the study if they had carotid stenosis  $\geq$  70% with indications for EAC. Patients were excluded in they exhibited carotid stenosis < 70% or occlusion of this arterial segment; indications for carotid angioplasty; autoimmune or systemic diseases; recent infections (< 1 month); or a recent CVA (< 1 month); hypersensitivity to and/or contraindications to use of hydrocortisone, or were taking glucocorticoids at the time of enrolment.

The study was approved by the Research Ethics Committee (Hearing No. 108.870) and patients signed free and informed consent forms.

### Preoperative analysis

Carotid stenosis was investigated using color Doppler ultrasonography and stenosis  $\geq$  70% was confirmed with angiography of cerebral and supra-aortic trunks, since we do not have access to angiotomography equipment.

A data collection form covering epidemiological data and cardiovascular risk factors was designed for the study and completed for all patients, as shown in Table 1. Systemic arterial hypertension (SAH) was defined as systolic pressure  $\geq$  140 mmHg, diastolic pressure  $\geq$  90 mmHg and/or use of antihypertensive medications. Diabetes mellitus (DM) was defined as fasting glycemia level  $\geq$  126 mg/dL or use of hypoglycemic medications. Smoking was assessed in terms of mean consumption at the time of enrollment. Hypercholesterolemia was defined as total cholesterol > 200 mg/dL or use of hypolipidemic medications. Obesity was defined as body mass index (BMI)  $\geq$  30 Kg/m<sup>2</sup>.

Patients were defined as symptomatic if they had suffered a TIA or CVA less than six months prior to EAC.<sup>11</sup>

Patients who were to undergo EAC were allocated to one of two groups: either a Control Group (n=11, 5 asymptomatic and 6 symptomatic patients), containing patients who would not be administered hydrocortisone, or Group 1 (n=11, 4 asymptomatic and 7 symptomatic patients) comprising patients who would be given 500 mg of intravenous hydrocortisone during induction of anesthesia. Randomization of patients was achieved by randomly distributing 22 sequential numbers into 22 envelopes numbered from 1 to 22. Patients who were drawn even numbers were allocated to the control group and patients drawn uneven numbers were allocated to Group 1. The envelopes were opened in numerical order before induction of anesthesia.

As can be observed from the data in Table 1, both groups studied were predominantly made up of male patients, with a high incidence of hypertense and diabetic patients, a low percentage of obese individuals and a majority of patients with <50% contralateral carotid stenosis. There were no statistical differences between patients in the control group and patients in group 1 in terms of epidemiological or clinical characteristics, cardiovascular risk factors, neurological symptomology or duration of carotid clamping.

### Endarterectomy of the carotid artery

All patients were treated with classic EAC, under general anesthesia. After administration of 5,000 units of intravenous heparin, the internal, external and common carotid arteries were clamped. After

**Table 1.** Clinical and laboratory characteristics of the patients.

Variables	Control group (11)	Group 1 (11)	p
Age (years)	69.09 ± 8.30	69.91 ± 10.20	0.718*
Sex	Male	72.70%	0.647 <sup>§</sup>
	Female	27.30%	
Systemic arterial hypertension	Yes	90.90%	0.306 <sup>§</sup>
	No	9.10%	
Diabetes mellitus	Yes	54.50%	0.170 <sup>§</sup>
	No	45.50%	
Smoking	Yes	36.40%	0.201 <sup>§</sup>
	No	63.60%	
Obesity	Yes	9.10%	0.269 <sup>§</sup>
	No	90.90%	
BMI (kg/m <sup>2</sup> )	26.64 ± 3.61	26.26 ± 5.14	0.669*
Total cholesterol (mg/dL)	189.45 ± 22.39	160.09 ± 33.43	0.023*
HDL (mg/dL)	47.55 ± 11.26	41.18 ± 13.56	0.200*
LDL (mg/dL)	110.64 ± 27.23	92.91 ± 27.53	0.212*
Triglycerides (mg/dL)	156.09 ± 50.08	156.91 ± 112.12	0.511*
Fasting glycemia (mg/dL)	129.36 ± 53.82	110.27 ± 32.23	0.869*
Duration of carotid clamping (minutes)	44.18 ± 7.22	48.36 ± 12.44	0.430*
Contralateral carotid stenosis	< 50%	54.50%	0.287 <sup>§</sup>
	50% a 69%	45.50%	
	≥ 70%	0%	
Neurological symptoms	Symptomatic	54.50%	0.670 <sup>§</sup>
	Asymptomatic	45.50%	
Coronary disease	Yes	36.40%	0.201 <sup>§</sup>
	No	63.60%	
Myocardial revascularization	Yes	36.40%	0.201 <sup>§</sup>
	No	63.60%	

Data expressed as mean ± standard deviation or percentage. BMI = body mass index; HDL = high density lipoprotein; LDL = low density lipoprotein. \*Mann-Whitney test. <sup>§</sup> Likelihood ratio test.

removal of the atheromatous plaques, primary arteriorrhaphy was conducted. Vascular shunt was not used. The mean duration of carotid clamping was 46.27 ± 10.16 minutes. None of the patients exhibited neurological ischemic events after EAC.

### Administration of the intravenous hydrocortisone

A 500 mg dose of hydrocortisone sodium succinate (SOLU – CORTEF), in the form of lyophilized powder for injectable solutions, was diluted in 4 mL of distilled water for injections and then added to 500 mL of 0.9% saline solution. The resulting solution at a concentration of 1 mg/mL was injected into a peripheral vein over an infusion time of 30 minutes.

Its effects are evident 1 hour after administration, it binds to a range of proteins and has a half-life in plasma of 1.5 to 2 hours and a half-life in tissues of 8 to 12 hours.

### Determination of serum hsCRP, TNF-alpha and VEGF

Blood samples were taken preoperatively (24 hours before EAC) and 1 hour, 6 hours and 24 hours after the carotids were unclamped. Blood samples were collected into three tubes. One of these contained gel and clot activator, for the hsCRP assay, and the other two had walls coated with EDTA anticoagulant, for the TNF-alpha and VEGF assays.

Samples with EDTA were centrifuged at 1,000 rotations per minute (rpm) for 15 minutes and then at 10,000 rpm for 10 minutes. Samples drawn into tubes containing gel were centrifuged at 4,500 rpm for 20 minutes. After centrifugation, the supernatants were aliquoted into Eppendorf tubes (300 to 500 µL) and stored in an industrial freezer at -70 °C. The reference value was hsCRP < 5mg/L. The detection limits were: TNF-alpha < 0.2 pg/mL and VEGF < 0.04 pg/mL.

### Determination of TNF-alpha and VEGF in the carotid plaques

After removal, the endarterectomy specimens were frozen in an industrial freezer at -70 °C. The biomarkers were determined after tissue lysis using a Cell lysis kit (Biorad) using the LUMINEX Methodology.

### Statistical analysis

The Statistical Package for Social Sciences, version 21.0, was used to calculate the results. Values were expressed as means ± standard deviations and percentages. The significance level was set at  $p < 0.05$ .

For clinical and epidemiological characteristics, parametric variables were evaluated using the Mann-Whitney test and nonparametric variables with the likelihood ratio test. The Mann-Whitney test was used to analyze serum and tissue biomarker results.

## RESULTS

### Comparison of serum inflammatory activity of hsCRP, of TNF-alpha and of VEGF for the control group versus group 1

At 1 hour and 6 hours after EAC, serum concentrations of hsCRP were higher in group 1 than in the control group. In both groups, peak hsCRP inflammatory activity occurred at 24 hours after EAC (Figure 1). There were no statistically significant differences between the groups in terms of hsCRP levels.

The inflammatory profiles of TNF-alpha concentrations were similar for the two groups, with a reduction in levels 1 hour after EAC and an increase at 6 hours and 24 hours after EAC. However, group 1 exhibited a lower concentration of TNF-alpha than the control group at all postoperative analysis times (Figure 2). The differences in TNF-alpha levels were statistically significant at 1 hour ( $p=0.031$ ), 6 hours ( $p=0.015$ ) and 24 hours ( $p=0.017$ ) after EAC.

Concentrations of VEGF exhibited similar inflammatory responses in both groups, with a reduction in concentrations 1 hour after EAC and increases at 6 hours and 24 hours after EAC. However, group 1 exhibited a lower concentration of VEGF than the control group at all postoperative analysis times (Figure 3). The differences in VEGF levels were statistically significant at 1 hour ( $p=0.006$ ) and at 6 hours ( $p=0.005$ ), after EAC.

Table 2 shows the comparison of serum inflammatory activity of hsCRP, of TNF-alpha and of VEGF, for control group against group 1, at the four observation times.

### Comparison of serum inflammatory activity of hsCRP, of TNF-alpha and of VEGF between symptomatic patients in control group and symptomatic patients in group 1

The symptomatic patients in the control group ( $n=6$ ) exhibited lower concentrations of hsCRP 1 hour after EAC, while at 6 hours and 24 hours after EAC,

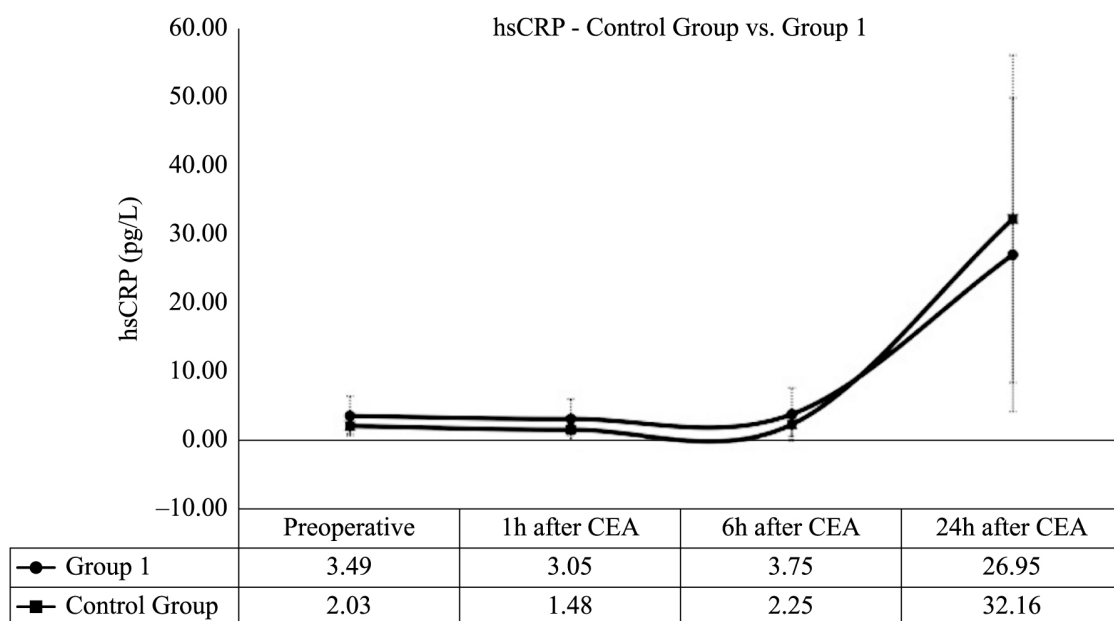


Figure 1. Serum inflammatory activity of hsCRP for control group and group 1.

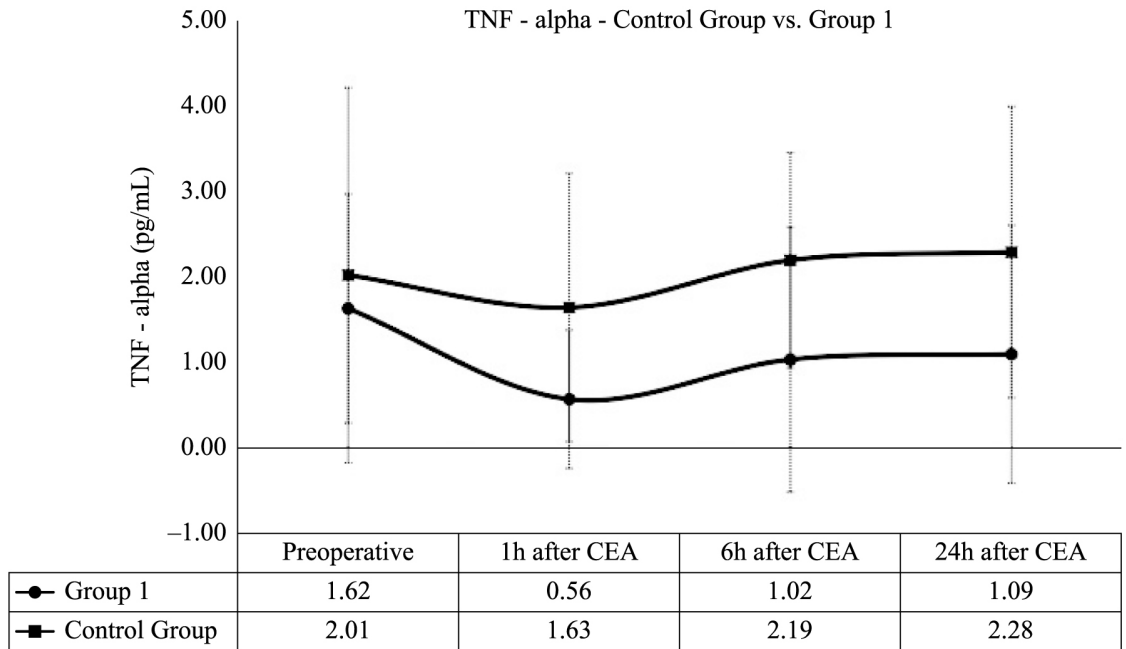


Figure 2. Serum inflammatory activity of TNF-alpha for control group and group 1.

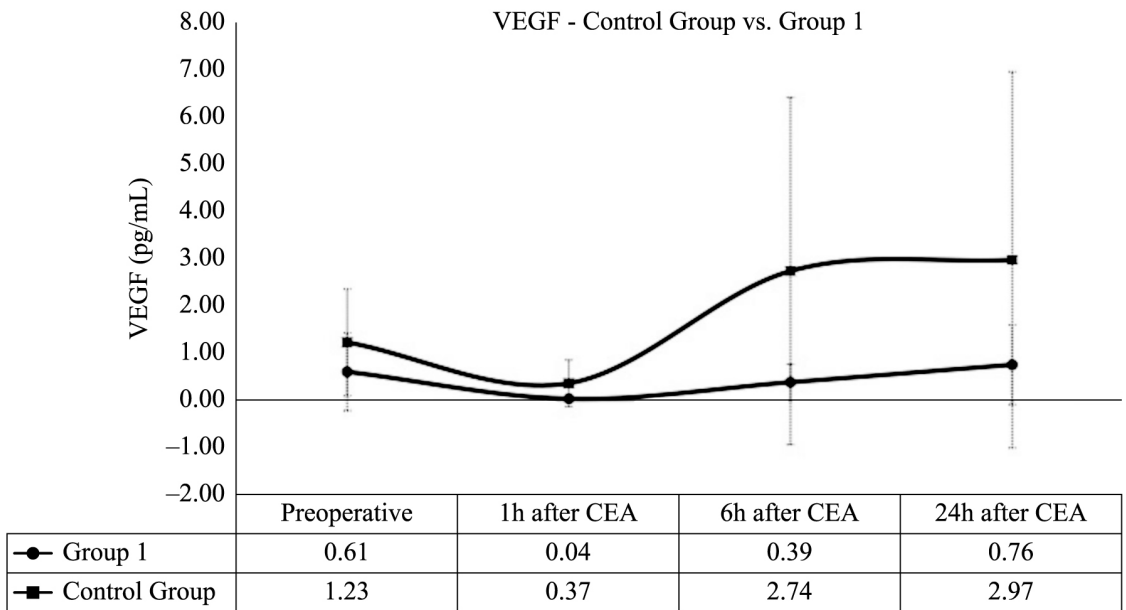


Figure 3. Serum inflammatory activity of VEGF for control group and group 1.

serum hsCRP concentrations were lower in group 1. The differences between these subsets' hsCRP levels were not statistically significant.

The TNF-alpha inflammatory activity in group 1 was lower than in the control group at all observation times. Differences in TNF-alpha levels attained statistical significance at 1 hour ( $p=0.011$ ) and 6 hours ( $p=0.042$ ) after EAC.

Symptomatic patients in group 1 exhibited lower concentrations of VEGF than symptomatic patients in control group at all postoperative analysis times. Only the differences in VEGF levels 1 hour after EAC attained statistical significance ( $p=0.011$ ).

Table 3 lists the comparisons of serum inflammatory activity results for hsCRP, for TNF-alpha and for VEGF, for the symptomatic patients in the control

group versus the symptomatic patients in group 1, at the four observation times.

### Comparison of serum inflammatory activity of hsCRP, of TNF-alpha and of VEGF between asymptomatic patients in the control group and asymptomatic patients in group 1

The asymptomatic patients in group 1 (n=4) exhibited higher hsCRP concentrations than the asymptomatic patients in the control group at all observation times. The differences between these subsets' hsCRP levels were not statistically significant.

Inflammatory activity of TNF-alpha was lower in group 1 than in the control group at all postoperative observation times. There were no statistically significant differences between the TNF-alpha levels of these subsets.

The asymptomatic patients in group 1 exhibited lower concentrations of VEGF at all observation times

than the asymptomatic patients in the control group. The differences between VEGF levels in these subsets were statistically significant at 6 hours (p=0.025) and 24 hours (p=0.011) after EAC.

Table 4 lists the comparisons of serum inflammatory activity results for hsCRP, for TNF-alpha and for VEGF, for the asymptomatic patients in the control group versus the asymptomatic patients in group 1, at the four observation times.

### Comparison of the inflammatory activity of TNF-alpha and of VEGF in tissue specimens from symptomatic and asymptomatic patients in the control group and in group 1

Symptomatic patients exhibited higher tissue concentrations of VEGF, whereas the asymptomatic patients exhibited higher tissue levels of TNF-alpha. There were no statistically significant differences

**Table 2.** Serum inflammatory activity of hsCRP, of TNF-alpha and of VEGF, for control group and group 1, at four different observation times.

		Control group (11)	Group 1 (11)	Total (22)	p
hsCRP (mg/L)	Preoperative	2.03 ± 1.14	3.49 ± 2.89	2.76 ± 2.27	0.189
	1h after EAC	1.48 ± 0.86	3.05 ± 2.88	2.27 ± 2.23	0.177
	6h after EAC	2.25 ± 1.72	3.75 ± 3.84	3.00 ± 3.00	0.576
	24h after EAC	32.16 ± 23.82	26.95 ± 22.84	29.56 ± 22.93	0.450
TNF-ALPHA (pg/mL)	Preoperative	2.01 ± 2.20	1.62 ± 1.34	1.82 ± 1.79	0.869
	1h after EAC	1.63 ± 1.57	0.56 ± 0.81	1.10 ± 1.34	0.031
	6h after EAC	2.19 ± 1.26	1.02 ± 1.55	1.61 ± 1.50	0.015
	24h after EAC	2.28 ± 1.70	1.09 ± 1.51	1.68 ± 1.69	0.017
VEGF (pg/mL)	Preoperative	1.23 ± 1.13	0.61 ± 0.82	0.92 ± 1.01	0.070
	1h after EAC	0.37 ± 0.49	0.04 ± 0.00	0.20 ± 0.38	0.006
	6h after EAC	2.74 ± 3.67	0.39 ± 0.38	1.56 ± 2.81	0.005
	24h after EAC	2.97 ± 3.97	0.76 ± 0.84	1.87 ± 3.02	0.069

**Table 3.** Serum inflammatory activity of hsCRP, of TNF-alpha and of VEGF, for symptomatic patients in the control group and in group 1, at four different observation times.

		Control group (6)	Group 1 (7)	Total (13)	p
hsCRP (mg/L)	Preoperative	2.38 ± 1.26	3.80 ± 3.31	3.15 ± 2.59	0.391
	1h after EAC	1.75 ± 0.96	2.83 ± 2.44	2.33 ± 1.91	0.390
	6h after EAC	2.68 ± 2.08	2.57 ± 2.04	2.62 ± 1.97	0.886
	24h after EAC	38.85 ± 24.66	25.77 ± 19.76	31.81 ± 22.25	0.253
TNF-ALPHA (pg/mL)	Preoperative	2.37 ± 2.60	1.62 ± 1.46	1.97 ± 2.01	0.774
	1h after EAC	2.47 ± 1.68	0.52 ± 0.86	1.42 ± 1.60	0.011
	6h after EAC	3.09 ± 0.47	1.36 ± 1.88	2.16 ± 1.63	0.042
	24h after EAC	3.19 ± 1.60	1.44 ± 1.80	2.25 ± 1.88	0.072
VEGF (pg/mL)	Preoperative	0.88 ± 0.65	0.65 ± 0.87	0.75 ± 0.76	0.283
	1h after EAC	0.22 ± 0.42	0.04 ± 0.00	0.12 ± 0.29	0.042
	6h after EAC	3.43 ± 4.92	0.51 ± 0.40	1.86 ± 3.53	0.062
	24h after EAC	1.20 ± 1.05	1.17 ± 0.80	1.18 ± 0.88	0.886

**Table 4.** Serum inflammatory activity of hsCRP, of TNF-alpha and of VEGF, for asymptomatic patients in the control group and in group 1, at four different observation times.

		Control group (5)	Group 1 (4)	Total (9)	p
hsCRP (mg/L)	Preoperative	1.60 ± 0.90	2.95 ± 2.31	2.20 ± 1.71	0.462
	1h after EAC	1.16 ± 0.69	3.45 ± 3.94	2.18 ± 2.74	0.387
	6h after EAC	1.74 ± 1.16	5.80 ± 5.67	3.54 ± 4.16	0.461
	24h after EAC	24.14 ± 22.59	29.03 ± 30.80	26.31 ± 24.85	0.462
TNF-ALPHA (pg/mL)	Preoperative	1.58 ± 1.78	1.63 ± 1.32	1.60 ± 1.49	0.902
	1h after EAC	0.64 ± 0.61	0.62 ± 0.84	0.63 ± 0.67	0.884
	6h after EAC	1.10 ± 1.01	0.43 ± 0.46	0.80 ± 0.84	0.096
	24h after EAC	1.20 ± 1.17	0.47 ± 0.54	0.87 ± 0.97	0.081
VEGF (pg/mL)	Preoperative	1.66 ± 1.49	0.54 ± 0.85	1.16 ± 1.32	0.140
	1h after EAC	0.55 ± 0.56	0.04 ± 0.00	0.32 ± 0.48	0.081
	6h after EAC	1.91 ± 1.35	0.17 ± 0.27	1.14 ± 1.33	0.025
	24h after EAC	5.11 ± 5.26	0.04 ± 0.00	2.86 ± 4.58	0.011

**Table 5.** Tissue inflammatory activity of TNF-alpha and of VEGF, for patients in the control group and in group 1.

		Control group (11)	Group 1 (11)	Total (22)	p
TNF-ALPHA (pg/mL)	Symptomatic	1.29 ± 0.33	1.16 ± 0.52	1.22 ± 0.43	0.550
	Asymptomatic	1.40 ± 0.46	1.16 ± 0.56	1.30 ± 0.49	0.521
VEGF (pg/mL)	Symptomatic	11.02 ± 17.85	5.33 ± 5.76	7.96 ± 12.57	0.886
	Asymptomatic	8.15 ± 13.13	1.78 ± 0.99	5.32 ± 9.89	0.327

in tissue concentrations of TNF-alpha or of VEGF between symptomatic and asymptomatic patients.

The asymptomatic and symptomatic patients in group 1 exhibited lower tissue concentrations of TNF-alpha and of VEGF than those in the control group. There were no statistically significant differences in tissue concentrations of TNF-alpha or of VEGF between patients in the control group and patients in group 1.

Table 5 lists the results for tissue inflammatory activity of TNF-alpha and of VEGF for patients in the control group and in group 1.

## DISCUSSION

This study has demonstrated for the first time that intraoperative administration of hydrocortisone interferes in the inflammatory responses of TNF-alpha and VEGF, reducing their concentrations over a period of 24 hours after EAC. Additionally, we also observed a tendency for hydrocortisone to have an effect not only at the serum level, but also at the tissue level, controlling inflammatory activity of TNF-alpha and of VEGF in the carotid plaque.

Hermus et al.<sup>7</sup> state that cellular inflammatory activity, expressed by serum and tissue production of inflammatory biomarkers such as interleukins, metalloproteinases, hsCRP, TNF-alpha and VEGF, contributes to progression and destabilization of the carotid plaque, resulting in TIA and CVA.<sup>7</sup> Additionally, the magnitude of the systemic and tissue inflammatory

process, assessed by measurement of these specific biomarkers may reflect the risk of postoperative complications and progression of the contralateral carotid disease.<sup>7</sup>

Raja et al.<sup>12</sup> claim that the systemic inflammatory response after cardiovascular surgery is due to ischemia-reperfusion injury to target organs caused by clamping of arteries and restoration of perfusion after release of the hemostatic forceps. Additionally, persistence of an elevated degree of postoperative inflammation can potentially be prejudicial to patients subjected to cardiovascular surgical interventions.<sup>12</sup> However, Rubens et al.<sup>13</sup> and Liguori et al.<sup>14</sup> have concluded that the systemic inflammatory response is variable and can be influenced by patient comorbidities; intraoperative non-pharmacological interventions; type of anesthesia; perioperative hemodynamic conditions; surgical aspects (surgical incision, duration of the procedure, duration of arterial clamping and need for blood transfusion); and postoperative recovery. In order to ensure that the same perioperative conditions prevailed, we standardized general anesthesia and classical endarterectomy. The serum hsCRP, TNF-alpha and VEGF assays were performed on samples taken after removal of carotid clamping, so that the results would reflect the maximum production of these biomarkers during carotid clamping. In contrast, the hydrocortisone was administered during induction of anesthesia, in order to assess its immunomodulatory

effect, which has a short half-life (1.5 to 2 hours), not only on the postoperative inflammatory activity of serum hsCRP, TNF-alpha and VEGF, but also on TNF-alpha and VEGF in the carotid plaque.

Previous publications have already demonstrated that glucocorticoids can interfere in the systemic inflammatory response, reducing concentrations of serum biomarkers and attenuating activation of the complement system and leukocyte and neutrophil adhesion,<sup>13,14</sup> but this effect has not been demonstrated with respect to hsCRP, TNF-alpha or VEGF, and the action of glucocorticoids on the behavior of biomarkers present in patients treated with EAC has not been assessed. After administering 30 mg/kg of intravenous methylprednisolone before surgery and before unclamping the thoracic aorta in 16 patients treated with revascularization of the myocardium, Kawamura et al.<sup>15</sup> observed reductions in plasma concentrations of interleukins IL-6 and IL-8 at 1 hour, 2 hours and 3 hours after the aorta was unclamped. Komori et al.<sup>16</sup> administered 1g of intravenous methylprednisolone to ten patients 2 hours before conventional surgical repair of infrarenal abdominal aortic aneurysms and detected lower concentrations of IL-6 after unclamping the abdominal aorta and on the first day after the operation and lower CRP levels on the first day after the operation, compared with patients who were not given the preoperative dose of methylprednisolone.

In our study, the symptomatic patients exhibited higher serum concentrations of hsCRP, TNF-alpha and VEGF than the asymptomatic patients for the preoperative measurement and the postoperative measurements, with the exception of the hsCRP concentration at 6 hours after EAC and the VEGF concentration at 24 hours after EAC. Koutouzis et al.<sup>17</sup> also observed higher preoperative concentrations of hsCRP and TNF-alpha in symptomatic patients; but the preoperative levels of hsCRP and TNF-alpha were lower in their symptomatic and asymptomatic patients than in our patients. In contrast, Puz et al.<sup>18</sup> reported higher preoperative TNF-alpha levels in both symptomatic patients (14.58 pg/mL) and in asymptomatic patients (13.93 pg/mL), when compared with our patients. We believe that these differences were the result of the characteristics of the patients analyzed, the methods used to assay these biomarkers, the methods for classification of patients according to presence or absence of prior neurological symptoms and to the interval of time that elapsed between symptoms and measurement of the biomarkers.

Of the biomarkers analyzed, hsCRP exhibited the greatest postoperative inflammatory activity, and the

maximum concentration was observed 24 hours after EAC. Alvarez Garcia et al.<sup>19</sup> identified an association between elevated hsCRP levels and a history of neurological events and presence of unstable carotid plaques, but did not demonstrate differences between the levels of this biomarker and the severity of the neurological ischemic event. Heider et al.<sup>20</sup> observed ischemic brain injuries in 22.4% of their patients after EAC, concluding that elevated preoperative levels of hsCRP are associated with a greater risk of cerebral ischemic injuries after EAC. In our study, serum hsCRP levels exhibited a discrete change in inflammatory behavior in response to administration of hydrocortisone, but significant reductions in their postoperative concentrations were not observed in symptomatic or asymptomatic patients. Morrow et al.<sup>21</sup> have previously concluded that serum hsCRP levels do not change in response to hormone changes or anti-inflammatory drugs. Possible explanations for the mild effect exerted by the hydrocortisone on serum hsCRP levels in our study are founded on the short half-life (1.5 to 2 hours) of hydrocortisone, its administration during induction of anesthesia and the long half-life of hsCRP (18 hours). Strategies that could be tested in future studies with the aim of reducing the inflammatory response of hsCRP include increasing the dose of hydrocortisone, using a corticoid with a longer half-life or administering the hydrocortisone 24 hours after EAC.

In contrast with hsCRP, we observed a considerable reduction in the postoperative concentrations of TNF-alpha, at all observation times, in response to administration of hydrocortisone. The lower inflammatory activity of TNF-alpha was still identifiable when symptomatic patients were analyzed separately, with a reduction in concentrations at 1 hour and 6 hours after EAC, in response to administration of hydrocortisone. TNF-alpha stimulates thickening of the vascular endothelium, provoking deposition of collagen by fibroblasts and contributes to hypotension, coagulopathy and renal dysfunction.<sup>22</sup> Welsh et al.<sup>23</sup> observed a correlation between TNF-alpha levels and the risk of recurrent CVA. Additionally, Profumo et al.<sup>24</sup> state that maintenance of elevated TNF-alpha levels after EAC is associated with progression of contralateral carotid disease.

A considerable reduction in the inflammatory activity of VEGF was also observed among the patients given the intraoperative dose of hydrocortisone. We identified reduced serum expression of VEGF 1 hour after EAC in symptomatic patients and at 6 hours and 24 hours after EAC in asymptomatic patients. VEGF is associated with histological vulnerability of carotid



plaques through the process of chemoattraction of monocytes and other inflammatory cells, since it induces expression of metalloproteinases and cellular adhesion molecules, promotes neoangiogenesis and stimulates expression and synthesis of thromboplastin.<sup>25</sup> Szabó et al.<sup>26</sup> assessed 82 patients treated with eversion EAC, observing that carotid restenosis occurred in patients who exhibited elevated serum VEGF levels four days after the operation.

We did not observe statistical differences between symptomatic patients and asymptomatic patients in terms of concentrations of TNF-alpha or VEGF in carotid plaques. Grufman et al.<sup>27</sup> also failed to detect statistical differences between tissue concentrations of TNF-alpha between symptomatic patients and asymptomatic patients. However, administration of the intraoperative dose of hydrocortisone did exhibit a tendency to reduce the concentrations of TNF-alpha and VEGF in the carotid plaque, suggesting an attempt to control the inflammatory activity in the carotid plaque.

This study suffers from certain limitations. While the hydrocortisone interfered in the inflammatory activity of the serum biomarkers, our results do not provide prognostic information about the patients in terms of progression of contralateral carotid disease and or carotid restenosis. Our results do however provide a basis for conducting further studies to assess the effects of intraoperative administration of hydrocortisone on the prognosis of patients treated with EAC.

In conclusion, the immunomodulatory effect of hydrocortisone reduces postoperative serum concentrations of TNF-alpha and VEGF, particularly in symptomatic patients. Intraoperative administration of hydrocortisone exhibited a tendency to reduce tissue concentrations of TNF-alpha and VEGF.

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