

Selective Cyclooxygenase-2 Inhibition Reduces Endothelial Dysfunction and Improves Inflammatory Status in Patients With Intermittent Claudication

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Introduction and objectives. Both endothelial dysfunction and a proinflammatory state are present during the early stages of atherosclerosis. In this context, increased expression of cyclooxygenase-2 (COX-2) results in higher levels of vasoconstrictive and proinflammatory substances. The aim of this study was to investigate the influence of COX-2 activity on endothelial dysfunction associated with peripheral arterial disease (PAD).

Methods. Brachial artery flow-mediated dilatation (BAFMD), endothelin and high-sensitivity C-reactive protein (hsCRP) levels, and the lipid profile were assessed in 40 patients with intermittent claudication. Of these, 20 were randomly assigned to a group in which they received the selective COX-2 inhibitor celecoxib for 1 week (Group 1), while the other 20 served as controls (Group 2).

Results. In Group 1, BAFMD increased significantly both 3 hours after the first dose of celecoxib (3.33% [4.11%] vs 6.97% [3.27%]; $P=0.008$) and 1 week after (3.33 [4.11] vs 7.09% [4.40%]; $P=0.001$). The endothelin level decreased significantly in Group 1 (2.92 [1.87] vs 1.93 [1.07] pg/mL; $P=0.018$), as did the levels of hsCRP (4.78 [2.73] vs 2.95 [2.11] mg/L; $P=0.023$) and low-density lipoprotein cholesterol (106.38 [18.89] vs 90.8 [28.58] mg/dL; $P=0.019$). In Group 2, none of these parameters changed significantly.

Conclusions. COX-2 products contribute to endothelial dysfunction and an inflammatory state in PAD. This study's findings provide evidence that these phenomena are implicated in the initiation of atherosclerosis and could prove a new means of investigating alternative approaches to the treatment of early-stage disease.

Key words: Atherosclerosis. Endothelium. Endothelin. Inflammation. Peripheral arterial disease.

La inhibición selectiva de la ciclooxigenasa-2 mejora la disfunción endotelial y disminuye el estado inflamatorio en pacientes claudicantes

Introducción y objetivos. En el origen de la arteriosclerosis, actúan la disfunción endotelial y un estado proinflamatorio. En esta situación, una expresión aumentada de ciclooxigenasa-2 (COX-2) produce un incremento de sustancias vasoconstrictoras y proinflamatorias. El objetivo de este estudio es conocer la contribución de la actividad de la COX-2 en la disfunción endotelial existente en la enfermedad arterial periférica (EAP).

Métodos. Estudiamos la dilatación de la arteria braquial mediada por flujo (DABMF), las concentraciones de endotelina, proteína C reactiva ultrasensible (PCRus) y el perfil lipídico de 40 pacientes claudicantes. Asignamos aleatoriamente a 20 pacientes a un grupo en el que se administró el inhibidor selectivo de la COX-2 celecoxib durante 1 semana (grupo 1), y otros 20 actuaron como controles (grupo 2).

Resultados. En el grupo 1, la DABMF aumentó significativamente 3 h después de la primera dosis de celecoxib (3,33% \pm 4,11% frente a 6,97% \pm 3,27%; $p=0,008$) y tras 1 semana (3,33% \pm 4,11 frente a 7,09% \pm 4,4%; $p=0,001$). Las concentraciones de endotelina disminuyeron significativamente en el grupo 1 (2,92 \pm 1,87 frente a 1,93 \pm 1,07 pg/ml; $p=0,018$), así como los de PCRus (4,78 \pm 2,73 frente a 2,95 \pm 2,11 mg/l; $p=0,023$) y colesterol de las lipoproteínas de baja densidad (106,38 \pm 18,89 frente a 90,8 \pm 28,58 mg/dl; $p=0,019$). Ninguno de estos parámetros cambió significativamente en el grupo 2.

Conclusiones. Los productos de la COX-2 contribuyen a la disfunción endotelial y el estado inflamatorio de la EAP. Los resultados de este estudio confirman la implicación de estos fenómenos en el origen de la arteriosclerosis. Esto puede suponer una nueva vía de estudio de posibles alternativas terapéuticas para los estadios incipientes de la enfermedad.

Palabras clave: Arteriosclerosis. Endotelio. Endotelina. Inflamación. Enfermedad vascular periférica.

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Received July 22, 2008.
Accepted for publication March 25, 2009.

INTRODUCTION

Atherosclerosis is a systemic inflammatory process involving a dysfunction of the endothelial cells of

ABBREVIATIONS

BAFMD: brachial artery flow-mediated dilation
COX-2: cyclooxygenase-2
hsCRP: high-sensitivity C-reactive protein
TG: triglycerides

blood vessel walls.¹ Prostaglandins are a family of lipidic mediators with a key role in inflammation.² Cyclooxygenase (COX) or prostaglandin H synthase is the enzyme that catalyzes the first step in the transformation of arachidonic acid into the different types of prostaglandin.³ This enzyme exists as 2 isoforms, COX-1 or constitutive COX, which is expressed constitutively in the majority of tissues, and COX-2 or inducible COX, which is expressed in response to different proinflammatory stimuli.^{4,5}

The presence of COX-2 has been demonstrated in atheromatous but not in healthy arteries, indicating it to have a role in the formation or maintenance of atheromatous plaques.⁶

A number anti-inflammatory drugs with different gastric and cardiovascular profiles selectively inhibit COX-2.⁷⁻¹⁰ The selective COX-2 inhibitor celecoxib has been shown to reduce atherosclerotic lesions,¹¹ and in patients with high blood pressure and ischemic cardiomyopathy it improves endothelial function and reduces the systemic inflammatory response.^{12,13}

The aim of the present work was to assess the action of an anti-inflammatory agent with no antiaggregant activity, the selective COX-2 inhibitor celecoxib, on endothelial function (measured in terms of brachial artery flow-mediated dilation [BAFMD] and the concentration of endothelin) and inflammation (estimated by the concentration of high-sensitivity C-reactive protein [hsCRP]) in patients with peripheral artery disease (PAD).

METHODS

This prospective study involved a group of 40 patients with chronic ischemia of the legs (grades IIA and IIB on the Fontaine scale) recruited at the outpatient angiology and vascular surgery consultation clinics of the Hospital Universitario de Getafe (Madrid, Spain).

To avoid bias, patients with poorly controlled blood pressure were excluded, as were those with insulin-dependent diabetes, advanced kidney failure, heart failure, and those receiving chronic treatment

with nitrates or anti-inflammatory drugs other than acetylsalicylic acid. Indeed, all the selected patients were being treated with acetylsalicylic acid.

The study was approved by the Ethics Committee of the Hospital Universitario de Getafe. All patients gave their signed, informed consent to be included in the study.

During their first consultation, all patients were subjected to detailed anamnesis, the determination of the ankle/arm index, and a blood analysis to determine their hsCRP concentration and lipid profiles. A sample of plasma was also retained from each patient for later endothelin determination. The diameter of the brachial artery was also measured by BAFMD. The patients were then randomly assigned to either a control or treatment group (n=20 for both) using a table of random numbers. Those in the treatment group received celecoxib 200 mg/12 h for 1 week (the dose described in earlier literature^{12,13}; the described protocol and follow-up procedure to observe changes in endothelial function due to celecoxib were also followed). The control group received no treatment.

Three hours after randomization to the different groups, BAFMD testing was performed again, as it was at 1 week; the analyses (hsCRP, lipid profile, and endothelin) performed at the first visit were also repeated at this time.

For BAFMD assessments, echo-Doppler visualizations of the brachial artery were made over a longitudinal section above the elbow skin fold. Three measurements of the brachial artery diameter were made between the intima and media interfaces. A cuff was then inflated to provide a pressure of 250 mm Hg for 5 min; 3 further measurements of the diameter were made 70 s after the release of this pressure. The BAFMD value was calculated using the following formula: the mean post-ischemic diameter minus the mean baseline diameter, divided by the mean baseline diameter. Results were expressed as percentages.¹⁴ All BAFMD and analytical determinations were made blind; the operator did not know which patients belonged to the experimental and control groups.

hsCRP values were determined by automated high sensitivity immunoanalysis¹⁵ (Roche Diagnostics); the lower detection limit was 0.2 mg/L and the coefficient of variation 4.2% at 4 mg/L and 6.3% at 1 mg/L.

Total cholesterol, high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured by molecular absorption spectrometry (Roche Diagnostics). Low density lipoprotein cholesterol (LDL-C) was estimated using the following formula: LDL-C = total cholesterol – HDL-C – TG/5 (where TG/5 is very low density lipoprotein cholesterol [VLDL-C]).

TABLE 1. Baseline Characteristic of the Control and Treatment Groups

	Treatment Group (n=20)	Control Group (n=20)	P
Women, n	2	2	1
Men, n	18	18	1
High blood pressure, n	14	16	.46
Dyslipidemia, n	10	11	.75
Smokers, n	11	8	.62
Ischemic cardiomyopathy, n	2	5	.21
COPD, n	1	2	.54
ACVA/TIA, n	2	3	.63
Grade IIA, n	14	17	.25
Grade IIB, n	6	3	.25
Age, mean (SD), y	64.5 (9.33)	61.2 (11.41)	.25
BAFMD, %	3.33 (4.11)	5.19 (2.68)	.10
LDL-C, mg/dL	106.38 (18.89)	114.75 (36.18)	.36
hsCRP, mg/L	4.78 (2.73)	3.31 (2.51)	.11
Endothelin, pg/mL	2.92 (1.78)	2.70 (1.72)	.69
ABI	0.61 (0.14)	0.65 (0.11)	.25

ABI, ankle/brachial index; ACVA/TIA, acute cerebrovascular accident/transitory ischemic accident; BAFMD, brachial artery flow-mediated dilation; COPD, chronic obstructive pulmonary disease; hsCRP, high-sensitivity C-reactive protein; LDL-C, low density lipoprotein cholesterol.

Endothelin levels were determined using samples of venous blood which were stored on ice immediately after their extraction and centrifuged on the same day. The plasma obtained was stored at -20°C until analysis by ELISA (Endothelin Assay Kit IBL) employing high specificity antibodies. The lower detection limit of the test was 0.36 pg/mL; the coefficient of variation was 3.1% at 27.15 pg/mL and 7.1% at 3.08 pg/mL.

Statistical Analysis

The required sample size was estimated using data from earlier pilot studies,^{12,13} in which the distribution and dispersion of the percentage change in the BAFMD values were superimposable, to provide a minimum statistical power of 80% to detect a change of 0.1 mm and a 2% change in the BAFMD value, for an α value of .05.

The normality of the variables was assessed using the Kolmogorov-Smirnov test. The means of parametric variables were compared using the Student *t* test for paired or independent samples as required. The Mann-Whitney *U* test was used to compare non-parametric variables. Dichotomous variables were compared using the χ^2 test. All calculations were performed using SPSS® v.15.0 software. Data were expressed as means (standard deviation [SD]). A *P* value less than .05 was considered significant (2 tailed analysis).

RESULTS

There were no patients lost to follow-up and therapeutic compliance was 100%. The only adverse effects recorded were mild gastrointestinal problems in 2 patients.

A comparison of the characteristics of the 2 patient groups showed them to be homogeneous in terms of cardiovascular risk, the degree of chronic ischemia, and the values of baseline study variables. In the main, the patients were in the first stages of PAD (Table).

The BAFMD value for the patients of the treatment group increased significantly by 3 h after the first dose of celecoxib (3.33% [4.11%] vs 6.97% [3.27%]; *P*=.008); this increase was maintained 1 week after starting treatment (3.33% [4.11%] vs 7.09% [4.4%]; *P*=.001) (Figure 1). The members of the treatment group also showed a significant reduction in the hsCRP value at 1 week (4.78 [2.73] vs 2.95 [2.11]; *P*=.023), as well as a reduction in plasma endothelin (PE) (2.92 [1.78] vs 1.93 [1.07]; *P*=.018) and LDL-C (106.38 [18.89] vs 90.8 [28.58]; *P*=.019). In the control group, no significant differences were seen between the values of these variables recorded at the initial visit and 1 week into the study (BAFMD, 5.19 [2.68] vs 3.81 [3.25], *P*=.069; hsCRP, 3.31 [2.51] vs 4.38 [3.28], *P*=.14; PE, 2.7 [1.72] vs 2.73 [1.93], *P*=.65; LDL-C, 114.75 [36.18] vs 120.83 [35.54], *P*=.26) (Figures 2-5).

Inter-group comparisons of the change in the values of the measured variables between the beginning

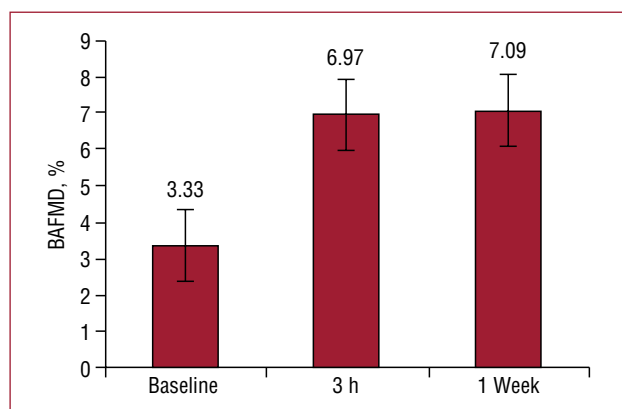


Figure 1. Comparison between baseline brachial artery flow-mediated dilation (BAFMD) values and those at 3 h and 1 week after celecoxib administration (treatment group) ($P=.008$ between baseline and 3h, $P=.001$ between baseline and 1 week).

and end of the study (ie, post-pre BAFMD, pre-post hsCRP, pre-post PE, and pre-post LDL-C) were also made. Compared to the controls, a significant increase was recorded in the treatment group for BAFMD (3.76 [4.25] vs -1.40 [2.96]; $P=.001$), and significant reductions were seen in hsCRP (1.83 [2.42] vs -1.07 [2.89]; $P=.002$), PE (0.99 [1.47] vs -0.03 [1.82]; $P=.010$) and LDL-C (15.58 [23.73] vs -6.1 [35.86]; $P=.011$) (Figures 6-9).

DISCUSSION

Earlier studies have shown the involvement of inflammation and endothelial dysfunction in atherosclerosis and PAD.¹⁶⁻¹⁹ The aim of the present work was to investigate the effect of modulating inflammation on inflammation itself and endothelial function during the initial stages of PAD. COX-2

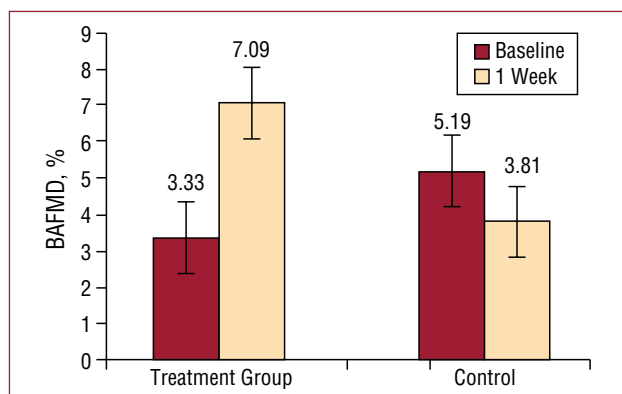


Figure 2. Comparison of brachial artery flow-mediated dilation values between control and treatment groups at baseline and at 1 week. The difference was significant in the treatment group (celecoxib administered) ($P=.001$); no significant differences were seen for the control group ($P=.069$).

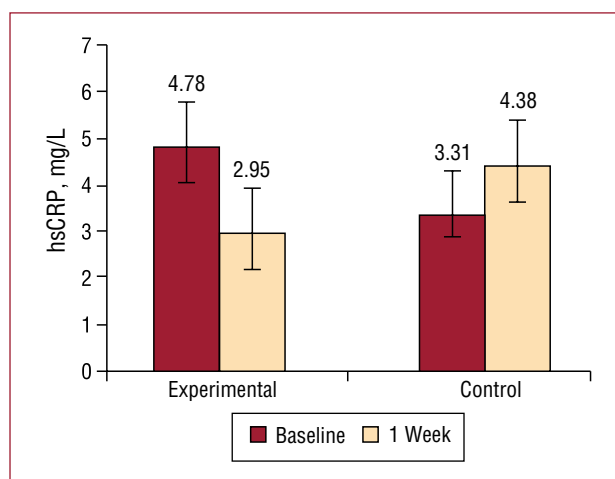


Figure 3. Comparison of high-sensitivity C-reactive protein levels (hsCRP) in the 2 groups at baseline and at 1 week. A significant difference was seen in the treatment group ($P=.023$) but not in the control group ($P=.14$).

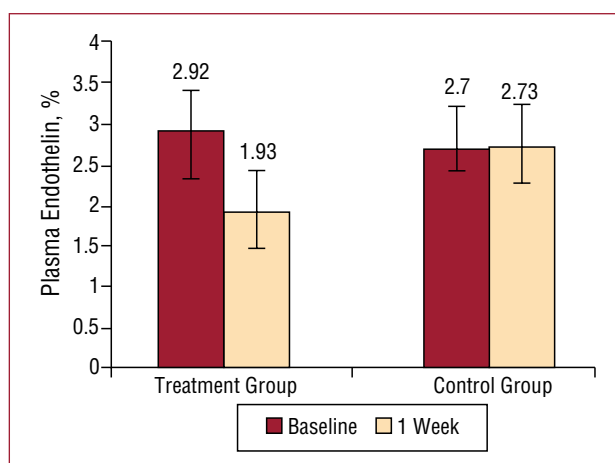


Figure 4. Comparison of plasma endothelin (PE) concentrations in the 2 groups at baseline and at 1 week. A significant difference was seen in the treatment group ($P=.018$) but not in the control group ($P=.65$).

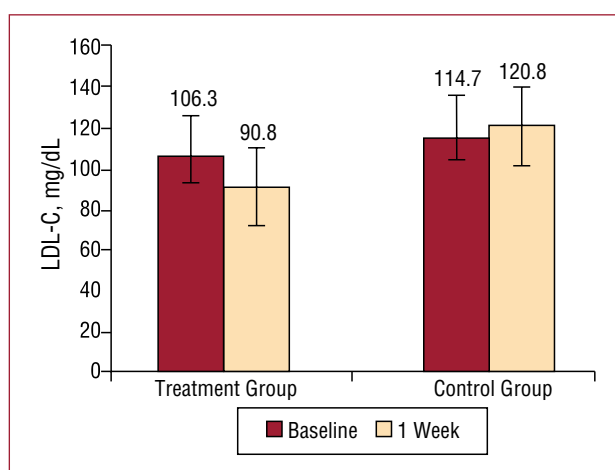


Figure 5. Comparison of low density lipoprotein cholesterol (LDL-C) concentrations in the 2 groups at baseline and at 1 week. A significant difference was seen in the treatment group ($P=.019$) but not in the control group ($P=.26$).

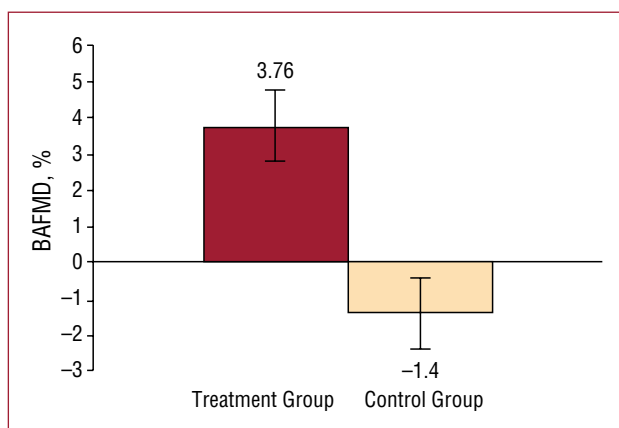


Figure 6. Comparison of change in the brachial artery flow-mediated dilation (BAFMD) values (BAFMD post-pre) ($P=.001$).

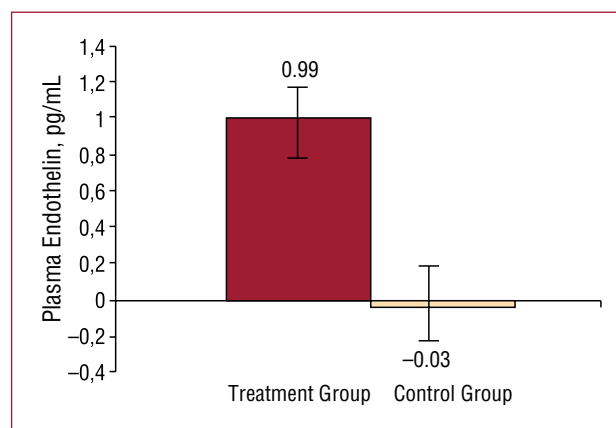


Figure 8. Comparison of the change in pre-post endothelin ($P=.010$).

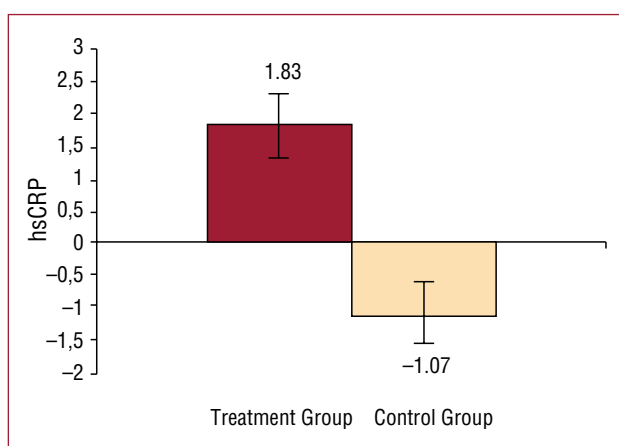


Figure 7. Comparison of the change in pre-post hsCRP ($P=.002$).

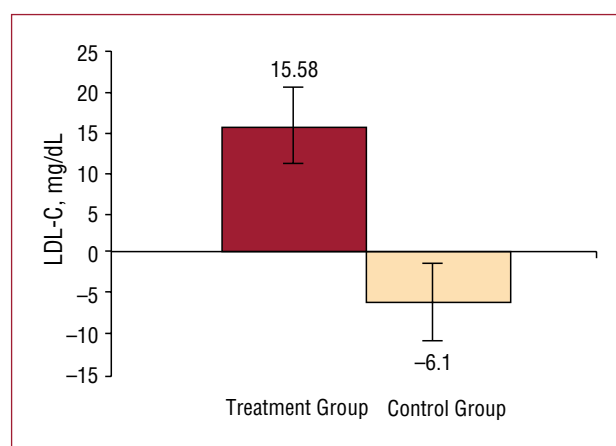


Figure 9. Comparison of the change in pre-post LDL-C ($P=.011$).

is involved in inflammatory processes and its selective inhibition with celecoxib (a specific anti-inflammatory agent with no ant-aggregant activity) allows the effect of the modulation of inflammation to be appreciated.

To the best of our knowledge, this is the first study to investigate the effect of selective COX-2 inhibitors in patients with PAD. The results obtained reinforce those recorded in patients with high blood pressure and heart disease, in whom inflammation and endothelial dysfunction have been implicated in atherosclerotic processes. The results open up new therapeutic and preventative possibilities.

In the present work, an improvement in dilation-dependent upon the endothelium—was seen in the patients administered celecoxib. This observation agrees with that reported in patients with high blood pressure, in whom a significant increase in BAFMD values were recorded at 3 h and 1 week after the first dose (7.9% [4.5%]; 9.9% [5.1%]; 10.1% [6.1%]; $P=.005$ and $P=.006$),¹² and in patients with

ischemic cardiomyopathy¹³—increases that cannot be explained by a vasodilatory effect of the drug.²⁰ This increased endothelium-dependent dilation might be explained in that the reactive oxygen species generated by COX-2 directly reduce the biological activity of nitric oxide and contribute towards lipid peroxidation.^{21,22} In a study involving patients with coronary artery disease, patients administered celecoxib showed a greater BAFMD for 2 weeks compared to those who received a placebo (3.3% [0.4%] vs 2% [0.5%]; $P=.026$). No changes in dilation were seen, however, with the administration of glycerol trinitrate (the action of which is independent of the endothelium) (9% [1.6%] vs 9.5% [1.3%]; $P=.75$).¹³

A reduction in the hsCRP value was also seen in the present work following celecoxib administration; hsCRP is a well-known marker of chronic inflammation involved in atherogenesis.^{16,19} This reduction might also improve endothelial function since it has been shown that this protein reduces the

bioavailability of nitric oxide, which, along with the systemic inflammatory response in itself, leads to a deterioration of endothelial function.^{23,24}

A number of proinflammatory cytokines (eg, interleukin 1 and tumor necrosis factor alpha), lipopolysaccharide (LPS), and growth factors (PDGF, EGF, FGF) induce the expression of COX-2 in monocytes,²⁵ promoting their transformation into activated macrophages. COX-2 is amply expressed in monocytes, macrophages, smooth muscle cells, and the endothelial cells of atherosclerotic plaques, but not in healthy arteries.⁶ Therefore, its selective inhibition allows one to study its effects in tissues affected by PAD. Macrophages that express COX-2 produce different cytokines, some with beneficial effects such as prostacyclin (PGI₂), while others are proinflammatory molecules.²⁶ It is known that when COX-2 is inhibited, the activity of COX-1 increases, probably leading to the production of just enough prostacyclin to ensure the protection of the cardiovascular system.^{27,28} Unlike PGI₂, PGE₂ induces the production of pro-inflammatory cytokines such as interleukin 6 and stimulates the release and activation of metalloproteinases, enzymes with an important role in the migration of macrophages. The chemotaxis of monocytes stimulated by LDL-C is also dependent on COX.²⁹

Other pro-atherogenic actions mediated by the production of prostaglandins via COX-2 include an increase in vascular permeability, the activation of chemotaxis, the migration of smooth muscle cells, and the synthesis of extracellular matrix, etc.²⁶

Activated macrophages also induce the production of cholesterol and induce plaque instability and endothelial dysfunction.

All these actions support the hypothesis that the inhibition of COX-2 should reduce atherogenesis, especially during the initial phases of the disease since an increase in LDL oxidases reduces the expression of COX-2, and mature foam cells do not produce this enzyme.^{26,30}

Endothelin is a protein produced by endothelial cells³¹ in response to different stimuli such as hypoxia, LDL-C, and procagulant factors. Increased endothelin concentrations have been reported in patients with coronary artery disease and PAD, especially in the early stages.³²⁻³⁴ The present results indicate there to be a reduction in PE in patients with intermittent claudication in its early stages (grade II) after the inhibition of COX-2 with celecoxib. This could be due to a reduction in LDL-C and the systemic inflammatory response. This would help explain the improvement seen in endothelium-dependent dilation-PE is a known marker of endothelial dysfunction. Nonetheless, at the present time there is no clinical evidence that treatment during the early stages of PAD directed at reducing

inflammation and endothelial dysfunction actually improves patient prognosis. Large prospective studies would be needed to determine this.

CONCLUSIONS

The results of this work confirm the involvement of inflammation and endothelial dysfunction in the pathogeny of atherosclerosis, and its reduction via the use of an anti-inflammatory agent in patients with PAD. Further studies should be performed to investigate the use of inflammation modulators in the etiologic treatment of early PAD.

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