Biglycan expression in hypertensive subjects with normal or increased carotid intima-media wall thickness

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1. Introduction

Proteoglycans are the prominent constituents of both extracellular matrix (ECM) and cellular basal membrane. They have recently become a focus of attention as they, besides playing a role in matrix formation, have been shown to be involved in many vascular and cellular functions. Biglycan (BGN), a small leucine rich proteoglycan that organizes the fibril collagen assembly [1] is considered an early initiator of arterial lesions, including wall thickening [2] by virtue of its capacity to bind and retain lipoprotein on ECM [3,4]. Biglycan has been found to affect cell migration [5] and growth [6] and cytokine activity [7]. Furthermore, BGN has been shown to bind recognition receptors on macrophages [8] that implicate BGN as a cell signalling molecule. Moreover, in macrophages from mice with acute inflammation, it acts as an endogenous ligand of toll like receptors (TLRs), TLR4 and TLR2, which mediate innate immunity and inflammation [9]. The expression of BGN has been detected in many human cell types; however, the biological function of this molecule remains yet poorly understood.

Activations of blood monocytes and increased levels of pro-inflammatory markers, including interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP) have been observed to stimulate hepatic release of CRP [15] and to modulate host defence [16] as well as systemic inflammation [17]. IL-6 has been reported to associate with insulin resistance [18] atherosclerosis [19] and hypertension [11,13,19]. Additionally, IL-6 seems to mediate the expression of BGN in macrophage cells of septic mice [9]. Similarly, TNF-α modulates inflammation [9]. The expression of BGN has been shown to convey pro-inflammatory signals. In the present study we investigated BGN expression and its correlation with plasma levels of inflammatory markers in hypertensive subjects with or without alteration of carotid intima media thickness (IMT).

Methods: We evaluated 123 untreated essential hypertensives with no additional risk factors for atherosclerosis nor signs of cardiovascular disease and 40 controls. Hypertensives were classified according to a normal (≤1 mm) or increased (>1 mm) IMT. BGN-mRNA and protein expression were measured in unstimulated, LPS- and Angiotensin II (Ang-II)-stimulated blood monocytes. Plasma concentrations of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and high sensitivity-C-reactive protein (hs-CRP) were also measured.

Results: We found increased levels of IL-6, TNF-α, hs-CRP, and BGN-mRNA and protein in hypertensives vs controls (1.72 ± 0.60 vs 1 n-fold, and 3.60 ± 0.75 vs 1 n-fold, both p<0.001). However, BGN expression was not significantly different between hypertensives with IMT ≤1 mm and >1 mm. Furthermore, in vitro addition of Ang II enhanced basal BGN-mRNA (in hypertensives: 3.57 ± 1.08 vs 1.72 ± 0.60 n-fold, p<0.001) and protein (in hypertensives: 4.92 ± 0.42 vs 3.41 ± 0.75, p<0.001) expression in monocytes.

Conclusions: Our data provide evidence of an enhanced expression of BGN in essential hypertension. In addition we suggest that Ang II can mediate monocyte BGN production.

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Keywords: Biglycan; Arterial hypertension; Carotid intima media thickness; Atherosclerosis; Angiotensin II; Inflammation; Monocytes

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ABSTRACT

Background: Biglycan (BGN), an extracellular matrix proteoglycan, has been shown to convey pro-inflammatory signals. In the present study we investigated BGN expression and its correlation with plasma levels of inflammatory markers in hypertensive subjects with or without alteration of carotid intima media thickness (IMT).

Methods: We evaluated 123 untreated essential hypertensives with no additional risk factors for atherosclerosis nor signs of cardiovascular disease and 40 controls. Hypertensives were classified according to a normal (≤1 mm) or increased (>1 mm) IMT. BGN-mRNA and protein expression were measured in unstimulated, LPS- and Angiotensin II (Ang-II)-stimulated blood monocytes. Plasma concentrations of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and high sensitivity-C-reactive protein (hs-CRP) were also measured.

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several pathways of inflammation [20], interacts with ECM molecules [7], induces apoptosis [21] and resistance to bacterial infection [22]. An association of TNF-α with arterial hypertension and its complications has been previously demonstrated [23,24]. Furthermore, in lipopolysaccharide (LPS) activated macrophages isolated from mice, BGN mRNA expression appeared to be associated with an enhanced synthesis of TNF-α [9]. These findings further supported the involvement of IL-6 and TNF-α in inflammation and immunity. CRP has been considered as a marker of CAD in a variety of clinical settings, furthermore in a few studies CRP was reported directly participate to the formation of arterial lesions [25] and up-regulation of Angiostensin II (Ang II) receptors [26]; Ang II is a vasoactive peptide involved in advancing arterial hypertension and inflammation. Although all these findings, and a great number of other studies, have demonstrated a significant association between these inflammatory molecules and CAD, their inner role is actually debated [27-29].

In the present study we investigated the BGN-mRNA and protein expression in monocyte/macrophage cells isolated from patients with essential hypertension with or without increased carotid intima media thickness (IMT) and/or plaques. We also examined the relationship between BGN-mRNA expression and plasma levels of IL-6, TNF-α and CRP.

2. Materials and methods

2.1. Subjects

All the procedures were in accordance with the Declaration of Helsinki and the protocol has been reviewed and approved by the Ethics Committee of the University of Messina. We examined 123 untreated essential hypertensive patients and 40 normotensive controls matched for sex and age. All hypertensives were newly diagnosed outpatients, while controls were recruited from hospital personnel. All participants were enrolled between January 2006 and March 2007 and informed consent was obtained from each of them. The blood samples were collected at the time of diagnosis and none of the patients was receiving medications, including antihypertensive drugs and 3hydroxy-3methyl glutaryl coenzyme A reductase inhibitors or statins. Furthermore, anti-hypertensive therapy was started after blood collection.

Blood pressure (BP) was measured after 15 min of quiet rest in the supported arm of the seated subjects with a mercury sphygmomanometer cuff-size adjusted for arm circumference. Phases I and V of the Korotkoff sounds were considered systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively. Three measurements performed with intervals of more than 2 min were averaged. Only subjects with SBP ≥140 mmHg and/or DBP ≥90 mmHg were included in the study. Patients with secondary hypertension, body mass index (BMI) ≥30, smoking habit, alcohol consumption, abnormal electrocardiographic and echocardiographic patterns, clinical history of CAD, diabetes mellitus, cholesterol levels ≥6 mmol/l and triglyceride levels ≥2.5 mmol/l, thyroid, liver or kidney diseases were excluded.

All hypertensives and controls underwent a carotid Doppler-ultrasonography and laboratory tests, including clinical chemistry and blood monocytes isolation. Plasma lipids and glucose levels were measured by routine methods. LDL-cholesterol was calculated by the Friedewald formula.

The ELISA technique was used to measure in duplicates the levels of hs-CRP (Immundiagnostik AG, IL-6 (Quantikin-HS) and TNF-α (R & D Systems-Ltd). Intra-assay variability was ± 5% for all the assays.

2.2. Ultrasound scanning procedure

High-resolution B-mode ultrasound images (Vivid 3 Expert, General Electric Company) with a 15 MHz linear array transducer were used to measure carotid intima-media wall thickness (IMT). The carotid arteries were examined bilaterally in the areas of the common carotid (1 cm proximal to the carotid bulb), the carotid bifurcation (1 cm proximal to the flow divider) and the internal carotid artery (1 cm distal to the flow divider). All measurements were determined manually in longitudinal and transverse planes with anterior, lateral and posterior approaches. From B-mode images, single video frames were selected for IMT measurements.

The IMT was defined as the distance between the lumen/intima and the media/adventitia interfaces. The mean value was calculated for each parameter. The mean IMT was used as the key variable for statistical analysis, because of its strong association with cardiovascular disease. Two independent readers, who were blinded with respect to patients’ clinical and laboratory profile, made the measurements. Patients were classified according to a normal (≤1 mm) or abnormal (>1 mm) IMT, including atheroma to positive plaques. The interobserver variability was evaluated on the measurements obtained from all subjects participating in the study. The interobserver variability of IMT measurements, as evaluated by comparing values obtained by two sets of scans evaluated by each reader, was 0.05 mm (coefficient of variation 3.58%). The intraobserver variability was 0.05 mm (coefficient of variation 2.13%).

2.3. Monocytes isolation

Peripheral blood mononuclear cells were isolated from heparinized venous blood. To minimize platelet contamination, the blood samples were centrifuged immediately at 500 g for 10 min at 4 °C. After removal of the plasma, the pellets were suspended in RPMI1640 (ICN, Biomedicals, Verona, Italy) supplemented with 2 mmol/L glucose, 0.5% streptomycin-penicillin-Fungizone and 10% Fetal Calf Serum (FCS) and centrifuged over Ficoll–Paque (Pharmacia, Fine Chemicals, Uppsala, Sweden). Gradient at 1000 g for 20 min. Mononuclear cells were collected from the interface Ficoll–Paque and suspended in RPMI1640 and incubated in 35 mm plastic dishes for 90 min at 37 °C, in 5% CO2 humid atmosphere. Monocytes were obtained by their adherence to dishes while the lymphocytes (non adherent cells) were removed by aspiration with Pasteur pipette and washing of the dishes with RPMI1640. Cell preparations were >98% monocytes, which was determined by flow cytometry and Giemsa staining. After isolation, cells were incubated at 1 × 10⁶ cells/ml in RPMI1640 at 37 °C, 5% CO2 for 6 h. Cultures were performed in presence or absence of LPS (Escherichia coli 0111:B4, Sigma, St. Louis, MI, USA) at final concentration of 0.1 µg/ml. At the end of the incubation period the cells (5 × 10⁶ cells/ml) were washed until the analysis.

2.4. Induction of monocyte biglycan expression by Angiostensin II

An aliquot of monocytes (5 × 10⁵ cells/ml in triplicate), isolated from 30 randomly chosen subjects (selection by “Random Generator” algorithm in order to obtain 10 controls, 10 hypertensives with IMT ≤1 mm and 10 hypertensives with IMT >1 mm), was incubated for 12 h in RPMI1640 at 37 °C. 5% CO2 in plastic dishes and cultured in presence or absence of Angiostensin II (Sigma Aldrich, Milan, Italy) (1 µM) or LPS (0.1 µg/ml) and then washed and subjected for the total RNA and protein extraction.

2.5. Biglycan-mRNA expression by real-time-polymerase chain reaction

Total RNA was extracted from 5 × 10⁶ cells and 1 µg was reverse transcribed; 0.25 µg of total cDNA was used to quantized the amount of BGN mRNA by real time polymerase chain reaction (PCR) method using β-actin cDNA as endogenous control for the final normalisation. The reactions were carried out by ready to use chemicals (Applied Biosystems, USA) and by mod.7500-PCR System, (Applied Biosystems, USA). The quantification was determined by standard curve method for both target and endogenous reference. In order to obtain the standard curves, a series of dilutions of BGN and β-actin cDNA clones were performed (obtained by TA cloning method, Invigen, USA).

After normalisation, the median of BGN target levels in unstimulated monocytes from control subjects was considered as calibrator (1 × sample) and the results were expressed as n-fold difference relative to normal controls [30].

2.6. Biglycan protein expression by Western blotting assay

We examined BGN protein expression—at baseline and after Ang II from about 10⁵ cells for each sample—in the 30 randomly selected subjects we evaluated for BGN-mRNA expression after Ang II addition. Each sample was treated with chondroitinase

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>All Hypertensives</th>
<th>Hypertensives with IMT ≤1 mm</th>
<th>Hypertensives with IMT &gt;1 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
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<td>123</td>
<td>62</td>
<td>61</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>18/32</td>
<td>61/62</td>
<td>28/34</td>
<td>31/28</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43±10</td>
<td>42±9</td>
<td>42±10</td>
<td>42±8</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>28.5±5.8</td>
<td>25.6±3.2</td>
<td>25.8±2.9</td>
<td>25.4±3.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>118±9.9</td>
<td>120.5±9.9</td>
<td>146.2±6.8</td>
<td>154.2±9.9</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>66±5.0</td>
<td>90.9±10.5*</td>
<td>91.3±8.7</td>
<td>90.5±11</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.35±0.50</td>
<td>4.37±0.68</td>
<td>4.36±0.60</td>
<td>4.39±0.76</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.39±0.21</td>
<td>1.4±0.37</td>
<td>1.45±0.38</td>
<td>1.35±0.35</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.06±0.20</td>
<td>1.04±0.31</td>
<td>1.01±0.33</td>
<td>1.07±0.29</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
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<td>2.49±0.68</td>
<td>2.44±0.54</td>
<td>2.54±0.8</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>4.8±0.51</td>
<td>4.7±0.58</td>
<td>4.8±0.57</td>
<td>4.68±0.59</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>214.7±26.7</td>
<td>254.9±51.4*</td>
<td>231.7±6.9</td>
<td>278.5±58.8*</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.70±0.21</td>
<td>1.50±0.95*</td>
<td>0.80±0.22</td>
<td>2.31±0.69*</td>
</tr>
</tbody>
</table>

Values are mean±SD. Abbreviations: BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; HDL-C: high density lipoprotein-cholesterol; TC: triglycerides; LDL-C: low density lipoprotein cholesterol. *p<0.001 versus controls and hypertension subjects with IMT ≤1 mm; p: two tailed significance level of NIPC test; p values are after Bonferroni's adjustment.
Values of interleukin-6, TNF-α, fibrinogen, glucose are mean±SD. Abbreviations: Hs-CRP: high sensitivity C-reactive protein, IL-6: inflammatory markers, TNF-α tumor necrosis factor alpha.

Table 1 shows the baseline characteristics of the study groups. Normotensive controls had IMT values similar to hypertensives with IMT ≤1 mm. Hypertensives with IMT ≥1 mm showed significantly higher SBP than hypertensives with IMT ≤1 mm (p<0.001). Fibrinogen levels were significantly higher in hypertensives with IMT >1 mm than in hypertensives with IMT ≤1 mm (p<0.001). Age, sex, BMI, lipid profile and plasma glucose levels were comparable among the study groups.

Table 2 shows the plasma levels of IL-6, TNF-α and hs-CRP. Mean values of IL-6, TNF-α and hs-CRP were significantly higher in each group; in this way the reliability of the results was guaranteed and the sampling results can be extended to the whole group population.

2.7. Statistical methods

Non-parametric approach was used since examined variables did not present normal distribution (verified by Kolmogorov Smirnov test); as permutation tests-based analysis was performed, data were expressed as mean and standard deviations. Biglycan mRNA and protein values are expressed as n-fold variation versus controls basal mean values. Ang-II angiotensin II. *p<0.001 versus basal expression. **p<0.001 versus controls. ***p<0.001 versus basal expression and versus stimulated controls. Comparisons between unstimulated and stimulated monocytes have been performed by NPC test for Repeated Measures.

3. Results

3.1. Characteristics of the study subjects

Table 1 shows the baseline characteristics of the study groups. Normotensive controls had IMT values similar to hypertensives with IMT ≤1 mm. Hypertensives with IMT >1 mm showed significantly higher SBP than hypertensives with IMT ≤1 mm (p<0.001). Fibrinogen levels were significantly higher in hypertensives with IMT >1 mm than in hypertensives with IMT ≤1 mm (p<0.001). Age, sex, BMI, lipid profile and plasma glucose levels were comparable among the study groups.
hypertensives compared to controls \((p<0.001)\). IL-6, TNF-α and hs-CRP levels were significantly higher in hypertensives with IMT ≤1 mm compared to controls \((p<0.001)\) and in hypertensives with IMT >1 mm compared to either controls and hypertensives with normal IMT.

### 3.2. Evaluation of biglycan-mRNA expression in unstimulated and LPS-stimulated monocytes

Fig. 1 shows basal and LPS-stimulated values of BGN-mRNA expression in monocytes. Basal and LPS-stimulated expression of BGN-mRNA was higher in hypertensives than in controls \((\text{basal: } 1.76 \pm 0.89 \text{ vs } 1 \text{ n-fold, and after stimulation: } 2.39 \pm 0.58 \text{ vs } 1.46 \pm 0.47 \text{ n-fold, both } p<0.001)\). No statistical difference was detected in BGN-mRNA expression between IMT ≤1 and IMT >1 mm hypertensives, in either unstimulated and LPS-stimulated monocytes.

### 3.3. Effect of Angiotensin II on biglycan-mRNA and protein expression

There was no significant difference between the clinical characteristics of the whole population and the randomly selected subjects, suggesting that this small group (30 subjects) was representative of our larger sample. Fig. 2 shows the values of basal and Ang II-stimulated BGN-mRNA expression in monocytes isolated from the randomly selected subjects. Addition of Ang II to monocytes isolated from hypertensives resulted in a higher expression of BGN compared to that of the control group \((3.47 \pm 1.08 \text{ vs } 1.72 \pm 0.35 \text{ n-fold, both } p<0.001)\). No statistical difference was detected in Ang II-induced BGN-mRNA expression between IMT ≤1 and IMT >1 mm hypertensives \((3.46 \pm 0.81 \text{ vs } 3.52 \pm 0.92)\).

Fig. 3 shows the values of basal and Ang II-stimulated BGN protein expression in monocytes isolated from the randomly selected subjects. Unstimulated and Ang II-stimulated monocytes from hypertensives showed significantly higher protein expression than controls \((3.41 \pm 0.75 \text{ vs } 1 \text{ n-fold, and } 4.92 \pm 0.42 \text{ vs } 2.14 \pm 0.71 \text{ n-fold, both } p<0.001)\), confirming that BGN-mRNA and BGN protein expression have the same behaviour pattern. No statistical difference was detected in Ang II-induced BGN protein expression between IMT ≤1 and IMT >1 mm hypertensives \((4.87 \pm 0.49 \text{ vs } 5.04 \pm 0.64 \text{ n-fold})\).

### 3.4. Relationship among statistical variables

Examining the bivariate interdependence among the considered variables, we found a significant correlation between BGN and SBP \((r_b=0.47, p<0.001)\), DBP \((r_b=0.28, p<0.005)\), IMT \((r_b=0.40, p<0.001)\), and with considered inflammatory markers \((\text{Fibrinogen: } r_f=0.30; \text{TNF-α: } r_f=0.60; \text{IL-6: } r_f=0.54, p<0.001; \text{CRP: } r_f=0.18, p<0.05)\). Significant correlations were found between SBP and IMT \((r_b=0.50, p<0.001)\), CRP \((r_b=0.35, p<0.001)\), TNF-α \((r_b=0.69, p<0.001)\), and IL-6 \((r_b=0.41, p<0.005)\); moreover, a strong correlation between IMT and CRP \((r_b=0.52, p<0.001)\), TNF-α \((r_b=0.70, p<0.001)\), IL-6 \((r_b=0.62, p<0.001)\), and BGN basal expression \((r_b=0.40, p<0.001)\) occurred.

Then, the contribution of the study variables \((\text{age, sex, BMI, SBP, DBP, TC, HDL-C, TG, glucose, fibrinogen, hs-CRP, IMT, IL-6, TNF-α})\) in determining BGN-mRNA expression was evaluated. We found a significant dependence of BGN-mRNA from TNF-α and IL-6 \((p<0.001)\). Moreover, when we estimated a dependence model for IMT, we found that IMT was significantly dependent from IL-6, CRP and TNF-α \((p<0.001)\), apart from SBP \((p<0.005)\), but not from BGN (Table 3).

### 4. Discussion

In the present study we found that plasma levels of IL-6, TNF-α and CRP were increased in hypertensive subjects with no additional risk factors and CAD signs compared to normotensive controls. We also observed that BGN-mRNA and protein expression were significantly higher in circulating monocytes from hypertensives subjects. By contrast, no difference in BGN expression was observed in monocytes from hypertensive subjects with or without IMT abnormalities.

Inflammatory features, such as enhanced monocytes response to in vitro stimulation \([10,14]\) and increased levels of circulating inflammatory molecules \([8–10,21]\), have been shown in individuals with high blood pressure. For these reasons, inflammation has been hypothesized to play a role in arterial hypertension and its vascular complications.

Hypertensive subjects with IMT ≤1 mm had significantly higher plasma levels of IL-6, TNF-α and CRP compared to those of hypertensives with IMT >1 mm. The regression model performed to evaluate the variables dependent on IMT increase, showed an association of IMT with inflammatory molecules, and with SBP. It has been recently suggested that the association between inflammatory molecules, hypertension \([27,28]\) and carotid IMT \([29]\), may reflect confounding factors rather than causal effects. We cannot prove that the inflammatory markers have a causal effect in the development of arterial lesions in hypertension nor can we exclude that such association is due to confounding factors, including genetic factors. Nevertheless our data are in agreement with those studies demonstrating an elevating of the levels of CRP, IL-6 and TNF-α in hypertensive subjects. \([11–13,24]\).

It has been recently reported that macrophage cells are able to produce BGN upon stimulation by pro-inflammatory factors \([31,32]\). Macrophages contained and released BGN in a model of renal inflammation \([31]\) and in human inflamed gingival \([32]\). Furthermore, Schafer et al. identified BGN as a secreted product of LPS activated mice macrophages \([9]\) and suggested a novel role of this protein as a signalling molecule and a crucial pro-inflammatory factor. They reported that LPS initially triggers IL-1β and IL-6 expression in isolated macrophages, leading to BGN expression and in turn to TNF-α release. Biglycan itself was able to modulate the immune and inflammatory sequences through the interaction with the TLRs signalling cascade. Therefore, during inflammation, BGN may be released from ECM or alternatively induced in macrophage cells by cytokines, to signal through the interaction with TLRs. Subsequent expression of TNF-α might result in increased recruitment of macrophages that release further BGN, favouring the creation of a cycle that is capable to drive an inflammatory response in both an autocrine and a paracrine manner.

We demonstrated that monocyte BGN-mRNA and protein expression were significantly higher in hypertensive subjects, than in normotensive. By contrast, no difference in BGN-mRNA values has been observed in monocytes from hypertensive subjects with or without IMT abnormalities, indicating an enhanced production of BGN in essential hypertension, which is not related to carotid thickness, at least at this very early stage of disease. Our results show that BGN is significantly associated with IL-6, TNF-α, Fibrinogen and CRP plasma levels, suggesting that BGN might be involved in vascular inflammatory process. Furthermore, we also suggest that undiscovered mechanisms may act in triggering the expression of BGN in monocytes.
It has been observed that Ang II receptors are expressed by human peripheral monocytes and that their stimulation may promote monocyte activation and adhesion [33]. Angiotensin II was reported to regulate BGN mRNA expression in rat cardiac fibroblasts [34] and arterial wall [35], as well as in human vascular smooth muscle cells [36]. A mechanism by which Ang II mediates BGN stimulation in fibroblasts, involving protein-kinase C and tyrosine-kinase, has been previously described [34]. Furthermore, Ang II stimulated BGN production in renal tissues of spontaneous hypertension rats [38], contributing to the development of structural and functional modifications of cardiovascular system. Although different cell types may have different signalling pathways, we tried to evaluate whether BGN-mRNA and protein expression could be influenced by in vitro addition of Ang II in monocytes isolated from hypertensive subjects. It was already observed that in human cultured monocytes, Ang II type 1 receptor antagonism resulted in an inhibition of the synthesis of tissue factor and plasminogen activator inhibitor-1 [37]. More recently, Dasu et al. reported that Ang II type-1 receptor blocker inhibited LPS induced TLR2 and TLR4 expression at mRNA and protein levels in monocytes isolated from healthy volunteers, indicating a novel mechanism by which Ang II could induce inflammatory effects [39].

In our study, in vitro addition of Ang II to monocytes caused enhancement of their expression of BGN-mRNA and protein, especially in monocytes obtained from hypertensive subjects. These findings suggest that Ang II can mediate monocyte BGN expression. Moreover, the association between BGN and several inflammatory markers, such as IL-6, TNF-alpha, CRP, fibrinogen, can indicate its involvement in inflammatory features in arterial hypertension. In the present study some limitations have to be considered. For example, we used only Ang II as potential stimulator of biglycan expression in monocytes, even if different molecules, including the cytokines transforming growth factor (TGF)-beta [40] and platelet-derived growth factor (PDGF) [41] and mechanical forces, such as shear stress, have been demonstrated to stimulate biglycan expression in different cell types. Further studies had to be performed to clarify the role of monocyte BGN in inflammation and cardiovascular disease.

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