2-Cyclopenten-1-one and prostaglandin J_2 reduce restenosis after balloon angioplasty in rats: role of NF-κB

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Abstract The aim of this study was to evaluate, using a rat model of balloon angioplasty, whether prostaglandin (PG) J_2 and 2-cyclopenten-1-one are able to reduce restenosis. We found that both PGJ_2 and 2-cyclopenten-1-one, administered by local application on carotid arteries, caused a dose-dependent inhibition of neointimal formation. Furthermore, both agents prevented vascular negative remodeling. The effect of these compounds on restenosis was correlated with an inhibition of nuclear factor-κB (NF-κB) activation as well as of intercellular adhesion molecule-1 (ICAM-1) protein expression in injured carotid arteries of control animals. Our results show that cyclopentenone PGs and their derivatives reduce restenosis and may have therapeutic relevance for the prevention of human restenosis.

Key words: Cyclopentenone prostaglandin; 2-Cyclopenten-1-one; Nuclear factor-κB; Restenosis

1. Introduction

Percutaneous transluminal coronary angioplasty (PTCA) is a widely used technique in the therapy of coronary artery stenosis where the occluded segment is dilated by inflating an angioplasty balloon catheter. Despite a primary success rate greater than 90%, the long-term efficacy of this procedure continues to be compromised by the re-narrowing of the vessel, a phenomenon known as restenosis, which affects 30–40% of patients [1,2]. Restenosis was originally considered due to an exaggeration of the normal reparative processes after balloon-induced vascular injury leading to the migration of vascular smooth muscle cells (VSMCs) from the media across the internal elastic lamina (IEL) to form a new intimal layer or 'neointima' followed by replication of VSMCs within the neointima [3–5]. Despite extensive characterization of the mechanisms underlying intimal hyperplasia, experimental strategies to inhibit neointimal formation have generally failed to prevent restenosis in clinical practice probably because other processes (e.g. vascular remodeling) are involved [6]. Remodeling refers to geometric redistribution of cells and matrix independent of net change in wall mass or cross-sectional area. Thus, cells and matrix reorganize either to increase wall diameter, while decreasing wall thickness (positive remodeling) or to decrease wall diameter, while increasing wall thickness (negative remodeling) [7]. Actually restenosis after balloon angioplasty is considered primarily due to negative vascular remodeling and only partially to VSMC proliferation [8]. Negative vascular remodeling is a complex and poorly understood process that involves alteration in the balance between cell proliferation and apoptosis [9], matrix protein production and degradation [10] as well as hemodynamic changing in blood flow [11,12] that cause a decrease of vessel diameter and lumen area. The exact mechanism of arterial neointimal thickening remains to be understood and may involve growth factors, vasoactive agents, cytokines and adhesion molecules. Most of these factors are regulated at transcriptional level by nuclear factor-κB (NF-κB). Activation of NF-κB has been found to occur also in balloon-injured rat carotid arteries and has been associated with neointima formation and expression of NF-κB-regulated genes such as cytokines, growth factors and cell adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) [13–15].

NF-κB is normally found in the cytoplasm of unstimulated cells as an inactive complex, whose predominant form is a heterodimer composed of p50 and p65 (Rel A) subunits, bound to inhibitory proteins of the I-κB family, usually I-κB-α [16]. NF-κB is activated in response to a variety of pathogenic stimuli, including inflammatory cytokines, endotoxins and oxidative stress. Several stimuli activate NF-κB by augmenting the activity of the I-κB kinase (IKK) complex, that phosphorylates I-κBs at sites that trigger their ubiquitination and proteasome-mediated degradation [17,18]. Freed NF-κB dimers translocate to the nucleus where they bind to specific B elements and activate transcription of a variety of genes encoding adhesion molecules, inflammatory and chemotactic cytokines, cytokine receptors, and enzymes that produce inflammatory mediators most of which are involved in development of restenosis [18,19]. Thus NF-κB seems to be an interesting therapeutic target for the development of molecules able to inhibit restenosis.

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Abbreviations: cyPG, cyclopentenone prostaglandin; EMSA, electrophoretic mobility shift assay; IKK, I-κB kinase; ICAM-1, intercellular adhesion molecule-1; IEL, internal elastic lamina; L-PDGS, lipocalin-type prostaglandin D synthase; NF-κB, nuclear factor-κB; PTCA, percutaneous transluminal coronary angioplasty; PPARγ, peroxisome proliferator-activated receptor-γ; VSMC, vascular smooth muscle cell

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Prostaglandins (PGs) are a class of naturally occurring cyclic 20-carbon fatty acids synthesized from arachidonic acid which are involved in the regulation of various physiological and pathological processes including cell proliferation and inflammation [20,21]. The type A and J PGs, characterized by the presence of a reactive α,β-unsaturated carbonyl group in the cyclopentane ring (cyclopentenone PGs, cyPGs), exhibit a wide array of biological activities, including antiproliferative and anti-inflammatory effects [22]. It has been shown that cyPGs are able to inhibit the phosphorylation and prevent the degradation of the NF-κB inhibitor I-κB-α in human cells and this inhibition is dependent on the presence of the cyclopentenone ring structure itself, 2-cyclopenten-1-one [22–24].

In the present study, using a rat carotid artery injury model, we show that local delivery of PGJ_2 or of 2-cyclopenten-1-one reduces restenosis. We also show that this effect is associated with the block of NF-κB activation in injured vessels and with decreased expression of ICAM-1, a gene regulated by NF-κB, in the rat carotid artery.

2. Materials and methods

2.1. Animals

Male Wistar rats (Harlan, Italy) weighing 280–300 g were used for this study. Animals were housed in propylene cages with food and water ad libitum. The light cycle was automatically controlled (on 07:00 h; off 19:00 h) and the room temperature thermostatically controlled to 22 ± 1°C. Prior to the experiment animals were housed in these conditions for 4–5 days to become acclimatized.

2.2. Balloon angioplasty

Animals were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg). Experimental rat balloon angioplasty of the right common carotid artery was performed according to the procedure described by Indolfi et al. [25]. 2-Cyclopenten-1-one (6.5, 13 and 26 μg/site) and PGJ_2 (25, 50 and 100 μg/site) were administered by local application on carotid arteries, after vascular injury, by using a solution of F127 pluronic gels prepared as described in [26]. Control animals received an equal volume of pluronic gel (200 μl). Some animals were subjected to anesthesia and surgical procedure without balloon injury (sham-operated rats). Rats were killed 14 days after vascular injury and carotid arteries were removed and processed as described below.

2.3. Morphology

After 14 days from angioplasty animals were anesthetized as described above and the carotid arteries were immediately removed and fixed in 10% buffered formalin. Sections were cut (6 μm thick) from the approximate midportion of the artery and stained with hematoxylin and eosin to demarcate cell types. Both the circumference and the cross-sectional area of external elastic lamina (EEL), IEL, lumen, media and neointima were measured as described by Ialenti et al. [27] by using an image analysis system (Qwin Lite 2.2, Leica).

2.4. Arterial remodeling

For each animal the ratio between EEL circumference of right injured artery (EEL_{CR}) and of EEL of left non-injured artery (EEL_{CL}) was calculated as arterial remodeling index (ARI^1) as previously described [28]. The same ratio was calculated using EEL dimension as cross-sectional area (ARI^2).

2.5. Preparation of whole tissue extracts

All the extraction procedures were performed on ice with ice-cold reagents. Separate carotid segments removed from rats treated as described above after 14 days from angioplasty were frozen in liquid nitrogen, immediately suspended in a volume of a high-salt extraction buffer and incubated on ice for 15 min as previously described by Ialenti et al. [27]. After centrifugation at 13 000 × *g* at 4°C for 5 min, the protein concentration in the supernatant was determined by the Bio-Rad protein assay kit, and then it was aliquoted and stored at −80°C.

2.6. Electrophoretic mobility shift assay (EMSA)

EMSA studies were performed on nuclear extracts as previously described [27]. Briefly, a double-stranded NF-κB consensus oligonucleotide probe (5′-AGTTGAGGGGATTTTCCCAGGC-3′) was end-labeled with [^{32}P-γ]adenosine triphosphate (ATP). Tissue extracts (12 μg protein for each sample) were incubated with radiolabeled oligonucleotides ($2.5–5.0 \times 10^4$ counts per minute (cpm)) in 20 μl reaction buffer containing 2 μg poly dI-dC, 10 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM dithiothreitol, 1 μg/μl bovine serum albumin (BSA), 10% (v/v) glycerol. To verify the specificity of protein–DNA complexes, in competition reactions nuclear extracts from injured carotid arteries were incubated with radiolabeled NF-κB probe in the absence or the presence of identical but unlabeled oligonucleotides (wild type, W.T. 50×), mutated non-functional κB probe (Mut. 50×) or unlabeled oligonucleotide containing the consensus sequence for Sp-1 (Sp-1 50×). Nuclear protein–oligonucleotide complexes were analyzed by gel electrophoresis. Gels were dried and autoradiographed with intensifying screen at −80°C for 20 h. Quantitative evaluation of NF-κB/κB complex formation was determined by densitometric analysis of the X-ray films using a GS 700 Imaging Densitometer (Bio-Rad) with the Molecular Analyst program (IBM).

2.7. Immunohistochemical localization of ICAM-1

Carotid sections were deparaffinized and permeabilized with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 10 min and rehydrated with PBS at room temperature for 45 min. Endogenous peroxidase was quenched with 0.3% H_2O_2 in 60% methanol for 30 min. Non-specific absorption was minimized by incubating the section in 1% BSA in PBS for 40 min. Sections were subsequently incubated overnight with primary anti-ICAM-1 (1:500 dilution). Controls included buffer alone or non-specific purified IgG. Specific labeling was detected with biotin-conjugated specific secondary antibody and avidin-biotin peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories).

2.8. Western blot analysis

The levels of ICAM-1 and β-actin were quantified in total extracts of whole carotid arteries by Western blot analysis as previously described [29]. Briefly, equivalent amounts of protein (20 μg) from each sample were mixed with gel loading buffer (50 mM Tris/10% sodium dodecyl sulfate (SDS)/10% glycerol/10% 2-mercaptoethanol/2 mg bromophenol per ml) in a ratio of 1:1, boiled for 3 min, centrifuged at 10 000 × *g* for 10 min and electrophoresed in an 8% discontinuous polyacrylamide minigel. The proteins were transferred onto nitrocellulose membranes, according to the manufacturer's instructions. The membranes were saturated by incubation at 4°C overnight with 10% non-fat dry milk in PBS and then incubated with anti-ICAM-1 monoclonal antibody or anti-β-actin (1:1000) monoclonal antibody, for 2 h at room temperature. The membranes were washed three times with 1% Triton X-100 in PBS and then incubated with anti-mouse immunoglobulins coupled to peroxidase (1:2000). The immune complexes were visualized by the enhanced chemiluminescence method. Subsequently, the relative expression of the proteins was quantified by densitometric scanning of the X-ray films with GS-700 Imaging Densitometer (Bio-Rad, Milan, Italy) with the Molecular Analyst computer program (IBM).

2.9. Statistic

Data are expressed as means ± S.E.M. of *n* rats. Statistical significance was calculated by one-way analysis of variance (ANOVA) and Bonferroni-corrected *P* value for multiple comparisons. The level of statistically significant difference was defined as $P < 0.05$.

2.10. Reagents

Bio-Rad protein assay kit and non-fat dry milk were from Bio-Rad, Milan, Italy. Oligonucleotides were synthesized by Roche Biomol, Milan, Italy. PBS was from Celbio (Milan, Italy). 2-Cyclopenten-1-one was from Fluka (Milan, Italy). DL-dithiothreitol, phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, pepstatin A, leupeptin and benzamidine were from Calbiochem (Milan, Italy). [^{32}P-γ]ATP was from ICN Biomedicals (Milan, Italy). Poly dI-dC was from Boehringer-Mannheim (Milan, Italy). Anti-ICAM-1 was from DBA (Milan, Italy). Anti-β-actin was from Sigma (Milan, Italy). All other reagents were from Sigma (Milan, Italy).

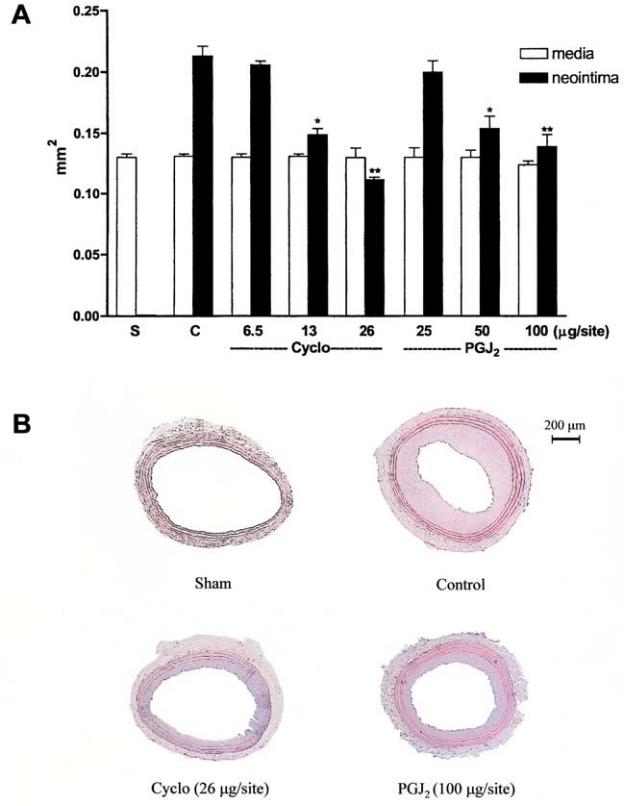

Fig. 1. A: Effect of 2-cyclopenten-1-one (Cyclo; 6.5, 13, 26 μg/site) and PGJ_2 (25, 50, 100 μg/site) on media (open bars) and neointima (solid bars) areas of injured carotid arteries. Results are expressed as mean ± S.E.M. $^*P < 0.05$, $^{**}P < 0.01$ vs. control group (C). S = sham-operated rats. B: Photomicrographs showing the neointimal formation in rat carotid arteries 14 days after balloon angioplasty. Representative cross-sections are from the carotid artery of sham-operated rat, control rat subjected to balloon injury and rat treated with 2-cyclopenten-1-one (Cyclo; 26 μg/site) or PGJ_2 (100 μg/site) subjected to balloon injury.

3. Results

3.1. Effect of 2-cyclopenten-1-one and PGJ_2 on neointimal proliferation

In sham-operated rats ($n = 5$) not subjected to vascular injury, no neointimal formation was observed. In balloon-injured carotid arteries from control rats ($n = 12$) the neointima was increased to an area of 0.213 ± 0.008 mm^2 (Fig. 1A). Treatment with 2-cyclopenten-1-one at a dose of 6.5 μg/site ($n = 9$) did not cause any alteration in the neointima area as compared to the control group, while doses of 13 and 26 μg/site caused a dose-dependent inhibition of neointimal formation by 30% ($n = 8$; $P < 0.05$) and 47% ($n = 9$; $P < 0.01$), respectively (Fig. 1A). Similar results were obtained with PGJ_2 given at equimolar doses (25, 50 and 100 μg/site). The neointima area was decreased by 28 and 35% at doses of 50 and 100 μg/site PGJ_2 respectively, while it was unmodified when this compound was given at a dose of 25 μg/site (Fig. 1A). The media area was not affected by 2-cyclopenten-1-one and PGJ_2 at any of the doses tested (Fig. 1A). Representative histological cross-sections of the carotid arteries are shown in Fig. 1B.

3.2. Effect of 2-cyclopenten-1-one and PGJ_2 on vascular remodeling

Vascular negative remodeling was observed in the control group after 14 days from angioplasty compared with sham-operated animals (Table 1). Interestingly, treatment of rats with both 2-cyclopenten-1-one and PGJ_2 prevented vascular negative remodeling. Thus, as shown in Table 1, 2-cyclopenten-1-one significantly ($P < 0.01$) increased the ARI at highest doses used (13 and 26 μg/site). Similar results were obtained with PGJ_2 at doses of 50 and 100 μg/site.

3.3. Effect of 2-cyclopenten-1-one and PGJ_2 on NF-κB activation

A low level of NF-κB/DNA binding activity was detected in nuclear protein extracts from carotid arteries of sham-operated rats ($n = 3$) (Fig. 2A). Conversely, a retarded band was clearly shown in injured carotid arteries from control rats ($n = 5$). Treatment of rats with 6.5 μg/site 2-cyclopenten-1-one ($n = 5$) or with 25 μg/site PGJ_2 ($n = 5$) did not modify NF-κB/DNA binding activity while either 13 μg/site 2-cyclopenten-1-one ($n = 6$) or 50 μg/site PGJ_2 ($n = 6$) caused a significant ($P < 0.05$) inhibition of NF-κB activation by about 30% (Fig. 2A). Treatment of rats with 2-cyclopenten-1-one (26 μg/site; $n = 6$) or with PGJ_2 (100 μg/site; $n = 6$) resulted in a complete inhibition of NF-κB activation in injured arteries (Fig. 2A). The specificity of the NF-κB/DNA binding complex was shown by the complete displacement of the NF-κB/DNA binding in the presence of a 50-fold molar excess of unlabeled NF-κB probe (W.T. 50×) in competition reactions (Fig. 2B). In contrast, a 50-fold molar excess of unlabeled mutated NF-κB probe (Mut. 50×) or of Sp-1 oligonucleotide (Sp-1 50×) had no effect on DNA binding activity.

Table 1
Arterial remodeling

	Control	2-Cyclopenten-1-one (μg/site)			PGJ_2 (μg/site)		
		6.5	13	26	25	50	100
EEL_{CR} (mm)	2.611 ± 0.022*	2.607 ± 0.020*	2.658 ± 0.040	2.672 ± 0.080	2.609 ± 0.020*	2.661 ± 0.040	2.668 ± 0.049
EEL_{CL} (mm)	2.668 ± 0.025	2.680 ± 0.026	2.684 ± 0.022	2.690 ± 0.025	2.686 ± 0.025	2.689 ± 0.022	2.686 ± 0.023
ARI^1	0.971 ± 0.022	0.972 ± 0.024	0.990 ± 0.025§	0.993 ± 0.015§	0.971 ± 0.017	0.989 ± 0.025§	0.993 ± 0.024§
EEL_R area (mm^2)	0.447 ± 0.006#	0.450 ± 0.010#	0.466 ± 0.024	0.474 ± 0.020	0.445 ± 0.006#	0.468 ± 0.024	0.475 ± 0.023
EEL_L area (mm^2)	0.475 ± 0.017	0.478 ± 0.015	0.474 ± 0.015	0.476 ± 0.011	0.475 ± 0.017	0.473 ± 0.015	0.478 ± 0.010
ARI^2	0.941 ± 0.020	0.941 ± 0.025	0.983 ± 0.022§	0.996 ± 0.020§	0.936 ± 0.025	0.989 ± 0.022§	0.993 ± 0.030§

EEL_{CR}, EEL circumference of right injured carotid artery; EEL_{CL}, EEL circumference of left uninjured carotid artery; ARI^1, EEL_{CR}/EEL_{CL}; EEL_R area, EEL area of right injured carotid artery; EEL_L area, EEL area of left uninjured carotid artery; ARI^2, EEL_C area/EEL_L area.
$^*P < 0.01$ vs. EEL_{CL}; $^{\#}P < 0.01$ vs. EEL_L area; §$P < 0.01$ vs. control.

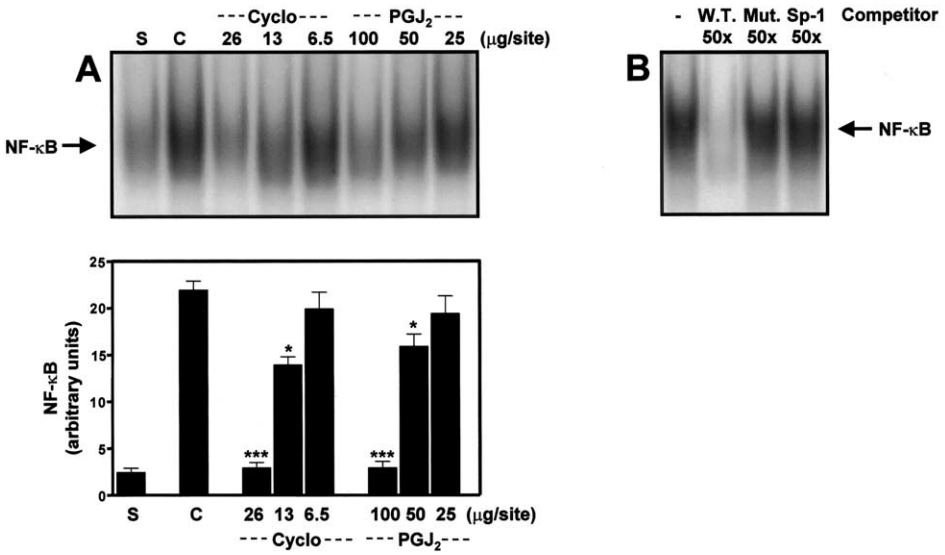

Fig. 2. A: Representative EMSA (upper panel) of NF-κB and densitometric analysis (lower panel) showing the effect of 2-cyclopenten-1-one (Cyclo; 6.5, 13 and 26 μg/site) and PGJ_2 (25, 50 and 100 μg/site) on NF-κB/DNA binding activity in nuclear extracts of injured rat carotid arteries 14 days after vascular injury. Results are expressed as mean ± S.E.M. of three separate experiments. $*P < 0.05$, $***P < 0.01$ vs. control group (C). S = sham-operated rats. B: In competition reactions, nuclear extracts were incubated with radiolabeled NF-κB probe in the absence or the presence of identical but unlabeled oligonucleotides (W.T. 50×), mutated non-functional κB probe (Mut. 50×) or unlabeled oligonucleotide containing the consensus sequence for Sp-1 (Sp-1 50×). Results illustrated are from a single experiment and representative of three separate experiments.

3.4. *Effect of 2-cyclopenten-1-one and PGJ_2 on ICAM-1 expression*

Staining of carotid tissue sections obtained from sham-operated rats with anti-ICAM-1 antibody showed a specific staining on endothelial cells and adventitia, demonstrating that ICAM-1 is constitutively expressed (Fig. 3). In the control groups subjected to vascular injury the staining intensity for ICAM-1 was substantially increased in the neointima 14 days after angioplasty as well as in VSMCs and adventitia (Fig. 3). Analysis of carotid sections derived from rats treated with either 2-cyclopenten-1-one (26 μg/site) or PGJ_2 (100 μg/site) revealed a down-regulation of ICAM-1 as compared to the control group (Fig. 3). These results were confirmed by Western blot analysis showing a significant level of ICAM-1

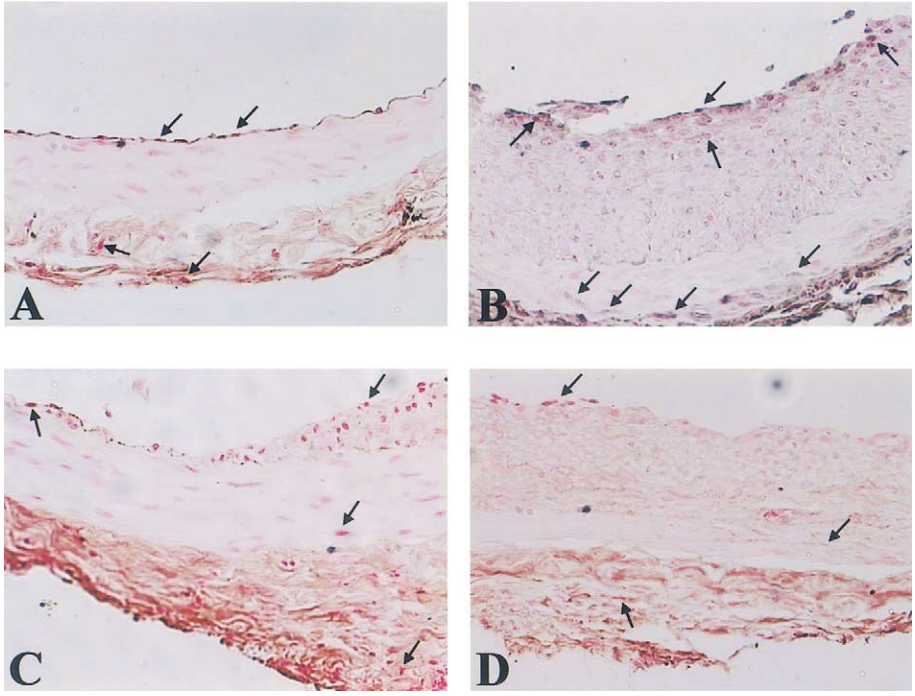

Fig. 3. Representative photomicrographs of sections of carotid arteries at 14 days after balloon angioplasty labeling for ICAM-1 (original magnification: ×250). Results illustrated are from a single experiment and are representative of three separate experiments. A: Sham-operated rat. B: Control animal. C: 2-Cyclopenten-1-one (26 μg/site). D: PGJ_2 (100 μg/site).

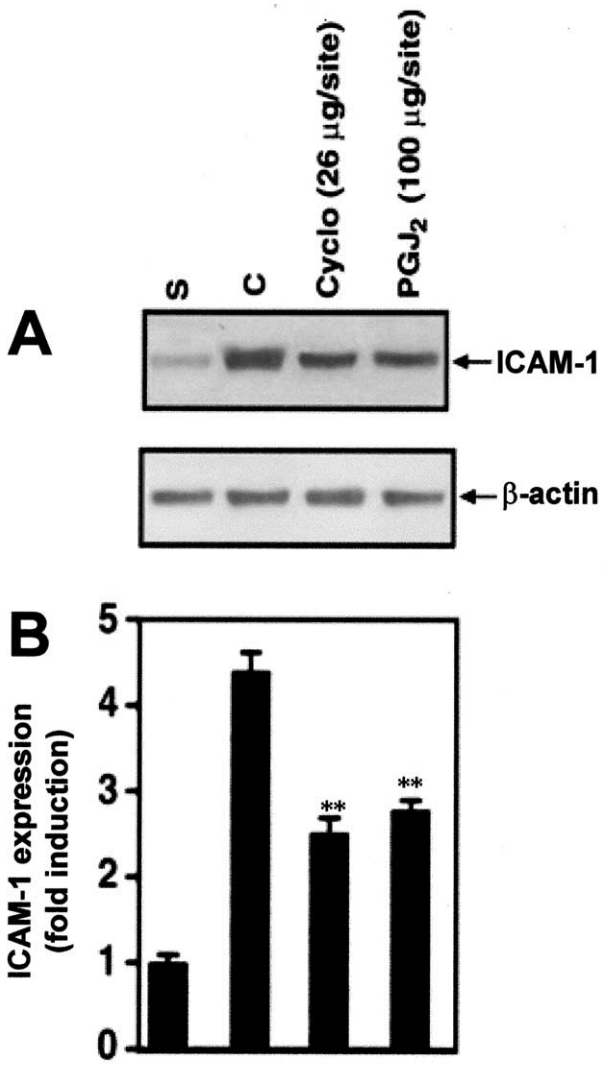

Fig. 4. A: Representative Western blot analysis of the effect of 2-cyclopenten-1-one (Cyclo; 26 μg/site) and PGJ_2 (100 μg/site) on ICAM-1 expression (upper panel) in carotid arteries. Equal loading was confirmed by β-actin staining (lower panel). B: Densitometric analysis of ICAM-1 expression. ICAM-1 levels in arbitrary units are expressed as fold induction of ICAM-1 levels in sham-operated rats. Results are expressed as mean ± S.E.M. of three separate experiments. $^{**}P < 0.01$ vs. control group.

protein expression in injured carotid arteries of control animals as compared to sham-operated rats (Fig. 4A). Treatment of rats with 6.5 μg/site 2-cyclopenten-1-one ($n = 5$) or with 25 μg/site PGJ_2 ($n = 5$) did not modify ICAM-1 expression that was faintly but not significantly reduced by either 13 μg/site 2-cyclopenten-1-one ($n = 6$) or 50 μg/site PGJ_2 ($n = 6$) (data not shown). Densitometric analysis showed that both agents only at highest doses used significantly ($P < 0.01$) reduced ICAM-1 protein expression by about 40% (Fig. 4B).

4. Discussion

In this study we show that local delivery of either the cyclopentenone prostanoid PGJ_2 or 2-cyclopenten-1-one, the active component of cyPGs, reduces restenosis by affecting favorably arterial remodeling as well as by inhibiting neointima formation. This effect was associated with inhibition of NF-κB activation that was triggered by vascular injury. As previously shown in rat-injured carotids [14,27], the NF-κB complex activated was found to be the heterodimer composed by the p50 and p65 subunits (data not shown). NF-κB inhibition resulted in a reduced expression of ICAM-1, one of various adhesion molecules that is activated via the NF-κB pathway and expressed in damaged vascular tissue [30]. In this study we observed an increase of ICAM-1 expression in endothelial cells, VSMCs and adventitia of injured carotids according to previous findings [31–33]. Several lines of evidence suggest that ICAM-1 could play an important role in the restenotic process. In fact, in a rabbit iliac artery balloon angioplasty model it has been demonstrated that a reduced expression of ICAM-1 was associated to a decreased restenosis [33]. Furthermore, it has been shown in ICAM-1-deficient mice that neointimal hyperplasia of vein bypass grafts was greatly reduced [31]. Although the importance of ICAM-1 in the mediation of cell adhesion to endothelium has been established, little is known about the role of ICAM-1 in VSMCs and adventitia. The expression of ICAM-1 on VSMCs may be relevant to the phenotypical change of VSMCs which is considered to be essential to the migration and proliferation of these cells to form neointimal layer [34]. On the other hand it has been observed in a rat model of vascular remodeling that an increase of ICAM-1 expression in adventitia contributes to the inflammatory infiltration leading to the perivascular fibrosis, an important event to wall shrinkage during pathological vascular remodeling [32]. Our results show that treatment with 2-cyclopenten-1-one or PGJ_2 actually inhibits important molecular events downstream of NF-κB activation leading to prevention of neointima formation as well as of arterial remodeling.

NF-κB is an attractive therapeutic target for the pharmacological control of vascular response to injury. In fact, it has been demonstrated that inhibition of NF-κB by an antisense oligonucleotide to the p65 subunit of NF-κB as well as by the antioxidant pyrrolidine dithiocarbamate, resulted in a reduction of neointima formation [13,27,35]. More recently it has been shown in a porcine balloon-injured coronary artery model that neointimal formation was reduced by using a double-stranded oligodeoxynucleotide with consensus NF-κB sequence demonstrating the feasibility of the 'decoy' strategy against NF-κB in the treatment of restenosis after angioplasty [36]. Consequently, the need for the development of effective NF-κB inhibitors with therapeutic efficacy is widely recognized. In this respect, cyPGs represent an interesting class of molecules.

PGs of the J family, including PGJ_2, Δ^{12}-PGJ_2 and 15-deoxy-$\Delta^{12,14}$-PGJ_2 are non-enzymatic metabolites of PGD_2 [37,38]. Recently it has been shown that the level of lipocalin-type PG D synthase (L-PDGS), which catalyzes the isomerization of PGH_2 to PGD_2 [39], was significantly increased in patients undergoing PTCA but not suffering of subsequent restenosis, whereas the level of L-PDGS remained unmodified in patients who developed restenosis [40]. It may be hypothesized that endogenous PGD_2 generated by L-PDGS could play a role in inhibiting the development of restenosis. The fact that the local administration of the PGD_2 metabolite PGJ_2 shown in this study is able to reduce neointima formation strengthens the hypothesis that generation of cyPGs in the vessels could be part of a physiological mechanism to

prevent the development of restenosis. The protective effect of cyPGs may arise from different biological activities characteristic of these molecules. CyPGs have in fact been considered as part of an endogenous defence mechanism by which cells preserve their homeostasis, and are known to possess cytoprotective properties via the activation of the heat shock transcription factor type 1 (HSF-1) and the induction of the synthesis of cytoprotective heat shock proteins [22]. Also, cyPGs are known to exhibit antiproliferative properties in a number of in vitro and in vivo tumor models [41]. It has also been reported that PGJ_2 prevents the proliferation of VSMCs, an important component of neointimal thickening following arterial balloon injury [42]. Moreover, cyPGs are characterized by a potent anti-inflammatory activity, due to their well-known effect as inhibitors of NF-κB activation during inflammation and viral infection [43–46]. CyPGs have been shown to act by blocking the phosphorylation and preventing the degradation of NF-κB inhibitor I-κB-α [24]. The IKK was identified as a selective molecular target for cyPGs, which bind directly to the cysteine-179 residue in the activation loop of the IKKβ subunit. This effect is dependent on the presence of a reactive cyclopentenoic moiety [45].

Apart of their direct effect as IKK inhibitors, the anti-inflammatory effect of cyPGs has also been attributed to their ability to activate peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor that interferes with the transcriptional activity of NF-κB [44,47] and whose activation inhibits VSMC proliferation [48]. Indeed, clinical observations indicate that treatment with fibrate PPARα activators lowers the progression of atherosclerotic lesions [49–51] and that the PPARγ agonist troglitazone decreases intimal thickness in human carotid arteries [52]. However, it has been demonstrated that the concentrations of selective PPARγ ligands required to mimic the effects of cyPGs are several orders of magnitude higher than that required to activate PPARγ [47]. Furthermore, it has been observed that cyPGs inhibit NF-κB activation in human cells expressing very low levels of PPARγ [24].

CyPGs are characterized by the presence of a reactive α,β-unsaturated carbonyl group in the cyclopentane ring (cyclopentenone) which appears to be the key structure for their effects since it enables this portion of the molecule to form Michael adducts with cellular nucleophiles, and covalently bind to cysteine residues of proteins [53]. The cyclopentenone structure itself (2-cyclopenten-1-one) has been shown to mimic the effect of cyclopentenone prostanoids in vitro, even though at higher concentrations than the natural compounds [23]. We have now shown that 2-cyclopenten-1-one also mimics the effect of the natural PGJ_2 inhibiting NF-κB activation in vivo, and that treatment with 2-cyclopenten-1-one is able to reduce neointimal formation in balloon-injured rat carotid arteries. It should be emphasized that 2-cyclopenten-1-one is 15–20 times less active than PGJ_2 in inhibiting NF-κB in an in vitro model in which human or murine cells are stimulated with either the mitogen TPA or tumor necrosis factor-α (data not shown). The similar activity of 2-cyclopenten-1-one and PGJ_2 in long-term experiments in vivo can be explained by the fact that PGJ_2 is known to be rapidly catabolized in vivo [37]. The difference in *c* log *P* values between 2-cyclopenten-1-one (0.03) and PGJ_2 (2.97) may also be influential in terms of dictating different rates of release of the two compounds from their reservoirs.

These results open new perspectives in the design and development of a novel class of molecules with potential activity in the prevention of restenosis. Alternatively, these compounds could also be useful in stent technology, in which the coating of stents with these agents may help to delay restenosis.

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