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Research Article

β-Adrenergic-induced CD40 overexpression on gingival fibroblasts: role of PGE_2

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Abstract

CD40, a member of the tumour necrosis factor-α receptor family, is constitutively expressed by cells of haematopoietic and non-haematopoietic origin, including fibroblasts. Signalling through this receptor molecule regulates inflammatory mediator secretion by many cell types. The work has been performed in healthy subjects and the authors studied, by cellular culture, flow cytometric analysis and ELISA assay, the expression of CD40 and PGE_2 (prostaglandin E_2) generation on gingival fibroblasts stimulated by β-AR (β-adrenoceptor) agonists. Herein, the authors demonstrate that β-AR subtype activation via their own specific agonists markedly increased CD40 expression on human gingival fibroblasts. This effect was prevented by β-AR subtype-specific antagonists. In addition, gingival fibroblast β-AR stimulation resulted in an increase in PGE_2 generation. The inhibition of PLA_2 (phospholipase A_2) and COX-1 (cyclo-oxygenase-1) but not COX-2 impaired β-AR increase of PGE_2, an effect that was restored by the addition of low concentrations of PGE_2, suggesting that PGE_2 generation is implicated in the mechanism underlying β-AR-agonist-mediated CD40 overexpression. Our work has revealed an endogenous β-AR mediator network involving gingival fibroblasts.

Keywords: β-adrenoceptor; CD40; fibroblast cell; prostaglandin E_2 (PGE_2)

1. Introduction

CD40 is a cell surface receptor glycoprotein, which is constitutively expressed by many cells of haematopoietic (Stamenkovik et al., 1989) and non-haematopoietic (Hollenbaugh et al., 1995) origin. CD40L is a ligand for this receptor molecule, which is principally expressed on activated T-cells (Armitage et al., 1992). CD40-CD40L-mediated contact is considered a crucial step in T-cell-dependent, B cell co-stimulatory pathways (Noelle et al., 1992) and in primary T-cell helper function (Van Essen et al., 1995). While significant progress has been made in the biological significance of CD40 expression on cells of haematopoietic origin (B or T-cells), much less is known about the role that the CD40-CD40L signalling system plays in the pathophysiology of non-immune cells.

Fibroblasts, as non-immune cells, have been shown to express CD40 both *in vivo* (Fries et al., 1995) and *in vitro* (Yellin et al., 1995). Furthermore, it has been demonstrated that CD40 ligation signals proliferation and up-regulates cytokine secretion by sinovial fibroblasts *in vitro* (Yellin et al., 1995). Fibroblasts participate in the histopathological changes associated with a number of chronic inflammatory diseases, such as rheumatoid arthritis (Arend and Dayer, 1990) and periodontitis (Schroeder and Page, 1972). Moreover, it has been suggested that the CD40-mediated interaction of fibroblasts with activated T-cells may play a role in regulating the contribution of these cells to connective tissue pathology. Expression of CD40 has been previously reported in gingival fibroblasts (Fries et al., 1995). It is possible that signalling through CD40 may play an immunopathogenic role in periodontitis by augmenting the inflammatory responses of gingival fibroblasts secreting considerable amounts of inflammatory cytokines and/or mediators (Irwin et al., 1994; Phipps et al., 1995; Phipps and Blieden, 1995; Dongari-Bagtzoglou and Ebersole, 1996).

β-ARs (β-adrenergic receptors) are expressed on a wide variety of tissues and are recognized as pivotal functional regulators of the cardiac, pulmonary, vascular, endocrine and central nervous systems. Although the expression of β-ARs in human fibroblasts has been described (Schallreuter et al., 1993), their functional significance in this cell type has been recognized only recently. The β_1-AR (Furlán et al., 2005), β_2-AR (McSwigan et al., 1981) and β_3-AR (Furlán et al., 2005) subtypes are expressed on the membranes of fibroblasts and are known to couple $G_{\alpha s}$, the 'classic' G_α protein (Rich and Karpen, 2002) which, upon activation, increases intracellular cAMP levels (Xiao and Lakatta, 1995). The role of the β-AR signalling system in cutaneous wound repair by decreasing keratinocyte migration to permit re-epithelization has been demonstrated (Pullar and Isseroff, 2005). In chronic inflammatory diseases, β-AR stimulation modifies the release of inflammatory mediators such as PGE_2 (prostaglandin E_2) (Straub and Härle, 2005) and triggers cAMP accumulation with a subsequent increase in PGE_2 production (Ehrlich and Wyler, 1983). Also, PGE_2 (Ehrlich and Wyler, 1983), nitric oxide (Zhu et al., 2001) and phosphodiesterase 4 inhibitors (McSwigan et al., 1981) increase intracellular cAMP levels and inhibit wound contraction by dermal fibroblasts (Kohyama et al., 2004). Additionally, noradrenaline–PGE_2 release in rabbit splenic fibroblasts via α-adrenoceptors involves influx of calcium and activation of PLA_2 (phospholipase A_2) (Brückner-Schmidt et al.,

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Abbreviations: AA, arachidonic acid; β-AR, β-adrenoceptor; COX, cyclo-oxygenase; FBS, fetal bovine serum; INDO, indomethacin; MoAbs, monoclonal antibodies; OBAA, 4-(4-octadecylphenyl)-4-oxobutenoic acid; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2.

1981a). The α-adrenoceptors involved had properties different from those clinically reported, so far, for the α_1-adrenoceptor (Brückner-Schmidt et al., 1981b).

COX (cyclo-oxygenase), also known as PG (prostaglandin) endoperoxide synthase, is an enzyme that catalyses the conversion of arachidonate to prostaglandins and other prostanoids (WL Smith et al., 1997). It has been shown that orbital fibroblasts are particularly susceptible to the up-regulation of endoperoxide synthase expression by certain pro-inflammatory cytokines and IL-1β (Wang et al., 1996). This induction, mediated through both transcriptional and post-transcriptional mechanisms, results in dramatic increases in the production of PGE_2 (Wang et al., 1996). Furthermore, it has been reported that PGE_2 generation is enhanced through the ligation of CD40 (Zhang et al., 1998). This action of CD40 ligand on PGE_2 production has not been characterized in human gingival fibroblasts.

Taking together these observations, we can consider: CD40 function in fibroblasts is important for pathogenesis of inflammatory conditions including periodontal disease; β-AR stimulation and the downstream production of PGE_2 is important for fibroblast function in wound repair and cytokines; and CD40 ligations increase PGE_2 synthesis via the COX enzyme.

The objective of this study is to examine the role of CD40 in β-AR-induced PGE_2 generation (or vice versa) and its biological outcome.

2. Materials and methods

2.1. Subjects

The study group comprised 25 subjects (22 males and 3 females) who were attending odontology clinics in the metropolitan area of Buenos Aires. The mean age of the subjects was 40 years (range, 25-58 years). Inclusion criteria for healthy individuals were no interproximal attachment loss and no signs of other inflammatory conditions or general diseases. None of the healthy control subjects had a known systemic disorder that could affect periodontal conditions; no patients had used antibiotics in the last 6 months. Smokers were excluded. Healthy control subjects consented to participate in the study. The study was conducted according to the tenets of the Declaration of Helsinki (1964). The Ethical Committee of the School of Dentistry of the University of Buenos Aires approved the study.

2.2. Cell culture

The gingival fibroblasts used throughout the experiments are from separate persons. Cell cultures of human gingival fibroblasts were established from human normal gingiva and grown in DMEM (Dulbecco's modified Eagle's medium) (GIBCO) supplemented with 10% FBS (fetal bovine serum), 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml) in a 5% CO_2 environment at 37°C, as previously described by Varani et al. (1990). The medium was replenished every 3–4 days. Confluent T-cells were subcultured to multiwell plates by scraping the monolayer. Assays were performed using adherent cultured cells. Cells of passages 6–8 were used throughout.

2.3. Cell treatment

During the subculture plating of cells to 24-well multiwell plates, equal numbers of cells (1×10^6 cells/well) were plated to each well. Before each treatment, serum-free media were added to each well overnight. The cells were then treated with 1×10^{-9} M dobutamine (β_1-agonist), soterenol (β_2-agonist) and ZD 7114 (β_3-agonist) alone or in the presence of 1×10^{-7} M atenolol (β_1-antagonist), butoxamine (β_2-antagonist) and SR 59230A (β_3-agonist) respectively. The β-AR antagonists were added to the cells 1 h before stimulation with β-AR agonists. In each experiment, the treatment groups were compared with the control group. In the control group, the cells were incubated in the same type of serum-free media and for the same incubation time as in the treatment group but did not receive any stimulation of β-AR agonists or any β-AR antagonists.

Activation or inhibition with agonist or antagonist drugs were performed on adherent culture cells; percentage of confluence for all assays was about 95–96%.

2.4. Flow cytometric analysis

After washing cells with PBS, flow cytometric analysis using adherent cultured cells was done. After incubation for 1 h at 4°C, the cells were washed and further incubated for 30 min with rabbit anti-human IgG FITC-conjugated $F(ab)'_2$ fragments (1:100). Cells were then fixed with 1% paraformaldehyde. The percentage of positive cells was determined by the threshold set using isotopic controls. Analyses of the expression of cell-surface CD40 protein were done using FITC-conjugated MoAbs (monoclonal antibodies) to human CD40 or isotype-matched controls (Becton Dickinson) in a standard staining procedure. Briefly, fibroblasts (1×10^6 cell/well), using the same protocol and identical reagents as in the passages of fibroblasts in culture, were incubated (or not incubated) with different β-AR agonists (1×10^{-9} M) for 90 min in the presence of isotype-matched control (negative controls). When different β-AR antagonists, INDO (indomethacin; 5×10^{-6} M) or PGE_2 (1×10^{-11} M) were used, they were added 10 min before agonists. Cells with the corresponding antibody were washed with PBS containing 2.5% FBS, fixed in 0.5% paraformaldehyde and analysed with a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson), with appropriate forward and side-scatter adjustment for human skin fibroblast cells. Mean fluorescence intensity values obtained by staining with specific MoAbs were corrected by subtraction of background values (isotype-matched control MoAb).

It is important to take into account that the proper way to assess the role of each drug would be to treat with each of the drugs at the concentrations cited in the text (treated group), with a vehicle only (basal); then wash the cells and stain with anti-CD40 and isotype control antibody (negative control).

2.5. PGE_2 assay

Human gingival fibroblasts (1×10^6 cell/well), treated as indicated above, were seeded in 48 cell plates. Thereafter, all procedures were those indicated in the protocol of the Prostaglandin E_2 Biotrak Enzyme Immuno Assay (ELISA) System (Amersham

Biosciences). PGE_2 results were expressed as microlitres per millilitres (μl/ml).

2.6. Drugs

Stock solutions of atenolol (specific β_1-antagonist), butoxamine (specific β_2-antagonist), SR 59230A (β_3-antagonist), dobutamine hydrochloride (specific β_1-agonist), soterenol (specific β_2-agonist), ZD 7114 (specific β_3-agonist), INDO (inhibitor of COX-1 and COX-2), PGE_2, SQ 22536 (adenylate cyclase inhibitor) and verapamil (calcium flux inhibitor) (Sigma Chemical Co.) and ZD 7114, SR 59230A, OBAA [4-(4-octadecylphenyl)-4-oxobutenoic acid], 1-[4,5-bis (4-methoxyphenyl)-2-thiazolyl] carbonyl-4-methylpiperazine hydrochloride (FR 122047) and 5-bromo-2-(4-urophenyl)-3-[4-(methylsulfonyl)phenyl]-tiophene (DuP 697) (Tocris Cookson Inc) were freshly prepared before each experiment.

2.7. Statistical analyses

The Student's *t* test for unpaired values was used to determine the significance level. ANOVA (analysis of variance) and a post hoc test (Dunnett's method and Student-Newman-Kuels test) were used when a pairwise multiple comparison procedure was necessary. Differences between means were considered significant if $P<0.05$.

3. Results

Figure 1 shows the comparatively maximal effects of β_1-, β_2- and β_3-adrenergic agonists on CD40 expression in human gingival fibroblast cultures. As shown in Figure 1(A), all three specific adrenergic agonists significantly enhanced the CD40 expression above basal levels (CD40 alone). The basal value of positive cells was 29 ± 3 and increased to 223 ± 20, 277 ± 27 and 305 ± 28 in the presence of 1×10^{-9} M soterenol, 1×10^{-9} M dobutamine, and 1×10^{-9} M ZD 7114 respectively (Figure 1B). There were no significant differences between the actions of the three specific adrenergic agonists. There are no differences between basal values and isotype-matched control (22 ± 3, $n=5$).

To assess whether CD40 overexpression resulted in specific functional postsynaptic β-AR subtype activation, we measured the CD40 expression in human gingival fibroblast cultures using specific β-AR blocker subtypes. As shown in Figure 2(A), the fluorescence intensity (positive cells) effects of dobutamine (1×10^{-9} M), soterenol (1×10^{-9} M) and ZD 7114 (1×10^{-9} M) were reduced in the presence of atenolol (1×10^{-7} M), butoxamine (1×10^{-7} M) and SR 59230A (1×10^{-7} M). Figure 2(B) shows the mean values of fluorescence intensity (positive cells) obtained in the presence of the β_1-, β_2- and β_3-AR agonists and after the treatment with their respective β_1-, β_2- and β_3-AR antagonists. When the β_1-, β_2- and β_3-AR blockers were used with the non-specific agonists, they were ineffective in our study system.

To elucidate whether the CD40 overexpression triggered by β-AR stimulation was dependent on the increment of PGE_2 generation, the effect of 5×10^{-6} M INDO was studied. Figure 3(A) shows that the inhibition of COX by INDO reduced the effect of dobutamine, soterenol or ZD 7114. Moreover, when exogenous

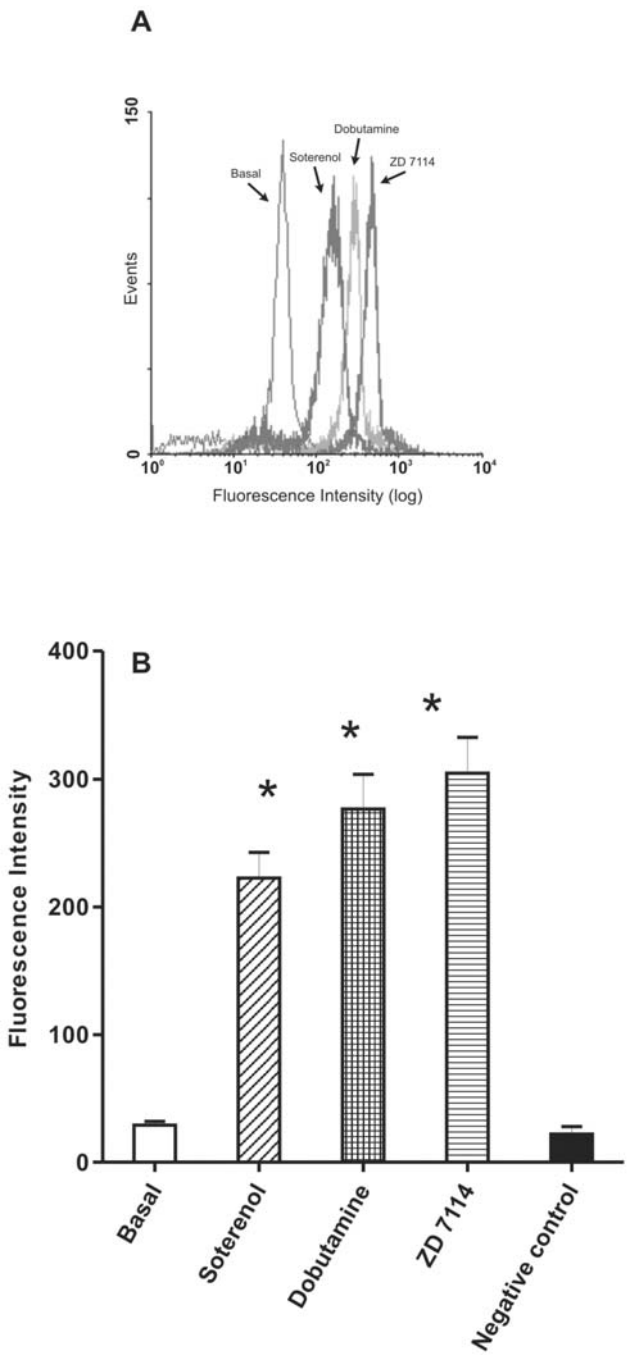

Figure 1 Effects of β-AR subtype agonists on CD40 expression in human gingival fibroblast cultures

(**A**) Representative flow cytometric analysis of CD40 expression. (**B**) Mean values of fluorescence intensity (positive cells) for each experimental group. Fibroblast cells were incubated in medium with CD40 alone (basal, vehicle only) or in medium with CD40 plus 1×10^{-9} M dobutamine, soterenol and ZD 7114. The isotope-matched control (negative control) is also shown. Values are the mean ± S.E.M. of six experiments from different donor samples in each group evaluated in triplicate. *$P<0.001$ compared with basal.

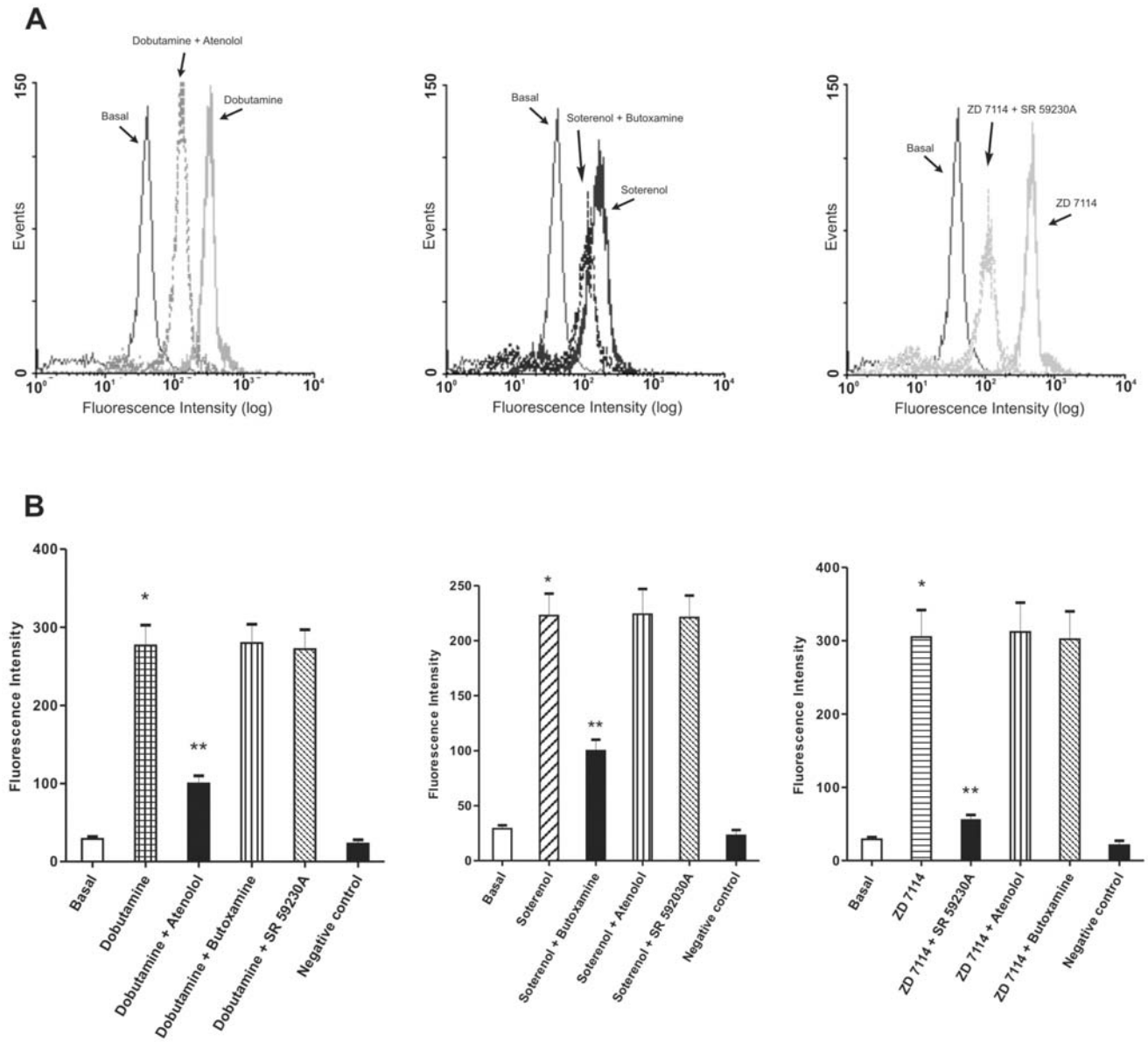

Figure 2 **Influence of specific β-AR subtype blockers on β-AR-agonist-stimulated CD40 expression in human gingival fibroblast cultures**
(**A**) Representative flow cytometric analysis of CD40 expression. (**B**) Mean values of fluorescence intensity (positive cells) for each experimental group. Fibroblast cells were incubated in medium with CD40 alone (basal, vehicle only) or in medium with CD40 that was previously in the presence of 1×10^{-9} M dobutamine with or without 1×10^{-7} M atenolol, butoxamine and SR 59230A (left-hand panel) or 1×10^{-9} M soterenol with or without 1×10^{-7} M butoxamine, atenolol and SR 59230A (middle panel) or 1×10^{-9} M ZD 7114 with or without 1×10^{-7} M SR 59230A, atenolol and butoxamine (right-hand panel). The isotype-matched control (negative control) is also shown. Values are the mean ± S.E.M. of six experiments from different donor samples in each group performed in triplicate. *$P<0.001$ compared with basal; **$P<0.001$ compared with the agonist values alone.

PGE_2 (1×10^{-9} M) was added, the inhibitory effect of INDO was restored. Figure 3(B) shows the mean values obtained for each β-AR agonist subtype studied. In order to discern which AA (arachidonic acid) cascade enzymes are implicated in the CD40 overexpression triggered by β-AR agonist-generated PGE_2, several inhibitors of this cascade reaction were used. It can be seen in Table 1 that inhibition of PLA_2 by OBAA (5×10^{-6} M) (Köhler et al., 1992) or COX-1 by FR 122047 (5×10^{-8} M) (Ochi and Goto, 2002) prevented the stimulatory action of dobutamine, soterenol and ZD 7114 induced CD40 overexpression, while COX-2 inhibition by DuP697 (5×10^{-8} M) was without effect.

To confirm if the β-AR stimulation is able to trigger PGE_2 generation by human gingival fibroblast cultures, we measured PGE_2 production in the presence of specific β-AR subtype agonists. It can be seen in Figure 4 that dobutamine (Figure 4A), soterenol (Figure 4B), and ZD 7114 (Figure 4C) were able to increase PGE_2 production in a dose-response fashion. The β_1-, β_2- and β_3-AR agonist dose-response curves were shifted to the

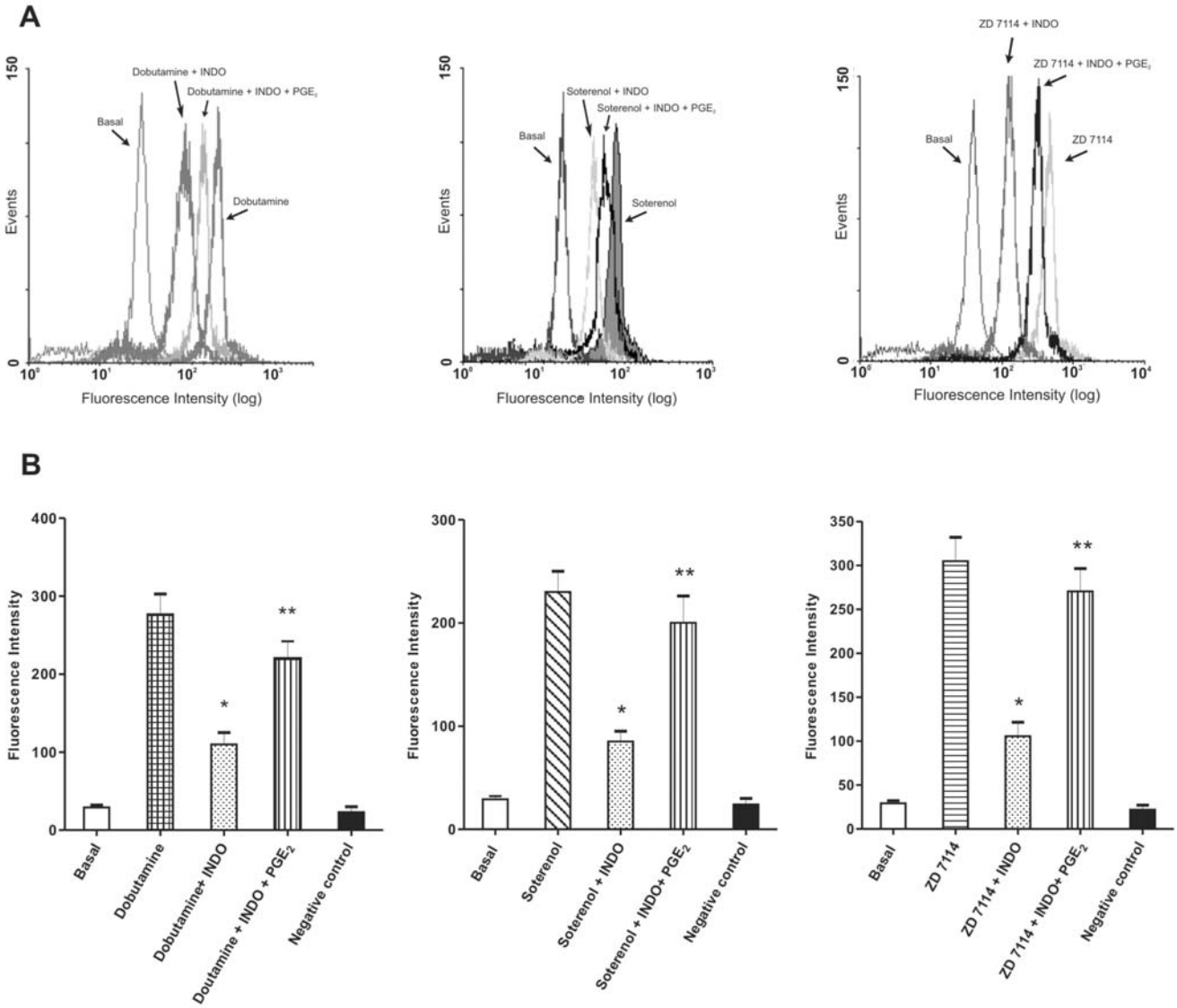

Figure 3 **Influence of INDO and exogenous PGE_2 on β-AR-agonist-stimulated CD40 expression in human gingival fibroblast cultures**

(**A**) Representative flow cytometric analysis of CD40 expression. (**B**) Mean values of fluorescence intensity (positive cells) for each experimental group. Fibroblast cells were incubated in medium with CD40 alone (basal, vehicle only) or in medium with CD40 plus 1×10^{-9} M dobutamine with or without 5×10^{-6} M INDO (left-hand panel) or 1×10^{-9} M soterenol with or without 5×10^{-6}M INDO (middle panel) or 1×10^{-9} M ZD 7114 with or without 5×10^{-6} M INDO (right-hand panel). In all cases, the reversion of PGE_2 (1×10^{-11} M) on the inhibitory effect of INDO is also shown. The isotype-matched control (negative control) is also shown. Values are the mean $\pm$ S.E.M. of five experiments from different donor samples in each group performed in triplicate. *$P<0.001$ compared with basal; **$P<0.001$ compared with the agonist plus INDO.

Table 1 **Action of AA-enzyme inhibitors on β-AR agonist-stimulated CD40 expression on human gingival fibroblast cultures**

Values are the mean $\pm$ S.E.M. of n number of experiments performed in triplicate. Basal values without OBAA and FR 122047 blocker are 31.2 ± 4 and 29.6 ± 7 respectively, and they were not modified by DuP 695 (5×10^{-8} M, 28.8 ± 3). All the experiments were conducted as described in the Materials and methods section.

	Fluorescence intensity (positive cells)			
Drug additions	**Dobutamine (1×10^{-9} M)**	**Soterenol (1×10^{-9} M)**	**ZD 7114 (1×10^{-9} M)**	***n***
None	285 ± 281	232 ± 25	298 ± 26	6
OBAA (5×10^{-6} M)	2 ± 10*	95 ± 9*	84 ± 8*	7
FR 122047 (5×10^{-8} M)	96 ± 10*	85 ± 9*	79 ± 9*	7
DuP697 (5×10^{-8} M)	279 ± 26	241 ± 27	309 ± 29	6

* $P<0.001$ compared with none.

right in the presence of atenolol, butoxamine and SR 59230A (all at 1×10^{-7} M; Figures 4A, 4B and 4C). CD40L augmented the PGE_2 generation (20 ± 3 μl/ml, $n=4$) mimicking the β-AR agonist effect.

To determine the mechanism by which β-AR stimulation induced CD40 overexpression, the effects of L-type calcium channel and adenylate cyclase inhibitors were explored. Table 2 shows that verapamil (1×10^{-6} M), but not SQ 22536 (5×10^{-6} M), at concentrations able to inhibit L-type calcium channel or adenylate cyclase activity respectively significantly reduced the CD40 overexpression triggered by the β-AR agonist subtypes. Every inhibitory agent at doses used had no effect in fibroblast cells (data not shown).

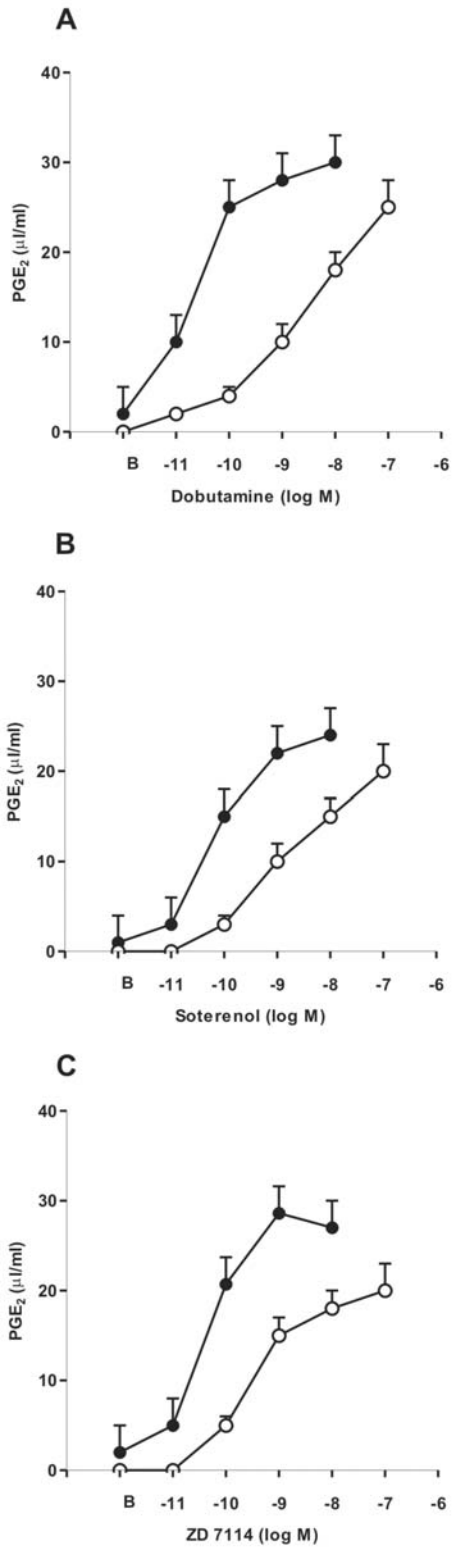

Figure 4 PGE_2 production triggered by β-AR-subtype agonists in human gingival fibroblast cultures

PGE_2 generation by increasing concentrations of: (**A**) dobutamine alone (●) or in the presence of 1×10^{-7} M atenolol plus dobutamine (○); (**B**) soterenol alone (●) or in the presence of 1×10^{-7} M butoxamine plus soterenol (○); and (**C**) ZD 7114 alone (●) or in the presence of 1×10^{-7} M SR 59230A plus ZD 7114 (○). Results are the mean ± S.E.M. of six experiments from different donor samples in each group performed in triplicate. *$P<0.001$ compared with basal.

Table 2 Action of adenylate cyclase and calcium blockers on β-AR agonist-stimulated CD40 expression on human gingival fibroblast cultures

Values are the mean ± S.E.M. of *n* number of experiments performed in triplicate. Basal values without adenylate cyclase inhibitor and calcium L-type channel blocker are 29.2 ± 2 and 27.8 ± 5 respectively, and they were not modified by SQ 22536 (5×10^{-6} M, 27.8 ± 3) and verapamil (1×10^{-6} M, 28.7 ± 4). All the experiments were conducted as described in the Materials and methods section.

	Fluorescence intensity (positive cells)			
Drug additions	**Dobutamine (1×10^{-9} M)**	**Soterenol (1×10^{-9} M)**	**ZD 7114 (1×10^{-9} M)**	***n***
None	277 ± 27	223 ± 20	305 ± 28	6
SQ 22536 (5×10^{-6} M)	267 ± 25	229 ± 21	298 ± 21	7
Verapamil (1×10^{-6} M)	92 ± 8*	89 ± 9*	91 ± 9*	7

* $P<0.001$ compared with none.

4. Discussion

This study demonstrated that the presence of active β-AR in human gingival fibroblasts modulates CD40 expression. We found that β_1-, β_2- and β_3-AR agonists are implicated in this effect. Thus, functional studies demonstrated that dobutamine, soterenol and ZD 7114 were able to enhance CD40 expression and triggered the production of PGE_2. The increase in PGE_2 levels was also observed with exogenous CD40L. Since all three β-AR subtypes cause CD40 overexpression, a receptor cross-activation could be possible. It is known that β-ARs are coupled to G_s, given a parallel increase in intracellular cAMP and in calcium influx (Emorine et al., 1989). The fact that the inhibition of the L-type calcium channel by verapamil, but not the inhibition of adenylate cyclase by SQ 22536, significantly reduced the CD40 overexpression triggered by the β-AR agonist subtypes suggests that the increase in calcium flux is a key factor in β-AR-induced CD40 expression. Alternatively, the rise in cytosolic calcium could activate PLA_2 with subsequent activation of COX-1 which induced PGE_2 generation.

These two features involving fibroblast β-AR activation (over-expression of CD40 and increased production of PGE_2) are consistent with the remodelling phenomena that occur in human gingival connective tissue during inflammatory processes. They potentially represent the molecular basis for gingival fibroblast activation in inflammatory processes and explain multiple aspects of the complex interplay between the immune system and connective tissue. The up-regulation for β-AR agonists on CD40 ligation is apparently dependent on the stimulation of PLA_2 and COX-1 with an increased production of PGE_2, as is evidenced by the lack of effect when OBAA, INDO and FR 122047 were present, and exogenous PGE_2 restored the β-AR agonist fibroblast response. All of these suggest that the interruption of the molecular consequences of the CD40/CD40L bridge occurs via some products of the AA cascade. This fact may account for the therapeutic benefit associated with the use of non-steroidal anti-inflammatory agents in chronic periodontal disease.

Thus, fibroblasts are known to participate in the histopathology changes associated with periodontitis (Schroeder and Page, 1972), and the inflammatory reaction observed during the course of experimental periodontitis could be the result of the large amounts of PGE_2 produced by gingival fibroblasts in response to sympathetic stimulation. Moreover, increase in sympathetic

activity in experimental periodontitis has been described (Busch et al., 2009). The fact that gingival fibroblasts functionally express the CD40 receptor, point to the role of CD40L-expressing cells in playing an immunopathogenic function in periodontitis. Thus, the signals delivered to gingival fibroblasts through CD40 over-expression by β-AR agonist activation may play a role in augmenting the inflammatory processes in periodontitis by up-regulating the PGE_2 increased secretion from these cells.

It had been reported previously that CD40 engagement on orbital fibroblasts results in an increase in the expression of IL-6 and IL-8 (Sempowski et al., 1998). Thus, it would appear that the expression of a number of potentially important genes and their products is enhanced through the CD40/CD40L bridge. Lung (Sempowski et al., 1997a), gingival (Sempowski et al., 1997b) and thyroid-derived (TJ Smith et al., 1997) fibroblasts express CD40, and this expression can be substantially up-regulated by CD40 ligation in lung fibroblasts (Sempowski et al., 1997a). In lung fibroblasts, IFN-γ enhanced the number of CD40 ligand-binding sites on the surfaces of these cells (Sempowski et al., 1997a, 1998).

CD40 expression is relevant on synovial membrane cells with fibroblast-like morphology in rheumatoid arthritis (Spicer et al., 1996), as well as on cultured fibroblasts from patients with periodontitis (Yellin et al., 1995), suggesting an association of fibroblast CD40 expression levels with inflammatory disorders.

As is known, β-AR agonists can stimulate proliferation and migration of fibroblasts and keratinocyte cells (Pullar et al., 2003) regulating skin wound healing (Pullar et al., 2006). Pretreating human gingival fibroblasts with specific β-AR antagonists prevented the β_1-, β_2- and β_3-AR-induced CD40 overexpression, thus modulating or contributing to repair of an inflammatory focus on the human gingiva. The role of the β-AR agonist/antagonist signalling system in cutaneous wound repair decreases keratinocyte cell migration, an essential step in wound re-epithelialization (Pullar et al., 2003).

Our work uncovers novel gingival fibroblast biology and a possible significant role for the adrenergic hormonal mediator network in the course of periodontitis and skin wound healing. Overall, the results of these findings suggest that β-AR activation induces PGE_2 generation, which is involved in CD40-CD40L activation, increase PGE_2 production.

Author contribution

César Furlán carried out the experimental work and created line diagrams and bar graphs. Leonor Sterin-Borda made the graphs, edited the manuscript and wrote the Discussion section. Enri Borda also helped in editing the manuscript.

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