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ORIGINAL ARTICLE

Platelet-derived growth factor (PDGF) isoform and PDGF receptor expression in drug-induced gingival overgrowth and hereditary gingival fibrosis

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OBJECTIVE: To investigate possible associations between platelet-derived growth factor (PDGF), PDGF receptor expression and macrophages in drug-induced and hereditary gingival overgrowth.

MATERIALS AND METHODS: Tissues from patients with drug-induced gingival overgrowth (DIGO) ($n = 10$) and hereditary gingival fibrosis ($n = 10$) were studied and compared with 'control' gingiva ($n = 10$). Expression of PDGF and its α and β receptors was investigated immunohistochemically and by RT-PCR. Macrophages were identified by immunostaining for CD68.

RESULTS: PDGF isoforms and receptors were detected in most cells within all specimens. There were no differences in the numbers of macrophages, or fibroblasts expressing PDGF or receptors, between groups. The level of PDGF expression by fibroblasts, determined by absorbance measurements, was similar between groups for PDGF A. Significantly lower levels of total PDGF and the receptors were detected in drug-induced overgrowth compared to those in hereditary fibrosis ($P < 0.004$) and control specimens ($P < 0.034$). All specimens expressed mRNA for PDGF A, PDGF B and α and β receptors.

CONCLUSIONS: These data do not support a pivotal role for macrophage-derived PDGF B in the pathogenesis of DIGO. They suggest that fibroblasts in drug-induced lesions have a lowered capacity to produce, and respond to, PDGF, a property not shared by fibroblasts associated with hereditary fibrosis.

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Keywords: gingival overgrowth; drug induced; hereditary gingival fibrosis; platelet-derived growth factor; platelet-derived growth factor receptors; immunohistochemistry; RT-PCR

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Introduction

Gingival overgrowth is a relatively common side effect of certain drugs, such as phenytoin, nifedipine and cyclosporin (Seymour *et al*, 1996). Clinically similar overgrowth is also seen as a usually autosomal dominant disorder, hereditary gingival fibromatosis (HF), which can occur alone or as part of a syndrome (Ramer *et al*, 1996). Both types of overgrowth are fibroepithelial in nature and are characterised histologically by a thickened, parakeratinised epithelium with elongated rete pegs and an increase in the extracellular matrix materials within the connective tissue (Collan *et al*, 1982; O'Valle *et al*, 1994).

Fibrosis is the result of a variety of biochemical signals from many cell types which stimulate chemotaxis and proliferation of fibroblasts and elaboration of extracellular matrix components by fibroblasts (Werner and Grose, 2003). Many of the events leading to fibrosis and gingival overgrowth are thought to be similar to those involved in normal wound healing and scar formation. PDGF is an important cytokine that is upregulated during both wound healing and fibrosis (Cochran and Wozney, 1999). In mammals there are three isoforms distinguished by their effects on chemotaxis, cell growth, expression with respect to other growth factors and receptor-binding characteristics. The three isoforms consist of two homodimers (AA and BB) and one heterodimer (AB) and are expressed by a variety of cell types including fibroblasts, macrophages and epithelial cells. However, it has been suggested that there is variation in which chain is expressed by which cell type (Sasahara *et al*, 1991; Pierce *et al*, 1995).

The effects of PDGF on cells is currently thought to be mediated via two receptors (α and β), which are distinguished by their ability to bind different PDGF chains (α binds both A and B chains, β only binds the B chain). Because each chain of a PDGF molecule has a receptor-binding site, receptor–ligand interaction causes dimerisation of the receptors with the nature of the

resulting receptor dimer being dependent upon the PDGF isoform. For example, interaction with PDGF AA results in $\alpha\alpha$ receptor dimer formation whereas PDGF BB allows the generation of $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$ receptor dimers (Heldin *et al*, 1998). Thus, changes in the relative amounts of B chain and/or α receptor would potentially have greatest effect on moderating the biological consequences of PDGF activity.

PDGF B expression has been shown to occur early in gingival wound healing (Green *et al*, 1997) and is increased in non-drug-induced fibrosis (systemic sclerosis; Xue-yi *et al*, 1998) and cyclosporin-induced nephrotoxicity in rat kidney (Shehata *et al*, 1994). The PDGF BB isoform is also known to cause proliferation of periodontal fibroblasts and modulate their production of extracellular matrix components (Mailhot *et al*, 1995). In addition, data published from one group (Nares *et al*, 1996; Plemons *et al*, 1996; Iacopino *et al*, 1997) have indicated that an important event in gingival overgrowth associated with either cyclosporin or phenytoin therapy is upregulation of PDGF B chain expression by gingival macrophages. Taken together these findings support a role for PDGF B in the pathogenesis of gingival overgrowth.

Less is known about the activities of PDGF A or changes in cell surface receptor expression. In early gingival wound healing, PDGF A expression appears to be greater than PDGF B and differential expression of receptors has been noted between different cell types (Green *et al*, 1997). Such linked changes suggest that in normal wound healing there is targeting of particular PDGF isoforms to different cell populations.

Although PDGF B has been implicated in the pathogenesis of drug-induced gingival overgrowth (DIGO), there have been no studies to determine the possible roles of PDGF A or local expression of its α and β receptors. Furthermore, no studies on PDGF have been reported on the clinically similar overgrowth seen in HF. Thus, the purpose of this immunohistochemical and RT-PCR study was to determine the presence and distribution of PDGF (A and B) and its receptors in control gingival tissues and those from patients with DIGO and HF.

Material and methods

Gingival specimens

Gingival tissues, excess to any required for diagnostic purposes, were obtained with informed consent from 30 subjects aged between 13 and 69 years (Table 1). Ten of these subjects were organ transplant recipients who were undergoing surgical reduction of their gingival overgrowth. All of these patients had been medicated with cyclosporin or cyclosporin in combination with other putative overgrowth-inducing antihypertensive drugs (e.g. nifedipine) for a minimum of 6 months. A further 20 specimens were obtained from patients who had never taken cyclosporin or other known overgrowth-inducing drugs. These included tissues from 10 patients undergoing surgical reduction for HF and 10 non-overgrowth control subjects who were undergoing a variety of procedures in which gingival tissue was removed (Table 1). Gingival tissues were fixed in 10% neutral buffered formalin (18–24 h) and processed via ethanol and xylene to paraffin wax (60°C), using a Shandon Hypercenter2 (Shandon Ltd, Astmoor, UK). Portions of some specimens were also snap frozen and stored in liquid nitrogen.

Immunohistochemistry

All immunostaining was performed on freshly cut, 5 μm thick, paraffin sections that had been pretreated by trypsin digestion (0.1% trypsin; Difco, Detroit, MI, USA, 1:250 grade, 30 min). Sections were stained using a biotin–streptavidin immunoperoxidase technique (StrAviGen, Biogenex, San Ramon, CA, USA) as previously reported (Mason *et al*, 2000; Wright *et al*, 2001) and rabbit anti-peptide, primary antibodies specific for PDGF A (Biotechnology Inc, Santa Cruz, USA, sc-128; 2 μg ml^{-1}; detects AA and AB forms of PDGF), PDGF A/B (Biogenex; 376P raised to a PDGF B peptide sequence sharing high homology with PDGF A; 2 μg ml^{-1}; detects AA, AB and BB forms of PDGF) and their α and β receptors (Santa Cruz; sc-338 and sc-339; 0.6 μg ml^{-1}). Bound peroxidase was visualised using 3,3′-diaminobenzidine reagent (5 min). When required, immunostained sections were lightly counter stained in Meyer's haematoxylin and mounted in Xam. All reagent dilutions and washings were performed in 0.01 M phosphate buffered saline (PBS), pH 7.6.

Immunostaining for each antigen was performed on all specimens at the same time to maximise comparability of staining between specimens. Negative staining controls included omission of the primary layer and substitution of the primary layer with PBS. Specificity of the PDGF A chain and α and β receptor antibodies was confirmed by checkerboard peptide blocking experiments. The working dilution of each antibody was incubated with 10-fold excess (by weight) of peptide (Santa Cruz) overnight at 4°C, prior to staining. In all cases, staining was abolished by homologous peptide (Figure 1) but unaffected by pre-incubation with peptides corresponding to other chain or receptor types.

Staining for the macrophage marker CD68 was performed using monoclonal antibody PG-M1 (1/200; Dako, Cytomation Ltd, Ely, UK) in a similar manner to that described for PDGF and its receptors except that the DAB reaction product was intensified using 0.5% $CuSO_4$ in saline (5 min) prior to mounting.

Examination of sections

Fibroblast counts were performed using a microscope fitted with an eyepiece graticule at a magnification of ×320. Fibroblasts were defined as cells showing tapering bipolar spindle-shaped morphology with or without additional cell processes. Incorporated into this definition were cells showing characteristics of both active (relatively abundant cytoplasm and large oval nucleus) and inactive (smaller nucleus and scant cytoplasm) fibroblasts. All fibroblast-like cells within the graticule area (0.1 mm^2) were counted and recorded as positive

Table 1 Clinical details of patients and controls

Number/age /sex	*Diagnosis*	*Degree of overgrowth*[a]	*Drug history*[b]	*Other information*	*Inflammation*[c]
1/32/F	Periodontal disease	–	–	Flap	++
2/38/M	Periodontal disease	–	–	Gingivectomy	++
3/39/F	Gingival health	–	–	Distal wedge	+
4/42/F	Periodontal disease	–	Ventolin	Gingivectomy	++
5/45/F	Periodontal disease	–	Oral contraceptive	Flap	++
6/49/F	Gingival health	–	HRT	Distal wedge	+
7/52/M	Gingival health	–	–	Crown lengthening	–
8/53/F	Gingival health	–	–	Crown lengthening	–
9/59/M	Periodontal disease	–	–	Flap	++
10/61/F	Periodontal disease	–	HRT	Flap	++
11/13/M	HF[d]	Moderate	–		–
12/27/F	HF	Moderate	–		–
13/28/F	HF	Moderate	–		+
14/35/F	HF	Moderate	–		–
15/36/F	HF	Moderate	–		–
16/39/F	HF	Severe	Propranolol		–
17/42/F	HF	Severe	–		+
18/42/F	HF	Severe	–		–
19/45/F	HF	Severe	HRT, ventolin		+
20/48/F	HF	Severe	HRT, ventolin, coproximol		–
21/39/F	DIGO	Mild	CSA, phenytoin, others	Renal transplant	–
22/62/M	DIGO	Mild/moderate	CSA, NF, AZ, asprin, cronolibrizol	Heart transplant	+
23/69/M	DIGO	Mild/moderate	CSA, AZ, PR, AM, asprin, insulin	Renal transplant and diabetes	++
24/16/M	DIGO	Moderate	CSA, NF, AT, AZ, PR, hydralazine	Renal transplant	++
25/21/M	DIGO	Moderate	CSA, AT, AM, AZ	Renal transplant	–
26/30/F	DIGO	Moderate	CSA, NF, CI, DO	Renal transplant	+
27/56/M	DIGO	Moderate	CSA, NF, DO, balsalazide	Heart transplant	++
28/15/M	DIGO	Severe	CSA, NF, others	Liver transplant	++
29/18/M	DIGO	Severe	CSA, NF, others	Liver transplant	++
30/20/M	DIGO	Severe	CSA, NF, AT, AZ, PR	Liver transplant	++

DIGO, drug-induced gingival overgrowth.
[a]Graded according to coverage of clinical crown: mild, up to 33%; moderate, 34–50%; severe, >50%. Buccopalatal thickness of the tissue was not assessed.
[b]CSA, cyclosporin; NF, nifedipine; AT, atenolol; AZ, azathioprine; AM, amlodipine; PR, prednisolone; CI, ciprofloxacin; DO, doxazosin; HRT, hormone replacement therapy.
[c]Histological presence of inflammatory cell infiltrate within gingival tissues scored subjectively: –, none; +, mild to moderate infiltrates; ++, extensive infiltrates.
[d]Hereditary gingival fibrosis.

or negative. Five randomly selected fields in areas free of inflammatory infiltrate were counted within each specimen, ensuring that a minimum of 200 fibroblasts were examined from each specimen. The total and mean number of positive and negative cells for each specimen were used to calculate the percentage of positive fibroblasts per specimen.

The absorbance of 40, randomly selected, positive fibroblasts from each non-counterstained specimen was determined using a Seescan prism 512 system (Seescan imaging Ltd, Unit 9, Gwydir St., Cambridge, UK), linked to a leitz Diaplan microscope and a modified black and white single chip TV camera. The camera modifications enable accurate and reproducible absorbance readings (coefficient of variation $<0.81\%$ over an absorbance range of 0.01–1.3) to be obtained over a wide range of background illumination levels (30–115 grey scale levels in the maximum range up to 128). Images were captured at a magnification of ×512 and absorbance measurements of individual, positive fibroblasts performed as previously described (Wright *et al*, 2001; Mason *et al*, 2003). The effects of any background extracellular staining were eliminated by isolating the cell under investigation within the captured image either by manual outlining or interactive thresholding of the cell prior to measurement.

Measurement of macrophage content of tissues

The area of tissue positive for CD68 was determined using Image Pro-plus (version 4.1, Media Cybernetics, Silver Spring, MD, USA) and a high-resolution camera (Coolsnap colour camera, Media Cybernetics, Silver Spring). Twenty randomly selected areas (0.07 mm^2 each) were analysed and then the stained area per mm^2 determined.

RNA isolation and RT-PCR analysis

Total RNA was extracted from the tissues using the RNeasy mini kit (Qiagen Ltd, Crawley, UK) as recommended by the manufacturer. Subsequently, 1–5 μg of DNase-digested total RNA was used for oligo-dT reverse transcription to generate single-strand cDNA using the Omniscript kit (Qiagen Ltd). Both RNA and cDNA concentrations were determined using a BioPhotometer

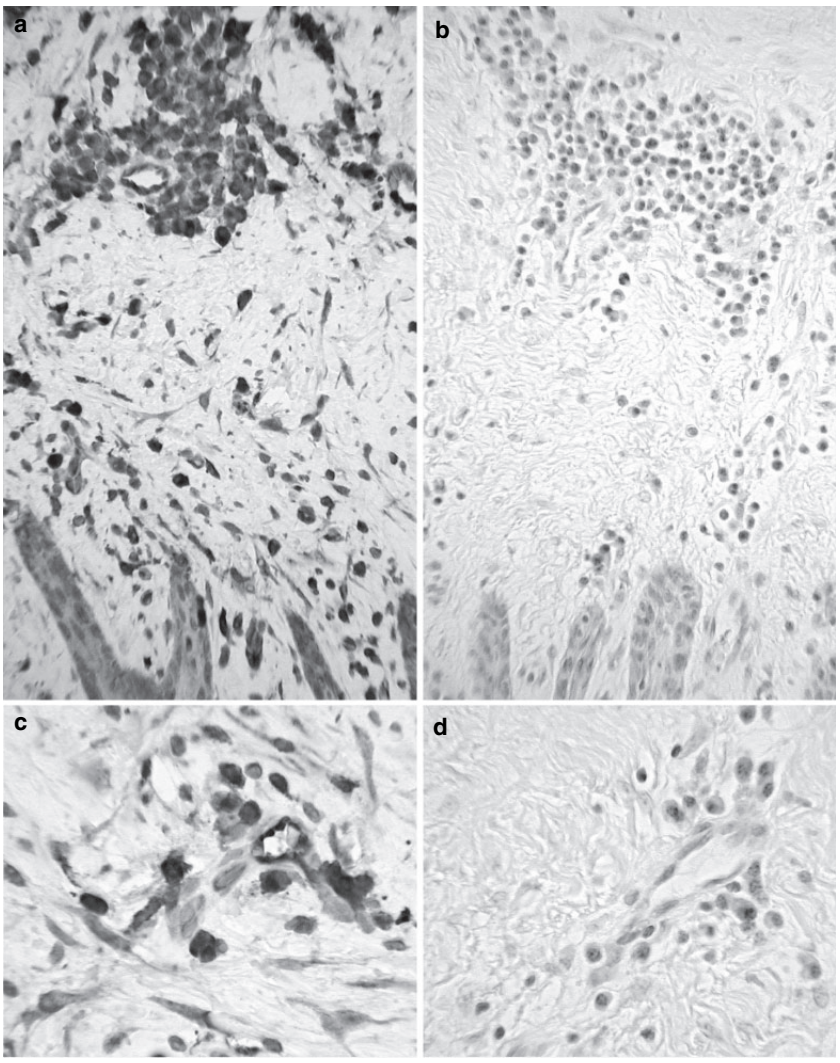

Figure 1 Expression of PDGF A (**a**) and PDGF receptor α (**c**) in a variety of cell types within a gingival specimen from a patient with chronic periodontitis. Pre-incubation of the primary antibody with its corresponding immunising peptide resulted in complete blocking of specific staining for PDGF A (**b**) and the α receptor (**d**). Haematoxylin-peroxidase

(Eppendorf, Histon, UK). The cDNAs were subsequently amplified in a 25 μl reaction mixture containing 12.5 μl of Sigma Red Taq (Sigma, Poole, UK) and 0.5 μl of each primer. The following primers were used in the detection of PDGF and its receptors: PDGF A sense, 5′-CCACACCTCCTCGCTGTAG-3′, antisense 5′-CAGCAGCCTGTGTGTTATC-3′ (NM_002607; 245 bp; 35cycles); PDGF B sense, 5-′-GTGGCTT-CTTTTCGTT-3′, antisense 5′-GAAAATGCAGGG-TAGGA-3′ (NM_002608; 235 bp; 35cycles); PDGF Rα sense, 5′-CTGGGTTTCCATCCTTGAG-3′, antisense 5′-TAGTAGGCTTCCTGCGTGG-3′ (M21574; 194 bp; 32cycles); PDGF Rβ sense, 5′-CATGGGGGTA-TGGTTTTGT-3′, antisense 5′-GTAAGGTGC-CAACCTGCAA-3′ (NM_002609; 192 bp; 32cycles). The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control: sense 5′-CCACCCATGGCAAATTCCATGGCA-3′, antisense 5′-TCTAGACGGCAGGTCAGGTCCACC-3′ (550bp; 27cycles). Oligonucleotide primers were designed using the primer 3 program (http//www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). After an initial denaturation of 5 min at 94°C, the designated number of amplification cycles of 94°C for 20 s, 62°C for 20 s and 72°C for 20 s were followed by elongation for 10 min at 72°C using an Eppendorf Mastercycler thermal cycler (Eppendorf, UK). Thereafter, PCR products were visualised on a 1.5% agarose gel containing 0.5 μg ml^{-1} ethidium bromide. Scanned gel images were imported into AIDA image analysis software (Fuji, London, UK) and the volume density of amplified products calculated and normalised against the GAPDH housekeeping gene control values.

Statistical analysis

All data was analysed using Minitab (ver. 9, Minitab Inc, State College, PA, USA) and the significance of differences across the three groups determined using the Kruskall–Wallis test. Where significant differences were found, comparisons between overgrowth groups or overgrowth groups and controls were performed using the Mann–Whitney *U*-test with Bonferroni correction. Differences in the proportions of cells positive for a particular isoform or receptor type were performed by the chi-squared test on total positive and negative cell numbers.

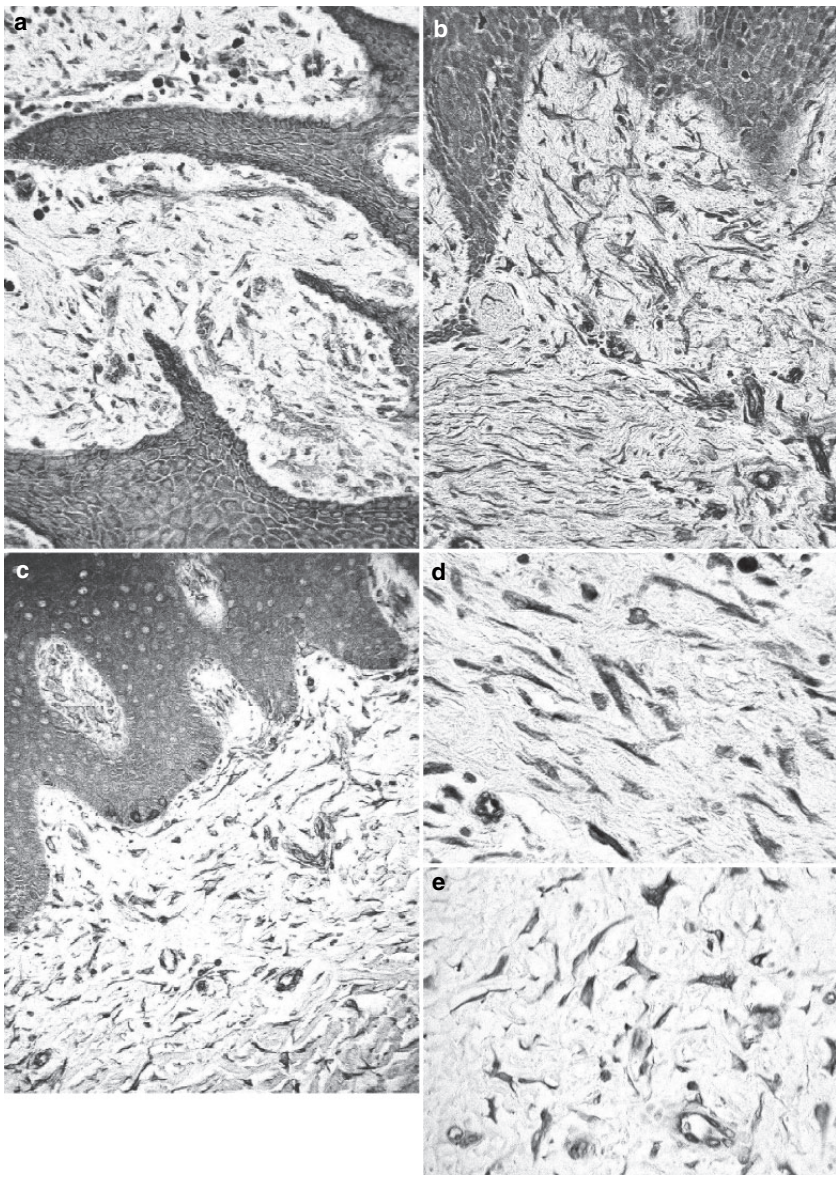

Figure 2 Expression of PDGF A (**a,d**) and PDGF A/B (**b,c,e**) by most cells in drug-induced gingival overgrowth (a,b,d) and hereditary gingival fibrosis (**c,e**). Fibroblasts and vascular endothelium within deep connective tissue (**d,e**) show similar reactivity to those in the superficial lamina propria. Haematoxylin-peroxidase

Results

Cytoplasmic staining for PDGF A was exhibited by most cell types within all gingival tissues irrespective of their source (Figures 1 and 2). Both crevicular and gingival oral epithelial cells were strongly positive except for the superficial parakeratinised layers which were generally negative. PDGF A expression was greatest in the granular layer associated with some parts of parakeratinised epithelium. Within connective tissue, PDGF A was expressed by vascular endothelium, inflammatory cells and fibroblasts. Most inflammatory cells and fibroblasts displayed variable staining intensities. Subjectively there appeared to be no differences in the cellular distribution and level of PDGF A expression between tissues from control, DIGO and HF groups.

The staining pattern for PDGF A/B was similar to that obtained for PDGF A (Figure 2). Overall, the intensity of staining was greater than that found for PDGF A and DIGO tissues appeared to show less intense staining for PDGF A/B than the other two specimen groups.

Cytoplasmic staining for both α and β PDGF receptors was seen in all cell types (Figures 1 and 3). Although the overall staining pattern for the receptors was similar, reactivity for the α receptor was always greater than that for the β receptor, especially within epithelial cells. All layers of epithelium, except the superficial layer, expressed the receptors as did fibroblasts, endothelial cells and inflammatory cells. The staining intensity of these connective tissue cells often appeared greater than that of epithelium. Receptor expression in HF and control tissues was similar and differed from that of DIGO tissues which displayed a generally lower, and in fibroblasts more variable, level of receptor expression.

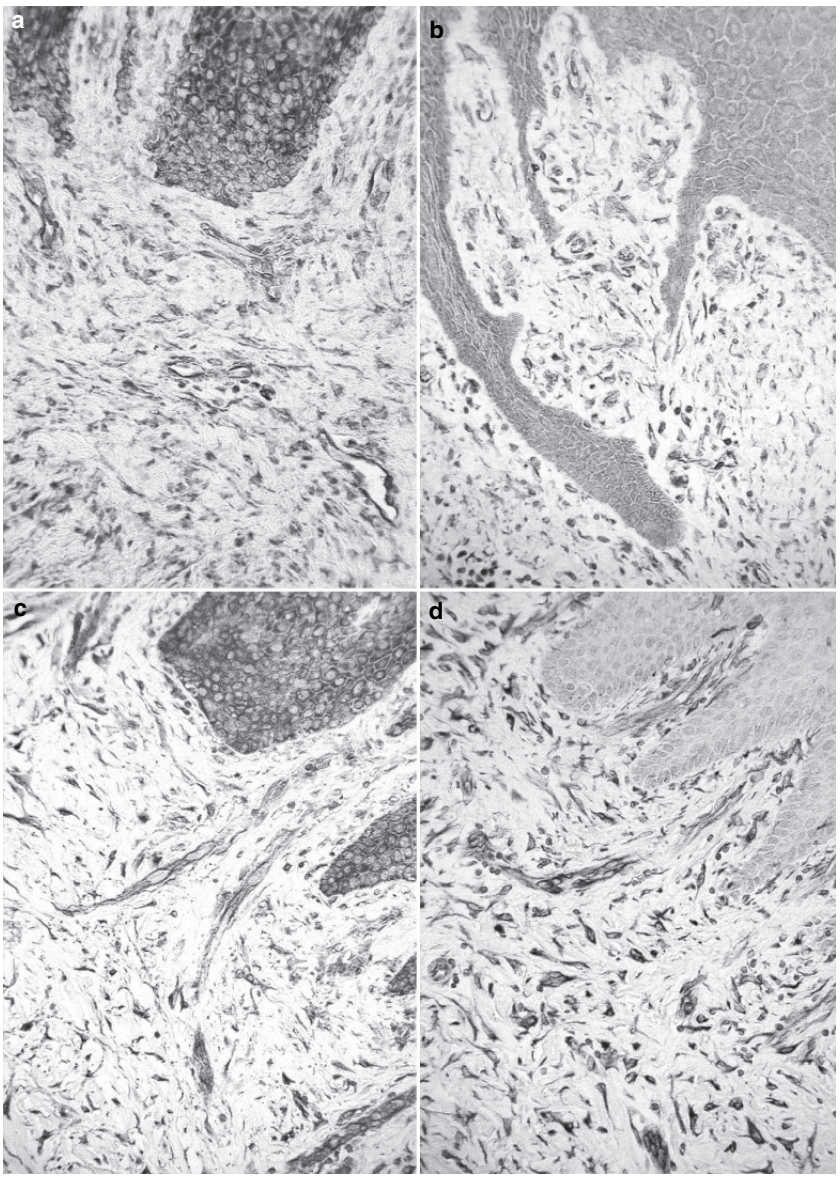

Figure 3 Cytoplasmic expression of PDGF receptors α (**a,c**) and β (**b,d**) by most cells in drug-induced gingival overgrowth (**a,b**) and hereditary gingival fibrosis (**c,d**). Note the relatively low expression of the β receptor by epithelium compared to cells within the lamina propria. Haematoxylin-peroxidase

In order to investigate possible differences in fibroblast expression of PDGF chains and its receptors between overgrowth and control tissues, cell counts and absorbance measurements were performed (Figure 4. There were no significant differences in the densities (Figure 4a) or proportion of fibroblasts positive (data not shown) for either PDGF A, PDGF A/B or the α and β receptors. Similarly, there were no differences in the level of PGDF A expression between control, DIGO and HF fibroblasts as determined by absorbance measurements (Figure 4b; $P = 0.35$). By contrast, absorbance measurements revealed differences in the level of PDGF A/B expression between tissue groups ($P < 0.0001$) with fibroblasts within DIGO specimens displaying lower levels than those from control ($P = 0.0024$) or HF groups ($P = 0.0006$).

Although 90–97% of fibroblasts expressed both α and β receptors, absorbance measurements (Figure 4b) demonstrated that there were significant differences in expression between the three tissue groups (α receptor, $P = 0.001$; β receptor, $P < 0.0001$). Both receptors were expressed at lower levels by fibroblasts in DIGO tissues compared with those in control (α receptor, $P = 0.034$; β receptor, $P = 0.0012$) or HF (α receptor, $P = 0.0039$; β receptor, $P = 0.0012$) specimens. There were no significant differences in the fibroblast receptor absorbance readings between the control and HF groups.

Because of the possible link between upregulation of the PDGF B gene by macrophages and DIGO[15], the CD68-positive cell (macrophage) content of sections from all specimens from each tissue group was determined. Although DIGO tissues had the highest mean macrophage content, analysis of the area measurements revealed no significant difference in the percentage area of lamina propria positive for CD68 between control

(1.85 ± 1.00), DIGO (2.69 ± 2.05) and HGF (1.69 ± 0.95) tissues ($P = 0.2$).

Semi-quantitative RT-PCR (sq-RT-PCR) analysis of whole tissue extracts demonstrated the presence of mRNA for PDGF A, PDGF B and both α and β receptors (Figure 5a). Densitometric analysis of sq-RT-PCR products indicated that mean mRNA expression levels were lower in DIGO tissues compared with control and HF specimens (Figure 5b). Expression of α receptor mRNA was significantly different between groups ($P = 0.038$) with DIGO expression being lower than that of controls ($P = 0.025$).

Discussion

This is the first study to investigate the expression of PDGF and its receptors in DIGO or HF at both the protein and mRNA level. The results demonstrate that most cell types within control and overgrown gingival tissues express PDGF A, PDGF B and both PDGF receptors and are in agreement with the fact that PDGF is produced by a variety of cell types (Sasahara *et al*, 1991; Pierce *et al*, 1995; Feliciani *et al*, 1996). Furthermore, the results show, for the first time, that while PDGF A protein expression by fibroblasts is not altered by drug use or hereditary factors, PDGF AB/BB production and receptor expression by fibroblasts is reduced in DIGO compared to HF and control tissues. This altered fibroblast protein phenotype was also reflected in reduced tissue expression of the α receptor gene in DIGO indicating differing roles for this family of growth factors and receptors in gingival overgrowth.

Relatively little is known about the presence and distribution of PDGF and its receptors within normal and diseased, overgrown tissues. The majority of work on DIGO, performed by Iacopino and co-workers (Nares *et al*, 1996; Plemons *et al*, 1996; Iacopino *et al*, 1997), has centred on PDGF B and its expression by macrophages. Both phenytoin and cyclosporin cause increased production of PDGF AB/BB by macrophages *in vitro* (Dill *et al*, 1993; Iacopino *et al*, 1997) and QC-RT-PCR analysis of RNA isolated from overgrowth tissues associated with both drugs is reported to have about a 47-fold greater expression of the PDGF B gene (Nares *et al*, 1996; Iacopino *et al*, 1997). Furthermore, limited analysis of both control and cyclosporin-induced overgrowth tissues by immunohistochemistry and *in situ* hybridisation revealed that PDGF B expression was restricted to some of the CD51-positive macrophages within the papillary lamina propria (Nares *et al*, 1996; Plemons *et al*, 1996). Thus, the overall conclusion from this series of reports has been that PDGF B specifically produced by, and upregulated in, a subpopulation of gingival macrophages is an important factor in the expression of DIGO (Boltchi *et al*, 1999).

While the data reported by Iacopino and co-workers appear convincing their immunohistological findings, using an anti-PDGF B antibody (Oncogene Science) that would detect both AB and BB forms of the PDGF dimer within tissues, are not supported by other works, including this study. Both PDGF A and PDGF B have

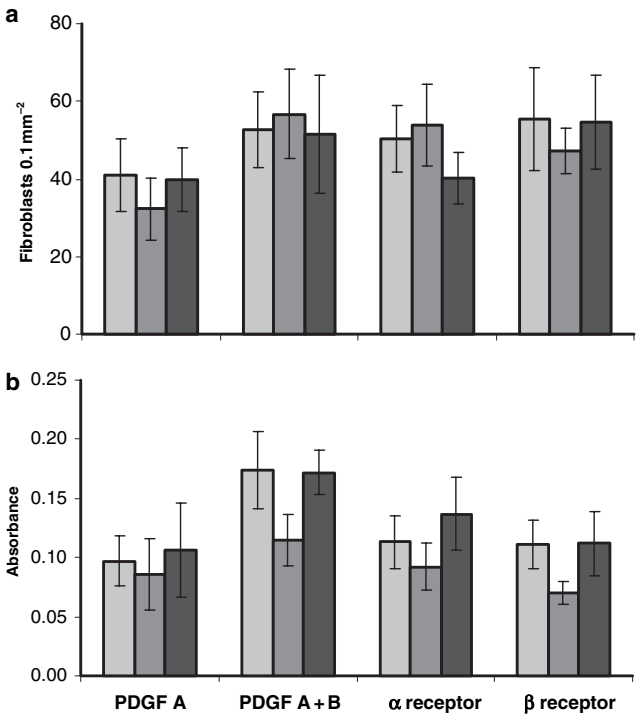

Figure 4 Histograms summarising PDGF and PDGF receptor expression by gingival fibroblasts within control (■), DIGO (■) and HF (■) tissues. (**a**) Density of positive cells and (**b**) absorbance measurements of positive cell population (mean ± s.d.)

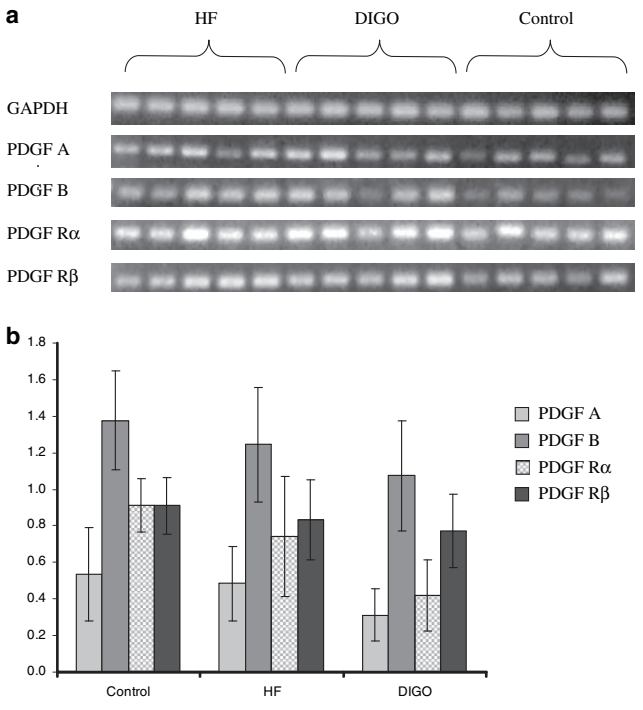

Figure 5 Semi-quantitative RT-PCR analysis of whole tissue extracts. Gel bands corresponding to RT-PCR products for PDGF A, PDGF B and the α and β receptors (**a**) and densitometric analysis normalised against the GAPDH housekeeping gene (**b**)

been immunolocalised, using antibodies reactive with various combinations of PDGF-chain dimers, throughout the epithelium and within blood vessel walls in inflamed gingiva and gingival wounds (Green *et al*, 1997; Pinheiro *et al*, 2003) indicating a more generalised expression pattern within these tissues, similar to our findings in DIGO, HF and control (inflamed and non-inflamed) gingival tissues.

Similarly, our sq-RT-PCR data support our immunohistochemical findings. Expression levels of both the PDGF A and PDGF B genes were found to be extensive and heterogeneous irrespective of the presence of gingival overgrowth or medication with overgrowth-inducing drugs. The disparity in these results compared with the published QC-RT-PCR data (Nares *et al*, 1996) suggesting that increased PDGF-B mRNA levels are attributable to infiltrating macrophages is difficult to explain as extensive DNA database searches demonstrate the specificity of both sets of primer sequences. Our recent unpublished Affymetrix gene array data (U133A), using the H400 oral epithelial cell line (Prime *et al*, 1990), also indicates that PDGF B mRNA is likely to be expressed in gingival specimens, irrespective of the presence of overgrowth or inflammation. In addition, this analysis indicates that these cells upregulate PDGF-B mRNA when challenged with inflammatory stimuli (unpublished observations).

The limited published data on PDGF receptor expression within gingival tissues generally show a similar distribution to that of the ligands (Green *et al*, 1997) although one report, which did not utilise positive controls, suggested that the α receptor is not expressed by any periodontal cells (Parkar *et al*, 2001). Results from our studies indicate extensive expression of α and β receptors within control and overgrowth gingival tissues at both the protein and mRNA levels suggesting that most cells could bind and respond to all PDGF dimer combinations.

PDGF A is thought to be involved in early wound healing and in gingival wounds it has been seen that the increased levels return to normal within 7 days (Green *et al*, 1997). That PDGF A expression was not altered in overgrowth tissues is not surprising, as all specimens studied represented well-established fibrosis requiring surgical reduction. It is possible that PDGF expression in the early phases of development would be different and that transient changes in PDGF A might occur and be important in initiation of the disease process. However, the lack of any change in PDGF A expression detected in this study is consistent with the findings in other chronic fibrotic conditions such as tubulonephritis and pancreatitis (Tang *et al*, 1996; Ebert *et al*, 1998). By contrast, PDGF B has been implicated in both wound healing and fibrosis (Green *et al*, 1997; Xue-yi *et al*, 1998) with increased fibroblast mRNA levels being reported in various fibrotic conditions, including scleroderma and membranous nephropathy (Xue-yi *et al*, 1998; Mezzano *et al*, 2000). Furthermore, cyclosporin-fed rats have been reported to display increases in PDGF B in the kidney tubules (Shehata *et al*, 1994).

Our data based on immunolocalised PDGF show either decreased or 'control' levels of PDGF B expression by fibroblasts in gingival overgrowth dependent upon aetiology. Thus, DIGO but not HF was characterised by reduced fibroblast expression of PDGF B and both PDGF receptors, suggesting a cellular response aimed at altering fibroblast reactivity to PDGF. This difference between overgrowth types could reflect a direct effect of cyclosporin on gingival fibroblasts or be due to other interacting factors. Previous studies have shown that both DIGO and HF are characterised by increased fibroblast expression of TGFβ1 (Wright *et al*, 2001) as well as high local levels of the growth factor in crevicular fluid (Wright *et al*, 2004). TGFβ1 is able to decrease PDGF α receptor expression by fibroblasts (Gronwald *et al*, 1989) and it is possible that TGFβ1 could regulate the differences in PDGF and receptor expression detected in DIGO. That high levels of TGFβ1 expression do not result in similar changes in HF fibroblasts might reflect the intrinsic defect predisposing them to fibrosis.

It is interesting to note that the mean α:β receptor absorbance ratio for DIGO fibroblasts (1:0.81; data not shown) differed from that of controls (1:0.98) indicating differing reactivity to PDGF dimers which could alter the downstream effects of PDGF signalling, perhaps tipping the balance in favour of fibrosis. This possibility is also supported by the fact that HF fibroblasts, although displaying higher levels of both receptors than those in DIGO, showed a similar imbalance in α:β receptor absorbance ratio (1:0.82).

In conclusion, the present study has documented the expression of PDGF isoforms and receptors in gingival tissues and the changes in isoform and receptor expression in DIGO and HF. The results do not support the suggested pivotal role for macrophage-derived PDGF B in the pathogenesis of gingival overgrowth. However, they do demonstrate subtle differences in PDGF isoform and receptor expression between DIGO and HF which may be implicated in the pathogenesis of both overgrowth types.

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