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J. Tolonen
M. Grönblad
J. Virri
S. Seitsalo
T. Rytömaa
E. O. Karaharju

Platelet-derived growth factor and vascular endothelial growth factor expression in disc herniation tissue: an immunohistochemical study

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J. Tolonen (✉) · M. Grönblad
J. Virri · S. Seitsalo · E. O. Karaharju
Research Laboratory,
Spine Research Unit,
Department of Orthopaedics
and Traumatology, University of Helsinki
Topeliuksenkatu 5,
FIN-00250 Helsinki, Finland
Tel. + 358-0-4717287;
Fax + 358-0-4717587

T. Rytömaa
Finnish Centre for Radiation
and Nuclear Safety
Helsinki, Finland

Abstract Angiogenesis is essential in tissue growth and regeneration. There are several factors that are able to stimulate vascular endothelial cell growth, including platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). Disc herniation tissue (DHT) contains vascular ingrowth, which promotes granulation tissue formation. In this study we observed 50 disc herniations for PDGF and VEGF immunoreactivity. PDGF immunopositivity was detected in 38 samples (78%). In 28 samples (56%) there were PDGF immunopositive capillaries, PDGF immunopositive disc cells were detected in 19 samples (38%) and PDGF immunopositive fibroblasts in 6 DHT samples (12%). VEGF immunopositive capillaries were identified in 44 DHT samples (88%). For neither growth factor was immunopositivity dependent on preoperative radicular pain duration. In extrusions (n = 25) VEGF immunopositive capillaries were detected in 23 samples (92%) and PDGF immunopositivity in 21 samples (84%). PDGF immunopositivity was more commonly associated with capillaries than with nuclei of disc cells. In sequesters (n = 20) VEGF immunopositive capillaries were identified in all samples and PDGF immunopositivity in 16 (80%). As in extrusions, PDGF immunoreaction was more prevalent in capillaries than in disc cells. Patient age did not relate to VEGF expression. In all age groups it was higher than 80%. Thus capillaries in disc herniation tissue are evidently newly formed and our results demonstrate that PDGF and VEGF participate in the neovascularization process. The presence of PDGF in fibroblasts and in disc cells suggests that this growth factor regulates the function of these cells, possibly the proliferation of the cells and the production of extracellular matrix components.

Key words Intervertebral disc · Disc herniation · Platelet-derived growth factor · Vascular endothelial growth factor

Introduction

Angiogenesis involves proliferation of endothelial cells, chemotaxis and enzymatic degradation of the basement membrane of local blood vessels [8, 12, 33]. This process is essential in tissue growth and regeneration. Several factors are able to stimulate vascular endothelial cell growth, including fibroblast growth factor (FGF) [10], angiogenin [7], transforming growth factor beta (TGF-beta) [25], platelet derived growth factor (PDGF) [26] and vascular endothelial growth factor (VEGF) [2, 4, 11, 16].

Platelet-derived growth factor (PDGF) is structurally a dimer of A chains (17 kD) and B chains (16 kD). It appears either in the form of an A-A or a B-B homodimer or

as an A-B heterodimer [15]. It stimulates the growth of vascular endothelial cells [26] and fibroblasts [29]. It also acts as a chemotactic signal for fibroblasts, even at very low concentrations [22]. In cartilage it has been demonstrated to increase the level of intracellular free calcium ions in chondrocytes [9]. Furthermore, it stimulates DNA and proteoglycan synthesis in cartilage tissue [9] and chondrocyte proliferation [13].

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is a dimeric, heparin-bonding protein with a molecular weight of 45 kD [4, 5]. Its expression has previously been demonstrated in brain, kidney, pituitary gland, lung, adrenal gland, heart, liver, stomach mucosa and ovary, as well as in some tumours [1, 6]. VEGF is actually a family composed of four different species of VEGF [6]. It regulates endothelial differentiation, blood vessel growth and vascular repair [21], and it shares homologies of about 21% and 24%, respectively, with the A and B chains of PDGF [30]. VEGF also regulates plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells [20]. It promotes blood vessel hyperpermeability, endothelial cell growth, angiogenesis and enhanced glucose transport [3]. Hypoxia has been shown to induce VEGF, which may in turn mediate hypoxia-initiated angiogenesis [28].

Intervertebral disc tissue may herniate in three basic patterns. In protrusions it bulges posteriorly, but the annulus fibrosus remains intact. In extrusions disc material is exposed to the epidural space; there is still, however, a continuity with disc tissue. In sequesters there is a separate fragment of disc tissue material in the epidural space.

Disc herniation tissue (DHT) contains vascular ingrowth [14, 17, 32]. This neovascularization promotes granulation tissue formation. In a previous study we demonstrated basic FGF expression in DHT [31]. The aim of this study was to investigate PDGF and VEGF expression in DHT and compare it with vascularization.

Materials and methods

Our disc material was obtained from 50 discectomy operations. The age of the patients ranged from 24 to 71 years. There were 26 male and 24 female patients (Table 1). All the tissue material was rapidly frozen in the operating theatre and 8 μ thick cryostat sections were cut (2800 Frigocut, Reichert-Jung). The sections were fixed in ice-cold acetone and stained by the avidin biotin complex- (ABC-) peroxidase immunohistochemical staining method (Vectastain, Vector Lab, Burlingame, Calif.). Disc material was also obtained from organ donor patients as a control. In these specimens there was no sign of autolysis.

We used a polyclonal platelet-derived growth factor antibody (1 mg/ml) at a dilution 1 : 100 (R & D Systems, Minneapolis, Minn.) and a polyclonal vascular endothelial growth factor antibody (1 mg/ml) at a dilution 1 : 500 (Santa Cruz Biotechnology, Santa Cruz, Calif.). Antigen absorption (1 : 10) was made to test the specificity of the antibodies. We used a polyclonal von Willebrand factor antibody (1 : 20 000) to visualize vascular endothelium (Dakopatts, Copenhagen). In addition to studying control discs, sections were also stained omitting the primary antibody. The immunohisto-

Table 1 Clinical characteristics of the patients sampled for disc herniation tissue

Patient no.	Age (years)	Sex	Preoperative pain duration (months)
1	39	M	2.5
2	32	F	2
3	55	F	3
4	37	M	2.5
5	44	F	0.5
6	27	F	6
7	28	M	6
8	37	F	3.5
9	71	M	3
10	68	M	3
11	45	M	2.5
12	37	M	2.5
13	42	F	2
14	47	M	2
15	47	F	3
16	39	F	2
17	56	M	0.75
18	49	F	5
19	63	F	4
20	31	M	2
21	45	M	3.5
22	39	M	12
23	30	M	1
24	46	F	6
25	45	F	0.5
26	46	F	0.75
27	56	F	6
28	33	F	12
29	29	M	5
30	29	M	6
31	58	F	0.25
32	31	M	6
33	55	F	0.5
34	47	M	12
35	53	M	8
36	52	F	2
37	44	F	6
38	24	F	1
39	40	F	3.5
40	31	M	2.5
41	42	M	4
42	39	M	2.5
43	34	F	3
44	39	F	9
45	47	M	24
46	42	M	12
47	46	F	4
48	58	M	2.5
49	31	M	6
50	56	M	4

Table 2 Platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) immunohistochemical staining results (*nr* not reported)

Patient (no.)	Prolapse (type)	PDGF (immunopositivity)			VEGF (immunopositivity)
		Nuclear	Blood vessel	Fibroblast	
1	nr	–	–	–	–
2	nr	–	–	–	–
3	Extrusion	+	–	–	–
4	Extrusion	–	–	+	+
5	Sequester	–	–	–	+
6	Protrusion	–	+	–	–
7	Sequester	–	+	–	+
8	Sequester	–	+	–	+
9	Extrusion	–	–	–	+
10	Sequester	–	–	–	+
11	Extrusion	–	+	–	+
12	Sequester	–	–	–	+
13	Extrusion	–	–	–	+
14	Sequester	–	+	–	+
15	Extrusion	–	+	–	+
16	Extrusion	–	+	–	+
17	Sequester	–	+	–	+
18	Extrusion	–	+	–	–
19	Protrusion	–	–	–	+
20	Sequester	–	+	–	+
21	Extrusion	–	+	–	+
22	Extrusion	–	+	+	+
23	Extrusion	–	+	–	+
24	Extrusion	–	+	–	+
25	Sequester	–	+	–	+
26	Sequester	–	+	–	+
27	Sequester	–	+	–	+
28	Extrusion	–	+	–	+
29	Extrusion	–	–	–	+
30	Protrusion	–	–	–	–
31	Extrusion	+	+	–	+
32	Extrusion	+	+	–	+
33	Sequester	+	–	+	+
34	Sequester	+	–	–	+
35	Sequester	+	–	–	+
36	Sequester	+	+	+	+
37	Sequester	+	–	–	+
38	Sequester	+	+	–	+
39	Extrusion	+	+	+	+
40	Sequester	+	+	+	+
41	Extrusion	+	–	–	+
42	Extrusion	+	+	–	+
43	Sequester	–	–	–	+
44	Extrusion	–	–	–	+
45	Sequester	+	–	–	+
46	Extrusion	+	+	–	+
47	Extrusion	+	–	–	+
48	Extrusion	+	–	–	+
49	Extrusion	+	+	–	+
50	Extrusion	+	+	–	+

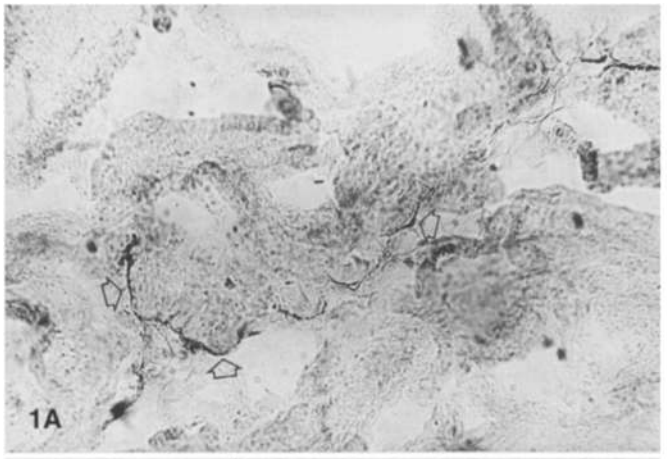
chemical results were then compared with the patient clinical data (Tables 1–4).

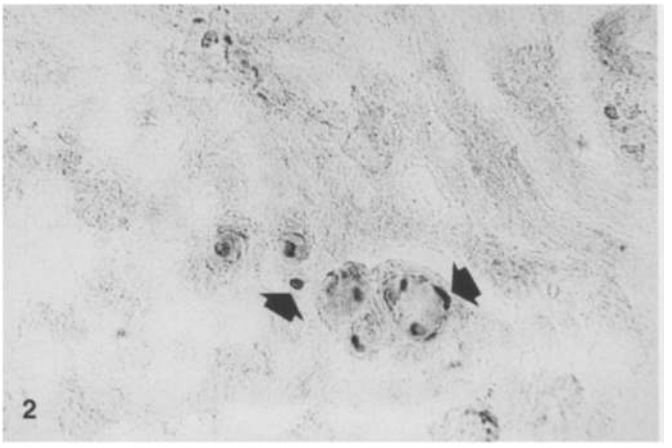
Results

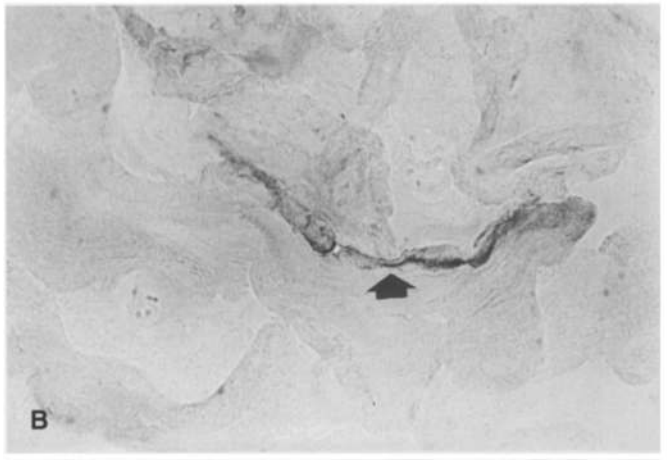
Immunohistochemical staining results are described in detail in Table 2. Altogether, 38 DHT samples (78%) showed PDGF immunopositivity. In 27 DHT samples (54%) this immunoreactivity was detected in blood vessels (Fig. 1 A) and in 19 (38%) it was found in nuclei of disc cells (Fig. 2). Six samples (12%) also showed PDGF immunopositive fibroblasts (Fig. 3). Forty-four DHT samples (88%) showed VEGF immunopositive capillaries (Fig. 1 B). In Fig. 1 C capillary endothelial cells are identified with von Willebrand factor antibody. The five studied control discs did not show any PDGF or VEGF immunoreactivity (Fig. 4). Sections stained omitting the primary antibody did not show any immunoreactivity.

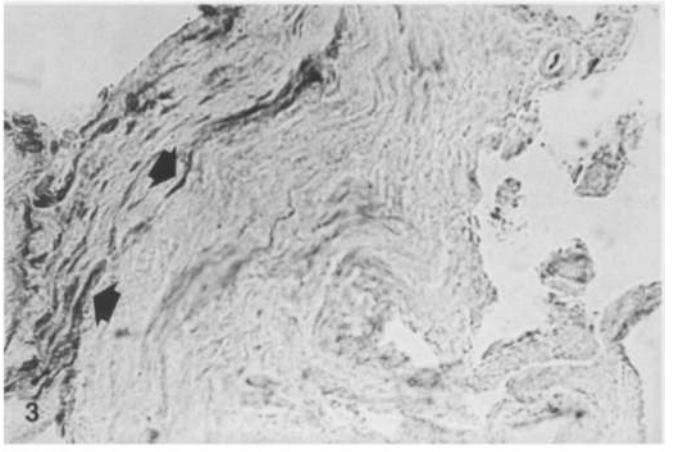
Mean preoperative pain duration for all patients was 4.12 months (median 3 months). VEGF and PDGF immunopositivity did not show any dependence on the duration of preoperative pain. Mean pain duration in the VEGF immunopositive group was 4.51 months (median 3 months). In the VEGF immunonegative group it was 4.08 months (median 4 months). Mean preoperative pain duration in the PDGF immunonegative group was 3.54 months (median 3 months); in the PDGF immuno-positive group it was 4.16 months (median 3 months). In the group where disc cell nuclei showed PDGF immunoreactivity the mean preoperative pain duration was 5.46 months (median 4 months). In the group where capillaries were immunoreactive for PDGF the mean preoperative pain duration was 4.13 months (median 3.5 months). Finally, in the group where fibroblasts stained for PDGF the mean preoperative pain period was 3.83 months (median 2.5 months). However, there were only six such DHT samples in the present material (Table 2).

Table 3 compares PDGF and VEGF immunoreactivity in the different prolapse types. Of the 25 extruded disc herniation samples studied, 23 (92%) showed VEGF immunoreactivity and 21 (84%) PDGF immunoreactivity. PDGF immunoreaction was located in disc cell nuclei in 11 samples (44%); in 16 samples (64%) it was associated with blood vessels and in 3 samples (12%) fibroblasts were PDGF immunopositive. Twenty of the DHTs were sequesters, VEGF immunopositive capillaries were detected in all of them. PDGF immunopositivity was detected in 16 samples (80%): in 8 of these immunoreaction was localized in disc cell nuclei, 10 (50%) showed PDGF immunopositive capillaries, and three (15%) PDGF immunopositivity in fibroblasts. In the three disc protrusions studied, one showed vascular PDGF immunoreactivity and VEGF immunoreactivity. For two of the tissue samples the prolapse type had not been defined by the operating spine surgeon.









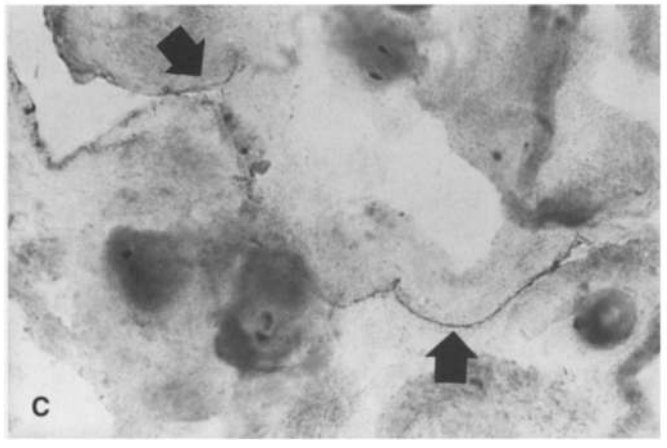


Fig. 1 **A** Platelet-derived growth factor (PDGF) immunopositivity in capillaries *(open arrows)* in an extrusion disc herniation tissue sample from a 39-year-old man. (Operation level L5–S1, avidin biotin complex- (ABC) immunostaining, haematoxylin counterstaining, original magnification × 370) **B** Vascular endothelial growth factor (VEGF) immunopositive capillaries *(black arrow)* in an extrusion from a 31-year-old man (Operation level L4–L5, ABC-immunostaining, haematoxylin counterstaining, original magnification × 250). **C** von Willebrand factor immunopositive capillaries *(black arrows)* in an extrusion disc herniation tissue sample from the same 39-year-old male patient as the sample in **A** (ABC-immunostaining, haematoxylin counterstaining, original magnification × 370)

Fig. 2 An extrusion from a 40-year-old woman. Note PDGF immunopositivity in the nuclei of disc cells *(black arrows)*. (ABC-immunostaining, haematoxylin counterstaining, original magnification × 370)

Fig. 3 PDGF immunopositive fibroblasts *(black arrows)* in a sequester from a 52-year-old woman (Operation level L5–S1, ABC-immunostaining, haematoxylin counterstaining, original magnification × 370)

Fig. 4 A normal control disc from a 50-year-old man. Note total lack of immunoreaction. (Level L4–L5, PDGF antibody, ABC-immunostaining, haematoxylin counterstaining, original magnification × 370)

Table 3 PDGF and VEGF immunoreactivity by prolapse type

Prolapse type[a]	PDGF immunopositivity				VEGF immunopositivity
	Total	Nuclear	Blood vessel	Fibroblast	
Protrusions (n = 3)	1/3	0/1	1/1	0/1	1/3
Extrusions (n = 25)	21/25 (84%)	11/21 (52%)	16/21 (76%)	3/21 (14%)	23/25 (92%)
Sequesters (n = 20)	16/20 (80%)	8/16 (50%)	10/16 (63%)	3/16 (19%)	20/20 (100%)

[a]Two samples were not classified

Table 4 PDGF and VEGF immunoreactivity by patient age group

Age group	PDGF immunopositivity				VEGF immunopositivity
	Total	Nuclear	Blood vessel	Fibroblast	
Below 40 years (n = 21)	14/21 (66.7%)	5/14 (23.8%)	13/14 (61.9%)	3/14 (14.2%)	17/21 (81.0%)
40–50 years (n = 17)	15/17 (88.2%)	7/15 (41.2%)	10/15 (58.8%)	1/15 (5.9%)	16/17 (94.1%)
Over 50 years (n = 12)	9/12 (75.0%)	7/ 9 (50.8%)	5/ 9 (41.6%)	2/ 9 (16.7%)	11/12 (91.7%)

Table 4 compares immunoreactivity for PDGF and VEGF among different age groups. VEGF immunoreactivity showed no clear age dependence and was present in more than 80% of all types of DHT (Table 4). Blood vessel associated PDGF immunopositivity was clearly more common than disc cell nuclear immunoreactivity in the youngest patients (under 40 years old). However, in the oldest age group PDGF immunoreaction appeared to be slightly more common in disc cells than in blood vessels (Table 4).

Discussion

DHT contains vascular ingrowth promoting granulation tissue formation [14, 17], PDGF is a potent chemotactic agent for fibroblasts [22] and it induces cellular growth of fibroblasts and stimulates their collagenase production [29]. PDGF is liberated from alpha-granules of platelets and macrophages [26, 29]. One finding of the present study was the demonstration of PDGF immunopositive fibroblasts in DHT, suggesting a regulative role for PDGF in fibroblast function, including in disc herniations. PDGF immunopositive disc cells were also demonstrated, the nuclei exhibiting strong immunoreactivity. The number of disc cells is greater in disc herniations than in normal discs (unpublished data). This could suggest that PDGF also regulates the function of disc cells and that in DHT PDGF stimulates extracellular matrix component production as has been observed for cartilage tissue [9, 23, 24]. PDGF may also be stimulative for disc cell proliferation. In a previous study we demonstrated disc cell cytoplasmic fibroblast growth factor (FGF) immunoreactivity [31], while in this study we noted disc cell nuclear PDGF immunoreactivity. This suggests that these growth factors are part of a greater network of growth factors and cytokines that contribute to overall disc tissue responses, governing repair of tissue damage.

DHT has a tendency to disappear or to decrease in size with time [18, 27]. Furthermore, it has been demonstrated that there is ongoing proteolytic enzyme activity in prolapsed discs [19]. PDGF stimulates collagenase production in fibroblasts [29] and has a chemotactic effect on them [22]. Our results may suggest a possible role for PDGF in regulation of proteolysis including in DHT. This proteolytic process is part of regeneration and may also act as a stimulator for angiogenesis.

Disc tissue is normally avascular and the appearance of capillaries is a sign of aging or tissue degeneration [32]. A neovascularization process also takes place following disc tissue injury and in disc herniation. The blood vessels in herniated disc tissue are mainly newly formed [32]. Several factors are able to stimulate such vascular endothelial cell growth. In this study we demonstrated PDGF and VEGF immunopositivity in disc herniation tissue capillaries. In a previous study we located FGF in these same capillaries [31]. These growth factors act synergistically in angiogenesis, and our results suggest that these factors also participate in the neovascularization process in DHT.

No relationship between VEGF or PDGF expression and the duration of preoperative pain before operation was noted. In the different prolapse types PDGF immunoreaction was detected slightly more often in capillaries than in nuclei of disc cells, but the difference was not marked (Table 3). VEGF immunopositive capillaries were highly prevalent. VEGF was expressed in all sequesters and in more than 90% of extrusions. In both sequesters and extrusions blood vessels were more often immunoreactive for VEGF than PDGF (Table 3).

VEGF expression in capillaries was similar across all age groups, whereas PDGF expression varied by age group, with a higher prevalence in capillaries than in disc cells being particularly characteristic of patients younger than 40 years (Table 4). In a previous study cellular FGF immunoreactivity was detected more often in younger pa-

tients and vascular FGF expression was found to be similar across all age groups [31].

Our results confirm that PDGF and VEGF participate in the neovascularization process. Furthermore, PDGF expression in disc cells and in fibroblasts suggests that this growth factor regulates the cellular function of these cells, mainly the production of the extracellular matrix components and proliferation of the cells.

Conclusion

The immunolocalization of the angiogenic agents PDGF and VEGF in capillaries of the herniated intervertebral disc supports the active neovascularization process. Furthermore, the presence of PDGF in disc cells suggests that this growth factor may be regulative for the production of extracellular matrix components and the proliferation of these cells. This disc cell activation is important in the pathogenesis of the intervertebral disc and needs further examination.

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