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ORIGINAL ARTICLE

Aklilu Habtemariam
Mats Grönblad
Johanna Virri
Olli Korkala

Blood clots in vessels surrounding symptomatic nerve roots in disc herniation patients: a pilot study

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A. Habtemariam · M. Grönblad (✉)
J. Virri
Research Laboratory,
Spine Research Unit,
University of Helsinki, Finland
e-mail: mats.gronblad@hus.fi,
Tel.: +358-9-47161609,
Fax: +358-9-47172354

M. Grönblad
Department of Physical Medicine
and Rehabilitation,
University of Helsinki, Central Hospital,
P.O. Box 340, 00029 HUS,
Helsinki, Finland

O. Korkala
Department of Surgery, City Hospital,
Lahti, Finland

Abstract Mechanical compression and biochemical influences, e.g. by various inflammatory cells and mediators, have been suggested to be involved in the pathophysiology of sciatica. In addition, it has been suggested, but so far not clearly demonstrated, that blood pooling in the venous blood vessels surrounding nerve roots may contribute to impaired nerve root function and sciatica. There is also more direct evidence from studies on an animal model that nucleus pulposus (NP) tissue may induce blood vessel thrombosis. In the present study, a specific monoclonal antibody to platelet membrane glycoprotein complex GPIIb-IIIa was used for direct visualization of blood clots in tissue samples removed from the vicinity of symptomatic nerve roots in 20 disc herniation (DH) patients. For 12 patients, the DH was primary, for 7 patients recurrent and for 1 patient the index operation was for root canal stenosis following a DH operation 3 months earlier. Blood clots immunoreactive to GPIIb-IIIa were observed in small blood vessels in 11/20 tissue samples (55%), in 5/12 patients (42%) with primary DH and in 5/7 patients (71%) with recurrent DH. The presence or absence of GPIIb-IIIa complex immunoreactivity did not show a statistically significant relationship with pain duration, DH type (primary or recurrent), gender or straight leg raising (SLR). Thus, though immunohistochemically demonstrable, the observed periradicular blood clots could not be established to have a clinical role in sciatica in this pilot study.

Keywords Herniated disc · Blood coagulation · Thrombosis · Sciatica

Introduction

Knowledge of the intricate mechanisms behind sciatica is rapidly expanding [25]. A previous view of mechanical compression as the sole or most important factor producing sciatica has been challenged with rapidly increasing new evidence of an involvement of various biochemical factors, including inflammatory mediators [2, 3, 16, 24], inflammatory cells [19], and matrix metalloproteinase enzymes [12]. Increased tissue acidity as a result of inflammation, injury or degenerative changes may alter the ion permeability in sensory neurons and produce both sustained neural activity and enhanced mechanosensitivity [31]. Recently it was shown that mechanical hyperalgesia produced by NP tissue placed on the nerve root depends on inflammatory cell infiltration [19]. The type of DH, whether noncontained or contained, determines the extent to which inflammatory mediators will affect the nerve root [24].

In addition to the mechanisms of sciatica mentioned above, there are clinical observations of circulatory stasis or swelling of epidural veins [20], as well as reports of intraneural (intraradicular) thromboses in patients with spinal degenerative disease [17]. In a recent study on perineural and extraneural tissues of patients with radiographically

proven DH, Cooper and co-workers [6] were unable to demonstrate inflammatory cells, whereas they observed vascular congestion and endothelial abnormalities, including luminal platelet adhesion, as well as both perivascular and perineural fibrosis. Thus, vascular pathology, including pathological alterations in the small vessels that surround nerve roots, may be clinically important in DH tissue-induced nerve root dysfunction and sciatic pain.

In the present study we sought to explore directly blood clots in tissue surrounding symptomatic nerve roots in patients operated for DH, by employing a specific monoclonal antibody to the glycoprotein (GP) IIb-IIIa complex, which is present in activated and aggregated platelets. All the patients studied had symptoms of sciatica at the time of the index operation when the tissue samples studied were obtained.

Control tissue from the vicinity of nonsymptomatic nerve roots could not, for practical reasons, be obtained for comparison. However, to study the possible clinical importance of obtained immunostaining results, they were compared with clinical data: tightness of straight leg raising (SLR), duration of pain preoperatively, gender and type of operation (primary or recurrent DH).

Material and methods

Patients

The 20 DH patients studied are described in detail in Table 1 and Table 2. In most patients, the DH had been diagnosed by computed tomography (CT), and in five patients by myelography only. For most of the patients (12 patients), the index operation was a primary DH operation, for 7 patients a recurrent DH operation and for 1 patient an operation for root canal stenosis following a DH operation 3 months earlier. Thus, in this particular patient there was no recurrent DH, but an interval of several months following a primary DH operation during which tissue pathophysiology may have developed. One patient had undergone two prior DH operations, all the other recurrent disc patients (six patients) only one prior DH operation.

Tissues studied

In patients with recurrent DH, the tissue taken for study was mostly scar tissue and/or adhesions on the nerve root. In primary DH patients with longer pain durations prior to the DH operation, the tissue removed for study was also mostly scar-type tissue, but in three patients (nos. 9, 16 and 20) DH tissue interfacing the nerve root comprised the material studied.

Immunohistochemistry

All tissue specimens were treated according to the same staining protocol. After removal at operation, the tissue was rinsed in physiological saline solution (PBS) and then frozen rapidly in liquid nitrogen. Specimens were kept in a freezer at –70°C until analyzed. Frozen sections 6 μm thick were cut on a cryostat, and sections were then subjected to indirect immunohistochemistry using the avidin-biotin-peroxidase complex (ABC) method [14]. Prior to immunostaining, slides were fixed in ice-cold (+4°C) acetone for 10 min. Before treatment with the primary antibody, nonspecific binding was blocked by treatment with non-immune serum (horse normal serum). Endogenous peroxidase was quenched by incubation in 0.3% H_2O_2 in 70% methanol-phosphate buffered saline at room temperature for 30 min.

Two random frozen sections were immunostained from each tissue sample. Two observers assessed the immunostaining results separately for each tissue sample studied. They classified each tissue sample as being either positive or negative for glycoprotein IIb-IIIa immunoreactivity. The two observers were in full agreement for all samples studied.

Primary antibody

Mouse anti-human platelet glycoprotein complex GPIIb-IIIa (CD41) monoclonal antibody was used at the dilution 1:300 (DAKO, Glostrup, Denmark, Code No. M 7057). According to the manufacturer manual, the antibody reacts with the glycoprotein IIb-IIIa complex present on human platelets [21, 23]. The antibody will detect platelets in human tissue and also labels megakaryocytes in sections or smears prepared from human bone marrow. Furthermore, the antibody will label platelets in peripheral blood smears.

Immunostaining control

For immunostaining controls, sections were stained as outlined above, but the primary monoclonal antibody was omitted and substituted by normal serum. Such control sections always lacked any immunostaining.

Statistics

The SigmaStat 1.0 statistical software program (Jandel Scientific GmbH, Erkrath, Germany) was used. Groups were compared by Fisher's exact test. The median was used as cut-off value when comparing continuous data (pain duration, SLR). Level of statistical significance was set at $P<0.05$.

Results

The results are presented in Table 1 and Table 2, which also show the patient characteristics. Immunoreactivity to glycoprotein complex GPIIb-IIIa was observed in blood vessels in 11/20 samples studied (55%). Such dense immunoreactivity was mostly present in blood vessels located in the periphery of the tissue section, immediately adjacent to the nerve root (*NRA* in Fig. 1 and Fig. 2). In contrast to the surrounding tissue, where scattered immunoreactive platelets could be observed (Fig. 1), some blood vessels appeared to be almost totally obliterated by clumps of aggregated platelets (Fig. 1, Fig. 2).

GPIIb-IIIa immunoreactivity was present in 5/12 primary DH patients (42%), in 5/7 recurrent DH patients (71%) and in a single patient who was operated on for root canal stenosis following a primary DH operation 3 months earlier (patient 15). There was no statistical difference for the occurrence of immunoreactivity to GPIIb-IIIa between primary and recurrent DH patients ($P=0.20$). Nor was there a difference between male (9/16, 56%) and female (2/4, 50%) patients.

Table 1 Clinical data of 20 disc herniation (DH) patients studied by immunohistochemistry with a monoclonal antibody to platelet glycoprotein (GP) complex IIb-IIIa (*SLR* straight leg raising test)

Pat. no.	Age/Sex	Duration of pain[a]	SLR[b]	Immuno-reactivity
1	42/M	4 weeks	60°/–	+
2	52/M	15 years	20°/10°	+
3	18/F	6 months	–/–	+
4	46/M	2 months	20°/–	+
5	53/M	1 week	–/40°	+
6	44/F	1 year	–/30°	+
7	42/M	3 months	45°/–	–
8	53/M	10 years	50°/–	–
9	44/F	10 years	45°/–	–
10	37/M	10 months	–/–	–
11	49/M	4 years	–/40°	+
12	53/M	2 years	–/60°	–
13	46/M	2.5 years	50°/–	+
14	66/M	11 years	45°/45°	–
15	49/M	6 months	-/40°	+
16	62/M	3 months	30°/50°	+
17	56/M	2 years	40°/–	+
18	37/M	2 months	–/40°	–
19	51/F	13 months	60/–	–
20	55/M	2 weeks	–/10°	–

[a]Duration of radicular pain prior to the index operation from which the study sample was obtained. Some pain durations are very long, because the index operation was for a recurrent DH

[b]Test results considered positive at <70°; -/- indicates bilaterally negative test. Note also occasional patients with a bilaterally positive SLR test

The median (range) duration of radicular pain was 11 months (1 week to 15 years). Immunoreactivity to GPIIb-IIIa did not relate to duration of pain (P=1.00). Nor was there a statistically significant relationship (P=0.07) between presence of GPIIb-IIIa immunoreactivity and tighter SLR (median=42.5°, range 10° to bilaterally negative).

Discussion

Platelets play an important role in acute myocardial infarction [9], in inflammatory diseases such as rheumatoid arthritis [33] and in bronchial asthma [15, 28]. According to the results of our study, platelets may also be involved in mechanisms of sciatica. Of note, platelets are a possible source of group II phospholipase A_2 (secretory PLA2: sPLA2), an enzyme that is involved in the amplification of both local and systemic inflammation [1, 7, 8], and has been suggested to be involved in disc pathophysiology and possibly also sciatica [27, 29]. Controlled studies have suggested that PLA2 levels are not pathologically high in disc herniation tissue or degenerated discs in comparison with control disc tissues [10, 11], and thus alternative sources of PLA2 enzyme, such as release from platelets, may be clinically relevant. PLA2 enzyme has been shown to have deleterious effects on nerve root function and structure in an animal model [5]. Platelets are known to contain both a low molecular weight (14 kDa, sPLA2) and a higher molecular weight (85 kDa, cytosolic: cPLA2) PLA2 enzyme, which are located in the α-granules and the cytosol of platelets, respectively. It has

Table 2 Additional clinical data for the patients shown in Table 1 (*CT* computed tomography, *Mye* myelography, *R* right-sided, *L* left-sided, *C* central)

Pat. no.	Imaging	Disc level	Herniation type/side[a]	Immuno-reactivity
1	CT	L5-S1	Primary/R	+
2	CT	L4-L5	Recurrent (2 yrs)/L	+
3	CT	L5-S1	Primary/R	+
4	CT	L4-L5	Recurrent (×2 previous DH)/R	+
5	CT	L4-L5	Recurrent (no. 1: cauda)/L	+
6	CT	L4-L5	Primary/L	+
7	CT	L5-S1	Primary/R	–
8	CT	L5-S1	Recurrent (1 yr)/C	–
9	CT, Mye	L4-L5	Primary/R	–
10	Mye	L4-L5	Primary/R	–
11	CT	L5-S1	Recurrent (3 yrs)/L	+
12	CT, Mye	L4-L5	Primary/L	–
13	CT	L5-S1	Recurrent (1 yr)/R	+
14	CT, Mye	L3-L4	Recurrent (11 yrs)/L	–
15	CT	L5-S1	Root canal stenosis (DH operated 3 months earlier)	+
16	Mye	L5-S1	Primary/C	+
17	CT	L4-L5	Primary/L	+
18	Mye	L5-S1	Primary/L	–
19	Mye	L4-L5	Primary/R	–
20	Mye	L4-L5	Primary/L	–

[a]Recurrent herniations: parenthesis shows time since previous DH operation when known: for patient no. 4 there were two prior DH operations; for patient no. 5 the prior DH was a cauda equina; for patient no. 15 the index operation was for root canal stenosis, not recurrent DH, but this patient had been operated for a DH 3 months earlier

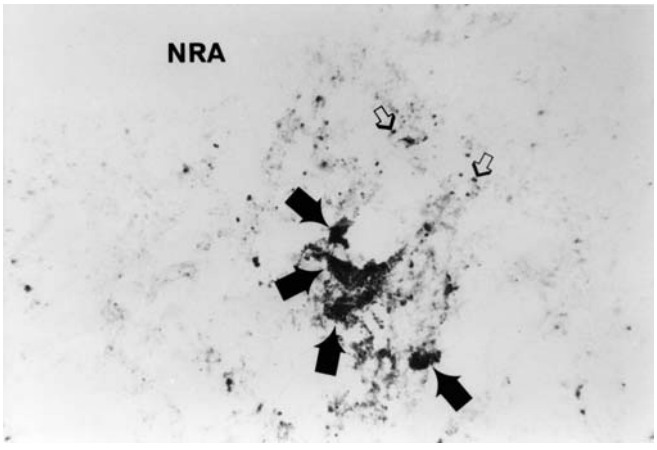

Fig. 1 Clogged up blood vessels (*black arrows*) seen after immunostaining with monoclonal antibody to platelet glycoprotein (GP) IIb-IIIa complex: an 18-year-old female patient (no. 3) with a primary disc herniation (DH) at the L5-S1 level. *Open arrows* point to nearby scattered platelets, *NRA* indicates the region where nerve root was located [avidin-biotin-peroxidase complex (ABC) immunostaining, hematoxylin counterstaining; original magnification ×480]

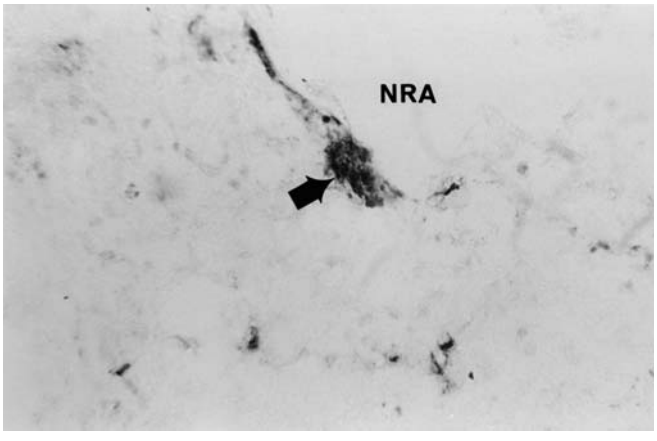

Fig. 2 Clogged up blood vessel near the periphery of the tissue section (*black arrow*), seen after immunostaining with monoclonal antibody to platelet GP IIb-IIIa complex: a 53-year-old male patient (no. 5) with recurrent DH (cauda equina) at the L4-L5 level (*NRA* region of nerve root; ABC immunostaining, hematoxylin counterstaining; original magnification ×480)

been suggested that sPLA2 and arachidonic acid are liberated when platelets become activated [1, 7, 8]. Interestingly, both these PLA2 enzymes were also recently demonstrated by immunohistochemistry in DH tissues [11].

Platelet activation follows adhesion, which is triggered by damage of the blood vessel wall [22]. The activation of platelets is associated with changes in their shape and activation of the glycoprotein (GP) IIb-IIIa receptor [22]. The platelet membrane GPIIb-IIIa complex is an essential mediator of platelet aggregation via its ability to bind fibrinogen. Pathways of aggregation are mediated by the release of endoperoxides, thromboxane A2, which is the precursor of thromboxane B2, and adenosine diphosphate. These bioactive substances amplify platelet aggregation, and it was also recently suggested that gelatinase A, thrombin and collagen could mediate platelet aggregation [22, 30]. Amplification of platelet aggregation by thromboxane A2 may be of interest in disc tissue pathophysiology, since it was shown by Nygaard and co-workers [24] that the concentration of thromboxane B2 is higher in non-contained than contained DH. Thus, inflammatory mediators present in increased concentrations in extruded disc tissue could contribute to the formation of blood clots adjacent to nerve roots, as observed in the present study. There is also one interesting experimental study [26] suggesting a triggering of thrombosis by nucleus pulposus (NP), even if the molecular mechanism producing such an effect is as yet unknown.

Recently, platelet-derived growth factor (PDGF) was demonstrated in 78% of DH tissue samples examined [32]. Interleukin-6 (IL-6) also has thrombopoietic activity, as shown in in vitro studies, and inflammatory disease is often coupled to reactive thrombocytosis [33]. Thus, recent reports of IL-6 in DH tissue [18] are of particular interest. IL-6 may contribute to the aggregation of platelets observed in the present study.

DH may lead to compression of epidural veins, with dilation of noncompressed veins. A decade ago, Hoyland and co-workers [13] presented observations on cadavers suggesting perineural vascular congestion, dilation, thrombosis and perineural and intraneural (intraradicular) fibrosis in association with DH. They suggested that the observed histopathological changes resulted from venous outflow obstruction by the DH tissue, with secondary thrombus formation, producing ischemic damage and fibrosis [13]. In addition, there is a rapid transport route from the epidural space to intraneural capillaries [4] that may directly carry products, e.g. inflammatory mediators released from clogged up blood vessels, into the nerve root.

In the present study, 11/20 tissue samples (55%) taken from the vicinity of symptomatic nerve roots exhibited blood vessels immunoreactive to GPIIb-IIIa complex. Comparing presence or absence of immunoreactivity in primary versus recurrent DH showed no statistically significant difference, and nor was any statistically significant relationship with pain duration or SLR observed. Thus, a clinical role in sciatica of the observed immunoreactivity indicating blood clots could not be established in this pilot study.

Conclusions

The present study explored a possible contributory mechanism behind sciatica that may operate in addition to better characterized mechanisms such as direct mechanical compression, effect of inflammatory cells and mediators and tissue-specific effects of nucleus pulposus (NP):

namely, the formation of blood clots in the small blood vessels adjacent to symptomatic nerve roots. Even though we were able to demonstrate such blood clots, their presence did not show a statistically significant relationship with clinical parameters of sciatica. Thus their role, if any, in the pathophysiology of sciatica remains unclear. It may be that similar blood clots previously reported to be present within nerve roots are clinically more relevant.

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