

Characteristic electron microscopic findings in the skin of patients with fibromyalgia—preliminary study

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Abstract This blinded study was done to determine if there are any abnormal electron microscopic (EM) findings in the skin of fibromyalgia syndrome (FMS) patients, which might contribute to or be due to the increased pain sensitivity seen in this condition. Skin biopsy samples were obtained from 13 FMS patients and 5 control subjects. All tissues were prepared for EM examination by immediate prefixation in 2.5% glutaraldehyde for 2 h and postfixation in 1% osmium acid for 24 h. Ultrathin sections on grids were stained by uranyl acetate and lead citrate. Biopsies were read by an individual without knowledge of participant status. Five skin biopsies from healthy controls showed relatively even distribution of variegated sized unmyelinated axons sheathed well by complicatedly folded Schwann cell membranes. In tissues from 9/13 FMS patients, unmyelinated Schwann cells were noted to be ballooned, whereas this finding was not noted in any controls ($p=0.029$). Axons in most patients trended towards being localized in the periphery of the unmyelinated Schwann cell sheaths ($p=0.002$). Particularly, peripheral

localization of axon in the unmyelinated Schwann cell sheath had a strong relationship with ballooning of Schwann cell ($p=0.042$), simplified folding of Schwann cell sheath ($p=0.039$) and smaller axon ($p=0.034$). Myelinated nerve fibers were unremarkable. The EM findings seen in the skin of FMS patients show unusual patterns of unmyelinated nerve fibers as well as associated Schwann cells. If these findings are replicated in a larger study, these abnormalities may contribute to, or be due to, the lower pain threshold seen in FMS patients.

Keywords Electron microscope · Fibromyalgia · Skin · Unmyelinated

Introduction

Fibromyalgia syndrome (FMS) is a chronic, generalized pain condition with diffuse tenderness characteristic tender points on physical examination, and is often accompanied by a number of associated symptoms such as sleep disturbance, fatigue, headache, irritable bowel syndrome, and mood disorders [1, 2]. The mechanisms responsible for symptom expression in FMS are complex. The most consistently detected objective abnormalities in FMS involve pain-processing systems [3, 4]. Psychophysical studies, functional neuroimaging, and many other lines of research have suggested that FMS patients display augmented central pain processing [5]. The amount of pressure stimuli required to cause cerebral activation in pain-processing regions of the brain was much lower in FMS in the study using functional magnetic resonance imaging (4). There are also data suggesting that FMS may be related to a decrease in the activity of descending, antinociceptive pathways (5). In addition to neurobiologic mechanisms, psychologic and behav-

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ioral factors also play a role in symptom expression in many patients [5].

There are many reasons that the augmented pain processing in FMS has been felt to be primarily due to abnormalities in central pain processing. First, the low pain threshold in FMS extends throughout the body, and is not just confined to areas such as tender points, or tissues that have underlying muscle [6]. Moreover, the fact that individuals with FMS are not just more sensitive to pressure but also to heat, noise, and auditory stimuli suggests a diffuse central nervous system defect in sensory processing. Second, FMS shares significant demographic, physiologic, and therapeutic response profiles with a number of other pain syndromes, especially visceral syndromes such as irritable bowel syndrome, interstitial cystitis, and noncardiac chest pain [7]. Finally, most previous studies of the peripheral tissues of FMS, especially the skeletal muscle, had been unremarkable [8]. However, Sprott's electron microscopic (EM) findings suggest that patients with FMS are characterized by abnormalities in muscle tissue that include increased DNA fragmentation and changes in the number and size of mitochondria [9].

Recently, investigators have questioned whether the peripheral nerves may be abnormal in FMS, either because these abnormalities contribute to the pain sensitivity, or alternatively are due to the decreased pain threshold [10–15]. Up to recently, central nervous system was a primary focus of investigations in FMS. With the recognition of abnormalities in skin of some FMS patients, it is now apparent that the role of peripheral nerve endings in FMS is much greater than previously thought. For example, Kim et al. reported that *N*-methyl-D-aspartate receptors subtype 2D expression was increased in the skin of patients with FMS versus controls and suggested that this could be indicative of a more generalized increase in other peripheral nerves [10]. Other investigators noted it was suggested that there were some differences in between the amino acid composition of skin proteins in FMS compared with controls, and found that the amount of collagen might be lower in skin from FMS patients, and collagen packing in the endoneurium might be less dense [11].

Several other studies support the notion that there may be increased exaggerated neurogenic inflammation in ory responses in patients with FMS, presumably reflective of increased activity of polymodal nociceptors of unmyelinated primary afferent nerves [12–15]. There are also reports supporting the hypothesis of neurogenic inflammation involvement in FMS (9–11). FMS skin biopsies have significantly higher IgG deposits in the dermis and vessel walls, as well as and also had a higher mean number of mast cells [13]. The detection of IL-1, IL-6, and tumor necrosis factor- α , and the finding of highly ordered cuffs of collagen around the terminal nerve fibers in skin of patients

with FMS by EM examination in FMS skin also support the presence of neurogenic inflammation [14, 15].

The aim of the present study was to investigate whether there were characteristic EM findings in skin from FMS patients when compared with skin from control subjects who were matched for age and gender.

Materials and methods

Patients were diagnosed as having FMS according to the American College of Rheumatology (ACR) criteria [1]. Skin biopsies from nontender left deltoid DJC region of 13 patients and 5 healthy 'age- and sex-matched' controls (Table 1) were performed after informed consent was obtained from participants. Table 2 shows the demographic characteristics of the patients with FMS. All work was previously approved by the institutional review board done under individual permission and the principle of experiment and research of Dongguk University College of Medicine.

All tissues were prepared for EM examination by immediate prefixation in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.3) for 2 h and postfixation in 1% osmium acid (0.1 M phosphate buffer, pH 7.3) for 24 h. After washed in buffer solution, fixed tissues were dehydrated through graded ethanol, and embedded in Epon. Ultrathin sections on grids were stained by uranyl acetate and lead citrate. Hitachi H-7500 and H-7100 transmission electron microscopes (Hitachi, Ibaraki, Japan) were used. Samples were read by two pathologists blinded to the study design. Unmyelinated fibers were scrutinized about sizes of axon and Schwann cell, the degree of the sheath folding, location of axons in sheath, and so on. Each pathologist was asked to assign a score of 0, 1, 2 for criteria including Schwann cell ballooning, axon in center, collagen crosslinks, etc. (Table 3). They showed 100% agreement regarding the characteristics of each sample ($\kappa=1$).

Statistical analysis was performed using SPSS for Windows (version 11.0; SPSS, Chicago, IL). Fisher's exact test was used to determine *P* values in all categorical data.

Table 1 Characteristics of the control subjects and patients with fibromyalgia

	Fibromyalgia patients (<i>n</i> =13)	Normal control (<i>n</i> =5)	<i>P</i> values
Age, mean±SD (years)	47.4±10.9	50.0±14.1	0.961 ^a
Sex, female/male	10:3	3:2	0.583 ^b

^a By Mann–Whitney *U* test

^b By Fisher's exact test

Table 2 Demographic characteristics of the patients with fibromyalgia

Patients/ age/sex	Disease duration at time of biopsy, years	Concurrent medication	Confounding diseases
1/58/F	0.5	G, E	
2/52/F	10	G, A, AP, C, ALP, T, GL, M	Diabetes
3/42/F	4	AM, TR, ALP, R, L	Hypertension
4/49/F	5	G, A, TR, ALP	Hypertension
5/47/F	6	G, A	
6/41/F	5	G, E	
7/53/M	3	CM	
8/73/M	2	G, TR, D	Diabetes
9/46/F	0.5		
10/40/F	0.5	P	
11/26/M	3	AM, AP+TR	
12/45/F	3	AM, EM, ET	
13/44/F	0.7	E, ET	

G Gabapentin, E Eperisone HCL, A Aceclofenac, AP Acetaminophen, C Carbamazepine, ALP Alprazolam, T Triazolam, GL Gliclazide, M Metformin, AM Amitriptyline HCL, TR Tramadol HCL, R Ramipril, L Lercanidipine HCL, CM Clematis mandshurica etc., D Diacerein, P Piroxicam, EM Etodolac micronized, ET Etizolam

Mann–Whitney *U* test was used to determine *P* value in age comparison of patients and controls. The associations were analyzed by means of Spearman’s rank correlation test. A *P* value of 0.05 was considered significant.

Results

The skin appeared normal on light microscopic findings in all specimens from patients and controls. The five skin biopsies from controls healthy persons for showed relatively even distribution of variegated sized axons sheathed well by complicatedly folded Schwann cell membranes (Fig. 1a–b).

In biopsied skins from 13 patients with FMS, most unmyelinated Schwann cells in 9 cases were ballooned ($p=0.029$). Axons in most patients trended to be peripheralized in their unmyelinated Schwann cell sheaths ($p=0.002$). Folding structures of the Schwann cell sheathed individual axons were simplified definitely in three cases. Particularly, pPeripheralization of axon in the unmyelinated Schwann cell sheath DJC occurred in association had a great relationship with ballooning of Schwann cell ($p=0.042$), simplified folding of Schwann cell sheath ($p=0.039$) and smaller axon ($p=0.034$). Collagen fibrils filling in endoneurium seem to be noted in similar frequency in not less in FMS (X/Y) and controls (X/Y) than in controls. Splitting of perineurium was not only observed in relatively equal frequency in FMS (X/Y) and controls (X/Y) but also found in controls. Three controls and four patients revealed collagen replacement of axon (Fig. 1c–f). Collagen crosslinks were detected in two cases of FMS but no controls (Fig. 1i).

Because of these findings, we performed a second Two skin biopsy in the forearm of one i patient to determine if the changes seen above were present in both a classic tender point

Table 3 Scores of the two pathologists for each criteria

	Ballooning of SC	Axon in center	Simplified folding of SC membrane	Replacement by collagen	Hydropic degeneration of axon	Small axon	Increased mitochondria	Collagen crosslink
Patient								
1	1/1	1/1	0/0	0/0	0/0	1/1	0/0	0/0
2	0/0	1/1	0/0	1/1	1/1	1/1	0/0	1/1
3	1/1	1/1	0/0	1/1	0/0	1/1	0/0	0/0
4	1/1	1/1	0/0	0/0	0/0	1/1	0/0	0/0
5	0/0	1/1	0/0	0/0	1/1	1/1	1/1	1/1
6	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0
7	0/0	1/1	1/1	0/0	0/0	0/0	0/0	0/0
8	1/1	0/0	1/1	0/0	1/1	1/1	0/0	0/0
9	1/1	0/0	1/1	0/0	0/0	1/1	0/0	0/0
10	1/1	1/1	0/0	1/1	0/0	1/1	0/0	0/0
11	1/1	1/1	0/0	1/1	0/0	0/0	0/0	0/0
12	1/1	1/1	0/0	1/1	0/0	1/1	0/0	0/0
13	1/1	1/1	0/0	0/0	1/1	0/0	0/0	0/0
Control								
1	0/0	2/2	0/0	0/0	1/1	0/0	0/0	0/0
2	0/0	2/2	0/0	0/0	0/0	0/0	1/1	0/0
3	0/0	2/2	0/0	1/1	1/1	0/0	0/0	0/0
4	0/0	2/2	0/0	1/1	1/1	0/0	0/0	0/0
5	0/0	1/1	0/0	1/1	0/0	1/1	0/0	0/0

First pathologist/second pathologist ($\kappa=1$)
SC Schwann cell

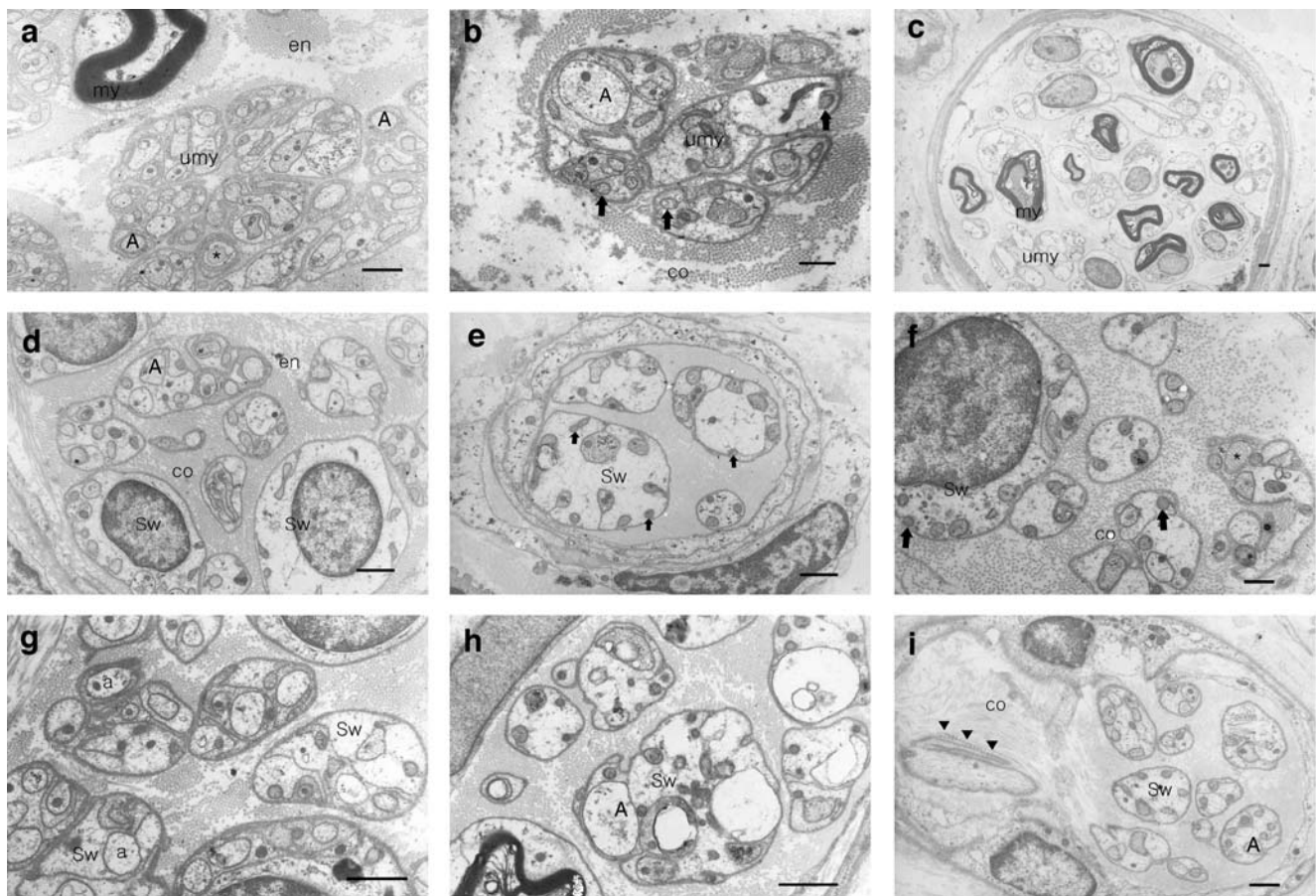


Fig. 1 Electron microscopic photographs taken from unmyelinated peripheral nerves in normal and fibromyalgia syndrome skin. **a** In normal skin, C-fiber (Remak) bundles of unmyelinated nerves (*umy*) near a myelinated nerve (*my*) show variegated sized axons (*A*) sheathed well by complicatedly folded Schwann cell (*Sw*) membranes. Collagen pockets (*asterisk*) replacing axons are present. **b** In other normal skin, small axons (*upward dark arrow*) are present. **c** Low magnification photograph from FMS skin shows ballooned Schwann

cells and peripheralized small axons of Remak bundles. **d** Collagen fibrils (*co*) in endoneurium (*en*) are not less than normal controls (**a** and **b**). **e**: A small nerve fiber shows small axons located in the peripheries of Schwann cells. **f**: Collagen pockets are also present. **g** Nontender area and **h** tender area show similar findings except more ballooned Schwann cells in tender areas. **i** Collagen cross-linking (*arrow head*) is noted near haphazard arranged collagen fibrils. (bar=1 μ m)

(the lateral epicondyle area) and nontender point (the deltoid region). These two sites showed qualitatively similar findings, es from different sites of a patient; tender area (forearm) and nontender area (trapezius region), showed similar electron microscopic findings, though the unmyelinated Schwann cells from the tender area were more ballooned than those from the nontender area (Fig. 1g and h).

The skin otherwise appeared normal on EM examination in all specimens from patients and controls, especially in myelinated fibers.

Discussion

This is the first study to note characteristic EM findings of unmyelinated nerve fibers in the skin of patients with FMS. The most striking finding was that it is unique that most unmyelinated Schwann cells in FMS skin were ballooned

compared with controls, and that the axons tended to be peripheralized in their unmyelinated Schwann cell sheaths. There is no report about these abnormalities of unmyelinated nerve fibers in other conditions including other chronic pain diseases. Two of the patients suffered from diabetes, and none of the control patients did not suffer from that disease. Due to the extensive effect of diabetes on peripheral nerves, this point is worthy of mentioning. Swollen and vacuolated intraepidermal nerve fibers were identified in patients with neuropathy by conventional and immuno-electron microscopy in Lauria's study [16]. We do not think these abnormalities are tissue fixation artifacts or normal variation within human skin because there was no ballooning of Schwann cell in other myelinated fiber and we could see microtubules in axon.

These findings differ somewhat from previous previous EM studies of FMS [11, 15]. In Ribbel-Madsen's study, skin biopsies were obtained from the thigh of 27 females who

fulfilled the ACR criteria [1] of FMS and from eight control subjects who were matched for gender, age and physical activity. The overall microscopic picture was normal. The lamellar structure of the perineurium and a deficiency in collagen packing in the endoneurium was observed more frequently and to a larger extent in FMS patients than in controls [11]. In Sprott's study, highly ordered cuffs of collagen were observed around the terminal nerve fibers by EM examination of biopsy tissue from all eight patients with FMS, but were not observed in any of the control skin samples [15].

We only noted the cIt is also characteristic that axons in most patients trended to be peripheralized in their unmyelinated Schwann cell sheaths. Collagen crosslinks, which Sprott et al. [15] reported were detected only in two cases of FMS. Unlike Ribell-Madsen et al.'s results, in which a deficiency in collagen packing in the endoneurium was observed more frequently and to a larger extent in FMS patients than in controls [11], collagen fibrils in endoneurium were not less than normal controls in our study (Fig. 1d). Despite these differences between our EM results and those of previous studies, these studies all suggest that there may be a number of identifiable abnormalities of the peripheral skin in FMS.

The meaning of these changes is unclear. The abnormalities that we identified could either be partly causing the increased pain sensitivity in FMS, or be due to it. For example, if FMS is primarily due to a decrease inIt is reasonable to speculate that morphologic changes of unmyelinated nerve fibers in skin may be the result of a decrease in the activity of descending, antinociceptive pathways in FMS patients [4], then peripheral nerves may depolarize more frequently because of the lack of inhibitory input in the dorsal horn, and could lead to such findings. In contrast, these changes could represent a fundamental problem in the peripheral nerves in FMS, and this could actually contribute to the increased pain sensitivity seen in this condition. In aggregate, these EM studies suggest that continued investigation of the peripheral nervous system may lead to useful insights into FMS.

The EM findings seen in the skin of FMS patients show unusual patterns of unmyelinated nerve fibers as well as associated Schwann cells. If these findings are replicated in a larger study, these abnormalities may contribute to, or be due to, the lower pain threshold seen in FMS patients.

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