The Morphology and Distribution of Sensory Nerve Endings in the Human Lateral Ankle Ligament

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ABSTRACT. Introduction : We examined the morphology of the sensory nerve endings in human lateral ankle ligaments electron microscopically and studied the three-dimensional distribution of these nerve endings.
Materials and Methods : Three sets of lateral ankle ligaments consisting of the anterior talofibular ligament(ATF), calcaneofibular ligament(CF) and posterior talofibular ligament(PTF) were obtained from cadavers donated for dissection or legs amputated due to injury. The ligaments were stained by a modified Gairns' gold chloride staining method. Serial sections were prepared, and the morphology and distribution of the sensory nerve endings in each section were observed under light and the electron microscopies.
Results & Conclusion : In the lateral ankle ligaments, three types of nerve endings were observed; namely, Ruffini's receptors, Golgi's tendon-like receptors and free nerve endings. In the ATF andCF, many nerve endings were distributed in the proximal part. In the PTF, there was an equal distribution of nerve endings in both the proximal part and the distal part. The nerve endings were more abundant on the lateral side than the medial side. The sensory nerve endings in the lateral ankle ligament may play a role in the movement control mechanisms of the ankle joint.

Key words : Sensory nerve endings — Mechanoreceptor — Ultrastructure — Lateral ankle ligament

Smooth joint movement is achieved by the ligament and articular capsule, which play a role in the static stabilizing mechanism, together with the muscles and ligaments that are associated with the dynamic stabilizing mechanism. Recently, morphological and electrophysiological studies have demonstrated that deep sensory receptors in the ligaments are associated with mutual regulation between the ligaments and muscles.

In 1972, Wyke *et al*[1-3] classified the deep sensory receptors into four broad types, their based on morphological and physiological features (Table 1). Subsequently, morphological studies confirmed that the deep sensory receptors include the Ruffini receptors, Golgi tendon-like receptors and pacinian corpuscles in the anterior cruciate ligament of the knee,[4-7] the coracoacromial ligament,[8,9] and the supraspinous interspinous ligament,[10] and reported on their distribution. Physiological studies, which have included examination of intraoperative electrical changes in the anterior cruciate

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TABLE 1. Classification of articular mechanoreceptors abridged from Wyke[3)]

Type	Morphology	Parent nerve	Physiology
I	Thinly encapsulated globular corpusclen in clusters of 3 to 6	Small myelinated	Low threshold, slow adapting Static and dynamic
II	Thickly encapsulated corpuscles in clusters of 2 to 4	Medium, myelinated	Low threshold, rapid adapting Dynamic
III	Thinly encapsulated fujiform corpuscles	Large, myelinated	High threshold, slow adapting Dynamic
IV	Plexuses and free nerve ending	Very small myelinated	High threshold Pain receptors

ligament of the knee joint,[11)] and examination of deep sensory receptors with regend to electrical changes as a result of stimulation of the ankle joint lateral ligament in cats, have also been conducted.

However, to elucidate the involvement of deep sensory receptors in ligaments in the motor control mechanism, detailed morphological studies including the ultrastructure of the deep sensory receptors are essential.[12)]

Michelson *et al*[13)] studied the ankle joint ligament and demonstrated the existence of deep sensory receptors belonging to Wyke's types I to III. To the best of our knowledge, there have been no reports, including electron microscopic studies on the morphology and distribution of deep sensory receptors in the lateral ligament of the human ankle joint. Ankle injuries are routinely encountered traumatic injuries. Most of them are injuries of the lateral ligament of the ankle caused by inversion stress. To elucidate the involvement of the deep sensory receptors in the lateral ankle ligament in the motor control mechanism, morphological studies including the ultrastructures of the deep sensory receptors in the lateral ligament of the ankle are indispensable.

In the present study, we attempted to examine the involvement of the deep sensory receptors in the lateral ligament of the ankle in the motor control mechanism by observing the fine structures of the deep sensory receptors using light and electron microscopy and by analyzing the distribution of the deep sensory receptors in the ligaments three-dimensionally.

MATERIALS AND METHODS

1. Materials

The ankle lateral ligaments used in this study were obtained from 11 limbs of 11 subjects (Table 2) with no previous ankle injury who underwent lower limb amputation in our hospital between 2001 and 2004, and 10 limbs of five doneted bodies for dissection. Thus a total of 21 limbs from 16 subjercts (9 males and 7 females) aged from 19 to 94 years (mean ; 57.9 years) were studied. The major causes leading to amputation were traffic accidents and occupational accidents, with one case of femoral synovial sarcoma. None of the subjects had peripheral neuropathy before surgery, and none had underlying diseases that might cause peripheral neuropathy. None of the cases had any gross ankle damage, and none had sustained

TABLE 2. Material

Age	Sex	Causes leading to amputation
19	F	Femoral synovial sarcoma
43	M	Occupational accident
35	M	Traffic accident
20	M	Traffic accident
51	F	Occupational accident
68	M	Occupational accident
64	M	Traffic accident
54	M	Occupational accident
36	M	Traffic accident
86	F	Traffic accident
53	M	Occupational accident

injury to the lateral ankle ligaments. These ligaments were considered to be normal.

The three ligaments that make up the lateral ankle ligaments ; the anterior talofibular ligament, calcaneofibular ligament and posterior talofibular ligament, were removed, and were collected en bloc as bone-ligament-bone samples.

2. Histological study

1) Light microscopic studies

The three ligaments that compose the lateral ankle ligaments were removed en block as bone-ligament-bone blocks, immediately stained by a modified technique of Gairns gold chloride staining (Table 3), and cryo-embedded. For each ligament, the fibula attachment side wes difined as the proximal side, the talus or calcaneus bone attachment side as the distal side, the skin as the lateral side, and the joint side as the medial side. Serial sections 25 to 30 μm in thickness were cut from the lateral side toward the medial side with a cryostat. The sections were examined by light microscopy to observe the morphology of the deep sensory receptors.

TABLE 3. Modified method of Gairns gold chloride staining

1. Immerse the specimen in a mixture of 88% formic acid and fresh lemon juice (ratio 1 : 3) in a dark room for 15 minutes.
2. Remove the sample and immerse in a 1% gold chloride solution, and place in a dark room for 20 minutes.
3. Transfer the sample into 25% formic acid and place in a dark room for 15 to 16 hours.
4. Wash the sample under running water for at least one hour, freeze, and cut into 30 μm serial sections using a cryostat.
5. Spread the sections on glass slides and mount.
6. Examine each section under a light microscope.

2) Electron microscopic study

An electron microscopic(EM) study was conducted to identify the ultrastructures of the deep sensory receptors in the ligaments. As pretreatment for EM, the ligament samples were fixed and placed under a stereomicroscope. The ligament was explored to identify the nerve fibers, and the surrounding tissues were trimmed to prepare EM blocks. To search for nerve endings by light microscopy, 1 μm serial sections were prepared from the blocks and stained with toluidine blue. After confirming the presence of nerve endings, the blocks were treated as shown in Table 4. Ultrathin sections, 60 to 80 nm in thickness, were cut and examined under a transmission electron microscope (JEM-2000EX, Japan Electronics Inc.).

TABLE 4. Method for electron microscopy

1. Fix the specimen in 2.5% glutaraldehyde in 1/15 M phosphate buffer (PB) for 30 minutes.
2. Confirm the nerve fibers by following entry of the nerves into the ligament, and trim blocks of approximately 1 to 2 mm^3 under a stereomicroscope.
3. Immerse in 2.5% glutaraldehyde in 1/15 M PB for two hours.
4. Rinse in 1/15 M PB for 10 minutes, four times.
5. Postfix in 1% OsO_4 in 0.1 M cacodylate buffer for two hours.
6. Rinse in 1/15 M PB for 10 minutes, two times.
7. Dehydrate in serial alcohol.
8. Embed in Epon resin.
9. Polymerize at 60°C.

3. Study of distribution of deep sensory receptors

Specimens were stained by a modified method of Gairns gold chloride staining. 30 μm serial sections were cut from the lateral side toward the medial side and examined light microscopically. The ligament was divided into three portions ; a fibula portion, an intermediate portion and a talus portion. In addition, each section was divided into a lateral portion, an intermediate portion and a medial portion. For each of the nine divided portions, the type and the number of deep sensory receptors were determined. To avoid duplication in counting, a deep sensory receptor showing gradual reduction of diameter in serial sections was counted as one receptor. The distribution of each type of deep sensory receptor was investigated three-dimensionally. We then compared regions and segments of ligaments using one-factor analysis of variance.

RESULTS

1. Sensory nerve endings in the lateral ankle ligament

Ruffini receptors, Golgi tendon-like receptors and free nerve endings were the three types of sensory nerve endings observed in the lateral ankle ligament. There were absolutely no pacinian corpuscles.

Ruffini receptors were encapsulated and measured approximately 200 to 400 μm in size, and the terminals were formed by branching of the nerve fibers (Fig 1-a).

Golgi tendon-like receptors had large spherical or oval terminals measuring 300 to 600 μm in size, and were covered by a thick capsule with a lamellar structure. The nerve fibers entered the capsule, and after branching formed a globular structure (Fig 1-b).

The free nerve endings measured approximately 100 to 200 μm, appeared filamentous and were not covered by a capsule (Fig 1-c).

2.Ultrastructure of sensory nerve endings in anterior talofibular ligament

The receptor complex is situated within several compartments of the capsular lumen, bounded by thin cytoplasmic processes of fibroblast-like septal cells. There are small subcapsular spaces (Fig 2).

3.Distribution in sensory nerve endings in each ligament

(1) Anterior talofibular ligament (Fig 3)

Among the three ligaments Ruffini receptors were most abundantly observed in the anterior talofibular ligament. All types of nerve endings were found more abundantly at the fibula attachment side (proximal side) than at the talus attachment side (distal side). When comparing the medial

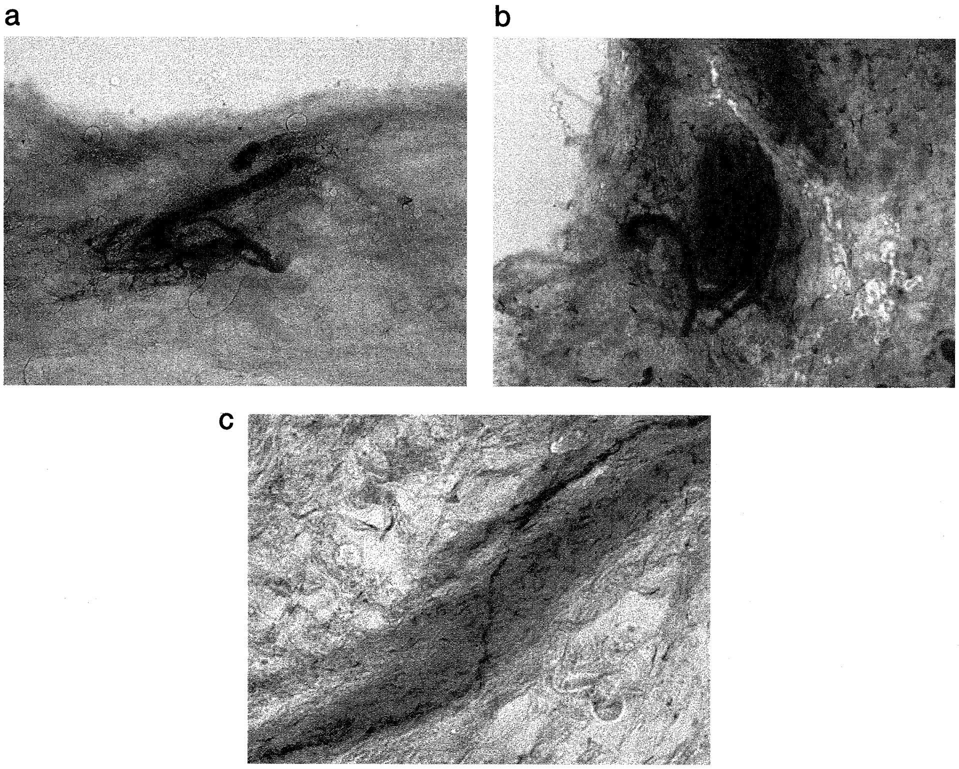

Fig 1. Sensory nerve endings in the lateral ankle ligament
Ruffini receptors (1-a), Golgi tendon-like receptors (1-b) and free nerve endings (1-c) were the three types of sensory nerve endings observed in the lateral ankle ligament. (Gairns gold chloride staining a ; ×200, b ; ×200, c ; ×400)

and lateral sides, more receptors were found on the lateral side. The intermediate portion of the ligament tended to have fewer nerve endings than the bone attachment sides.

Fig 2. Ultrastructure of sensory nerve endings in the ligament
The receptor complex (thin arrow) is situated within two compartments of the capsular lumen (thick arrow). There are small subcapsular spaces (S). Bar 1 μm.

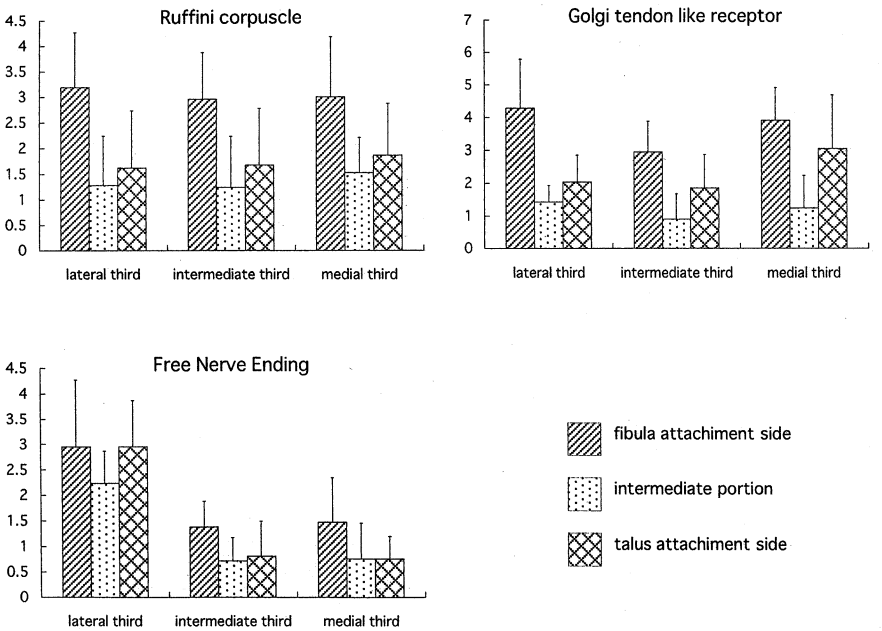

Fig 3. Distribution in the anterior talofibular ligament
All types of nerve endings were found more abundantly at the fibula attachment side (proximal side) than at the talus attachment side (distal side).

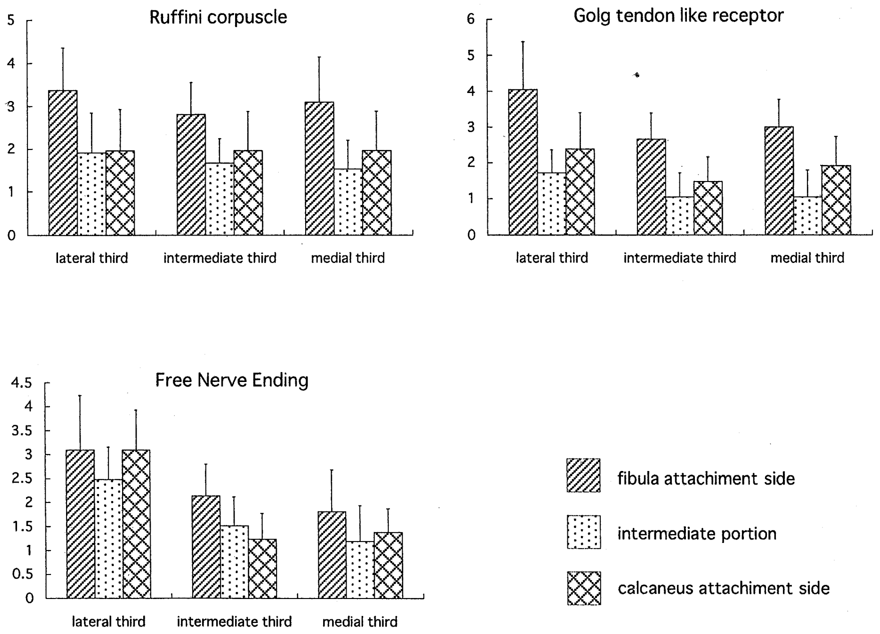

Fig 4. Distribution in the anterior talofibular ligament

Among the three ligaments, the calcaneofibular ligament had the smallest number of nerve endings. The distribution of nerve endings resembled that in the anterior talofibular ligament.

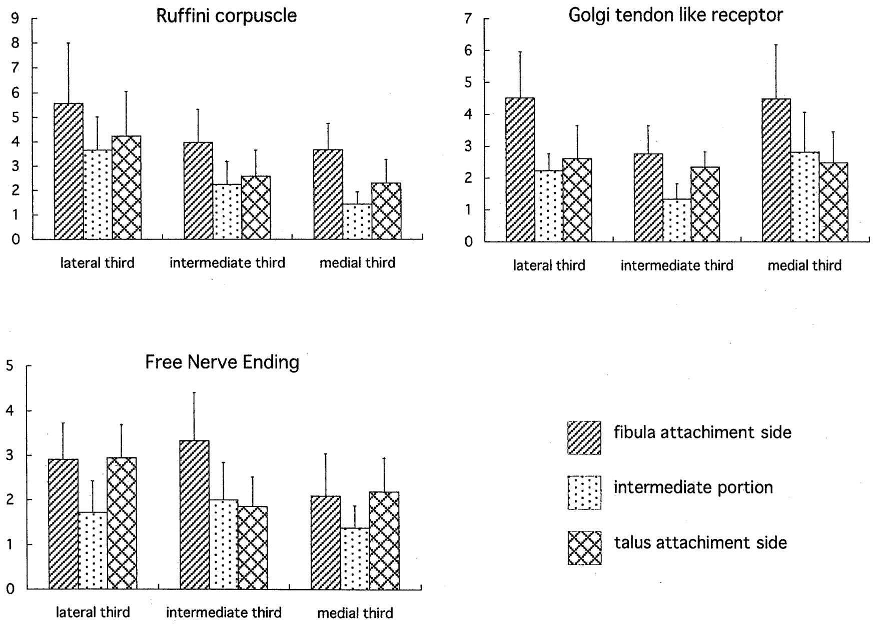

Fig 5. Distribution in the anterior talofibular ligament

The largest number of nerve endings was observed in the posterior talofibular ligament. The distribution of nerve endings differed from that in the anterior talofibular and calcaneofibular ligaments.

(2) Calcaneofibular ligament (Fig 4)

Among the three ligaments, the calcaneofibular ligament had the smallest number of nerve endings. The distribution of nerve endings resembled that in the anterior talofibular ligament, with Ruffini receptors being most abundant. All three types of nerve endings were present in greater numbers at the fibula attachment side than at the calcaneal bone attachment side, and the numbers of nerve endings were in the order of lateral side, medial side and intermediate portion.

(3) Posterior talofibular ligament (Fig 5)

The largest number of nerve endings was observed in the posterior talofibular ligament. The distribution of nerve endings differed from that in the anterior talofibular and calcaneofibular ligaments. The nerve endings were present in equal numbers at the fibula attachment side and talus attachment side, and there was no difference between the medial and lateral sides.

DISCUSSION

In recent years, morphological and electrophysiological studies have led to the recognition that the deep sensory receptors in ligaments are involved in the mutual regulation of ligaments and muscles. Morphological studies of the deep sensory receptors in human ligaments have reported the presence of four types of deep sensory receptors ; Ruffini receptors, Golgi tendon-like receptors, Pacinian corpuscles and free nerve endings in ligaments such as the anterior cruciate ligament of the knee,[4-7] the oracoacromial ligament[8,9] and the supraspinous interspinous ligament.[10] Furthermore, Wyki *et al*[1-3] classified sensory receptors into four types morpho-physiologically. Their classification consisted of four types of deep sensory receptors ; Ruffini receptors as type I receptors, Pacinian corpuscles as type II, Golgi tendon-like receptors as type III, and free nerve endings as type IV. Type I to type III receptors are small mechano-receptive endings (Table 1). Michelson *et al*[13] studied the ankle ligament and confirmed the presence of deep sensory receptors classified as Wyke's types I to III. They observed many type II and III sensory receptors and significantly small number of type I mechano-receptors. In the present study, however, we found large numbers of Ruffini receptors and Golgi tendon-like receptors classified as type I and type III, respectively, in Wyke's classification.

Electron microscopic studies of receptors have demonstrated an "inner core" in the extensive subcapsular space. This feature has been considered to be an important criterion in differentiating the encapsulated Ruffini receptors from Golgi tendon-like receptors.[14-16] In our study, we were able to examine the ultrastructures of Ruffini receptors. Since collagen bundles pass through the corpuscles of Ruffini receptors and Golgi tendon-like receptors, these receptors have been described as stretch receptors that respond to altered tension of the collagen in the surrounding tissues.[17-20] Vandenabeele *et al*[21] observed the ultrastructures of Ruffini receptors in human lumbar facet joints and reported a central network of highly aborising nerve terminals in their Ruffini receptors. These features resemble the structures we observed in the Ruffini receptors, and the two may share common structural characteristics.

We observed no Pacinian corpuscles, classified as type II mechanoreceptor, in our samples. Electrophysiological studies suggest that Pacinian corpuscles are sensory receptors that perceive acceleration and deceleration during initiation and termination of movements.[22,23] The fact that we observed no Pacinian corpuscles in the lateral ankle ligament may suggest that the ankle joint has a small range of motion, there is little need to sense acceleration during motion because compared with the knee and shoulder joint. However, the ligament samples we used included ones from the donated bodies of elderly subjects. According to Morizawa *et al*[17] it impossible to completely rule out the effects of aging-related changes of the nerve endings or underlying diseases. Akiyama *et al*[24] demonstrated Pacinian corpuscles in the fat tissue and synovial membrane of the tarsal sinus. These observations suggest that not only the ligaments but also other surrounding soft tissues external to the ankle joint are involved in the motor control mechanism of the ankle joint, especially to maintain overall balance against the inversion stress of the ankle joint.

Michelson *et al*[13] reported no observation of free nerve endings that respond sensitively to nociceptive stimuli in the anterior talofibular and calcaneofibular ligaments. We demonstrated the presence of free nerve endings in all three lateral ligaments of the ankle. These findings suggest that these nerve endings give rise to pain during injury of the ankle lateral ligament and chronic pain after ligament injury (Fig 6).

The presence of these deep sensory receptors in the lateral ankle ligaments also suggests the possibility that they are involved in the motor control mechanism of the ankle joint, The patterns of distribution of deep sensory receptors differed in various ligaments composing the lateral ankle ligaments, suggesting an influence of different stress distribution in various ligaments.

Unfortunately, Vandenabeele *et al*[21] reported on nerve endings are often confusing. Some difficulty is experienced in being sure that various researchers are referring to the same nerve ending. This situation may have arise from misinterpretation of nerve endings by some researchers, or from physiological diversity or interspecies variations of the nerve ending. Delineating the specific ultrastructural characteristics of deep sensory

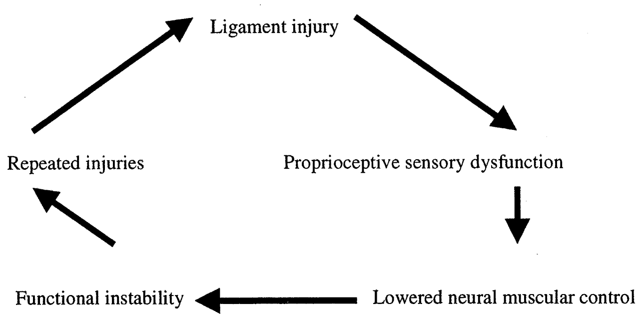

Fig 6. Vicious cycle of repeatede injuries caused by ligament injury and accompanying proprioceptive sensory dysfunction

receptors is important to deduce their possible physiological functions and their specific functional significance for particular joints.

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