J. Vet. Malaysia (2000) 12(2): 71-74

CHANGES IN NEUROGENIC COMPONENTS IN OSTEOARTHRITIC AND CONTRALATERAL JOINTS OF DOGS

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SUMMARY

Osteoarthritis is a common problem in dogs. The present study showed that there is a neurogenic component involved in the pathophysiology of arthritis. The general pattern of innervation was studied using antibody against protein gene-product 9.5 (PGP 9.5), the sensory components was studied using antibody against calcitonin gene-related peptide (CGRP) and the sympathetic components using neuropeptide Y (NPY). There was a marked reduction in the immunoreactive fibres (IRF) in osteoarthritis.

Keywords: Osteoarthritis, neuropeptides, dog, innervation

INTRODUCTION

Osteoarthritis is a chronic form of arthropathy, which is characterised by extensive destruction of the articular cartilage leading to a painful, limited motion in the affected joints. Osteoarthritis may occur either as monoarticular or polyarticular (Stanley and Robins, 1967; Whittick, 1990).

Although the pathogenesis of arthritis is poorly understood, a neuronal component was indicated by early studies in which peripheral nerve section was shown to attenuate the severity of experimental arthritis. Subsequent work has resulted in a large body of evidence in support of the peripheral nervous system playing an active role in the degenerative disease process (Levine *et al.*, 1984; Fitzgerald, 1989).

While it is often assumed that the only function of primary afferents is to transmit information from the periphery to the central nervous system, a local motor functions of the peripheral sensory terminal have been widely documented (Holzer, 1988). This sensory neuron belongs to the unmyelinated, peptide-containing, C-fibre class of primary afferents. Its release of neuropeptides from the peripheral terminals that give the effects of local motor actions (Holzer, 1988),

In normal synovial membrane, nerves containing SP and CGRP have been detected by immunohistochemical staining in rat (Levine *et al.*, 1984; Konttinen *et al.*, 1992), man (Gronblad *et al.*, 1988; Kidd *et al.*, 1989; Mapp *et al.*, 1994), cat and horse (Bowker *et al.*, 1993). These peptides have also been detected in normal synovial fluid by radioimmunoassay and the levels are elevated in naturally occurring or experimental arthritis in man and rats (Levine *et al.*, 1984; Joyce *et al.*, 1993). Thus it appears that the C-fibre afferents, in addition to transmission of sensory information such as pain, play an active role in the pathogenesis of arthritis.

Sympathetic fibres innervating the synovial membrane contain neuropeptide Y (NPY) (Kidd *et al.*, 1989). The local actions of NPY, if it is released into synovial fluid, are not known. However, there is evidence that classical neurotransmitter, the noradrenaline, plays an active role in arthritis (Levine *et al.*, 1986).

Although various studies had been carried out on the involvement of neuropeptides in rheumatoid arthritis, little studies were done on the neurogenic components in osteoarthritis. Therefore, the current work was undertaken to study the neurogenic components in osteoarthritis in dogs. The objectives of the current work were to identify the neurogenic components in osteoarthritis and to observe the changes of the neurogenic components in ipsilateral and contralateral joints.

MATERIALS AND METHODS

Samples

Samples of synovial membrane were obtained from ipsilateral and contralateral joints of 20 dogs either undergoing surgical treatment or were euthanised for joint disease (osteoarthritic joints). The control samples were obtained from 10 animals, euthanised for reasons not involving joint problem. These samples were obtained from both left and right stifle and carpal joints.

Immunohistochemistry technique

Tissues were fixed by immersion in 4% paraformaldehyde in 0.1M sodium cacodylate and the immunohistochemistry technique was carried out using

the standard technique (Stemberger, 1979). Briefly, 7μm thick cryostat sections were incubated in primary antisera at 4°C overnight. The primary antisera used were polyclonal raised in rabbits. Sensory fibres were identified by staining for the neuropeptide CGRP and sympathetic fibres by staining for NPY (Peninsula Lab., UK) and the general pattern of innervation was demonstrated by staining for protein gene product 9.5 (PGP 9.5) (Ultraclone Ltd., UK). The secondary antisera were biotinylated goat anti rabbit IgG (Sigma Chemicals Co. Ltd., UK). Avidin biotinylated peroxidase complex (Amersham International Plc.) was used as the third layer and the sites of antibody binding were visualised using glucose oxidase-nickel-diaminobenzidine substrate (Shu *et al.*, 1988)). The density of immunoreactive fibres was scored using a subjective scheme outlined by Shanthi (1997).

RESULTS

The synovial membranes from the control dogs consisted of two layers, a superficial intimal and a deeper subintimal layers (Fig. 1). The intimal layer contained villi that extended into joint cavity while its surface was covered by an incomplete layer of synovial cells. The subintimal layer consisted of loosely packed coarse collagen fibres and blood cells. The synovial membranes obtained from cases of osteoarthritis showed severe changes. In all cases, there was a clear hyperplasia of synovial cells while in some cases there were focal collection of inflammatory cells.

Fig. 1. Normal synovial membrane. The intinial layer (arrow) consists of one to two layers of synovial cells. HE x175

Using antisera against PGP 9.5, nerve fibres were found in the intimal (Fig. 2) and subintimal layers (Fig. 3) of the synovial membrane of the control dogs. The fibres were more abundant in the subintimal layer, organised into coarse bundles closely associated with blood vessels. In the intimal layer, the innervation was sparse.

CORP- and NPY- immunoreactive fibres (IRF) were found throughout the synovial membrane. They were most abundant in the subintimal layer (Figs. 4 and 5). The distribution was similar to the pattern revealed by staining for PGP 9.5 with variations in the density of innervation. The PGP 9.5 IRF were widely distributed compared to the CGRP or NPY fibres.

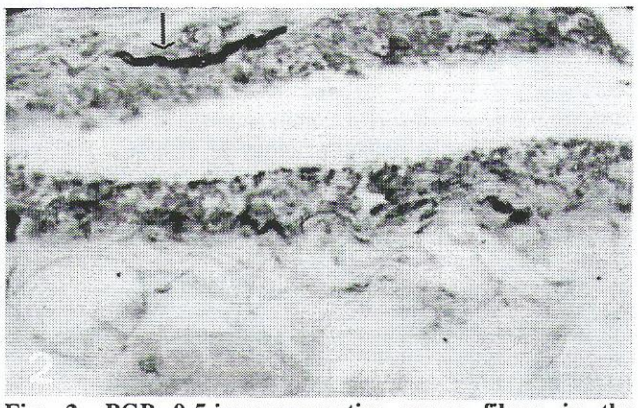

Fig. 2. PGP 9.5-immunoreactive nerve fibres in the intimal layer (arrow) of a normal synovial membrane close to the joint space. x160

Fig. 3. PGP 9.5-immunoreactive nerve fibres in the subintimal layer (arrow) in close association with blood vessels of normal synovial membrane. x160

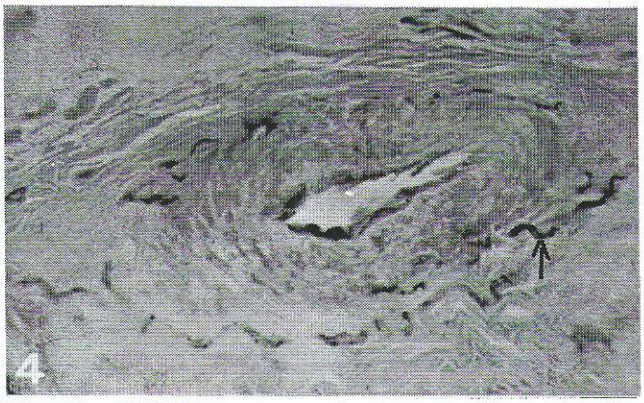

Fig. 4. CGRP-immunoreactive nerve fibres in the subintimal layer (arrow) of a nonnal synovial membrane. x165

Samples from osteoarthritis showed a general reduction in detectable nerve fibres. All antisera used in this study failed to detect nerve fibres in the intimal layer. There was a general reduction of the IRF in the subintimal layer. The fibres were lost, particularly from areas that were heavily infiltrated by inflammatory cells (Fig. 6).

Samples obtained from the joint contralateral to the arthritic joint had reduced number of immunoreactive fibres (Fig. 7). Changes found in these joints were similar to the changes observed in the ipsilateral joints.

Fig. 5. NPY-immunoreactive nerve fibres in the subintimal layer (arrow) of a normal synovial membrane. x165

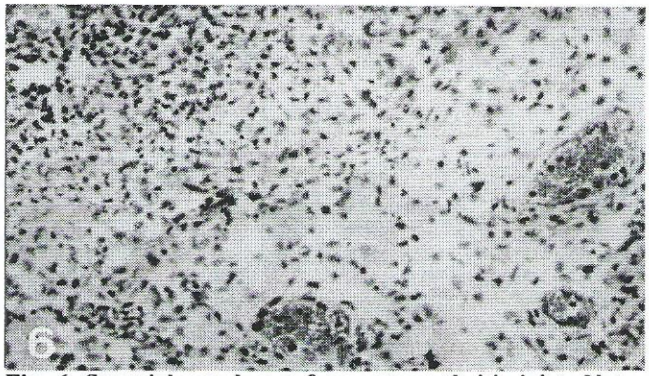

Fig. 6. Synovial membrane from osteoarthritic joint. Note the absence of PGP 9.5-immunoreactive nerve fibres in areas that are heavily infiltrated by inflammatory cells. x170

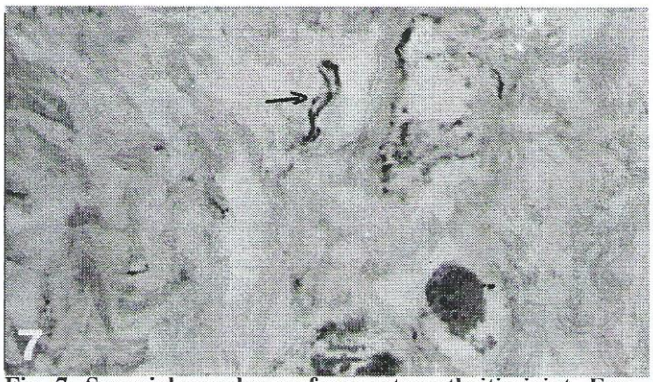

Fig. 7. Synovial membrane from osteoarthritic joint. Few PGP 9.5-immunoreactive fibres (arrow) are present. x160

DISCUSSION

The only report on the innervation of synovial membrane from cases of osteoarthritis (OA) is on human tissues (Gronblad *et al.*, 1988). They concluded that there was no loss of IRF, which is in contrast to the present findings. However, the human samples were characterised as non-inflammatory, whereas the majority of canine samples observed in this study showed clear evidence of inflammatory changes.

In this study, there was a huge reduction of IRF in the synovial membrane with OA. The factors underlying the axonal loss in affected synovial membrane are unknown, but it has been noted that nerve fibres are lost particularly from areas heavily infiltrated by inflammatory cells (Konttinen *et al.*, 1992), a feature seen also in the present study. In three rat models of arthritis, denervation was seen only in those that had inflammatory reactions in the membrane (Mapp *et al.*, 1994). Thus, it is proposed that inflamed synovial membrane may create a cytotoxic environment for the axons, possibly by the release of agents from active inflammatory cells (Mapp *et al.*, 1994).

The depletion of CGRP- and NPY-immunoreactivity from cases of OA indicates that both sensory and sympathetic motor fibres are affected. The observation that the depletion in neuropeptide immunoreactivity was more extensive than loss of PGP 9.5 IRF raises the possibilities of either excessive release of neuropeptides from the remaining nerve fibres or selective destruction of peptidergic fibres.

In this study, the uninjured or contralateral joints were found to show some depletion in the IRF. The result did not indicate if reduced number of IRF in the contralateral joint preceded arthritic changes or *vice versa*. However, intra-articular injection of noxious agents into one knee joint of rat resulted in significantly elevated levels of CGRP. The CGRP in synovial fluid of contralateral joints remained elevated until the end of the experiment 24 hours later (Biliviciute *et al.*, 1993).

The mechanism, which induced the contralateral changes observed in this study is not clear. It is possible that altered loading on the unaffected joint stimulated arthritic changes. However, there is accumulating evidence for neurogenic component in this symmetrically bilateral spread of disease.

In rats, inflammation of one hindpaw (Levine *et al.*, 1985) or monoarthritis induced in the knee (Kidd *et al.*, 1995) or in the ankle by local adjuvant injection (Donaldson *et al.*, 1995) resulted in spread of inflammatory changes to the contralateral limb. However, spread of inflammation to the contralateral paw was reduced by sympathectomy (Levine *et al.*, 1985) and also by ablation of pain afferent, using capsaicin. It has been suggested that there is a direct link at the spinal cord level (Kidd *et al.*, 1989) and the

sympathetic system provided the cross spinal bridge (Kidd *et al.*, 1995).

To explore further the role of sensory fibres and neuropeptides in osteoarthritis, a study involving the use of perineuronal capsaicin to ablate pain afferents projecting to the injured or contralateral limb is required.

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RINGKASAN

PERTUKARAN KOMPONEN NEUROGENIK SENDI OSTEOARTRITIS DAN KONTRALATERAL ANJING

Osteoartritis merupakan masalah lazim pada anjing. Kajian ini menunjukkan terdapat komponen neurogenik yang terlibat dalam patofisiologi artritis. Corak pensarafan am dikaji menggunakan antibodi terhadap produk gen protein 9.5, komponen deria dikaji menggunakan antibodi terhadap peptida berkait gen kalsitonin dan komponen simpatetik menggunakan neuropeptida Y. Terdapat pengurangan yang nyata dalam serat imun haiwan berpenyakit osteoartritis.