Contribution of sensory nerves within osteochondral channels to pain in human and rat knee osteoarthritis

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Purpose

Knee osteoarthritis (OA) is a common cause of pain and disability and pain is the most common reason sufferers seek medical help. We previously shown that subchondral pathology, including increased nerve growth factor (NGF) expression in osteochondral channels, was associated with symptomatic knee OA. NGF sensitises sensory nerves, and can also stimulate sensory nerve growth. Sensory nerve densities have been associated with pain in other diseases, but it is unclear whether sensory nerve growth at the osteochondral junction contributes to OA pain. The purpose of this study was to identify contributions of CGRP-immunoreactive (IR) sensory nerves at the osteochondral junction to OA pain in human and rat OA, and whether blocking NGF activity by inhibiting tropomyosin receptor kinase A (TrkA) prevented any OA-associated increase in CGRP-IR sensory nerves.

Methods

Eleven symptomatic chondropathy cases were selected from patients undergoing total knee replacement (TKR) for OA. Twelve asymptomatic chondropathy cases who had not presented with knee pain and 11 control cases who had macroscopically normal articular cartilage or only mild chondropathy were selected from post-mortem (PM) cases. Zamboni's-fixed frozen and formalin-fixed wax-embedded sections of the middle third of medial tibial plateaux were analyzed for CGRP-IR sensory nerves, histological grade for chondropathy, osteochodral channels and subchondral fibrovascular replacement of subchondral bone marrow.

OA was induced in rat knees by meniscal transection (MNX) and non-osteoarthritic (Sham-operated) rats were used as controls. Oral doses (30 mg/kg) of TrkA inhibitor (AR786) or vehicle (5% Gelucire 50/13) were administered twice daily from before to day 28 after OA induction. Pain behavior was assessed as weight-bearing asymmetry and as paw withdrawal threshold to punctate stimulation of the hindpaw. Alterations in subchondral bone and cartilage were examined by macroscopic visualization of articular surfaces and

histopathology.

Results

Macroscopic chondropathy in asymptomatic and symptomatic chondropathy were higher than in PM control, and similar between asymptomatic and symptomatic OA (Table 1). Total Mankin score in symptomatic OA was higher than in PM control, and cartilage surface integrity in asymptomatic and symptomatic OA were higher than in PM control (Table 1). Percentage of osteochondral channels containing CGRP-IR nerves in symptomatic chondropathy was higher than in asymptomatic chondropathy. OA structural changes were more severe in MNX- than in sham-operated rat knees, and were not significantly affected by AR786 treatment (Table 2). Percentage of osteochondral channels containing CGRP-IR nerves was higher in MNX-operated knees from rats treated with vehicle than in sham-operated knees, and treatment with AR786 prevented this increase in CGRP-IR nerves. Nerve density in subchondral bone marrow spaces did not differ between groups. Percentage of osteochondral channels containing CGRP-IR nerves was significantly associated with weight-bearing asymmetry (Spearman's $r = 0.40$, $p<0.05$), and with paw withdrawal threshold (spearman's $r = -0.56$, $p<0.01$).

Conclusions

We have identified CGRP-IR sensory nerves within osteochondral channels, associated with symptoms in human knee OA and pain behaviour in MNX-induced rat knee OA. In rats, blocking NGF activity by inhibiting TrkA prevented the OA-induced increase in osteochondral channels containing CGRP-IR nerves. This was associated with and might contribute to reduced pain behavior. Increased NGF expression in osteochondral channels was associated with symptomatic knee OA independently of chondropathy and synovitis in our previous study. NGF-induced growth of sensory nerves at the osteochondral junction might have a pivotal role in generation of chronic bone pain in knee OA. Inhibition of nerve growth in osteochondral channels might reduce OA pain.

	PM Control (n=11 cases)	**Asymptomatic chondropathy (n=12 cases)**	**Symptomatic chondropathy (n=11 cases)**
Age	50 (47, 65)	86 (78, 89)**	61 (58, 733)[#]
Gender (Male, %)	70	50	67
Macroscopic chondropathy score (0-100)	20 (17, 26)	68 (62, 83)*	73 (66, 79)*
Total Mankin score (0-14)	6 (5, 8)	9 (6, 11)	11 (9, 12)*
Cartilage surface integrity (0-6)	3 (2, 3)	5 (3, 6)**	6 (4, 6)**

Chondrocyte appearance (0-3)	2 (2, 3)	3 (3, 3)	3 (3, 3)
Tidemark integrity (Yes, %)	55	30	30
Proteoglycan loss (0-4)	1 (1, 1)	2 (1, 2)	2 (2, 2)
Channels breaching tidemark (%)	20	50	55
Subchondral bone marrow replacement (%)	45	67	64
Osteochondral channel density (/mm)	4.4 (3.9, 4.7)	3.7 (3.0, 5.0)	4.1 (3.3, 6.6)
Proportions of osteochondral channels containing CGRP-IR nerves (%)	1.2 (0, 2.9)	0 (0, 1.9)	4.7 (2.5, 4.7) ##

Table 1: Details of patient demographics and histology

Data displayed as median (IQR). *p<0.05 versus PM control, **p<0.01 versus PM control, #p<0.05 versus asymptomatic OA, ##p<0.01 versus asymptomatic OA

	SHAM+Vehicle (n=10 rats)	**MNX+Vehicle (n=10 rats)**	**MNX+AR786 (n=10 rats)**
Macroscopic chondropathy score	0 (0, 0.8)	3 (3, 3)**	3 (3, 4)**
Cartilage damage score in MTP (0-15)	0 (0, 0)	5 (3, 8)	6 (5, 10)**
Osteophyte score (0-3)	0 (0, 0)	1 (0, 3)	1 (0, 2)
Osteochondral channel density (/mm)	3.1 (2.9, 3.3)	2.5 (2.2, 3.6)	3.5 (2.5, 4.6)
Subchondral bone marrow replacement (%)	0	50	66.7*
Pain withdrawal threshold (Day 28)(g)	15 (11, 15)	6 (5, 6)**	13 (10, 15)**
Weight bearing asymmetry (Day 28)(%)	1.2 (0.1, 1.9)	25.2 (20.6, 27.4)**	1.5 (0.6, 3.8)**

Proportion of osteochondral channels containing CGRP-IR nerves (%)	2.8 (0.5, 7.4)	10 (8, 13.7)[#]	4.2 (3.2, 5.6)

Table 2: Joint pathology and pain behavior in rats

Data displayed as median (IQR). *p<0.05 versus SHAM+Vehicle, **p<0.01 versus SHAM+Vehicle, [#]p<0.05 versus SHAM+Vehicle and MNX+AR786.