Intervertebral discs which cause low back pain secrete high levels of proinflammatory mediators


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Herniated intervertebral disc tissue has been shown to produce a number of proinflammatory mediators and cytokines, but there have been no similar studies using discs from patients with discogenic low back pain.

We have compared the levels of production of interleukin-6 (IL-6), interleukin-8 (IL-8) and prostaglandin E2 (PGE2) in disc tissue from patients undergoing discectomy for sciatica (63) with that from patients undergoing fusion for discogenic low back pain (20) using an enzyme-linked immunoabsorbent assay.

There was a statistically significant difference between levels of production of IL-6 and IL-8 in the sciatica and low back pain groups (p < 0.006 and p < 0.003, respectively).

The high levels of proinflammatory mediator found in disc tissue from patients undergoing fusion suggest that production of proinflammatory mediators within the nucleus pulposus may be a major factor in the genesis of a painful lumbar disc.

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The pathophysiology of discogenic low back pain is incompletely understood. The changes which occur as a disc degenerates are well documented, but are unhelpful in determining whether a degenerate disc will cause pain. It is known that disc tissue from patients undergoing discectomy for sciatica synthesises proinflammatory mediators and cytokines. Sequestrated and extruded discs produce higher levels of these mediators than specimens in which the annulus is intact.

To date, there have been no studies of the production of inflammatory mediators in disc tissue from patients undergoing operations for discogenic low back pain. It has been shown, however, that degenerate discs in these patients contain more nociceptive nerve endings in the endplates of the disc and in the nucleus pulposus than do degenerate discs which do not cause low back pain.

We have therefore compared levels of production of the proinflammatory mediators tumour necrosis factor alpha (TNFα), interleukin-1beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and prostaglandin E2 (PGE2), in disc tissue from patients undergoing discectomy for sciatica with those from patients undergoing fusion for discogenic low back pain.

Patients and Methods

We obtained specimens of intervertebral disc from 63 patients undergoing primary lumbar discectomy for sciatica. Intraoperative assessment of the morphology of the disc herniation revealed 25 in which the annulus was intact (AI), 30 in which a nuclear extrusion was present (EXT) and eight in which the nucleus was sequestrated (SEQ). The mean ages were 42 years in the AI group, 39.5 years in the EXT group and 42 years in the SEQ group. The male:female ratio in the AI, EXT and SEQ groups was 17:8, 20:10 and 6:2, respectively. Three specimens were from the L3/L4 level, 28 from the L4/L5 level and 32 from the L5/S1 level.

We also obtained disc specimens from 20 patients undergoing primary lumbar interbody fusion for discogenic low back pain, which had been confirmed by discography. There were six men and 14 women with a mean age of 38.5 years. Twelve specimens were from the L4/L5 level and eight from the L5/S1 level. Information regarding the morphology of the disc was available for 13 specimens; four AI and nine EXT.

We excluded patients with degenerative spinal stenosis, tumours, infections, previous lumbar surgery and those who had had an epidural injection of corticosteroids within six months of operation.

Tissue culture. The degenerate and control disc specimens...
were freshly obtained at the time of surgery and stored in normal saline solution at 4°C until transported to the laboratory, within six hours. All specimens were prepared for culture by the principal author (JGB). The specimens were washed with normal saline to remove blood contaminants and, when possible, the nucleus pulposus was identified and separated from the other disc tissues. Great care was taken to exclude fragments of bone, cartilage and granulation tissue from the cultures. Only tissue which appeared morphologically to be nucleus pulposus was cultured.

The tissue was diced and 200 mg specimens were incubated in 3 ml of Neumann-Tytell serum free medium (Gibco, Cambridge, UK) at 37°C for 72 hours in a humidified atmosphere of 5% CO₂ in air, which is a modification of the method described by Kang et al. Penicillin (100 units), streptomycin (100 μg) and amphotericin B (2.5 μg) were added to the medium as prophylaxis against microbial infection (Sigma-Aldrich Co Ltd, Poole, UK). At the end of the incubation period the medium was harvested, aliquoted and stored at -80°C for biochemical analysis. Contamination of the medium by micro-organisms and cellular growth from the disc tissue were outruled by light microscopy and culture.

**Biochemical analysis.** Levels of TNFα, IL-1β, IL-6 and IL-8 in the media were determined by enzyme-linked immunoabsorbent assay, using commercially available kits (R and D Systems, Minneapolis, Minnesota), according to the manufacturers’ instructions. Levels of PGE₂ were measured using a commercially available competitive binding assay (R & D Systems). The TNFα, IL-1β, IL-6, IL-8 and PGE₂ kits were sensitive to concentrations of 4.4, 1, 0.7, 10 and 36.2 pg/ml, respectively.

**Statistical analysis.** Statistical analysis of the data was carried out using SPSS (SPSS Inc, Chertsey, UK) statistical software for non-parametric analysis by the Mann-Whitney U test. Significance was assumed at p < 0.05.

**Results**

Significant quantities of IL-6, IL-8 and PGE₂ were produced by both the sciatica and low back pain groups (Fig. 1). None of the specimens produced TNFα or IL-1β. There was no significant difference in age- or gender-matching of the groups, but there was a predominance of men in those with sciatica. Figure 2 and Table I show and compare the production of mediator according to the morphology of the disc herniation in the two groups. Figure 3 shows the percentage of disc specimens in each group which produced each mediator. Figure 4 shows the production of IL-6 and IL-8 in the individual disc specimens from the group with low back pain. There was a linear relationship between the production of IL-6 and IL-8 (Pearson correlation coefficient 0.744; Fig. 5). The Pearson correlation coefficients for IL-6 and PGE₂ and IL-8 and PGE₂ were 0.24 and 0.3, respectively.

**Discussion**

In recent years, attention has begun to focus on the cellular and molecular activity of intervertebral disc tissue in the search for an understanding of the pathophysiology of sciatica and discogenic low back pain. It is clear from imaging studies that radicular pain is not simply a mechanical phenomenon. It has been shown that degenerate disc tissue from patients with sciatica synthesises IL-6 and PGE₂ and that the quantities of these substances increase with increasing exposure of the nucleus. Our study confirms these findings. We have recently shown that human nucleus pulposus also produces IL-8. IL-1β and TNFα have been isolated from homogenates of human disc material by Takahashi et al. We have found no evidence of production by the disc of either of these mediators, even in those specimens producing high levels of other proinflammatory mediators. There are no previous
Graphs showing production of a) IL-6, b) IL-8 and c) PGE$_2$ according to the morphology of the disc.
studies in the literature comparing the levels of production of inflammatory mediators in degenerate discs which cause sciatica with those which cause low back pain.

Our study has shown that significantly more IL-6, IL-8 and PGE\textsubscript{2} are produced by discs from patients with low back pain compared with discs from patients with sciatica. There was a trend towards less exposure of the nucleus pulposus in the group with low back pain only compared with those with sciatica introducing a bias towards higher levels of mediator production in the latter.

Figure 2 illustrates the difference between the two groups. The effect of increasing exposure of the nucleus pulposus on the production of mediators is not significant in the group with low back pain, but marked in those with sciatica. Within each category of abnormality of the disc there are significant differences in the production of mediators between the two groups. Figure 3 shows the number of disc specimens in each group producing each mediator. The rates of production of IL-6 and IL-8 in the AI and EXT categories of discs in low back pain are much higher than those found in those with sciatica. These, however, are known to be infiltrated with macrophages and T-cells, which may contribute to the levels of production of mediators. The disc material in sequestrated herniations is also in a different anatomical location to the contained or semicontained specimens in the group with low back pain only. The reasons for increased production of inflammatory mediators by the nucleus pulposus in patients with discogenic low back pain are unknown. A recent study has shown that few inflammatory cells are found in these discs\textsuperscript{23} and therefore the source of the mediators must be cells from the nucleus pulposus itself. It is known that such tissue can produce a range of proinflammatory cytokines.\textsuperscript{3-12} We suggest that as some discs degenerate the cells of the nucleus pulposus may be exposed to a proinflammatory stimulus leading to a form of inflammatory degeneration which gives rise to low back pain. The nature of this stimulus is currently unknown.

Discs which cause low back pain have higher concentrations of sensory nerves than are seen in those which do not cause such pain.\textsuperscript{14,15} The sensory nerves in the former are found in the endplates and in the nucleus pulposus and lose their normal relationship with blood vessels. The ingrowth of nerves into degenerate discs which cause low back pain may be mediated by chemotactic substances released by the degenerating disc.\textsuperscript{24} A combination of the innervation of the nucleus pulposus and increased production of proinflammatory mediators suggests that the mechanism for discogenic low back pain may be the induction of hyperalgesia in the newly innervated degenerating nucleus pulposus. Both IL-8 and PGE\textsubscript{2} are known to induce hyperalgesia.\textsuperscript{25}

Micromovement may occur between the vertebral bodies, anteriorly, in the presence of a solid posterior fusion. Weatherley, Prickett and O’Brien\textsuperscript{26} have published a series in which discogenic pain persisted postoperatively despite a solid posterior fusion. These patients were cured by the addition of an anterior fusion. Butterman et al\textsuperscript{27} confirmed these findings and correlated the failure of posterior fusion alone with the presence of Modic changes\textsuperscript{28} (inflammatory

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<th>Group comparisons</th>
<th>Mediator</th>
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<tr>
<td>AI sciatica v EXT sciatica</td>
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Table 1. p values of comparisons of mediator production by intervertebral discs from the different patient groups (sciatica and low back pain (LBP)) and the different morphology groups (AI, EXT and SEQ)
bone marrow changes adjacent to a degenerate disc) at the symptomatic level. When micromovement is sufficient to cause pain which responds to excision and fusion of a disc, the mechanism of the generation of the pain cannot be attributed to instability, but is consistent with hyperalgesia induced in an innervated nucleus pulposus by inflammatory mediators.

Figure 4 shows the production of mediators by individual discs in low back pain. Only 65% of these discs produced mediators and therefore this is not a homogenous group. It is possible that the 35% of discs in low back pain which did not produce mediators may produce pain by some other mechanism. Alternatively, the diagnosis of discogenic pain in these patients may be incorrect, or the culture process may not have detected an inflammatory region of the disc. However, the production of relatively high levels of mediator is a strong argument in favour of the occurrence of an inflammatory form of disc degeneration which causes the pain.

The linear correlation between the production of IL-6 and IL-8 (Fig. 5) supports the theory that individual discs can produce an inflammatory response and suggests that the stimulus provoking production of these mediators is the same. The rather poor correlation between the production of PGE₂ and that of IL-6 and IL-8 suggests that a different stimulus may be responsible for the former. This, combined with the smaller differences between levels of production of PGE₂ in the group with sciatica and those with low back pain only may indicate that it is not of major importance in defining the different disc pathologies at a cellular and molecular level.

Provocative discography is currently the method of choice for diagnosing discogenic low back pain. It is a subjective test relying on the radiologists’ and patients’ perceptions to determine the result. Many patients with such complaints have associated psychological or psychiatric disturbances which may or may not be associated with medicolegal factors. All of these decrease their ability to give an accurate opinion as to whether the pain produced at discography is that of which they are complaining.
There clearly remains a need for an objective diagnostic test for discogenic low back pain. Our study has indicated that there are differences between degenerate discs which cause such pain and those which cause sciatica. It may be possible to exploit these differences to develop an objective diagnostic test for discogenic low back pain and to manipulate the biology of the degenerate disc to develop nonsurgical treatments for inflammatory discogenic pain.

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References