Why do intervertebral discs degenerate? -
Presentation of the European research project EURODISC

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Abstract:
Back pain is a very common burden that almost everybody experiences at least once in his lifetime. It leads to considerable loss of productivity in the working population, which together with direct health and benefit costs, make it one of the expensive health problems in the western world. The causes of back pain are poorly understood but in most cases correlate strongly with problems caused by disc degeneration. Relatively little basic research has been carried out into the causes of disc degeneration and fundamental questions remain unanswered. Why do intervertebral discs degenerate? Is this problem genetically determined? What is the impact of age, nutrition and physical loading on degeneration? The purpose of EURODISC was to investigate these questions through a multidisciplinary research consortium (Figure 1).

Figure 1: The Eurodisc consortium, a multidisciplinary research network focussing on the intervertebral disc. The locations of the 7 project partner groups from 6 European countries is shown.
EURODISC consists of 7 research groups from 6 European countries viz. UK (Oxford and Oswestry), Holland (Eindhoven), Finland (Helsinki), Greece (Athens), Israel (Haifa) and Germany (Ulm), funded by the European Union in 2003 for a duration of three years. Each group has expert knowledge and experience in different disciplines, including engineering, cell biology, genetics, radiology, biochemistry and spinal surgery. Close collaborations between the project partners has created a multidisciplinary network focussing on the intervertebral disc. Tissue samples from patients undergoing disc surgery are collected and exchanged between the partners from all EURODISC research groups and studied via histology, biochemistry and cell biology. In parallel, DNA from blood samples of all donors and matched ‘controls’ is assayed for genetic variations possibly associated with disc degeneration. Questionnaires with information regarding the back pain history of all individuals are collected and studied. The data gathered from all aspects of the study is entered into a common database for statistical analysis of correlations between tissue and cell behaviour and genetic polymorphisms.

The multidisciplinary nature of the consortium enables different aspects of degenerative change to be studied on specimens from the same patient. Results of histological and biochemical investigations provide evidence for changes in tissue structure, cellular parameters and composition of matrix macromolecules during degeneration. One if the most noticeable changes is the degradation and loss of proteoglycan, a major macromolecule of disc matrix responsible for its hydration and load-bearing properties. The fall in proteoglycan concentration appears to permit the greater degree of vascularisation and innervation observed in degenerated discs. Cellular changes have been observed, such as an increased degree of senescence in herniated discs. Other factors also influence cellular behaviour; mechanical loading affects synthesis and degradation of the intervertebral disc matrix via enhancement or inhibition of gene expression for the respective proteins involved in these processes. Mechanical forces also stimulate disc cells to release signalling molecules that influence proliferative activity of disc cells. In parallel to the analysis of disc tissue, cells, and blood samples, a finite element model that includes proteoglycan-dependent osmotic swelling and collagen fiber orientation properties has been developed. This mathematical model can be used for predicting changes of cellular environment of EURODISC samples. Genetic factors have a very strong influence on disc degeneration as demonstrated by twin studies. DNA-genotyping of blood samples from EURODISC patients and controls will allow the identification of variations (single nucleotide polymorphisms) in genes associated with different aspects of disc degeneration.

It is hoped that these investigations may contribute to a better understanding of the factors influencing intervertebral disc degeneration. This should give rise to the development of preventative concepts, more objective diagnostic schemes and improved, targeted treatments for intervertebral disc diseases and hence, hopefully, of back pain.

Introduction

Intervertebral discs play an important role in the biomechanical function of the spine. They provide flexibility of the spine and allow flexion, extension and lateral bending. These complex functions depend on the morphological structure of the disc, its matrix composition and cellular components. Histologically, IVDs consist in the central nucleus pulposus that is surrounded by the fibrous annulus lamellae. The major components of the disc matrix are water, collagen and proteoglycans. Although disc cells occupy only one percent of the whole tissue, the annulus and nucleus cells produce and maintain all of the matrix molecules i.e. each disc cell is responsible for a large volume of matrix. As discs are avascular, the transport of oxygen and nutrients, as well as degradation products, occurs via diffusion over distances of up to 8 mm. In addition, since the discs are subjected to mechanical loading at all times, disc cells are also exposed to multiple physical stimuli including tension, compression and also fluid flow (because discs loose and regain about 25% of their fluid during a diurnal cycle). A consequence of hydration and dehydration of the disc are changes in the physicochemical environment of the disc, as concentrations of matrix molecules, ions and hence osmolarity are influenced by these processes. All these factors are thought to affect activity of cells of the disc and play an important role in the maintenance of a balance between matrix forming and degrading processes.
During degeneration, matrix degradation is increased and matrix formation decreases, leading to an imbalance in matrix turnover. These processes are assumed to be influenced by ageing and genetics but also environmental factors such as nutrition, mechanical factors, innervation and vascularization.

What are the reasons for these degenerative changes?

All of the factors mentioned may influence matrix turnover and may contribute to degenerative pathways. The purpose of the EURODISC project is to analyze the effect and interactions of these factors with regard to their contribution to intervertebral disc degeneration. The different aspects and research tools of the various research groups as well as the initial results of the project are reviewed here.

**Age-related changes in disc morphology, histology and matrix composition**

Morphologically, intervertebral disc structure changes with age. This process begins at a rather young age; the decreased hydration capacity of disc matrix results in a fall in water content of the nucleus pulposus from more than 85% in juvenile discs to less than 70% and even lower in adult and senile discs. Many of the age related changes are similar to those seen in disc degeneration.

The EURODISC research group in Oswestry, England (Dr. Sally Roberts and Helena Evans) is studying disc morphology, in relation to age and degeneration via histological methods. The purpose of these investigations is to correlate the changes with degeneration and the back pain of the donor patients. A link between the presence of open annular tears and predisposition of back pain has been detected (Prof. Videman).

Besides matrix changes there are also alterations in the cells of the disc: they form clusters increasingly with degeneration (Figure 2) or can demonstrate ‘senescence’ (see later). For improved classification of the different regions of the disc, markers have been identified that help to phenotype annulus and nucleus cells (Dr Jill Urban, Oxford). It is hoped that all these data will contribute to the development of a better grading and classification of disc degeneration.

**Nutrition and blood supply**

As mentioned above, intervertebral discs are avascular and transport of nutrients and metabolites occurs mainly via diffusion (Figure 3). Dr. Jill Urban and her collaborators at Oxford University, England, study the influence of nutrient supply on disc cell activity. They found that disc cells require critical concentrations of oxygen and glucose and also a distinct pH to survive and to be metabolically active.

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*Figure 2: Clusters of cells, particularly in the nucleus pulposus, are frequently found in disc degeneration; this is generally more common in older individuals than in younger ones.*
Nutrients and metabolites that supply disc cells via diffusion from blood vessels in bone have to pass through the cartilage endplate[17]. Scoliotic and degenerate discs show a considerable degree of calcification that may decrease endplate permeability and prevent passage of nutrient molecules into the disc[12]. The EURODISC group in Oxford has developed methods for in vivo measurements of endplate permeability of patients and for measurements of oxygen concentrations undergoing discectomy. By examination of lactate production, oxygen consumption and cell viability the nutrient requirements of intervertebral discs has been analyzed. Results to date provide evidence that in the centre of many degenerated discs the nutrient supply is insufficient resulting in a low cell viability. This finding may have a very strong impact on disc degeneration. They also have found osmolarity greatly influences disc cell requirements for oxygen and glucose. Therefore, diurnal changes of hydration and hence osmolarity appear to be involved in the regulation of disc matrix turnover via alteration of disc cell metabolism and gene expression.

Biochemistry
Age-related changes in disc matrix composition are studied in the EURODISC lab in Haifa, Israel (Prof. Alice Maroudas and Dr. Sarit Sivan et al). Two of the major proteins of the human intervertebral disc matrix i.e. collagen and aggrecan were separated and studied for their turnover rate through two natural processes, i.e. racemization of aspartic acid and non-enzymatic glycation (pentosidine). After maturity (>20 years), racemization of

Figure 3: Schematic showing a longitudinal section through an intervertebral disc with superior vertebra. Blood vessels terminate in the cartilage endplate. Thus, metabolites pass through the disc tissue via diffusion.

Figure 4: Scheme showing age-related degradation of aggrecan macromolecules (from Verzijl et al., Matrix Biology (2001), 20:409417.)
Aspartic acid (D-Asp) and pentosidine content increase with age in both proteoglycans and collagen. Due to repair processes occurring in degenerative tissues, D-Asp accumulation is higher in normal tissue (PG’s and collagen) compared to degenerate one. Recent findings indicate that the non-aggregating PG’s in the matrix are degradation products rather than newly synthesized fragments[14]. Present results show age-related degradation of these proteins. These findings explain that the decreased hydration capacity of degenerated discs might be caused by alteration of matrix macromolecules like aggrecan that lead to shorter molecules (Figure 4) with a lower charge density and thus decreased water-binding capacity. The EURODISC group in Israel has estimated the true fixed charge density of the tissue by osmotic stress and low-angle X-ray scattering techniques allowing the determination of the proportion of intrafibrillar water. Moreover, from a force balance, it would appear that collagen tension plays only a minor role in the equilibrium of the human intervertebral disc under load, in contrast to articular cartilage, where collagen tension is important for load-bearing[15].

These alterations of macromolecules not only contribute to decreased hydration of the disc matrix but may also play an important role in disc vascularisation[9]. Fractions of these separated components are used to study their effects on nerve and blood vessel ingrowth into the disc by the group in Oswestry, as shown in the next paragraph.

**Vascularisation and Innervation**

Normal intervertebral discs are avascular. Recent results from Dr. Eustace Johnson, Oswestry, support the hypothesis that aggrecan, the predominant disc proteoglycan, inhibits ingrowth of nerves and blood vessels into the disc matrix. Degeneration associated changes of proteoglycan structure and decreased proteoglycan concentration may therefore support ingrowth of vessels and nerves into degenerated discs (Figure 5).

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**Figure 5**: A schematic representing the hypothesis being tested: that the high concentration of proteoglycans in normal discs inhibits ingrowth of blood vessels and nerves into the central region of the disc, whilst loss of these proteoglycans seen in degenerate discs diminishes this inhibition.

Cell culture studies have shown that endothelial cells are inhibited by proteoglycan coated cell-culture surfaces. The presence of pleiotrophin, an angiogenic and neurotrophic factor, was demonstrated in degenerated discs and its effects on innervation and vascularisation are currently being investigated. It is still unknown, whether mechanical factors contribute to vascularisation by induction of angiogenic factors that might be released from disc cells in response to loading – either at normal or abnormal loading ranges. This aspect is being studied also in current experiments.
Mechanical forces on disc cells

The disc is always exposed to mechanical loads – not only due to body weight and daily exercise but also during sleeping as even muscular activity of breathing causes measurable changes in intradiscal pressure as shown by in vivo measurements. Loading of the disc causes multiple physical stimuli that influence intervertebral disc cells by tensile strain, compression, hydrostatic pressure and fluid flow. The EURODISC group in Ulm (Dr. Cornelia Neidlinger-Wilke, Karin Würtz et al.) has developed methods to apply mechanical loads to disc cells within wide physiological loading ranges (Figure 6). With these methods the effects of cyclic strain and hydrostatic pressure on gene expression of intervertebral disc cells grown in three-dimensional scaffolds have been studied. Present findings suggest that disc matrix turnover is influenced by mechanical forces via alterations of gene expression of matrix forming or matrix degrading proteins[11].

![Figure 6: A schematic showing the experimental procedure of mechanical stimulation of intervertebral disc cells: after isolation from the disc tissue, cells are cultured in three-dimensional collagen scaffolds. The cell-seeded scaffolds are exposed to either hydrostatic pressure or cyclic strain by with specially developed devices for the application of mechanical forces to cell cultures.](image)

The effects of mechanical forces are influenced by the magnitude, duration and frequency of the applied load as well as by the tissue origin of the disc cells (nucleus or annulus). Preliminary results suggest that also variation of the osmolarity of the culture medium can alter disc cell responses to mechanical stimulation. These findings support that complex interactions of different factors such as mechanics, hydration and nutrition are involved in the regulation of disc matrix turnover.

Signalling and repair

As disc is an avascular tissue tissue maintenance and repair processes depend on the secretion of autocrine and paracrine growth factors. In addition, an important question for understanding the role of mechanical forces in disc homeostasis is the ability of the latter to induce growth factor secretion. To
this end, the research group of Dr. Dimitris Kletsas at the Institute of Biology in NCSR “Demokritos”, in Athens, Greece, study the role of exogenous and autocrine growth factors on disc cell proliferation and the signalling cascades involved. Known growth factors, whose expression was identified in discs by immunocytochemical studies, such as PDGF, basic FGF, IGF-I and TGF-β, were found to regulate cell proliferation; their effect seems to be determined by the presence of extracellular matrix components. Intracellular signaling pathways mediating these actions, e.g. MAPK, PI-3-K/Akt or Smads, have been also studied. Preliminary data indicate the central role of the PI-3K/Akt pathway in the proliferative response of disc cells. Furthermore, they showed that extreme stressful conditions of pH, osmolarity or oxygen tension strongly inhibit disc cell proliferation. Concerning the effect of mechanical forces, the mitogenicity of conditioned media collected from cultures subjected to stimulation by cyclic strain or hydrostatic pressure have been analyzed. Initial data indicate that disc cells exposed to distinct mechanical strain produced mitogenic factors; however, this response was donor-dependent. These cellular mechanisms might be involved in repair and degenerative pathways of the disc.

**Cell senescence**

Cellular senescence is assumed to contribute to tissue ageing and degeneration. The purpose of this part of the Eurodisc project was to determine the degree of cell senescence in vivo and to characterize the features of the senescent disc cells and their possible role in disc degeneration. It has been found that herniated discs exhibit an increased percentage of senescent cells, as judged by senescence associated β-galactosidase staining (SA-β-gal) staining (Fig. 7). Furthermore, in vitro senescence by serial subculturing is also being studied. Preliminary data with bovine disc cells indicate a rather extended in vitro lifespan, which seem to be donor-specific. Senescent cells were determined by their inability to proliferate, SA-β-gal staining and telomere shortening. The molecular alterations in these cells are currently investigated.

![Figure 7](image_url): Young (left) and in vitro senescent (right) nucleus pulposus cells, as determined by senescence associated β-galactosidase staining

**Electromechanical properties**

Based on detailed experimental data[7, 8] a finite element model that predicts electromechanical responses of the disc at the tissue and cell level as a function of external loading was the goal of this project part:
To reach this aim the EURODISC group of Jacques M. Huyghe, Eindhoven, The Netherlands, has developed a hydrogel that mimics swelling pressure and fixed charges of the disc. Disc responses to compression and degeneration have been simulated. Axial stresses predicted by the model are in agreement with data from Mc Nally et al.[1]. The model illustrates that even without external mechanical load, the disc is subject to substantial mechanical stresses, and existing cracks grow as degeneration progresses. The phenomenon of growing cracks in an unloaded disc is likened to the cracks growing in a (loaded or unloaded) oaken beam while it loses turgor with age. Hence, the model explains why the correlation between damage in the disc and external mechanical load is so poor[5] (Figure 9).

Figure 8: Deformed mesh of the disc FE model equilibrated in a physiological salt solution. Top: intradiscal pressure while disc is placed between the vertebra. Bottom: intradiscal pressure with no vertebra on top to constrain the swelling. None of the two states are stress-free, none of the states are subject to any external mechanical load.

Figure 9: In the degenerating disc stress concentrations develop at crack tips as degeneration develops, because of decreasing osmotic prestressing. As a result cracks open. The axial stresses shown arise without any external mechanical loading on the disc tissue and result from the decreasing fixed charge density with age.

Heredity

Over the past half century, disc degeneration has commonly been attributed to environmental effects, such as excessive mechanical loading and associated tissue damage. Yet, contrary to this view, only modest effects of extreme physical loading conditions on disc degeneration have been found in highly
controlled studies of monozygotic twins grossly discordant for lifetime physical loading. Instead, much of the variance in disc degeneration observed was attributable to familial aggregation, suggesting a strong genetic component[3, 4, 19] Research on familial aggregation of MRI findings of disc degeneration was the first step in investigating possible genetic influences. In order to estimate the overall magnitude of genetic influences on disc degeneration, the Twin Spine Study group of the University of Helsinki, Finland, and the University of Alberta, Canada, subsequently used a classic twin study design based on 147 monozygotic and 153 dizygotic twin pairs. Classic twin studies have demonstrated a dominant genetic component in lumbar disc degeneration, providing heritability estimates up to 74%[13]. Although the complex contributions and interactions between genes and environmental and behavioral factors are currently unknown, work has begun in this area, and is likely to become increasingly common as more genotypes associated with spine degeneration are identified. Specific gene polymorphisms or mutations of the vitamin D receptor gene, different collagen genes, metalloproteinase, aggrecan and interleukin genes have been identified to date and more are sure to follow[2, 10, 16, 20] The identification of such gene forms will further enhance the next step – to study gene-environment interactions, which may eventually provide key insights into underlying mechanisms.

The Twin Spine Study team of the Universities of Helsinki and Alberta has been searching for additional associated gene forms using a candidate gene approach. These efforts have been enhanced with improved phenotypic measures using quantitative measures of degenerative findings on spine MRI using a custom-made image analysis program. As a partner in the EURODISC project these studies have been extended to a wider population and to additional phenotypes of disc degeneration and pathology. Blood samples of patients being seen for disc surgery from all EURODISC countries are undergoing DNA genotyping to further investigate the role of identified polymorphisms in cellular and biochemical outcomes, as well as imaging findings, related to disc degeneration.

It is certain that both environmental and constitutional factors have some role in disc degeneration, and that their magnitude can vary[6]. The purpose of the study is to enhance knowledge of this important area, through a better understanding of the genetically determined ‘natural progression of disc degeneration’ and to what degree and how it is modified by behavioral and environmental factors[18].

Clinical relevance and overall objectives of the EURODISC project

The overall objectives of the EURODISC project are to investigate the aetiopathology of disc degeneration, to provide information on the interactions between disc degeneration and ageing, genetics and environmental factors. It is to be hoped that the results of the project will provide an objective diagnostic scheme for clinical use and will support the development of new diagnostic treatments of disc degeneration and spinal stenosis.

References