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Basic Science

Severity and pattern of post-traumatic intervertebral disc degeneration depend on the type of injury

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Abstract

BACKGROUND CONTEXT: The burst fracture of a vertebra is the result of a complex loading procedure and is often associated with intervertebral disc (IVD) degeneration. Likewise, the presumed etiologies are (i) the structural perturbation of the IVD/end plate, (ii) the impact of loading energy alone, and (iii) the depressurization of the nucleus pulposus.

PURPOSE: To describe the pathogenesis of post-traumatic disc degeneration (DD) by comparing the severity and patterns of degeneration with different injury models.

STUDY DESIGN: New data from an in vitro organ culture study are compared with the previous work on the same model system.

METHODS: To investigate in detail the contribution of each factor (i–iii) to DD, we extended our previous work to compare three different segmental trauma processes in a rabbit full-organ in vitro model: burst fracture (Group A, etiologies i–iii), equienergetic loading without a fracture (Group B, ii), and endplate puncturing (Group C, iii). DD markers (apoptosis, necrosis, matrix remodeling, inflammation) were monitored up to 28 days posttrauma. Gene transcription data were subjected to principal component analysis and agglomerative hierarchical clustering to identify and compare pathologic patterns.

RESULTS: Only Group A showed the full profile of DD: reduced glycosaminoglycan content, increased caspase-3/7 and lactate dehydrogenase (LDH) activity, and elevated messenger RNA of catabolic (matrix metalloproteinase-1, -3, -13) and proinflammatory (tumor necrosis factor-alpha, interleukin [IL]-6, IL-8, and monocyte chemotactic protein-1) genes. In Group B, only catabolic and proinflammatory genes were slightly upregulated. In Group C, LDH but not caspase-3/7 activity was increased. Catabolic and proinflammatory genes were upregulated, although less compared with Group A. Principal component analysis revealed different transcription patterns for Group C.

CONCLUSIONS: The structural perturbation of the end plate/IVD, but not the loading energy or nuclear depressurization, promotes DD. In addition, end-plate puncturing triggers a different pathogenesis, consistent with a more continuous matrix remodeling process.

Keywords: Disc degeneration; In vitro; Post-traumatic; Etiology; Burst fracture; Pattern

FDA device/drug status: Not applicable.

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Introduction

Burst fractures of the vertebra account for approximately 15% of all major spinal fractures [1,2]. Treatment, if any, focuses on preventing or limiting neurologic injury and correcting spinal deformity by stabilizing the vertebra (ie, spondylodesis, vertebroplasty) and decompressing spinal canal stenosis (eg, by laminectomy). Despite a possible concurrent degeneration of the involved intervertebral disc (IVD) [3,4], this is not treated in the primary setting, until it becomes painful in the later course. In this context, tissue engineering approaches (IVD replacements [5], annulus fibrosus [AF] closure [6]) and drug administration (anti-tumor necrosis factor-alpha [TNF-α] [7], anti-interleukin [IL]-1 [7,8], growth factors [9]) are discussed as early interventions. However, their application is primarily focused on degenerative disc disease [10]; therefore, their utilization in burst fracture scenarios is not established. Regenerative medicine approaches would require multiple methods, as multiple anatomic compartments (endplate, nucleus pulposus [NP], and AF) are affected [11]. Until now, the etiopathology of post-traumatic DD is insufficiently investigated; thus, the development of regenerative approaches is only slowly progressing. Until recently, post-traumatic DD investigations were hampered by the lack of an appropriate impact trauma model. Instead, DD was alternatively induced either enzymatically by digestion of the NP [12] or mechanically by perforating the endplate [13,14], by static overloading [15,16], or by annular stab incision [17]. Although these represent valid models for degenerative disc disease, they lack the dynamic character of impact loading and the concomitant massive structural impairment. Haschtmann et al. [18] presented the first in vitro burst fracture trauma model to study post-traumatic DD. In a previous study, exploiting this model, we showed that a single impact load without perturbation of the endplate is not sufficient to promote DD, although the same energy was applied (equienergetic) as for the burst fracture [19]. In this study, we further investigate whether nuclear depressurization, which inevitably parallels burst fractures, is sufficient to promote DD without any compression force. Depressurization is induced by endplate puncturing. This trauma is not intended to model any in vivo situation; it merely serves to isolate one characteristic factor in the potential etiology of post-traumatic DD. Furthermore, we compare in detail the three different pathogeneses (burst fracture, equienergetic loading without endplate fracture, and nuclear depressurization), using principal component analysis (PCA) and cluster analysis to compare transcription patterns of DD marker genes for the different traumas.

We hypothesize that the structural perturbation of the IVD/endplate, which is unique to the burst fracture model, is the major factor causing post-traumatic DD and that different etiologies cause different pathogenesis.

Methods

This study combines the results from previously performed trauma experiments on rabbit spinal segments in our group with the new experimental data. Earlier published data from burst fractures (Group A) and equienergetic loaded specimens without fractures (Group B) [19] were compared with new data from an endplate perforation trauma model (Group C), which was conducted at a later time point. In total, data from 99 specimens were compared and statistically evaluated in this study (Table 1). Whereas Group A comprises three putative etiologies (structural perturbation of the endplate, loading energy, and depressurization of the NP), Group B (loading energy) and Group C (depressurization of the NP) comprise only one each (Table 2). Clearly, no model could be designed that comprises only structural perturbation but not loading energy or nuclear depressurization. Nevertheless, this study design allows determining the contribution of each factor to post-traumatic DD by mutual exclusion.

Full-organ IVD culture model and trauma induction

Intervertebral disc/endplate specimens were harvested as previously described [19,20]. Briefly, specimens from T10/11 to L6/S1 (10 per animal) were isolated within 12 hours after sacrifice from New Zealand white rabbits (3–4 kg, 6 months old). Group A and Group B specimens were dissected as spinal segments (IVD/endplate with ~5 mm of the adjacent vertebral bodies). Group C specimens were prepared as IVD/endplate specimens as this allows to better control perforation depth. It was previously verified that

Table 1
Number of specimens assigned to the different assays for the endplate puncturing experiment (Group C)

	Day 1		Day 7*		Day 28	
Assay	Puncture	Control	Puncture	Control	Puncture	Control
Real-time qPCR, caspase-3/7	6	6	3	3	3	3
GAG/DNA, hydration	2	4	3	3	3	3
Histology	2	2	2	2	2	2

GAG, glycosaminoglycan; qPCR, quantitative polymerase chain reaction.

Note: Burst fracture (A) and equienergetic loading (Group B) were performed previously in a separate experiment. The same assays were performed. For the specimen group assignment of groups A and B, see Dudli et al. [19].

* Day 3 for qPCR and caspase-3/7 assay.