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Intervertebral Disc Cell Death in the Porcine and Human Injured Cervical Spine After Trauma

A Histological and Ultrastructural Study

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Study Design. Histologic and ultrastructural study of disc cell death after traumatic injury to the human cervical spine and postmortem (p-m) in the porcine cervical spine.

Objective. To determine the changes in disc cell morphology, viability, and manner of cell death after trauma in human discs and p-m in porcine discs.

Summary of Background Data. Similarities in the morphology of human and porcine spine have been shown in many histologic and biomechanical investigations. It is known that compressive or traumatic injuries to cartilage and intervertebral discs can result in cell death by necrosis or apoptosis. An additional form of apoptosis, chondroptosis, has been reported in articular cartilage, but not to date in the disc.

Methods. The anterior portion of intervertebral discs and endplates of 30 patients with traumatic injuries to the cervical spine were studied histologically (including trypan blue exclusion and TUNEL staining) and ultrastructurally. Fractures were classified according to Magerl and degeneration of the intervertebral disc according to Thompson and Benneker. Similar studies of disc and endplate were undertaken on porcine cervical spine 0 to 24 hours p-m.

Results. Electron and light microscopy showed up to 75% of human disc cells die within the first 24 hours of trauma, mainly by necrosis, similar to that seen in pig discs p-m. This study reports on 2 morphologies, chondroptosis and balloon cells, previously not described in the disc. Chondroptosis had been significantly higher and ballooned cells were exclusively seen in discs from fractures with compression, where apoptosis was also most common. Porcine samples revealed comparable rates of apoptosis and chondroptosis as fractures with less compression. Glycogen was commonly found in disc cells after trauma.

Conclusion. Traumatic injuries of the human cervical spine lead to rapid changes in disc cell morphology and cell death, particularly *via* necrosis. The type of fracture and load seems to influence cell death.

Key words: intervertebral disc, trauma, ultrastructure, necrosis, apoptosis, chondroptosis, balloon cell. **Spine 2009;34:131–140**

The intervertebral discs are vital to the functioning of the spine in terms of its movement, load bearing, and protection of the spinal cord. The discs in turn are dependent on the cells within them to produce and maintain a fully functioning extra cellular matrix. However, little is known about what influences the vitality and causes death of disc cells, or by what mechanism they may die. Trauma has been demonstrated in articular cartilage to lead to apoptosis (so called programmed cell death) as in human thoracolumbar intervertebral discs.[1–8] Loading of the intervertebral disc in the mouse tail also leads to apoptosis.[9–11] In the human scoliotic disc (where there may be abnormal loading) there is increased death of disc cells, although whether this occurs *via* apoptosis or the less controlled manner of cell death, necrosis, has not been identified.[12,13]

Apoptosis, where the nuclear contents are "packaged" into apoptotic bodies, is generally believed to minimize damage to the surrounding matrix. It involves condensation of the cell nucleus, margination of chromatin, and shrinkage of the cell. There is then ruffling of the plasma membrane before "budding" and formation of apoptotic bodies.[14–17] In necrotic cells, in contrast, there is loss of integrity of the plasma (and sometimes nuclear) membrane leading to swelling of the cell, vacuolization of cell organelles, and eventual rupture of the cell. This can lead to an inflammatory reaction.[18–22] A further type of cell death, chondroptosis, has been described in cartilage by Roach *et al*.[23–25] Cells undergoing chondroptosis have patchy condensed chromatin, increased endoplasmic reticulum, and Golgi apparatus with autophagic vacuoles, but no true apoptotic bodies. Ghadially FN[26] has described an additional morphology in articular cartilage chondrocytes: poorly visualized chromatin in a homogenous nucleus, contained within an obvious dark, sharp border of the nuclear membrane surrounded by normal cytoplasm in which large amounts of glycogen are stored. An appropriate description would be ballooned cells.

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The study and recruitment procedures were approved by the Ethics Committee, MUI (formerly Ethics Committee of the Medical Faculty, University of Innsbruck). All participants gave written informed consent after full explanation of study procedures.

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Table 1. Details of Patients Whose Discs Were Used in the Study Together With Histologic Observations

Patients No.	Age	Sex	Fracture Type (Magerl)	Radiological Deg. Grade	Lesion	Surgery Performed Posttrauma (D)	Cause of Injury	Cluster >10 Cells	Cluster 4–10 Cells	Vessel Ingrowth	Ruptures/ Haematoma
1	17	M	A 3.3.	I	C5/6	0	Skiing accident				oAF-iAF
					C6/7						iAF
2	26	M	A 3.3.	II	C6/7	0	Paragliding accident				iAF
					C7/Th1				iAF		iAF
3	47	F	A 3.3.	II	C4/5	0	Stair fall				oAF-iAF
4	34	M	A 3.3.	I	C3/4	0	Car accident				oAF-iAF
					C4/5						oAF
					C5/6				NP	oAF	
5	62	M	A 3.3.	IV	C6/7	0	Car accident		iAF		oAF-iAF
6	17	M	A 3.1.	I	C6/7	1	Fall		iAF		oAF-iAF
7	29	M	A 3.3.	I	C5/6	1	Skiing accident		iAF		iAF
					C6/7						
8	26	M	A 3.3.	II	C4/5	3	Skiing accident	iAF			iAF
					C5/6						
9	25	M	A 3.3.	II	C6/7	3	Snowboard accident				oAF-iAF
					C7/Th1				iAF		iAF
10	30	M	A 3.3.	I	C6/7	3	Dived into a lake				
					C7/Th1						
11	20	M	B 1.2.	I	C3/4	0	Snowboard accident				iAF
12	50	M	B 1.1.	II	C4/5	0	Skiing accident				iAF
13	76	M	B 2.3.	IV	C6/7	0	Fall		NP		iAF
14	77	M	B 3.1.	III	C5/6	0	Fall after hit by a car			oAF-EP	iAF
15	60	M	B 3.1.	III	C4/5	0	Skiing accident		iAF		iAF
16	49	M	B 2.2.	III	C6/7	1	Fall				iAF
17	69	M	B 3.	IV	C4/5	1	Skiing accident	NP		oAF	oAF-iAF
18	31	M	B 1.2.	II	C6/7	3	Snowboard accident		NP		iAF
19	51	M	B 3.	IV	C5/6	3	Fall with bicycle	iAF			iAF
20	35	M	B 3.2.	II	C6/7	3	Fall with bicycle				oAF-iAF-NP
21	66	M	C 2.2.	II	C6/7	0	Skiing accident		iAF		iAF
22	55	F	C 2.1.	III	C6/7	0	Skiing accident		iAF		oAF-iAF
23	40	M	C 2.1.	II	C4/5	0	Skiing accident	NP			oAF-iAF-NP
24	58	M	C 2.1.	II	C6/7	1	Skiing accident		iAF	oAF	oAF-iAF
25	45	M	C 2.1.	II	C6/7	1	Skiing accident				iAF-NP
26	33	F	C 2.1.	II	C6/7	1	Fall from a horse		iAF	oAF	oAF-iAF
27	30	M	C 2.1.	I	C4/5	1	Snowboard accident		NP		iAF-NP
28	53	M	C 2.2.	III	C6/7	2	Fall with bicycle	iAF			iAF-NP
29	16	F	C 2.1.	I	C4/5	2	Fall from a horse				oAF-iAF
30	37	M	C 2.1.	I	C6/7	2	Skiing accident		iAF-NP		iAF

Ultrastructural observations are the most definitive way of differentiating these cells morphologies but other techniques have been used at the light microscope level. For example, terminal deoxynucleotidyl transferase (TDT)-mediated dUTP nick end labeling (TUNEL) is often attributed with labeling apoptopic cells. However, it seems not to be specific for this as it will label any cells with a break in the DNA such as occurs in cell necrosis.[9,27,28] There is also a suggestion that staining for proliferating cell nuclear antigen and TUNEL codistribute.[29–31]

Patients who have undergone traumatic injuries to the cervical spine with subsequent surgical stabilization provide an ideal population in whom to study the effect of trauma on the cells of the human intervertebral disc. In this study, we have examined the response of disc cells in patients with different types of trauma between 0 and 3 days after injury. For comparison, morphologic changes in porcine discs were observed histologically and ultrastructurally between 0 and 24 hours postmortem (p-m). We have demonstrated that up to 75% of human disc cells die within the first 24 hours of trauma, mainly by necrosis, similar to that seen in pig discs p-m.

■ Materials and Methods

Samples of human intervertebral discs were obtained from 30 patients, aged 17 to 77 years (mean 41.5), undergoing routine stabilization 0 to 3 days after trauma. All patients had lesions of the lower cervical spine (C3–C7) caused by different accidents (Table 1). Injuries were categorized into 3 groups, each containing 10 patients, using Magerl classification according to their appearance on anterior-posterior and lateral radiographs and CT scans.[32–34] These are either (A) compression injury, (B1, B2) flexion combined with compression in the anterior portion and (B3) extension, or (C) a rotation injury. For A-fractures total corporectomy had to be performed with 2 adjacent discs excised and available for study in most cases. C-fractures accompanied by injuries of the vault of the cranium were excluded from this investigation. Degrees of degeneration of the lower cervical spine were recorded by 2 investigators from radiograph, CT scans, and/or MRT.[35,36]

Anterior segments (7–9 mm wide), including the endplates, the outer anulus fibrosus (oAF), inner anulus fibrosus (iAF), and sometimes nucleus pulposus (NP), were removed during operation and immediately dissected into 4 portions. These were processed for electron and light microscopy (vital staining with trypan blue and TUNEL staining).

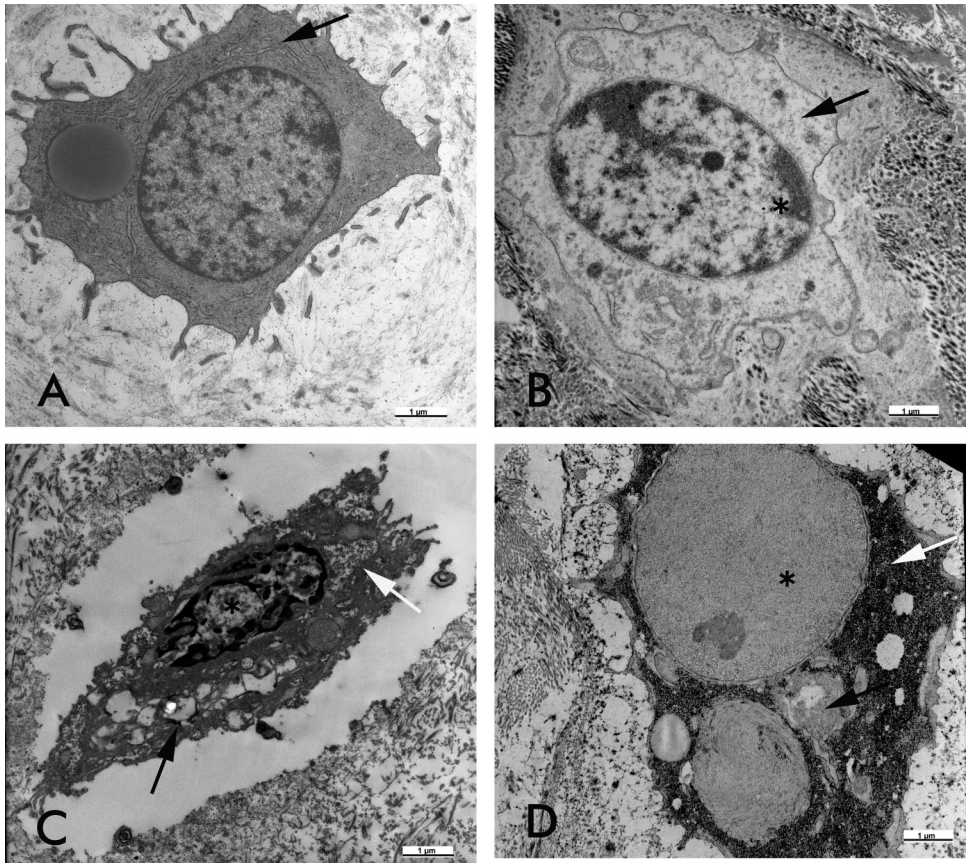

Figure 1. Examples of cells with typical morphologies of (**A**) a healthy cell, (**B**) necrosis, (**C**) chondroptosis, (**D**) balloon cell. **A, Healthy human sample:** patient 16: 49 years old with B2.2 fracture/DD III (C6/7) at 1 day p-m. Healthy cell of iAF with intact cell membrane, good structure of rER (arrow), and few mitochondria 8000×. **B, Necrotic human sample:** patient 21: 66 years old with C2.2. fracture/DD II (C6/7) on the day of trauma. Necrotic cell in oAF, with loss of ER, Golgi apparatus, and mitochondria (black arrow). There is diminished staining for chromatin (asterisk). This morphology is normally a rare appearance in traumatic injured discs, but frequent in p-m changes in pig. Parallel aligned microfibers can be seen close to the disc cell membrane. 8000×. **C, Chondroptosis and necrotic human sample:** patient 1: 17 years old with A3.3. fracture/DD I (C5/6) on the day of trauma. Chondroptotic cell with patchy condensed chromatin (asterisk) and some necrotic features, such as complete loss of cell organelles in cytoplasm in iAF (white and black arrow) 8000×. **D, Balloon cell human sample:** patient 12: 50 years old with B1.1. fracture/DD II (C4/5) on the day of trauma. Balloon cell with homogenous gray chromatin (asterisk) within the nucleus and large amounts of glycogen (white arrow) in the cytoplasm in iAF. This cell also shows some features of necrosis, such as loss of cell organelles (black arrow). 8000×.

Samples of endplate and intervertebral disc were obtained from 6 × 4 to 6-month-old domestic pigs (suis familiaris), which were killed *via* an intraocular captive bullet. Within 20 minutes of death the lower portion of the cervical spine (C3–C7) was sampled and used to study different time points p-m (1, 6, 12, and 24 hours).[37] The spine, with its surrounding muscles but not skin, was stored at 24°C between each time point when another sample of intervertebral disc was removed ventrally and dissected as for the human samples (*i.e.*, oAF and iAF). NP was very gelatinous and fluid, not similar to the human samples studies, and so was excluded from the investigations.

Ultrastructure Studies

Samples were dissected into cubes, from the outer, mid, and inner anulus, and sometimes human NP. These were fixed in 2.5% glutaraldehyde in cacodylate buffer for 12 hours, rinsed 3 times in buffer, and postfixed with 2% osmium tetroxide. After embedding in Araldite, semi-thin (6 μm) and ultra-thin (0.9 μm) sections were cut and stained with uranyl acetate and lead citrate and examined in a Zeiss transmission electron microscope, EM 10.[38,39]

The morphology of the cells was identified as healthy, apoptotic, necrotic, chondroptotic, or ballooned according to specific features (see also Figure 1, Table 2).[20,21,25,26] At least 23 to 27 cells were examined in each sample of the oAF, 20 to 23 in the iAF, and 10 to 13 cells in the NP.

Morphology at Light Microscope Level

Samples were fixed with Schaffer solution for 2 days. Dehydration with ethanol was followed by embedding in methylmethacrylate. Resin blocks were cut at 4 to 6 μm thickness.[40]

Sections were stained with Goldner and morphologic features of the disc and endplate were noted such as cracks, bleeding, edema, or swelling of the cells, in addition to the general integrity of the tissues. Cell clusters, defined as 4 or more cells grouped together within a common capsule, were noted in all sections; 2 sizes of cluster were recorded: those containing 4 to 10 cells and those with >10 cells in.

Cell Viability

Cell viability was assessed by trypan blue.[40,41] Samples were placed in trypan blue for 90 minutes before fixing in Bouin solution for 12 hours, and processed for paraffin. Cells that were viable at the time of immersion into trypan blue had intact membranes and excluded the dye, whereas dead cells allowed the dye to enter the cytoplasm and nucleus. Ten micron sections were counterstained with eosin. Minimum 40 cells per sample were counted in oAF, iAF, and NP and the ratio of live:dead cells was determined.

Apoptosis via *TUNEL*

Apoptosis *via* TUNEL staining was carried out on samples which were fixed in 0.4% paraformaldehyde for 4 hours and paraffin embedded in a routine manner. Ten micron serial sec-

Table 2. TEM Investigations of Disc Cell Morphologies

	Cell Membrane	Cytoplasm	Nucleus and Chromatin Structure
Necrotic cell (see Fig. 1B)	Ruptured	Loss of rER, swollen or loss of ER, and organelles	Diminished chromatin
	Traumatic lesion: Ruptured	Traumatic lesion: swollen or loss of ER andorganelles, condensed cytoplasm masses	
Apoptotic cell	Blebbing of apoptotic bodies and vacuoles	Vacuolated, but structured	Confluent and condensed chromatin, strongly osmiophilic
	Traumatic lesion: Ruptured, blebbing of apoptotic bodies and vacuoles	Traumatic lesion: Vacuolated with condensed necrotic areas in between	
Chondroptotic cell (See Fig. 1C)	Blebbing of cytoplasmic vacuoles	Slightly vacuolated	Patchy condensed chromatin, strongly osmiophilic
	Traumatic lesion: Ruptured with some blebbing of cytoplasmic vacuoles	Traumatic lesion: Slightly vacuolated with condensed necrotic areas in between	
Ballooned cell (See Fig. 1D)	Without interruptions	Well structured, rER, Golgi apparatus, Mitochondria, often having large glycogen deposits	Homogenous grey chromatin, nucleolus mostly visible
	Traumatic lesion: Ruptured	Traumatic lesion: Condensed necrotic cytoplasm, few identifiable organelles mostly swollen, mostly glycogen storage	Traumatic lesion: Homogenous grey chromatin, rare visible nucleolus

rER indicates rough endoplasmatic rediculum; ER, endoplasmatic rediculum.

tions were cut. Slices were dewaxed and after that exposed to Triton X-100 for 8 minutes and washed in PBS buffer before incubation with the TUNEL reaction mixture. *In situ* cell death detection kit (Roche, Mannheim) was applied to the sections according to the manufacturer's instructions. Human tonsils were used as both positive and negative controls. For quantification, same regions were chosen for counting cells within 1 mm^2 on both hematoxylin-eosin stained sections and TUNEL stained serial sections. Each human and porcine sample was investigated in triplicate.

Statistical Analysis

Summary statistics were performed using SPSS (version 15.0) software; the Shapiro-Wilk test was used to test for normality, to determine if parametric or nonparametric tests were appropriate. Differences between 2 unpaired groups were evaluated with Student *t* test or Mann-Whitney *U* test, and for more than 2 unpaired groups with ANOVA or Kruskal-Wallis test, as appropriate. If necessary, a further subinvestigation was done with a *post hoc* analysis or Mann-Whitney *U* test. Differences between paired groups were calculated with the Wilcoxon test. Correlation analyses were performed by using the Pearson or the Spearman correlation coefficient. A correlation coefficient of 0.2 to 0.49 was interpreted as low, 0.5 to 0.69 as moderate, 0.7 to 0.9 as high, and greater than 0.9 as very high. All reported *P*-values were 2-sided; a type 1 error level of 5% and a statistical power of 80% were used.

■ Results

Porcine Discs

Histology of porcine discs demonstrated intact tissue at the light microscopic level at all time points (1, 6, 12, and 24 hours) p-m. However, electron microscopy demonstrated ultrastructural changes in the matrix, with swelling of the collagen fibers obvious 24 hours p-m. Blood vessels were present in the longitudinal ligament and oAF. There were changes in the cell population p-m and obvious differences between regions (with most cells in the oAF compared with the iAF). The number of trypan blue-positive cells increased with time p-m reaching 70% to 80% in the annulus at 24 hours (See Figure 2) (oAF: 1 hour *vs*. 12 hours $p = 0.043$; 1 hour *vs*. 24 hours $p = 0.042$; iAF 1 hour *vs*. 24 hours $p = 0.027$). TUNEL-positive cells were more variable, with 0.0% to 3.3% of cells at all time points in the oAF being TUNEL-positive. In the iAF this increased to 5.6% to 17.4% of cells at 6 hours p-m. Surprisingly at 12 hours there had been a slight, but not significant decrease in the iAF with a slight increased number of TUNEL-positive cells at 24 hours in all pigs investigated. No clusters of cells were seen in any porcine discs.

T.E.M results showed similar trends with the proportion of healthy cells, with well retained rough endoplasmic reticulum (rER) but few mitochondria, decreasing from 60% immediately p-m to only 30% by 12 hours p-m (oAF $p = 0.043$; iAF $p = 0.027$). The iAF cells differed from those in the outer AF by having less rER but more mitochondria and sometimes were surrounded by a lacuna composed of thin collagen fibers. The number of necrotic cells, some of which were present in all samples, increased with time p-m (1 hour *vs*. 24 hours: oAF $p = 0.043$; iAF $p = 0.028$) (Figure 3). Cells with classic apoptotic ultrastructural features, in contrast, were much less frequent, whereas there were more cells with a chondroptotic appearance, particularly in the inner AF. Although numbers of both apoptotic and chondroptotic cells were greater at 6 to 12 hours p-mcompared with 1 hour, the difference was not statistically significant. Gly-

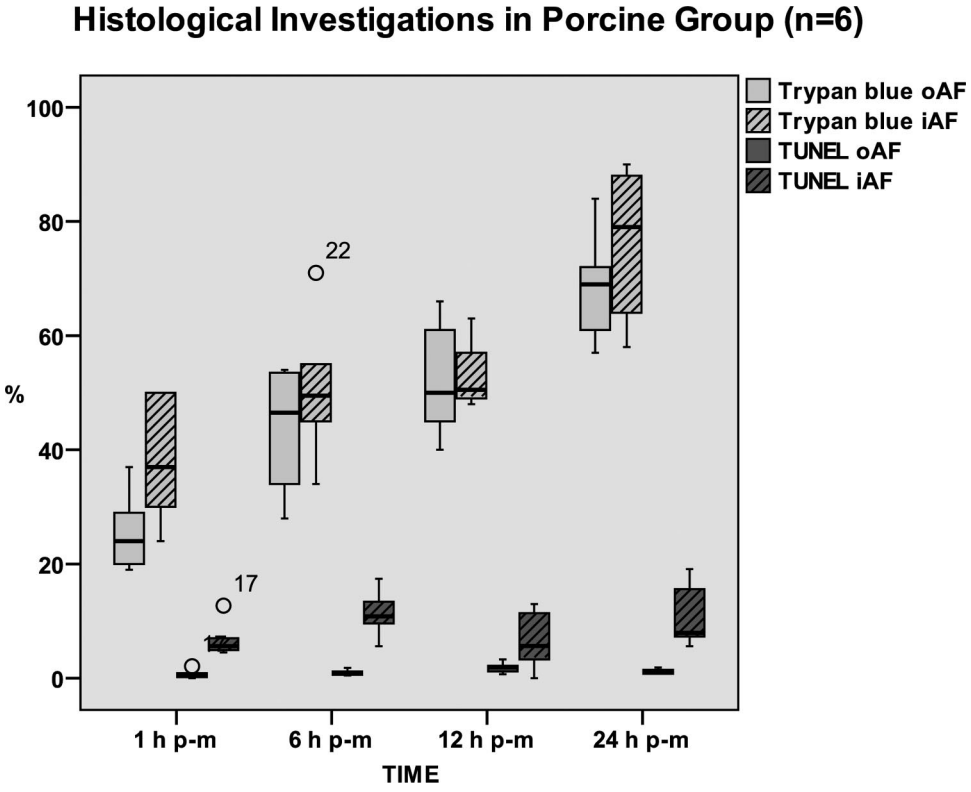

Figure 2. The percentage of trypan blue-positive cells in porcine intervertebral discs increased with time p-m, whereas TUNEL-positive cells were much fewer and showed no such relationship. The box and whisker plot shows the median value (horizontal line), 25% and 75% percentiles and the bars show upper and lower extremes.

cogen storage was only rarely seen (2/1261 cells) and there were no ballooned cells in the porcine discs.

Human Discs

The integrity of the disc matrix was damaged in nearly all samples, with ruptures or fissures which were often hematomous. Annular lamelas were disorganized in comparison to the porcine discs. Fragments of mineralized bone, bone marrow, and cartilage endplate could all be seen in some samples. The vertebral bone was often also abnormal, with necrotic cells within the marrow cavities in place of healthy bone marrow cells. Cell density diminished from the outer disc regions toward the center. Cell death, as shown by trypan blue positivity, was common with 61% of the cells "dead," regardless of fracture types, location, or time posttrauma (Figure 4).

TUNEL-positive cells in contrast were most common in A-fractures, particularly in the NP. Few cells were TUNEL-positive for B1-/B2-fractures and less again for B3- and C-fractures, the iAF always having more TUNEL-positive cells than the oAF. Cell clusters were also a common feature, being present in 67% of samples, the incidence showing an increasing trend with radiologic degeneration (grade I: 49%, II: 75%, III: 60%, and IV: 100%, respectively). Clusters were present in the iAF or more commonly in the NP, but there were none in the oAF. In a third of those discs, clusters were large, con-

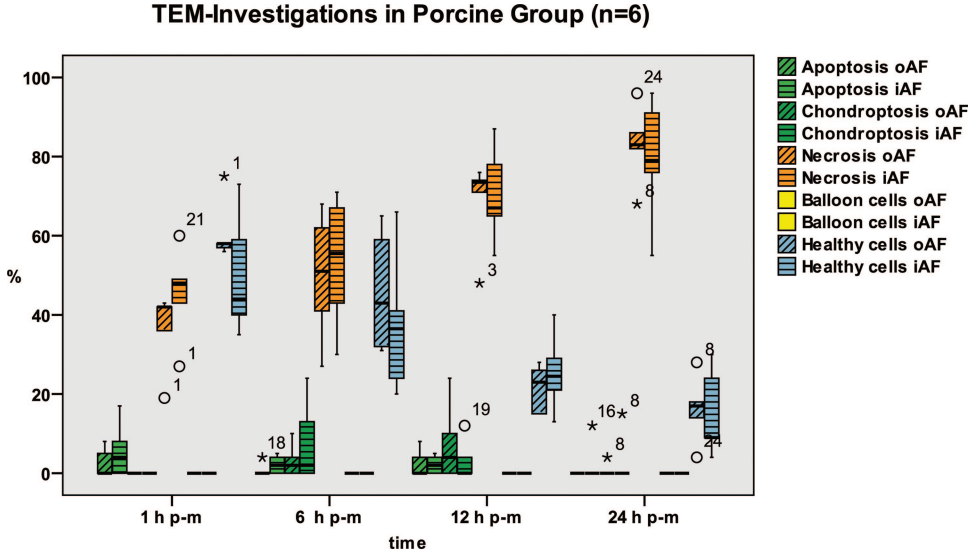

Figure 3. Box and whisker plots of the incidence of different cell morphologies identified by ultrastructural appearance in the inner and oAF of porcine intervertebral discs at different times p-m. Box shows the 25% and 75% percentile and median (horizontal line) values. Bars show upper and lower extremes.

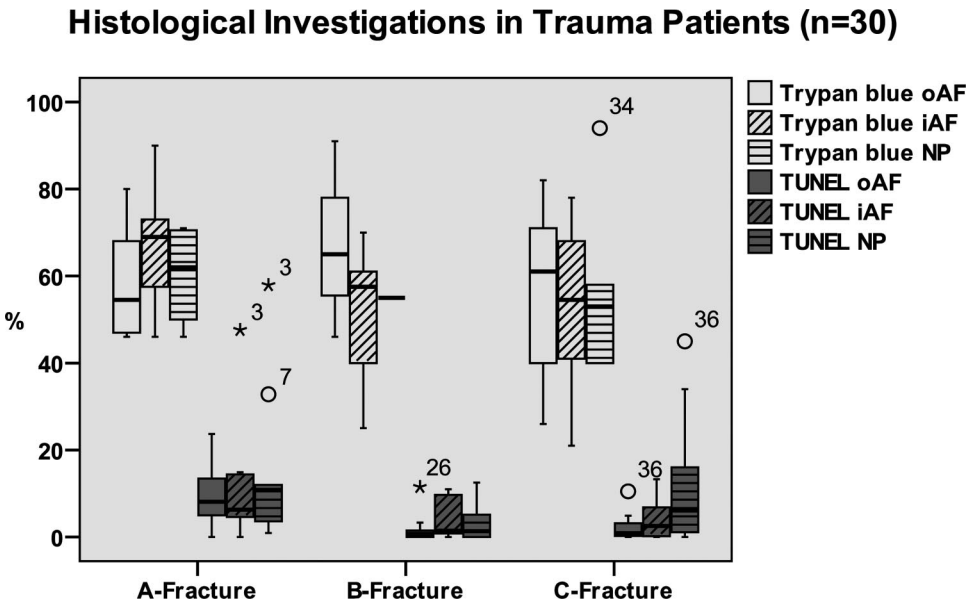

Figure 4. The incidence of trypan blue- and TUNEL-positive cells in the annulus fibrosus and NP of human discs with different types of fracture. The box and whisker plot shows the median value (horizontal line), 25% and 75% percentiles and the bars show upper and lower extremes.

taining more than 10 cells per cluster. Blood vessels were only present in 16% of samples, usually in the oAF, and bore no relationship to degeneration, but hematoma was present in all discs examined.

When combining the results from those discs that are thought to have resulted from a primarily compressive injury (type A-, B1-, and B2-fractures) and comparing them to those from discs with more shear or tensile loading (type B3- and C-fractures), there are some significant differences in cell morphologies. Discs with compression-related fractures had significantly higher numbers of TUNEL-positive cells compared with those with less compression (oAF: 9% for type A-, 3% for B1- and B2-fractures, compared with 1.8% for type B3- and C- fractures; $p = 0.011$). For the iAF, corresponding incidences of TUNEL-positive cells were: 10.7% (A-Fracture), and 6.4% (B1- and B2-fracture), and 3.6%, respectively in fractures with less compression (type B3- and C-fractures) $p = 0.005$.

The ultrastructural appearance of the cells within the human discs suggested that healthy cells were a rarity, with the majority of cells appearing necrotic, particularly in the outer regions (60% in the oAF and 51% in the NP). The cell membrane was poorly identifiable and, in contrast to necrotic cells in the porcine samples, the human necrotic cells had very few cell organelles visible, with only dark masses of fused cytoplasm rests (Figure 1, Table 2). Glycogen deposits were a common finding in the human disc samples, particularly those which had not been subjected to compressive loading. Glycogen deposits occurred in 35% to 70% of cells; they were found in both cells which were trypan blue positive or negative (*i.e.*, "alive,") but they tended to be more frequent in cells with an unhealthy appearance.

The type of fracture, but not the time postinjury, appeared to influence some aspects of disc cell health and death. Healthy cells were most common in type C-fractures and least in type A, particularly in the oAF (Figure 5). The incidence of necrosis and apoptosis was similar in all fracture types in the oAF, but there were more necrotic cells in the iAF and NP in the C-fracture group than in A or B. Chondroptosis and ballooned cells, in contrast, were most common in patients with type A-fractures and least common in those with type C.

There were significantly more cells which were apoptotic or chondroptotic in the discs with compressive injuries (type A- and B1-, B2-fractures; n = 15) than in those with types B3- or C-fractures ($p = 0.002$ for all regions, oAF, iAF, and NP, n = 15). The frequency with which ballooned cells were present was also greater in all regions of the discs with compressive injury, but it was only significant in the iAF and NP (Table 3). The percentage of cells which were necrotic differed less consistently, being higher (but not significantly) in the compressive injured discs in the oAF, but lower in these discs in the iAF and NP than in discs with more shear and tensile load (only significant for the iAF: $p = 0.003$).

■ Discussion

There have been several studies investigating death of intervertebral disc cells, particularly in response to mechanical load, in animals, but little is known of the effect in human discs.[9,28,42,43] Intervertebral discs from patients who have undergone traumatic injuries to their spine, thus provide a unique opportunity to study cell death in humans. They were compared with porcine discs which had not undergone mechanical injury, but have similar morphologic features as the human cervical spine and could be sampled in a controlled manner at different time intervals p-m.[44] The most likely "injury" to porcine disc cells would be due to ischemia with the resultant reduction in nutrient supply and anoxia, in addition to a possible build up of metabolic waste products. Many biomechanical studies and morphologic analyses

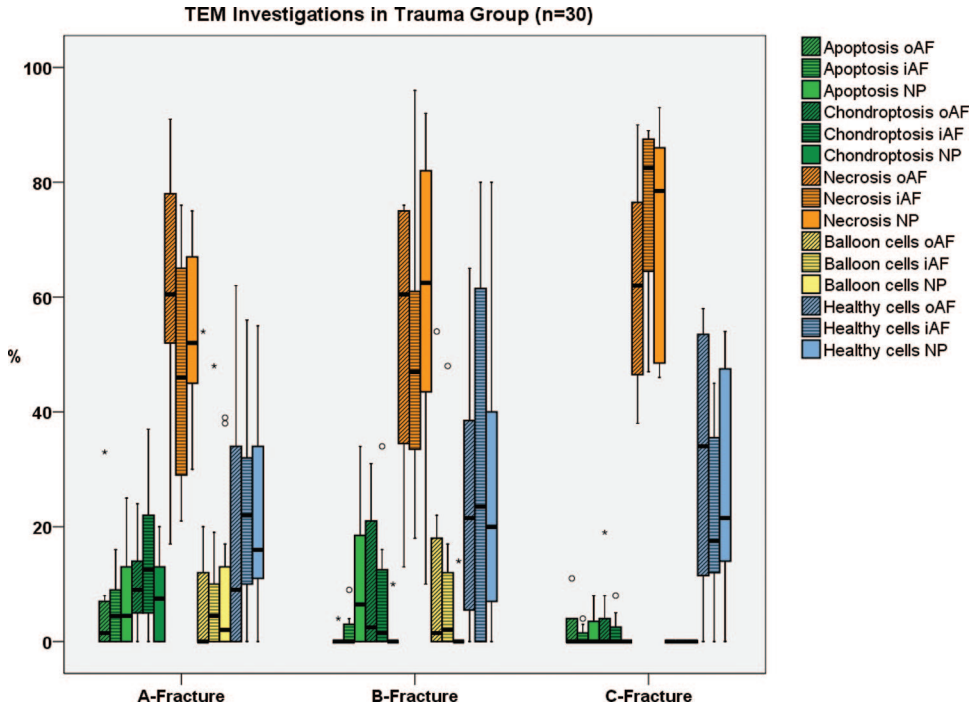

Figure 5. Box and whisker plot showing the incidence of different morphologies observed by T.E.M in human disc cells in patients with varying fracture types. Box shows the 25% and 75% percentile and median (horizontal line) values. Bars show upper and lower extremes.

have been based on porcine models.[44–47] The traumatized human discs had similar average number of dead cells (trypan blue positive) as were found at 12 hours p-m in pigs *e.g.*, 50% to 60%. However, the human discs differed from the porcine discs in that slightly more dead, trypan blue-positive cells were found in the oAF in humans whereas for the pigs there were more dead cells in the iAF. (Although the time interval between trauma and excision of the disc varied between 0 and 3 days for the humans, no trend was seen with this, possibly because of relatively low sample numbers.). In other tissues ultrastructural investigations have revealed necrosis to be the main cause of cell death after periods of ischemia, for example in the kidneys of rats. Different stages of necrosis could be defined according to the length of time of the ischemia. The changes described, such as swelling of cell organelles and loss of ribosomes at the ER, are similar to that seen in our porcine samples with ongoing time p-m.[48,49]

Biomechanical studies revealed that compressive and flexion-extension load is spread equally in the nondegenerated disc in the lumbar spine.[50,51] There were differences, however, in the thoracic spine and in degenerative discs of lumbar and thoracic spine with stress peaks in oAF.[51] Our investigations found similar numbers of trypan blue-positive cells across the cervical disc in A-fractures whereas T.E.M and trypan blue investigations of B1-, B2-fractures with higher degeneration degrees revealed more dead cells in the oAF. The lordosis of cervical spine and lumbar spine could suggest a similar behavior of these 2 areas of the spine. Other investigations showed the different behavior of nondegenerated and degenerated discs under compression.[52] The same compressive load was transferred differently in oAF and iAF, and resulted in more tensile stress for degenerative discs in both areas compared with nondegenerate. This could be a possible reason for different rates of disc cell

Table 3. Incidence of Cell Morphologies, Identified by TEM, in Human Discs From Different Types of Injury

	Compressive Injury (A + B1 + B2 Fractures)	Mean Cells (%)	Less-Compressive With More Shear and Tensile Loading (B3 + C fractures)	Mean Cells (%)	*P*
Apoptotic and Chondroptotic					
oAF	16/18 (89%)	15.2	6/13 (46%)	4.5	0.002
iAF	17/23 (74%)	16.8	4/15 (26%)	3.3	0.002
NP	19/23 (82%)	15.3	5/14 (36%)	2.8	0.002
Necrotic					
oAF	18/18 (100%)	59.0	13/13 (100%)	54.4	NS
iAF	23/23 (100%)	45.7	15/15 (100%)	67.9	0.003
NP	23/23 (100%)	51.1	14/14 (100%)	56.7	NS
Ballooned					
oAF	8/18 (44%)	9.9	2/13 (15%)	1.9	NS
iAF	14/23 (61%)	11.0	1/15 (7%)	0.3	0.001
NP	11/23 (47%)	7.1	0/14 (0%)	0	0.003

pathologies seen in peripheral and central areas of the disc in our investigations. Shear loads as in rotation injuries, are transferred mainly by the periphery. The disc pressure was much smaller than in compression loads,[53] which might indicate the different disc cell death seen in fractures with more shear but less compression compared with more compressive loading in the present study.

P-m investigations in mouse intervertebral disc showed degenerative changes proceeding from oAF toward NP, in contrast to our p-m studies in pigs. Mouse disc cells contain glycogen particles in the iAF, which were rarely seen in our investigated porcine samples.[37] As the amount of glycogen indicates the more anaerobic metabolism of a cell, the high incidence of glycogen in human discs posttrauma (compared with virtually no glycogen in porcine discs) could suggest altered metabolism in these human disc cells. Apart from muscle and liver cells glycogen is generally not found in large amounts in most cell types. However, articular cartilage chondrocytes are known to be able to contain significant quantities of glycogen, perhaps also reflecting a shift to anaerobic metabolism.[54] Certainly glycogen has been reported previously in human disc cells[55] with the potential of being used as an energy pool.[56]

As in the pig discs, ultrastructural observations showed necrosis to be much more common than apoptosis in the human trauma discs. Hence if trauma or other changes in the disc cell environment triggers apoptosis, it seems to be a very transient response, being rapidly succeeded or replaced by necrosis. It is interesting that the human discs contain 2 cell morphologies that are rarely, if ever, found in the pig disc cells: that of chondroptosis and balloon cells. The reason for this is unclear. However, there are basic differences in that the pig samples were from relatively young and healthy samples. In addition, although not used in this study the cells of the NP of the pig, unlike humans, include notochordal cells which are known to influence the metabolism of other disc cells.[57] The type of injury that the human and porcine disc cells have received is also different, possibly primarily deficient nutrition for the porcine but mechanical loading for the human disc cells. These could trigger different pathways, for example compressive load has been shown to trigger production of caspase 9 in disc cells,[8,42] which is known to be present in chondroptotic human chondrocytes.[25] There are many similarities between the intervertebral disc and articular cartilage, such as little exposure to phagocytic cells. Hence it would not be surprising that disc cells should undergo a similar cellular process such as chondroptosis to deal with cells which may have reached the end of their lifespan.

It is to be expected that TUNEL would label not only apoptotic cells, but also chondroptotic cells.[25] Likewise, several investigations have shown TUNEL to be less specific than ultrastructural investigations with late necrosis also resulting in TUNEL positivity.[9,27–30,58–61] In this study, similar proportions of apoptotic and chondroptotic cells were found according to ultrastructural morphology (by T.E.M) as the incidence of TUNEL-positive cells, particularly for the AF, although there was not a significant correlation between the 2 methods.

Animal studies applying a compressive load to mice tails have shown the amount of resultant cell death depends on the size of load and how long it was applied for.[62] Results from the present study suggest that the type and size of mechanical injury also alters the cell response in human discs. Apoptotic, chondroptotic, and balloon cells were seen most commonly in discs which had been subjected to primarily high compressive loading (resulting in either A- or B1-, B2-fractures), compared with discs from patients with less compressive but more tensile and shear loading (B3- and C-fractures). The appearance of homogenous nuclei as seen in ballooned cells could possibly indicate an increased activity status of these cells. Tumor cells, such as in multiple myeloma, present a similar homogeneous nucleus and are known to be very active.[26] These cells have a high rate of RNA synthesis, a high mitosis rate, and in this disease an enhanced protein synthesis. Whether ballooned cells in the disc would be as active as such tumor cells with high levels of protein synthesis, must be considered and investigated in further studies.

The exact environmental factor which triggers the cell response and frequently cell death in the traumatized human discs is not known. The changes in vasculature, *e.g.*, damage to blood supply and/or hematoma, are likely to change the concentration of serum protein, glucose, oxygen, and metabolites in the cell environment. Many of these have been shown to influence cell viability and proliferation.[63,64] Compressive loading in animal studies resulting in disc cell death, especially *via* apoptosis, is well investigated. However, this is the first report of chondroptosis or ballooned cells in the human intervertebral disc and the importance and relevance of their presence to disc metabolism and physiology is unclear.

Cells of the intervertebral disc are believed to exist in an environment which would be noxious to most other cell types.[65] They have a relatively slow metabolic rate in the normal disc, resulting in a very slow turnover of the matrix (*e.g.*, the half-life of the main extracellular components, aggrecan and collagen, is 8 to 12 years and 50 to 100 years, respectively).[66,67] The present study suggests that despite this, cells will respond to certain stimuli by going down apoptopic and particularly necrotic pathways very rapidly, within 24 hours of injury. To what extent the changed disc cell morphology might influence the survival or degeneration of the disc is not known. Whether disc cells and disc matrix will partially recover after trauma, and whether there will be a possible proliferation of disc

cells and remodeling of disc matrix must be investigated in further studies.

■ Key Points

- Traumatic injury to the cervical spine causes fracture of the vertebrae and lesion of the intervertebral disc followed by rapid and extensive disc cell death.
- Up to 75% of the cells in the oAF were Trypan blue positive within 3 days of trauma; approximately the same rate was seen in porcine samples 12 hours p-m.
- Disc cell death is dependent on fracture type and load. Fractures with a high degree of compressive loading resulted in disc cells with different ultrastructural morphologies compared with fractures with less compressive loading.
- Necrosis seems to be the main cause of disc cell death after trauma in humans and p-m in pigs.
- Chondroptosis has been described in intervertebral discs for the first time, particularly in discs from patients with compressive fractures.
- Balloon cells, such as can be seen in some tumors, where only seen in human discs from patients with compressive fractures.

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