Spine

SPINE Volume 40, Number 8, pp E450-E457

BASIC SCIENCE

Apoptosis in Human Compressive Myelopathy Due to Metastatic Neoplasia

Rowena E. A. Newcombe, PhD, MBBS,* John W. Finnie, FRCVS, PhD,† Anna V. Leonard, PhD,* Jim Manavis, BSc (Hons),† Peter L. Reilly, FRACS, MD,* Robert Vink, DSc,‡ and Peter C. Blumbergs, MBBS, FRCPA†

Study Design. Immunohistochemical assessment of apoptotic markers in human cases of compressive myelopathy due to neoplastic compression.

Objective. To characterize the role of apoptosis in neoplastic compressive myelopathy in human postmortem tissue with extramedullary tumor involvement.

Summary of Background Data. Neoplasms, whether primary or metastatic, may lead to compression of the spinal cord and development of a compressive myelopathy syndrome. Apoptotic processes of cell death are thought to contribute to cell death in chronic compressive myelopathy because of degenerative spondylosis, but this has not previously been described in neoplastic compression.

Methods. Six postmortem cases of human neoplastic compressive myelopathy were assessed for apoptosis using a panel of immunohistochemical markers including Fas, B-cell lymphoma 2 (Bcl-2), caspase-3 and 9, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), poly (ADP-ribose) polymerase (PARP), apoptosis-inducing factor (AIF), and terminal deoxynucleotide transferase dUTP Nick End Labeling (TUNEL).

Results. Apoptosis was maximal at the site of tumor compression. Glial cells, predominantly oligodendrocytes, were immunopositive for DNA-PKcs, PARP, AIF, and TUNEL. Axons were immunopositive for caspase 3, DNA-PKcs, and AIF. Neurons were immunopositive for DNA-PKcs, PARP, AIF, and TUNEL.

Conclusion. The current study demonstrates that apoptosis plays a role in human neoplastic compressive myelopathy. Necrosis dominates the severe end of the spectrum of compression. The prominent oligodendroglial involvement is suggestive that apoptosis may be important in the ongoing remodeling of white matter due to sustained compression.

Key words: apoptosis, neoplastic, compressive myelopathy, axonal injury.

Level of Evidence: 4

Spine 2015;40:E450–E457

Compressive myelopathy denotes spinal cord injury secondary to compressive forces of varying etiology (including intervertebral disc protrusion, chronic spondylosis, neoplasia, and vertebral fracture and subluxation). The compressive forces may be of varying magnitude and duration and may act either continuously or intermittently, leading to a spectrum of parenchymal damage of differing severity.

Spinal vertebrae are a common site for metastasis in systemic neoplasia often resulting in eventual spinal cord compression (SCC).[1,2] Almost any systemic tumor can metastasize to the spinal column, and extradural (epidural) compression (EDC) has been reported as a complication of every major type of systemic neoplasm, the frequency of occurrence related to the incidence of the given tumor in the general population, and its propensity for bony spinal involvement. Prostate, breast, and lung cancers are responsible for 15% to 20% of patients with EDC, with lymphoma, myeloma, and renal neoplasms, accounting for a further 5% to 10% of patients. Ninety percent of patients with prostate cancer have vertebral metastases at autopsy, 74% with breast cancer, 45% with lung cancer, 29% with lymphoma or kidney cancer, and 25% with gastrointestinal cancer. Although most cases of EDC develop in patients previously diagnosed with a primary tumor elsewhere, 8% to 34% have spinal involvement as the initial clinical presentation. In patients with terminal cancer, 2% to 5% develop EDC in the final 2 years of life, the nature of the primary tumor and degree of neurological deficit being the most important factors determining survival times.

*School of Medical Sciences, University of Adelaide, Adelaide, Australia; †SA Pathology, Hanson Institute Centre for Neurological Diseases and Schools of Medical and Veterinary Science, University of Adelaide, Adelaide, Australia; ‡Division of Health Sciences, University of South Australia, Adelaide, Australia.

Acknowledgment date: June 19, 2014. Revision date: October 14, 2014. Acceptance date: December 1, 2014.

The manuscript submitted does not contain information about medical device(s)/drug(s).

The Neil Sachse Foundation, Australia, funds were received to support this work.

Relevant financial activities outside the submitted work: consultancy, employment, expert testimony, grants, patents.

Address correspondence and reprint requests to Rowena E. A. Newcombe, PhD, MBBS, Brain and Mind Research Institute, 94 Mallett St, Campderdown, New South Wales, Australia, 2050; E-mail: rowena.newcombe@sydney.edu.au

DOI: 10.1097/BRS.0000000000000821

Clinical signs include pain and deficits in motor, sensory, and autonomic function.[3–7]

SCC secondary to EDC may be due to vertebral collapse with posterior extension leading to compression of the anterior aspect of the cord and tumor extension into the extradural space.[3–6] SCC occurs in the thoracic region in 60% to 80% of cases (predominantly due to its natural kyphosis and larger intrathecal cross-sectional area), lumbosacral region in 15% to 30% of cases, and cervical region in less than 10% of cases. Up to 50% of patients have multiple sites of compression.[3–6]

In SCC, 2 principal modes of cell death are recognized, namely, necrosis and apoptosis, but the contribution of the latter to the resulting myelopathy is incompletely understood. The few studies that have been conducted have largely addressed the molecular pathways determining the survival and progression of spinal neoplasms and the associated inflammatory response rather than on the spinal cord. Necrosis and apoptosis represent the 2 extremes of a spectrum of cell death and there is a continuum between these extremes, with many injurious insults producing both types.[8,9] Moreover, the mechanisms of apoptosis are varied, with multiple intrinsic and extrinsic pathways involved in this active, energy-requiring process of programmed cell death.[10–12]

In the present study, archival cases of human metastatic neoplasia-induced SCC were examined in an attempt to determine the role of apoptosis in the development of the resulting myelopathy.

MATERIALS AND METHODS

Six cases of archival human neoplastic SCC were obtained from the South Australian Brain Bank (Adelaide, Australia). All 6 cases had extradural compression. The tumor type varied in each case, but all were metastatic, and the clinical course ranged from 25 days to 5 years. The duration of the cord compression was defined as the time of onset of clinical signs suggestive of SCC to death. Details of these cases (age, tumor type, levels of cord compression, and clinical presentation and duration) are shown in Table 1.

Immunohistological Assessment

Spinal cords were immersion-fixed in 40% formalin for a minimum of 10 days and examined according to standard neuropathological protocol. Individual cord segments were cut transversely, and the segmental level was determined by counting the segments above and below the ventral nerve root at T2, a landmark selected because of the marked anatomical disparity between T1 and T2 nerve roots. After macroscopic examination, cord segments were processed to paraffin wax, 5-μm sections cut and stained with hematoxylin and eosin (H&E). Duplicate sections were stained with Weil's stain for myelin and immunohistochemically with antibodies to amyloid precursor protein (APP) and a range of markers of apoptosis (Table 2). APP is normally transported by fast axoplasmic transport and disruption to this movement leads to its aggregation in amount detectable by light microscopy. It is the most sensitive early marker of axonal injury.

With regard to the identity of immunoreactive cells in these spinal cords, neurons, astrocytes, and oligodendrocytes were determined on the basis of well-described morphological and immunohistochemical characteristics. Oligodendrocytes in H&E-stained, immersion-fixed human neural tissue are small cells with prominent, dark, rounded nuclei and a clear cytoplasm (a "fried egg" artifact appearance). These cells stain negatively with glial fibrillary acidic protein (GFAP). However, our experience with some of the immunocytochemical oligodendroglial markers suitable for human tissue (*e.g.*, Olig-2, P25, MOG, MOSP, MBP) is that they stain subsets of oligodendroglia and may cross-react with astrocytes. Astrocytes were distinguished from oligodendrocytes on H&E staining by their larger, vesicular nuclei and lack of cytoplasmic staining. Reactive fibrous astrocytes show a spectrum of increased cytoplasmic staining with radiating processes from the cell body and positive GFAP immunoreactivity. Anterior horn cells were identified by their location, size, pyramidal shape, and large nuclei with prominent nucleoli. Axonal swellings (spheroids) were identified immunohistochemically by APP immunopositivity, these spheroids not being labeled by MAP-2 (a dendritic marker).

A panel of immunohistochemical markers of apoptosis (Table 2) was selected to evaluate the contribution of this mode of cell death to the observed myelopathy in these cases. Tissue from human lymphoma cases was used as a positive control for caspase-3 and -9, DNA-PKcs, poly (ADP-ribose) polymerase (PARP), Bcl-2, first apoptosis signal (Fas), and terminal deoxynucleotide transferase dUTP Nick End Labeling (TUNEL). Human corpus callosum from traumatic brain

TABLE 1. Neoplastic Compressive Myelopathy Clinical Data

Case	Age, yr	Sex	Type	Vertebral Level	Spinal Cord Segment	Clinical Duration	Clinical Deficit
1	50	Male	Small cell carcinoma lung	T2–T4	T2–T5	25 d	Paraplegia
2	75	Female	Fibrous histiocytoma	L2	S1 Conus medullaris	1 mo	Paraplegia
3	79	Male	Adenocarcinoma, extramedullary	C3	C2–C5	6 wk	Quadriparesis R > L
4	59	Male	Osteogenic sarcoma	T11	L2–L3	6 wk	Paraplegia
5	24	Male	Ewing sarcoma	C5–C6	C4–C6	2 mo	Quadriparesis
6	72	Male	Prostate carcinoma	C2–C7	C2–C8	3 mo	Incomplete quadriplegia

TABLE 2. Antibodies Used to Detect Apoptosis After Neoplastic Compressive Myelopathy

Antibody	Clone/Catalogue	Dilution	Antigen Retrieval	Specificity	Source
Bcl-2	124 (MM)	1/150	Citrate	Human Bcl-2 protein	DAKO, United States
Fas	NCL-Fas-310 (MM)	1/1000	EDTA	Human Fas (CD 95)	Novocastrian, United Kingdom
Caspase-3	3015-100 (RP)	1/500	EDTA	Human P17 fragment active Caspase-3	Bio Vision, United States
Caspase-9	3149-100 (RP)	1/1000	EDTA	Cleaved caspase-9 protein 37 kDa	Bio Vision, United States
DNA-PKcs	AHP318 (RP)	1/10,000	TRS	Human DNA-PKcs	Serotec, United Kingdom
PARP	A6.4.12 (MM)	1/1000	Citrate	Human poly (ADP-ribose) polymerase 116 kDa	Serotec, United Kingdom
AIF c-terminus	AB16501	1/1000	Citrate	517-537a.a. of apoptosis-inducing factor molecule	Chemicon International, United States
TUNEL	S7101	Kit	Kit	3′-OH terminus single and double strand DNA	Intergen, United States

MM indicates mouse monoclonal; EDTA, ethylenediaminetetraacetic acid; RP, rat polyclonal; TRS, target retrieval solution; TUNEL, terminal deoxynucleotide transferase dUTP Nick End Labeling.

injury cases was used as a positive control for APP, whereas lesion-free human spinal cord served as a negative control.

All sections underwent a similar immunohistochemical procedure, with all antibodies incubated at room temperature and phosphate buffered saline washes applied between each antibody. Briefly, sections were de-waxed, dehydrated, and placed in methanol with 30% hydrogen peroxide. Specified microwave antigen retrieval was performed as required and sections incubated for 45 minutes in 3% normal horse serum. Primary antibody was added overnight before specific biotinylated secondary antibody (Vector, 1:250) was added for 30 minutes. Tertiary streptavidin peroxidase conjugate (SPC; Pierce, 1:1000) was added for 1 hour and the immunocomplex visualized using 3,3'diaminobenzidine (DAB; Sigma) as a chromogen in the peroxidase reaction. Generated slides were scanned at high resolution using a Hamamatsu Nanozoomer and viewed using the associated proprietary viewing software (NDP.view v1.1.27, Hamamatsu).

Apoptotic Markers

To detect apoptotic activation along multiple stages in both intrinsic and extrinsic pathways, a broad panel of classical apoptotic markers was used, including caspase-9, caspase-3, bcl-2, Fas, PARP, DNA-PKcs, and apoptosis-inducing factor (AIF).

First Apoptosis Signal

Fas/APO-1/CD95 (36 kDa) is a member of the tumor necrosis factor receptor family of transmembrane receptors. The Fas molecule is an important mediator of apoptotic cell death as well as being involved in inflammation. Signaling by receptors from the tumor necrosis factor family in response to external triggers contributes to a wide range of molecular processes, including apoptosis and inflammation. This family comprises at least 32 receptors and of those, Fas is primarily involved in programmed cell death.[13] It has now been well established that Fas-mediated apoptosis may occur after compressive spinal cord injury,[14] with Fas deficiency resulting in reduced oligodendroglia cell death and improved behavioral and histological outcome.[15,16]

B-Cell Lymphoma 2

The B-cell lymphoma 2 (Bcl-2) family is key protective regulator of the mitochondrial pathway of apoptosis. Bcl-2 is located within the outer mitochondrial membrane, endoplasmic reticulum, and nuclear envelope. In mammalian cells, they have been shown to act upstream of caspases and assist in determining the release of proapoptotic molecules such as cytochrome-c from the mitochondria.[17] Bcl-2 confers a protective effect on the cell by inhibiting pathways of apoptosis and its expression is increased under apoptotic conditions.[18] SCC has been shown to induce Bcl-2 immunoreactivity within axons, which intensified with increased compression severity.[19]

Caspase-3 and 9

Caspases are a family of cysteine proteases that act as central mediators to the process of apoptotic cell death for intrinsic, mitochondrial, and extrinsic pathways. They bind to aspartyl residues resulting in cleavage *via* oligomerization of specific proteins. Caspase-3, in its active form, is a cysteine-aspartic acid protease recognized as a major effector molecule of morphological changes in apoptosis.[20] The caspase-3 gene encodes a proenzyme that undergoes processing by initiator caspases at Asp28 and Asp175 to form 2 dimerized subunits that form active caspase-3, which binds to caspase-8 during apoptosis. It is thought that the translocation of caspase-3 from cytoplasm to the nucleus represents a key morphological change during apoptosis and that this is an active process.[21–23]

Cysteine aspartyl protease precursor, caspase-9 was chosen as a key immunological marker of the intrinsic apoptotic pathway[24] and subsequently leads to the activation of caspase-7 and -3, the latter thought to be the key executioner caspase for cell death.[11] Caspase-9 is itself resultant from activation of cytochrome-c and apoptotic peptidase activating factor (Apaf-1) within the apoptosome.[25] Pathways both preceding and superseding the activation of caspase-9 are regulated by a process of phosphorylation multiple sites, which can affect the activation of caspase-3.[26–28]

DNA-Dependent Protein Kinase Catalytic Subunit

The efficient and adequate repair of DNA strand breaks is crucial for the integrity of the cellular genome. DNA-dependent protein kinase (DNA-PK) is involved in the repair of double-stranded DNA breaks in mammalian cells. The DNA-PK molecule contains a heterodimeric DNA-binding subunit (Ku70/80) and a catalytic subunit of 465 kDa known as DNA-PKcs.[29,30] DNA-PKcs is a serine/threonine protein kinase activated *via* the Ku heterodimer, where DNA fragmentation exists. DNA-PKcs is inactive on its own and relies on the Ku component to trigger the kinase activity. DNA-PKcs is preferentially degraded after exposure to apoptotic agents, accompanied by reduced DNA-PK activity.[31] In a model of spinal cord ischemia, DNA-PKcs was found to decline during reperfusion,[32] indicating that ischemic injury overwhelms DNA repair processes, enabling apoptotic processes to develop.

Poly (ADP-Ribose) Polymerase

PARP are a family of nuclear proteins found in eukaryotic cells. The functions of PARP include the formation of mitotic spindles,[33] centromere and centrosomal function, telomere function *via* the action of Tankyrase, movement of endosomes, DNA strand break detection and repair,[34,35] and cell death along the spectrum of both apoptosis and necrosis.[36,37] PARP contributes to cell death by its activation in both the caspase-dependent and, more recently, caspase-independent pathways of apoptosis. Mild genotoxic stressors may facilitate DNA repair and cell survival; however, severe cytotoxic stimuli result in a rapid increase in PARP and failure to maintain the genomic structure.[38,39]

Apoptosis-Inducing Factor

A pathway of cell death usually independent of caspase-activation was identified involving activation of a key molecule known as apoptosis-inducing factor (AIF). AIF is a flavoprotein 57 kDa in length, encoded by a nuclear gene on the X chromosome. AIF is released during apoptosis and transported from the mitochondrial intermembrane space *via* the cytosol to the nucleus, where it causes large-scale DNA fragmentation of approximately 50 kilobase pairs and secondary chromatin condensation. Positive charges on the surface of the AIF molecule allow it to interact with DNA, with preferential binding to single-stranded rather than double-stranded DNA.[40] AIF has been found to play a significant role in the PARP-mediated pathway of cell death, primarily *via* the activity of PARP-1.[41]

Terminal Deoxynucleotide Transferase dUTP Nick End Labeling

TUNEL (Intergen, ApopTag S7101) staining was used as a biochemical marker of apoptotic DNA fragmentation. The TUNEL technique enzymatically labels the free DNA 3′-OH ends of specific DNA strand breaks that are characteristic, although not pathognomonic, of apoptosis. TUNEL may detect early-stage apoptosis prior to the condensation of chromatin.[42]

RESULTS

Morphological Features of Human Neoplastic Compressive Myelopathy

The macroscopic appearance of the spinal cord from each case is described in Table 3. All cases showed EDC and 2 cases (cases 2 and 3) in addition showed intradural extramedullary malignant infiltration. The Ewing sarcoma (case 5) involving the upper cervical vertebrae resolved with treatment, and no macroscopic tumor was found at postmortem, although there was clear evidence of compression deformity and white matter damage C4–C6.

Microscopically, in H&E-stained sections, SCC was characterized by cystic necrosis, preferentially involving central regions of gray and white matter. Necrosis was attended by phagocytic macrophages. There were numerous axonal swellings (spheroids) in all cases, being especially widespread in cases with loss of anterior horn cells and central chromatolysis of neurons. There was marked myelin loss at the site of maximal compression and patchy loss in adjacent segments. All 7 cases showed APP immunoreactivity (Figure 1) not only in axonal profiles of predominantly enlarged diameter but also in normal diameter axons indicative of axonal injury.

Immunoreactivity of Apoptotic Markers

Assessment of immunopositivity of apoptotic markers within each case is shown in Table 4. Positive immunoreactivity was found within glial, axonal, and neuronal regions as demonstrated in Figure 2.

Fas and Bcl-2 Immunoreactivity

Fas immunoreactivity was not present at any level of spinal cord in any cases of neoplastic compressive myelopathy. Only minimal Bcl-2 immunoreactivity was observed within oligodendrocytes of only 1 case.

Caspase-3 and 9

Immunopositivity to caspase-3 and 9 was seen exclusively in axons. Caspase-3 demonstrated greater immunoreactivity with 4 positive cases, whereas caspase-9 was equivocally observed in only 1 case. Caspase-3 axonal immunoreactivity was seen frequently at the site of compression and was greatest in regions of APP axonal immunoreactivity.

DNA-PKcs

DNA-PKcs immunoreactivity was predominantly present within oligodendrocytes and axons, with minimal neuronal

TABLE 3. Macroscopic and Microscopic Pathology

Case	Vertebral Column Metastases	Spinal Cord
1	T2, T3, T4 (paraspinal extension)	Extradural compression T2–T5. Maximal at T3 with almost complete necrosis of gray and white matter and numerous foamy macrophages. Axonal swellings, partial loss of AHCs and vacuolation neuropil in T2, T,4 and T5 segments
2	T12, C7, T2, T7, T4, T12, L2	Extra- and intradural compression lower spinal cord and cauda equina by circumferential plaque of hard, gray-white tumor. Necrosis (maximal S1 segment) with numerous foamy macrophages. Axonal swellings, partial loss of AHCs and neuropil vacuolation adjacent spinal cord segments
3	C3	Extra and intradural compression by tumor compressing and displacing C2–C5 segments to opposite side of dural sheath. White matter vacuolation and numerous axonal swellings maximal side of compression. Partial loss AHCs, central chromatolysis
4	T11 (large paraspinal extension)	Extradural compression with L2–L3 posterolateral white matter damage with numerous axonal swellings, AHC "acute ischemic cell change," central chromatolysis, vacuolation of neuropil
5	Cervical vertebrae (radiological) PM—no residual tumor postradiotherapy	Extradural compression C4–C6 maximal C5 lateral white matter with cystic necrosis, numerous macrophages, axonal swellings, vacuolation neuropil, loss of AHCs
6	C4–C7	Extradural compression C3–C8 maximal C8 with cystic change in lateral white matter, partial loss of AHCs C3–C8, central chromatolysis C4, C5, C7

AHCs indicates anterior horn cells; PM, post mortem.

positive staining (Figure 2). Oligodendrocytes demonstrated nuclear-specific immunoreactivity and were frequently found within the subpial region. Axonal immunopositivity was found within the most severely damaged regions of the white matter.

Poly (ADP-Ribose) Polymerase

PARP immunoreactivity was observed consistently in oligodendroglial nuclei of all cases, with neuronal staining observed in more than half of the cases (Figure 2).

Apoptosis-Inducing Factor

AIF immunopositivity was observed in oligodendrocytes, astrocytes, and neurons in all cases assessed, whereas axonal immunoreactivity was observed in 4 of 6 cases (Figure 2). AIF immunoreactivity was observed in the cytoplasm of cells, congruent with staining using an antibody against a mitochondrial protein.

Terminal Deoxynucleotide Transferase dUTP Nick End Labeling

TUNEL immunoreactivity was present exclusively in oligodendrocytes and neurons (Figure 2), maximal at the site of compression and margins around areas of necrosis. No positive axonal immunoreactivity was observed.

DISCUSSION

The results of this study demonstrated that apoptosis plays an important role in the pathogenesis of SCC produced by metastatic neoplasms. Apoptosis of glia, particularly oligodendrocytes, and, to a more limited extent, neurons, occurred maximally at the site of compression but also often in contiguous segments above and below this site. Apoptotic markers showing the most robust immunopositivity were PARP together with positive TUNEL biochemistry, whereas caspase-3 immunoreactivity was confined to axons in 3 cases, and Bcl-2 and Fas immunopositivity was rare or not detected.

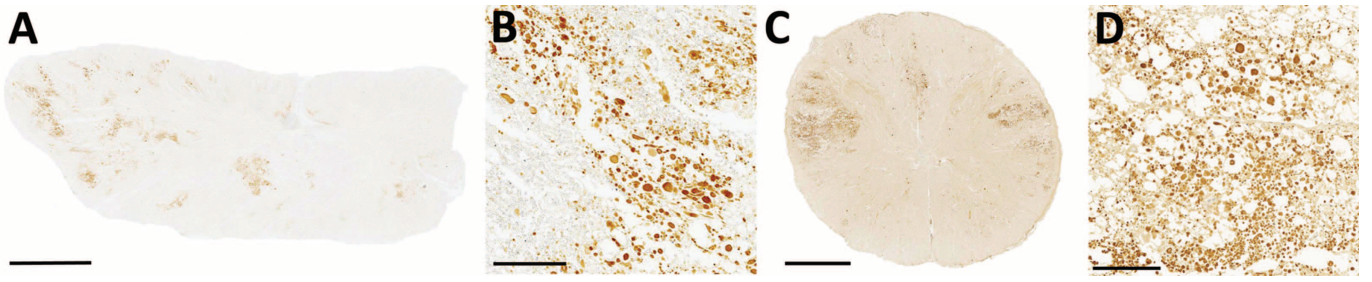

Figure 1. Amyloid precursor protein immunoreactivity within the compressed (**A** and **B**) and adjacent spinal cord segments (**C** and **D**) is evident with both enlarged and normal diameter axons observed at higher magnification (**B** and **D**). Scale bar A and C = 2 mm, B and D = 200 μm.

TABLE 4. Results of Apoptotic Marker Immunoreactivity Within Cellular-Specific Regions

Case	1	2	3	4	5	6
Fas	…	…	…	…	…	…
Bcl-2	…	…	…	…	…	Oligos
Caspase-3	Oligos Axons	Oligos	Oligos	Axons	Oligos Axons	…
Caspase-9	…	…	…	…	…	…
TUNEL	Oligos	Oligos Neurons	Oligos Neurons Axons	Oligos Neurons	Oligos Neurons	Oligos Neurons
PARP	Oligos	Oligos Neurons	Oligos Neurons	Oligos Neurons	Oligos	Oligos
AIF	Oligos Neurons Axons	Oligos Neurons Axons	Oligos Neurons Axons	Oligos Astrocytes Neurons Axons	Oligos Astrocytes Neurons Axons	Neurons Oligos Astrocytes
DNA-PKcs	Axons	Oligos	Oligos Axons	Oligos Axons	Oligos Axons	Oligos

TUNEL indicates terminal deoxynucleotide transferase dUTP Nick End Labeling; PARP, poly (ADP-ribose) polymerase; AIF, apoptosis-inducing factor.

The contribution of AIF was difficult to ascertain as immunopositivity was always cytoplasmic, with no nuclear translocation evident. Axonal injury in the form of APP-positive profiles was widely distributed in all cases, not only at the site of compression but also above and below this site, suggesting that axonal damage with disruption to axonal transport contributes to the pathology of SCC.

In the only previous human studies of apoptosis in chronic SCC, Yamaura *et al*[43] found TUNEL-positive glia of oligodendroglial lineage in a single case caused by ossification of the posterior longitudinal ligament, whereas Yu *et al*[44] observed FAS-mediated apoptosis of neurons and oligodendrocytes in 8 patients with SCC produced by cervical spondylotic myelopathy. In rodents models of SCC, Li *et al*[45] found TUNEL and FAS-positive, but not Bcl-2 immunoreactive, glia with a morphology compatible with oligodendroglia, whereas Casha *et al*[46] also noted TUNEL and FAS-positive oligodendrocytes. Liang *et al*[47] found neuronal

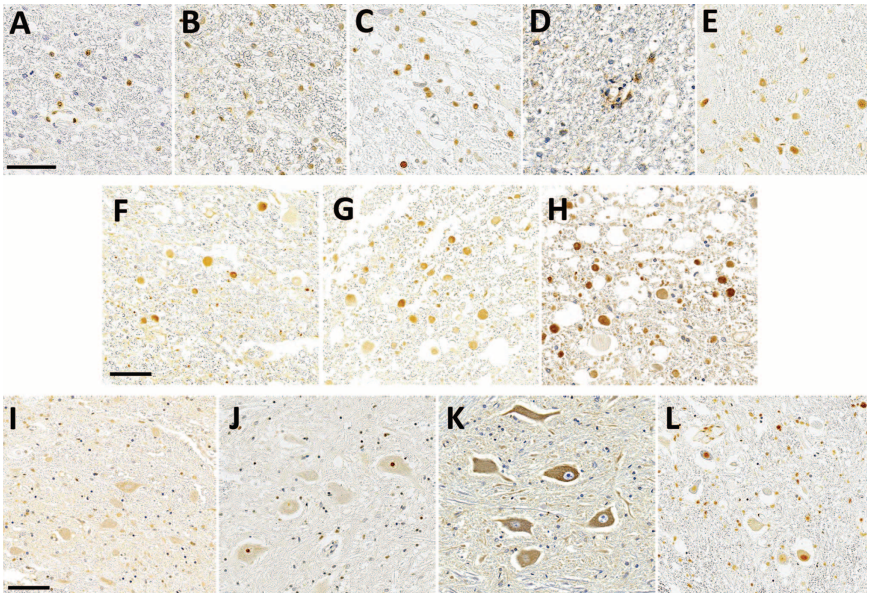

Figure 2. Immunoreactivity of apoptotic markers within oligodendrocytes **(A–E)**, axons **(F–H),** and neurons **(I–L)**. A range of apoptotic markers were found to be immunopositive within oligodendrocytes, including Bcl-2 **(A)**, DNA-PKcs **(B)**, PARP **(C)**, AIF **(D)**, and TUNEL **(E)**. Axonal immunoreactivity was observed after caspase-3 **(F)**, DNA-PKcs **(G)**, and AIF **(H)**. Neurons were faintly immunopositive for DNA-PKcs **(I)**, whereas PARP **(J)** staining was prominent within many neuronal nucleoli. AIF **(K)** immunoreactivity was observed within the cytoplasm only of neurons whereas nuclei were immunoreactive after TUNEL **(L)** staining. All scale bars = 50 μm.

caspase-12 immunoreactivity in their model, and Takenouchi *et al*[48] observed upregulation of caspase-3 in neurons and glia. Uchinda *et al*[49] found evidence of apoptosis in neurons and oligodendroglia and Yamaura *et al*[43] TUNEL positivity in the latter.

Because the spinal cord lies within a confined space in the vertebral canal, it has been suggested that mechanical compression might locally compromise the vascular supply, leading to ischaemia-hypoxia, and constitute a contributing factor to the pathophysiology of chronic compressive myelopathy.[50] SCC probably results from a combination of pressure applied to the neural parenchyma and blood vessels of supply.[4,51]

Previous studies[51] have shown that myelin seems to be especially susceptible to mechanical pressure. Swelling of myelin sheaths leading to spongy degeneration of the white matter is an early pathological change, followed by necrosis and cavitation, particularly in the center of the cord, and degeneration of ascending and descending fiber tracts. In segments above the site of compression, fiber loss occurs in posterior columns, spinothalamic and spinocerebellar tracts while, below the compression site, corticospinal tracts degenerate. In more chronic cases, the cavitating lesion is replaced by glial scarring of gray and white matter, with some axons being preserved.[51]

Although the contribution of apoptosis to the pathology of SCC has not hitherto received much attention, the results of the present study suggest that this form of cell death contributes to the final expression of spinal cord injury and neurological dysfunction. A better understanding of the role of apoptosis in SCC may lead to the development of more carefully targeted therapeutic intervention strategies, particularly if some of the events comprising this process of cell death, and the cascade of secondary events that follow SCC, are potentially reversible.v

➢ Key Points

- ❑ Apoptosis was maximal at the site of compression with glial cells, predominantly oligodendrocytes, immunopositive for DNA-PKcs, PARP, AIF, and TUNEL.
- ❑ In addition to glial cells, axons were immunopositive for caspase-3, DNA-PKcs, and AIF, whereas neurons were immunopositive for DNA-PKcs, PARP, AIF, and TUNEL.
- ❑ The prominent oligodendroglial involvement is suggestive that apoptosis may be important in the ongoing remodeling of white matter due to sustained compression.

Acknowledgment

This work was awarded the Spine Society of Australia Award for Spinal Research.

References

1. Harrington KD. Metastatic disease of the spine. *J Bone Joint Surg Am* 1986;68:1110–5.
2. McLinton A, Hutchison C. Malignant spinal cord compression: a retrospective audit of clinical practice at a UK regional cancer centre. *Br J Cancer* 2006;94:486–91.
3. Gabriel K, Schiff D. Metastatic spinal cord compression by solid tumors. *Semin Neurol* 2004;24:375–83.
4. Prasad D, Schiff D. Malignant spinal-cord compression. *Lancet Oncol* 2005;6:15–24.
5. Spinazze S, Caraceni A, Schrijvers D. Epidural spinal cord compression. *Crit Rev Oncol Hematol* 2005;56:397–406.
6. Taylor JW, Schiff D. Metastatic epidural spinal cord compression. *Semin Neurol* 2010;30:245–53.
7. Morgen SS, Lund-Andersen C, Larsen CF, et al. Prognosis in patients with symptomatic metastatic spinal cord compression: survival in different cancer diagnosis in a cohort of 2321 patients. *Spine (Phila Pa 1976)* 2013;38:1362–7.
8. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol* 1995;146:3–15.
9. Hotchkiss RS, Strasser A, McDunn JE, et al. Cell death. *N Engl J Med* 2009;361:1570–83.
10. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol* 2007;35:495–516.
11. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol* 2008;9:231–41.
12. Hongmei Z. Extrinsic and intrinsic apoptosis signal pathway review. In: Ntuli TM, ed. *Apoptosis and Medicine*. Rijeka, Croatia: InTech; 2012:3–22.
13. Muppidi JR, Tschopp J, Siegel RM. Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. *Immunity* 2004;21:461–5.
14. Yu WR, Liu T, Fehlings TK, et al. Involvement of mitochondrial signaling pathways in the mechanism of Fas-mediated apoptosis after spinal cord injury. *Eur J Neurosci* 2009;29:114–31.
15. Yoshino O, Matsuno H, Nakamura H, et al. The role of Fas-mediated apoptosis after traumatic spinal cord injury. *Spine (Phila Pa 1976)* 2004;29:1394–404.
16. Casha S, Yu WR, Fehlings MG. FAS deficiency reduces apoptosis, spares axons and improves function after spinal cord injury. *Exp Neurol* 2005;196:390–400.
17. Golstein P. Controlling cell death. *Science* 1997;275:1081–2.
18. Molica S, Mannella A, Dattilo A, et al. Differential expression of BCL-2 oncoprotein and Fas antigen on normal peripheral blood and leukemic bone marrow cells. A flow cytometric analysis. *Haematologica* 1996;81:302–9.
19. Li GL, Brodin G, Farooque M, et al. Apoptosis and expression of Bcl-2 after compression trauma to rat spinal cord. *J Neuropathol Exp Neurol* 1996;55:280–9.
20. Erhardt P, Tomaselli KJ, Cooper GM. Identification of the MDM2 oncoprotein as a substrate for CPP32-like apoptotic proteases. *J Biol Chem* 1997;272:15049–52.
21. Woo M, Hakem R, Soengas MS, et al. Essential contribution of caspase 3/CPP32 to apoptosis and its associated nuclear changes. *Genes Dev* 1998;12:806–19.
22. Zheng TS, Schlosser SF, Dao T, et al. Caspase-3 controls both cytoplasmic and nuclear events associated with Fas-mediated apoptosis in vivo. *Proc Natl Acad Sci U S A* 1998;95:13618–23.
23. Kamada S, Kikkawa U, Tsujimoto Y, et al. Nuclear translocation of caspase-3 is dependent on its proteolytic activation and recognition of a substrate-like protein(s). *J Biol Chem* 2005;280:857–60.
24. Budihardjo I, Oliver H, Lutter M, et al. Biochemical pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 1999;15:269–90.
25. Riedl SJ, Salvesen GS. The apoptosome: signalling platform of cell death. *Nat Rev Mol Cell Biol* 2007;8:405–13.
26. Schafer ZT, Kornbluth S. The apoptosome: physiological, developmental, and pathological modes of regulation. *Dev Cell* 2006;10:549–61.
27. Allan LA, Clarke PR. Apoptosis and autophagy: regulation of caspase-9 by phosphorylation. *FEBS J* 2009;276:6063–73.
28. Allan LA, Clarke PR. Phosphorylation of caspase-9 by CDK1/cyclin B1 protects mitotic cells against apoptosis. *Mol Cell* 2007;26 :301–10.

29. Anderson CW, Lees-Miller SP. The nuclear serine/threonine protein kinase DNA-PK. *Crit Rev Eukaryot Gene Expr* 1992;2:283–314.
30. Anderson CW, Carter TH. The DNA-activated protein kinase—DNA-PK. *Curr Top Microbiol Immunol* 1996;217:91–111.
31. Song Q, Lees-Miller SP, Kumar S, et al. DNA-dependent protein kinase catalytic subunit: a target for an ICE-like protease in apoptosis. *EMBO J* 1996;15:3238–46.
32. Shackelford DA, Tobaru T, Zhang S, et al. Changes in expression of the DNA repair protein complex DNA-dependent protein kinase after ischemia and reperfusion. *J Neurosci* 1999;19:4727–38.
33. Chang P, Jacobson MK, Mitchison TJ. Poly(ADP-ribose) is required for spindle assembly and structure. *Nature* 2004;432:645–9.
34. D'Amours D, Sallmann FR, Dixit VM, et al. Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. *J Cell Sci* 2001;114:3771–8.
35. D'Amours D, Desnoyers S, D'Silva I, et al. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem J* 1999;342(pt 2):249–68.
36. Burkle A. Poly(ADP-ribose). The most elaborate metabolite of NAD+. *FEBS J* 2005;272:4576–89.
37. Koh DW, Dawson TM, Dawson VL. Mediation of cell death by poly(ADP-ribose) polymerase-1. *Pharmacol Res* 2005;52:5–14.
38. Skaper SD. Poly(ADP-ribosyl)ation enzyme-1 as a target for neuroprotection in acute central nervous system injury. *Curr Drug Targets CNS Neurol Disord* 2003;2:279–91.
39. Virag L. Structure and function of poly(ADP-ribose) polymerase-1: role in oxidative stress-related pathologies. *Curr Vasc Pharmacol* 2005;3:209–14.
40. Vahsen N, Cande C, Dupaigne P, et al. Physical interaction of apoptosis-inducing factor with DNA and RNA. *Oncogene* 2006;25:1763–74.
41. Chen M, Zsengeller Z, Xiao CY, et al. Mitochondrial-to-nuclear translocation of apoptosis-inducing factor in cardiac myocytes during oxidant stress: potential role of poly(ADP-ribose) polymerase-1. *Cardiovasc Res* 2004;63:682–8.
42. Migheli A, Attanasio A, Schiffer D. Ultrastructural detection of DNA strand breaks in apoptotic neural cells by in situ end-labelling techniques. *J Pathol* 1995;176:27–35.
43. Yamaura I, Yone K, Nakahara S, et al. Mechanism of destructive pathologic changes in the spinal cord under chronic mechanical compression. *Spine (Phila Pa 1976)* 2002;27:21–6.
44. Yu WR, Liu T, Kiehl TR, et al. Human neuropathological and animal model evidence supporting a role for Fas-mediated apoptosis and inflammation in cervical spondylotic myelopathy. *Brain* 2011;134:1277–92.
45. Li GL, Farooque M, Olsson Y. Changes of Fas and Fas ligand immunoreactivity after compression trauma to rat spinal cord. *Acta Neuropathol* 2000;100:75–81.
46. Casha S, Yu WR, Fehlings MG. Oligodendroglial apoptosis occurs along degenerating axons and is associated with FAS and p75 expression following spinal cord injury in the rat. *Neuroscience* 2001;103:203–18.
47. Liang YJ, Sun SQ, Wang KJ, et al. [Apoptosis and caspase-12 expression in progressive compressive spinal cord injury: experiment with rats]. *Zhonghua Yi Xue Za Zhi* 2007;87:1063–5.
48. Takenouchi T, Setoguchi T, Yone K, et al. Expression of apoptosis signal-regulating kinase 1 in mouse spinal cord under chronic mechanical compression: possible involvement of the stress-activated mitogen-activated protein kinase pathways in spinal cord cell apoptosis. *Spine (Phila Pa 1976)* 2008;33:1943–50.
49. Uchida K, Nakajima H, Watanabe S, et al. Apoptosis of neurons and oligodendrocytes in the spinal cord of spinal hyperostotic mouse (twy/twy): possible pathomechanism of human cervical compressive myelopathy. *Eur Spine J* 2012;21:490–7.
50. Karadimas SK, Gatzounis G, Fehlings MG. Pathobiology of cervical spondylotic myelopathy [published online ahead of print March 14, 2014]. *Eur Spine J*. doi:10.1007/s00586-014-3264-4.
51. Hughes JT. Disorders of the spine and spinal cord. In: Adams JH, Duchen LW, eds. *Greenfield's Neuropathology*. 5th ed. New York, NY: Oxford University Press; 1992:1089–90.