TOPOGRAPHY AND QUANTITATION OF AXONAL INJURY AS DEFINED BY AMYLOID PRECURSOR PROTEIN AND THE SECTOR SCORING METHOD IN LATERAL AND OCCIPITAL HEAD IMPACTS: A PRELIMINARY REPORT.

Blumbergs PC, Scott G, Manavis J, Wainwright H, McLean AJ, Kloeden C, Simpson DA.
Neuropathology Laboratory, Institute of Medical and Veterinary Science and NHMRC Road Accident Research Unit, University of Adelaide.

ABSTRACT

Axonal injury (AI), as defined by amyloid precursor protein (APP) positive axonal swellings, was recorded on a series of line diagrams of standard brain sections divided into 116 sectors to provide an Axonal Injury Sector Score (AISS) ranging from 0 to 116. In this preliminary report the sector scoring method of recording axonal damage and providing a topographic and semiquantitative overview of AI was applied to a series of 11 lateral (4 pedestrian, 1 car occupant and 6 falls) and 7 occipital head impacts (3 pedestrian, 4 falls) for which the biomechanical data was sufficient to estimate peak linear acceleration and impact velocity as a measure of the magnitude of the impacting forces acting on the head. AI was present in all 18 cases and consisted of a complex mixture of focal and diffuse axonal damage arising by different pathophysiological mechanisms. Lateral impacts produced significant AI at linear accelerations lower than for occipital impacts. Widespread AI was present in all 10 fall cases irrespective of the height of the fall. The lowest AISS was found in the concussive mild head injury case (AISS 5).

RECENT STUDIES (summarised by Povlishock, 1992) have modified the original concept that axonal retraction balls represent extrusions of axoplasm from the torn ends of mechanically ruptured axons and have led to the concept that traumatic injury sets in train a series of ill understood events resulting in axonal swellings culminating in physical separation of axons. This process of secondary axonal degeneration has been termed secondary axotomy (Maxwell et al 1993) in contrast to primary axotomy which occurs when the forces are of sufficient magnitude to physically rupture axons (Maxwell et al 1993). The concept of a sequential evolution of axonal damage leading to secondary axotomy is of great potential clinical significance in that therapeutic intervention might minimise or prevent axonal damage if carried out before irreversible secondary axotomy is established.

Experimental studies (Gennarelli et al 1982, Povlishock et al 1983, Jane et al 1985, Erb and Povlishock 1988) have shown a spectrum of axonal injury (AI) depending on the severity of the head impact. Clinicopathological studies in humans (Oppenheimer, 1968; Pilz, 1983; Adams et al, 1989, Blumbergs et al, 1989 and 1994) have also shown a spectrum of AI.

AI may be pathologically classified as **focal, multifocal, diffuse** or any combination of these patterns of involvement.

Diffuse axonal injury (DAI) has been defined as the presence of microscopic axonal damage in the cerebral hemispheres, corpus callosum, brain stem and cerebellum (Adams et al, 1989). The AI around **focal and multifocal** lesions such as infarcts and haemorrhages presumably results from mechanisms different to those responsible for the diffuse axonal damage initiated by mechanical forces acting at the time of impact (secondary axotomy). Thus axonal swellings, the marker for AI, may result from different pathophysiological mechanisms

Morphological quantitation of AI is difficult because the distribution and extent of axonal damage is not uniform or symmetrical (Strich, 1961; Adams et al, 1977) and requires the systematic microscopic study of the whole brain.

The Neuropathology Section of the IMVS and the University of Adelaide NH&MRC Road Accident Research Unit has developed a sector scoring method where the presence of AI, as defined by amyloid precursor protein (APP), positive immunoreactive axonal swellings, is recorded on a series of line diagrams of standard "whole brain" sections divided into 116 sectors to provide an Axonal Injury Sector Score (AISS) ranging from 0 to 116 (Blumbergs et al, in press; Ryan et al, 1994). The sector scoring method provides in summary form a detailed topographic map of AI in a given brain.

APP AS A MARKER FOR AI

APP is a membrane spanning glycoprotein of nerve cells originating from a gene on chromosome 21 (Kang et al 1987). APP is transported by fast axoplasmic transport (Koo et al 1990) and is located at both pre and post-synaptic sites (Schubert et al 1991, Shigematsu et al 1992). APP is an excellent marker for axonal injury in experimental animals (Nakamura et al 1992, Otsuka et al 1991) and around cerebral infarcts and other pathological processes in humans (Ohgami et al 1992), including traumatic AI (Gentleman et al 1993, Sheriff et al, 1994). APP accumulation is thought to occur after cytoskeletal disruption (Otsuka et al 1991) resulting in inhibition of axoplasmic flow (Shigematsu and McGeer 1992).

It has been recognised since the early studies of Strich (1961) that axonal retraction balls take some time to develop and the question whether they can be recognised during the first 12 hours post-trauma has been controversial. Standard silver impregnation techniques require a survival time in excess of 18 hours to reliably demonstrate DAI (Adams 1992). Grady et al (1993) found that they could identify traumatically induced reactive axonal changes 6 hours post-injury using antibodies against the 68 kDa neurofilament subunit which by 12 hours progressed to complete axotomy. Focal axonal increase in the 68 kDa neurofilament subunit has been documented 1 hour after injury in the rat (Yaghmai and Povlishock 1992) and ubiquitin immunopositive axonal swellings were noted after 6 hours in a cat model of DAI (Schweitzer et al 1993). The advantages of APP over the other immunocytochemical markers of AI are its early detection of AI and that it can be performed on formalin fixed, paraffin embedded tissue where it only labels injured axons.

Positive APP immunostaining of axons has been demonstrated 1.75 hours after the traumatic event (Blumbergs et al, in press) and unlike traditional silver techniques for axons and immunocytochemical methods for phosphorylated neurofilament protein (NFP) does not stain normal axons.

There is evidence that lateral impacts may produce more brain damage than frontal or occipital impacts and in a subhuman primate experimental model DAI was most readily produced by lateral non-centroidal rotation of the head (Gennarelli et al, 1987).

In this preliminary report the topography of AI and the AISS were recorded in a series of 11 lateral head impacts (6 falls, 4 pedestrians and 1 car occupant) and 7 occipital impacts (4 falls and 3 pedestrians). The peak linear acceleration and impact velocity were estimated from biomechanical data and served as a measure of the magnitude of the impacting forces. The available information was not sufficiently detailed to calculate peak angular acceleration or change in angular velocity.

The hypotheses to be tested were that AI would increase commensurate with increasing severity of head impact in both lateral and occipital head impact groups and that for equivalent forces lateral impacts would be more injurious (ie have a higher AISS) than for occipital impacts.

MATERIALS AND METHODS

The NHMRC Road Accident Research Unit has developed methods of estimating the severity of impacts to the head of fatally injured pedestrians (Ryan et al, 1994) and car occupants (Simpson et al, 1991). The severity of the head impact sustained by fatally injured victims of falls is estimated from the fall height and the stiffness of the object struck by the head (Manavis et al, 1991). 18 cases from these ongoing series were selected for APP immunostaining on the basis of well defined lateral and occipital head impacts. The occipital impact group comprised 4 falls (2 from own height and 2 from greater than the victim's own height) and 3 pedestrians, one of these pedestrians sustained his head injury from a road impact and not from the car and as such had much in common with the fall cases. The lateral impact group consisted of 6 falls (3 from own height and 3 from greater than the victim's height), 5 pedestrians and 1 car occupant. The clinical details of the lateral and occipital head impact cases are summarised in tables 1 and 2.

Complete post-mortems were performed in every case. The formalin fixed brains from the head injury cases were examined according to a standard protocol (Ryan et al, 1994) which includes the microscopic examination of paraffin embedded sections of the cerebral hemispheres at 11 coronal levels separated by 10 mm intervals, cross sections of the brain stem (midbrain, pons, medulla) and one of each cerebellar hemisphere. AI and other types of macroscopic and microscopic brain damage are recorded on a series of line diagrams of these standard sections. The line diagrams are divided into 116 sectors including the brain stem and cerebellum which is a slight modification of our previously described method (Ryan et al, 1994) in which only the cerebral hemispheres were assessed.

Briefly, the cerebral cortex and underlying white matter of each of the eleven cerebral hemisphere sections is divided into 8 radial sectors. The left and right central grey matter are designated separately and are present in six of the coronal slices. The corpus callosum is also specified separately and is present in seven of the coronal sections, and the portions of the temporal poles adjacent to the Sylvian fissures are assigned four separate sectors.
When the two sectors allocated to the left and right cerebellum and the three brain stem sectors are included, the brain is divided into a total of 116 sectors.

All sections were incubated overnight with a monoclonal antibody to APP (Gift Prof Colin Masters) at a dilution of 1:3000 and stained with 3,3 diaminobenzidine tetrahydrochloride (DAB - Sigma) using avidin-biotin peroxidase (Vector stain ABC kit) and counter stained with haematoxylin.

The detection of any positive APP immunostaining of axons in a sector resulted in a positive score for AI. No attempt was made to separate AI around focal lesions from the more widespread patterns of involvement as we were interested in the total burden of AI in a given brain. By identifying those sectors in which there is evidence of axonal damage an overview of the pattern of axonal injury in a particular brain is readily seen and by counting the number of sectors in which AI is present, an Axonal Injury Sector Score (AISS), ranging from 0 to 116, is obtained. As the neuropathological examinations were conducted over a number of years using slightly different protocols some brains did not have all the sections available for examination. The number of sectors with injury in these cases has been standardised to 116 sectors for each brain by multiplying the number of sectors involved over the number of sectors available by 116. For example in case 3 where the AISS is 75/104, indicating that 75 out of a total of 104 sectors were positive for AI, the corrected AISS is 84/116 (75/104 x 116). This introduces a degree of correction bias which is felt not to be very significant as the AISS, is at best, an approximate method of describing extent and severity of AI. All the sections were also stained with haematoxylin-eosin and Weil stains and the brain stem, cerebellum and selected blocks of the corpus callosum were also stained with the Palmgren silver technique.

HEAD IMPACT CALCULATIONS

Falls

Head impact velocities and accelerations for the fall cases were calculated from the height of the fall and the nature of the surface struck by the head. In most of these cases the struck surface was effectively rigid and so the combined stiffness of the head/struck surface has been taken as the stiffness of the head, which we have estimated to be 1,300 kN/m (Ryan et al, 1989). The resulting acceleration of the head on impact was calculated using the formula

$$a = v\sqrt{\frac{K}{m}}$$

where v is the estimated head impact velocity, K is the stiffness and m is the mass of the head (5 kg).

Pedestrians and Car Occupants

The head impact velocities and linear acceleration for the pedestrian cases were calculated on the basis of vehicle impact velocity, site of head contact on the vehicle and the location of the head impact as previously described (Ryan et al, 1989; Simpson et al, 1991; Ryan et al, 1994).

RESULTS

Injuries other than AI, such as contusions, intracerebral haematomas, hypoxic-ischaemic damage, raised intracranial pressure, acute subdural haematomas and skull fractures noted in the lateral and occipital head impact groups are summarised in tables 3 and 4.

AI and APP immunostaining

AI, as defined by positive axonal APP immunostaining, was apparent as single stained axons randomly scattered among unlabelled axons, collections of stained axons running in parallel zig-zag collections of positive fibres suggestive of "stress lines" and around focal lesions such as haemorrhages, infarcts and tissue tears. AI was present in all 18 cases. The AISS ranged from 5 to 116 and the AISS and topography of APP immunostaining for lateral and occipital impacts is summarised in tables 5 and 6. The AISS and biomechanical data for the lateral and occipital impacts are provided in tables 7 and 8.

The graphs plotting AISS against linear acceleration are shown separately for lateral and occipital impacts (figs 1 and 2). There is no obvious relationship between AISS and linear acceleration for the lateral impacts (fig 1). The highest AISS scores were seen in the two cases with the lowest linear accelerations. The data do not support the hypothesis that AI increases with increasing linear acceleration above 1000 m/s^2 in lateral impacts.

In occipital impacts the concussive mild head injury (case 1) with the lowest linear acceleration demonstrated only mild AI (AISS 5) in the absence of any vascular, contusional or hypoxic-ischaemic damage and the 3 "talk and die" patients all had lower AISS than the 3 patients with no lucid intervals and GCS of 3 - 4 (see table 6). GCS (Glasgow Coma Scale) is a numerical rating system of functional impairment of speech, motor functions and eye movements ranging from 3 - 15. Although the numbers are very small it would appear that head impacts with linear accelerations of about 3000 m/s^2 or greater produce severe AI (AISS > 100) (fig 2). Thus in the case of occipital impacts there is some evidence that AI increases with increasing linear acceleration to the head.

AI in lateral and occipital head impacts

AI was present in all lateral and occipital cases including the "concussion case". Comparison of the cumulative sector score involvement for the lateral and occipital impacts showed no difference in the distribution pattern of AI (see fig 3).

AI and Falls

AI was present in all the fall cases. The AISS ranged from 39 to 87 for falls from their own height and 28 to 107 for falls greater than the victim's own height (see tables 7 and 8). The lateral and occipital head impact falls all showed widespread AI in the white matter of the cerebral hemispheres, corpus callosum and brain stem irrespective of whether they were falls from their own height or greater than their own height (see tables 5 and 6).

DISCUSSION

HETEROGENEITY OF AI

The total AI in a given brain may be due to a complex mixture of focal and diffuse axonal damage arising by different pathophysiological mechanisms. In some of our cases most of the AI was multifocal related to haemorrhages or areas of ischaemic damage secondary to perfusion failure in different vascular territories. The AI in these situations may not be the result of mechanical forces acting directly on the axons but due to mechanisms similar to those operating in the production of axonal damage in non-traumatic cerebrovascular disease. The relevance of this to our study is that axonal swellings produced by different pathophysiological mechanisms cannot at present be distinguished by histological or immunocytochemical methods and can only be distinguished by the systematic assessment of the total neuropathologic picture. Thus the AI documented in our cases represents a heterogeneous mixture of different types of axonal damage.

AISS as a measure of AI

In a previous study it was shown that the AISS correlated with the GCS in that patients with mild head injury (GCS 13 - 15) had a lower AISS than those with severe head injury (GCS 3 - 5) (Blumbergs et al, in press). The AISS is only an indirect measure of the extent of AI in that any unequivocal axonal APP immunoreactivity in a sector would result in a positive score irrespective of whether there was extensive or mild axonal injury within the sector. This means that the same AISS may occur in patients with markedly different amounts of AI as the AISS emphasises extent more than severity of AI.

AI in lateral head impacts

The lack of correlation of AISS with increasing linear acceleration supports the experimental studies showing that AI does not correlate well with linear acceleration (Gennarelli et al, 1987). These studies have demonstrated that DAI is most readily produced by angular acceleration (Gennarelli et al, 1987). Nevertheless, the linear acceleration of the head is a measure of the severity of the head impact and it would appear that values of 1000 m/s^2 or greater are associated with AI. If the 3 "talk and die" patients, where focal lesion such as ASDH are believed to be more significant than diffuse injuries, are excluded then lateral linear accelerations of the head greater than 1000 m/s^2 produce widespread AI (AISS greater than 80/116) (see fig 1).

AI in occipital head impacts

Although the numbers are small there is a suggestion that AISS increases with increasing linear acceleration and that linear acceleration of about 3000 m/s^2 or greater produces severe AI (AISS > 100) (see fig 2).

AI and falls

AI in a pattern consistent with DAI as defined by widespread microscopic involvement in cerebral hemispheres, corpus callosum, brain stem and cerebellum (Adams et al, 1989) was present in 4/10 fall cases irrespective of whether they occurred from a great height or from a distance not more than the person's own height. The other 6 cases also showed widespread AI involving the cerebral hemispheres, corpus callosum and brain stem, but not the cerebellum, irrespective of the height of the fall. However, the AISS, in general, was greater for falls from a considerable height.
A previous study found that DAI rarely, if ever, occurred as a result of a fall unless the patient had fallen a distance considerably greater than their own height (Adams et al, 1984). However, a recent report has also documented DAI as the result of a simple fall (Imago and Kazee, 1992).

AISS and "Talk and Die" patients

The 6 "talk and die" patients all had lower AISS than patients with no lucid intervals and equivalent linear head accelerations in keeping with the concept that "talk and die" patients have significant focal lesions and less severe diffuse injuries (Reilly et al, 1975).

AISS and concussive head injury

The concussive mild head injury (case 1 occipital series) demonstrated only mild axonal damage (AISS 5) in the absence of any vascular, contusional or hypoxic-ischaemic damage and as such represents the only case in the series uncomplicated by any focal vascular damage.

CONCLUSION

These are preliminary results. They are based on a small number of cases and the significance of the AISS has yet to be evaluated and the accuracy of the acceleration estimates is constrained by the nature of the data. The main purpose of this paper has been to demonstrate the application of the sector scoring method to the study of AI in human head injury.

TABLE 1 - LATERAL IMPACTS - CLINICAL DETAILS

Patient	Age	Sex	Survival	GCS	Duration of unconsciousness	Cause of death
1 Pedestrian	16	Male	5 days	3	5 days	Pulmonary oedema cardiac arrhythmias
2 Car occupant	86	Male	3 days	3	3 days	Raised intracranial pressure
3 Fall (own height)	74	Male	2 days	3	2 days	Raised intracranial pressure
4 Pedestrian	69	Male	8 days	3	8 days	Bronchopneumonia
5 Fall (own height)	76	Female	5 days	3	5 days "talk & die"	Raised intracranial pressure
6 Fall (own height)	66	Male	12 days	14	5 mins initially followed by 12 days "talk & die"	Bronchopneumonia
7 Pedestrian	14	Female	2 days	3	2 days	Raised intracranial pressure
8 Pedestrian	8	Male	1.75 hrs	3	1.75 hrs	Raised intracranial pressure
9 Fall (> own height)	65	Male	27 hrs	12	27 hrs	Myocardial infarction
10 Fall (> own height)	71	Male	9 days	4	9 days "talk & die"	Pulmonary emboli acute bronchitis
11 Fall (> own height)	53	Male	6 days	3	6 days	Raised intracranial pressure

TABLE 2 - OCCIPITAL IMPACTS - CLINICAL DETAILS

Patient	Age	Sex	Survival	GCS	Duration of unconsciousness	Cause of death
1 Pedestrian	81	Male	3hrs 45 mins	15	<40 mins	Haemorrhage from ruptured mesentery
2 Pedestrian (road impact)	84	Male	13 days	8	13 days "talk and die"	Haemorrhage from lacerated right lung
3 Fall (own height)	80	Female	3 days	5	3 days "talk and die"	Raised intracranial pressure
4 Fall (own height)	77	Male	2 days	10	3 mins "talk and die"	Bronchopneumonia, cardiac arrest
5 Pedestrian	39	Male	15 hrs 15 mins	4	15 hrs 15 mins	Raised intracranial pressure
6 Fall (> own height)	46	Male	5 days	3	5 days	Bronchopneumonia
7 Fall (> own height)	25	Male	2 days	4	2 days	Raised intracranial pressure

TABLE 3 - LATERAL IMPACTS - NEUROPATHOLOGY

Patient	AI	Hypoxic/ Ischaemic damage	Contusion	Haematoma > 3 cms	ASDH	ICP	Skull Fractures
1 Pedestrian	+	+++	++	O	O	not elevated	+
2 Car occupant	+	+++	+	O	L ASDH	↑ICP	O
3 Fall (own height)	+	+++	+	O	R ASDH craniotomy	↑ICP	O
4 Pedestrian	+	+++	+++	O	L ASDH craniotomy	not elevated	+
5 Fall (own height)	+	++	+++	O	R ASDH	↑ICP	+
6 Fall (own height)	+	+	++	O	O	↑ICP	+
7 Pedestrian	+	+	+++	O	O	↑ICP	+
8 Pedestrian	+	O	+++	O	small L ASDH	not elevated	+
9 Fall (> own height)	+	++	+++	O	O	not elevated	+ depressed
10 Fall (> own height)	+	+++	+++	O	R ASDH craniotomy	↑ICP	+
11 Fall (> own height)	+	++	+++	O	small L>R ASDH	not elevated	+

TABLE 4 - OCCIPITAL IMPACTS - NEUROPATHOLOGY

Patient	AI	Hypoxic/ ischaemic damage	Contusion	Haematoma > 3 cms	ASDH	ICP	Skull Fracture
1 Pedestrian	+	O	O	O	O	-	O
2 Pedestrian (road impact)	+	++	+++	Bifrontal (craniotomy)	O	↑ICP	+
3 Fall (own height)	+	+	O	O	L ASDH	↑ICP	O
4 Fall (own height)	+	+	+	O	O	not ↑	+
5 Pedestrian	+	+++	+++	O	O	↑ICP	+
6 Fall (> own height)	+	+++	+++	O	small SDH EDH 100 mls	↑ICP	+ (compound)
7 Fall (> own height)	+	+++	+++	O	O	↑ICP	+ (depressed)

TABLE 5 - LATERAL IMPACTS - AISS AND TOPOGRAPHY OF APP IMMUNOSTAINING

Patient	AISS	AISS corrected	White matter hemispheres	CC	Brain stem	Cerebellum
1 Pedestrian	107/116	107/116	+	+	+	+
2 Car occupant	106/106	116/116	+	+	+	+
3 Fall (own height)	75/104	84/116	+	+	+	+
4 Pedestrian	86/103	97/116	+	+	+	+
5 Fall (own height)	62/96	75/116	+	+	+	O
6 Fall (own height)	51/100	60/116	+	+	+	+
7 Pedestrian	98/108	105/116	+	+	+	+
8 Pedestrian	76/100	88/116	+	+	+	+
9 Fall (> own height)	91/116	91/116	+	+	+	O
10 Fall (> own height)	26/107	28/116	+	+	+	O
11 Fall (> own height)	85/107	92/116	+	+	+	O

TABLE 6 - OCCIPITAL IMPACTS - AISS AND TOPOGRAPHY OF APP IMMUNOSTAINING

Patient	AISS	AISS corrected	White matter hemispheres	CC	Brain stem	Cerebellum
1 Pedestrian	5/90	6/116	+	+	O	O
2 Pedestrian (road impact)	41/114	42/116	+	+	O	O
3 Fall (own height)	87/116	87/116	+	+	+	O
4 Fall (own height)	35/105	39/116	+	+	+	O
5 Pedestrian	94/99	110/116	+	+	+	+
6 Fall (> own height)	106/116	106/116	+	+	+	+
7 Fall (> own height)	107/116	107/116	+	+	+	+

TABLE 7 - LATERAL IMPACTS - AISS AND BIOMECHANICAL DATA

Patient	AISS	AISS corrected	Linear acceleration m/s^2	Impact Velocity (m/s)	Impact Surface	Skull Fracture
1 Pedestrian	107/116	107/116	1160	13.88	Windscreen	+ (very soft)
2 Car occupant	106/106	116/116	1440	4.16	Car door	O (hard)
3 Fall (own height)	75/104	84/116	2422	4.75	Wooden dresser	O (hard)
4 Pedestrian	86/103	97/116	2890	8.33	A-pillar (hard)	+
5 Fall (own height)	62/96	75/116	2968	5.82	Concrete	+
6 Fall (own height)	51/100	60/116	3006	5.91	Concrete	+
7 Pedestrian	98/108	105/116	3680	13.88	Windscreen	+ (medium)
8 Pedestrian	76/100	88/116	3700	13.88	Bonnet	+ (medium)
9 Fall (> own height)	91/116	91/116	4064	7.97	Concrete	+
10 Fall (> own height)	26/107	28/116	4370	8.57	Concrete	+
11 Fall (> own height)	85/107	92/116	5451	10.69	Concrete	+

TABLE 8 - OCCIPITAL IMPACTS - AISS AND BIOMECHANICAL DATA

Patient	AISS	AISS corrected	Linear acceleration m/s^2	Impact Velocity (m/s)	Impact Surface	Skull Fracture
1 Pedestrian (concussion)	5/90	6/116	1280	15.27	Windscreen (very soft)	O
2 Pedestrian (road impact)	41/114	42/116	2942	5.77	Bitumen (hard)	+
3 Fall (own height)	87/116	87/116	2942	5.77	Wooden Jarrah Frame (hard)	O
4 Fall (own height)	35/105	39/116	3090	6.06	Tiles bathroom (hard)	+
5 Pedestrian	94/99	110/116	3310	12.5	Windscreen edge (medium)	+
6 Fall (> own height)	106/116	106/116	7000	13.73	Concrete (hard)	+ compound
7 Fall (> own height)	107/116	107/116	10534	20.66	Rocks (hard)	+ depressed

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