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Is β-APP a marker of axonal damage in short-surviving head injury?

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Abstract β-Amyloid precursor protein (β-APP), a normal constituent of neurons which is conveyed by fast axonal transport, has been found to be a useful marker for axonal damage in cases of fatal head injury. Immunocytochemistry for β-APP is a more sensitive technique for identifying axonal injury than conventional silver impregnation. This study was designed to determine how quickly evidenc of axonal damage and bulb formation appears. Using this method a variety of brain areas were studied from 55 patients who died within 24 h of a head injury. Immunocytochemical evidence of axonal injury was first detected after 2 h survival, axonal bulbs were first identified after 3 h survival, and the amount of axonal damage and axonal bulb formation increased the longer the survival time.

Key words Head injury · Axonal bulbs · β-amyloid precursor protein (β-APP) · Immunocytochemistry · Diffuse axonal injury

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Introduction

Diffuse brain damage in patients who sustain a fatal non-missile head injury is often due to diffuse axonal injury (DAI) [1, 2]. Until recently, the histological diagnosis of DAI depended on the detection of axonal bulbs by silver impregnation techniques [1]. Using this method, axonal injury could be seen in approximately 30% of patients dying at least 15 h after sustaining the head injury. Axonal injury could not be detected in cases of shorter survival using this technique. However, more recently, immunocytochemistry for β-amyloid precursor protein (β-APP) has been shown to detect axonal injury [3] in patients surviving as little as 3 h [4–6]. Other axonally transported proteins have been investigated as potential markers for axonal damage [4], but β-APP immunocytochemistry was found to be the most sensitive.

The aims of this study were to determine how soon after injury immunocytochemistry for β-APP could detect axonal injury, the frequency with which axonal damage occurred with increasing survival time, the time-course of axonal bulb formation, the amount of axonal damage that occurred with increasing survival time and the distribution of axonal damage in different parts of the brain.

Materials and methods

The study was carried out using 55 fatal head injury cases provided by forensic pathologists at various hospitals in the West of Scotland, including the Institute of Neurological Sciences, Glasgow between 1978 and 1993. All patients died within 24 h of sustaining the head injury. A control group of cases was not included in this study, as a previous study [6] has shown that β-APP immunoreactivity can be detected in patients who die with no related intracranial disease, as an age-related phenomenon, and also in patients who die as a result of a variety of different intracranial disorders, with or without raised intracranial pressure.

Each brain was fixed in 10% formol saline for 3–5 weeks prior to dissection and histology was performed on paraffin-embedded blocks. Sections from the corpus callosum, parasagittal white matter, brain stem, internal capsule and thalamus were cut and immunostained as previously described [6].

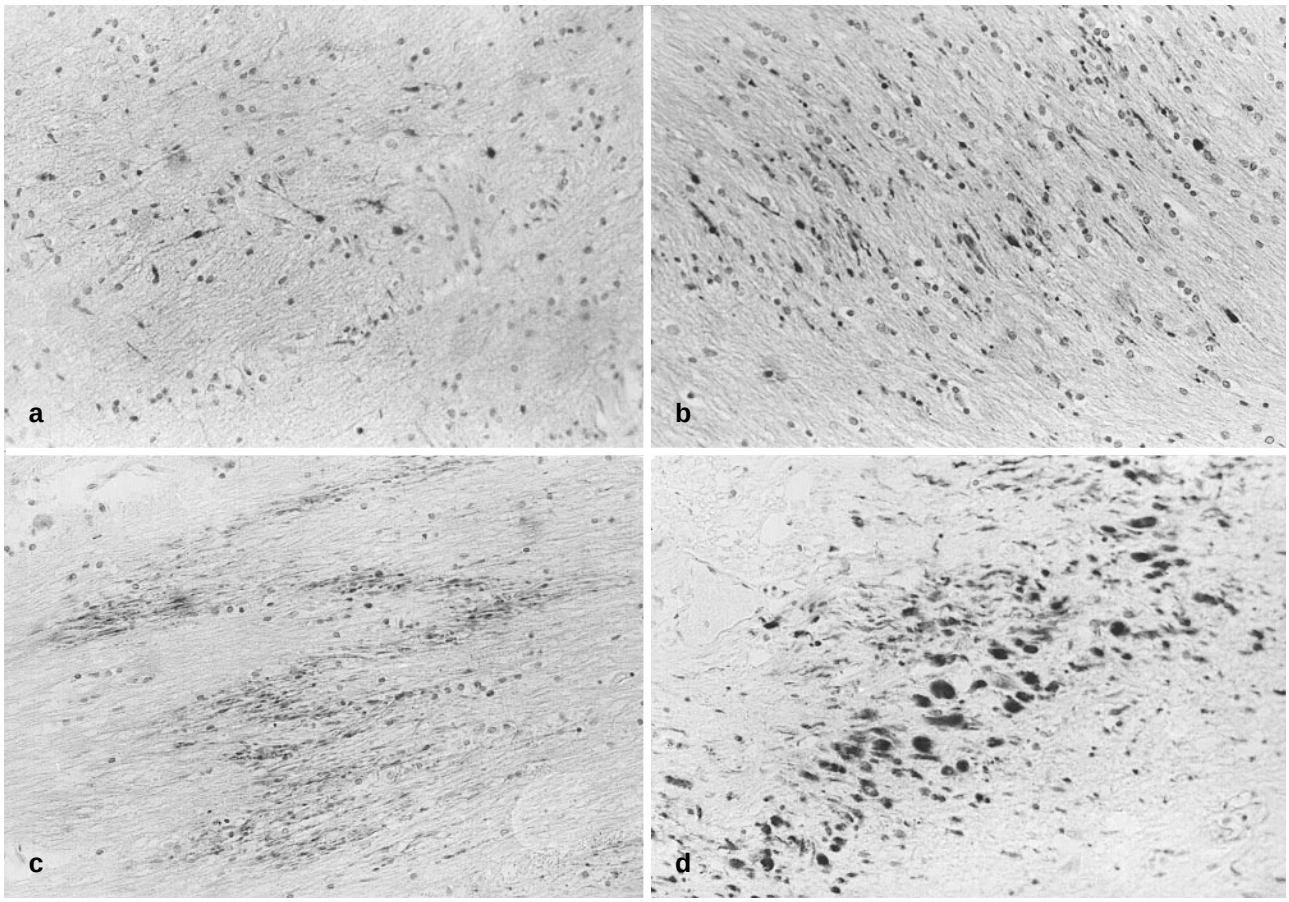

Fig. 1 a–d Demonstration of axonal injury using β-amyloid precursor protein (β-APP) immunocytochemistry. **a** Rating score 1 (3 h survival). **b** Rating score 2 (6 h survival). **c** Rating score 3 (12 h survival). **d** Rating score 3 with prominent bulb formation (24 h survival)

Immunostained sections were examined without prior knowledge of the survival period or diagnosis. Various changes were observed, ranging from accumulation of β-APP in slightly dilated axons, to accumulation of β-APP in swollen and tortuous axons or axonal bulb formation. These features were assessed for various survival periods, in several brain areas, and given a score rating, as previously described [6]. If none of the features was present, a rating of 0 was given. If there was any staining of axons, however, slight, the case was given a rating of 1. When there were scattered areas of axonal damage, a rating of 2 was given. When cases had extensive damage throughout large areas of white matter a score of 3 was given. The presence or absence of axonal bulbs was also noted (Fig. 1). There was no immunoreactivity in normal axons using this method.

Results

The 55 cases of fatal head injury consisted of 40 road traffic accidents, 10 falls, 2 assaults, 1 train accident and 2 cases where the cause was not known. The age range was 8 weeks to 77 years and all cases had a survival period of up to 24 h. Of the 55 cases, 46 had a fracture of the skull, 46 had contusions, 5 cases showed brain swelling and 21 had pathological evidence of raised intracranial pressure using the structural criterion of pressure necrosis in one or both parahippocampal gyri [7]. There was intracranial haemorrhage in 35 of the cases, comprising 4 extradural haematomas, 20 subdural haematomas, 2 intraventricular haemorrhages, 1 subarachnoid haemorrhage and 20 intracerebral haematomas, of which 15 were in the brain stem.

Examination of the β-APP-immunostained sections revealed β-APP accumulation, indicative of axonal damage, in 39 of the 55 cases (71%). The incidence of axonal damage increased to 80% if cases of instantaneous death were excluded, and to 83% if cases of less than 2 h survival were excluded (Fig. 2).

Analysis of the time-course of β-APP immunostaining showed that positive staining, and therefore evidence of axonal damage, could first be detected at 2 h survival. All of the cases of less than 2 h survival were negative. Detection of axonal damage increased substantially at 3 h survival, with over 80% of cases showing β-APP immunoreactivity. There was then only a gradual increase in the detection of axonal damage with increasing survival periods, to almost 90% at 24 h (Fig. 3).

Axonal bulbs were first detected at 3 h survival. The frequency of bulb formation increased with time and appeared to show two peaks. The first occurred between 3 and 9 h survival, with 55–60% of cases showing axonal bulb formation. There was an apparent second peak between 10 and 24 h when over 80% of cases showed axonal bulbs (Fig. 3).

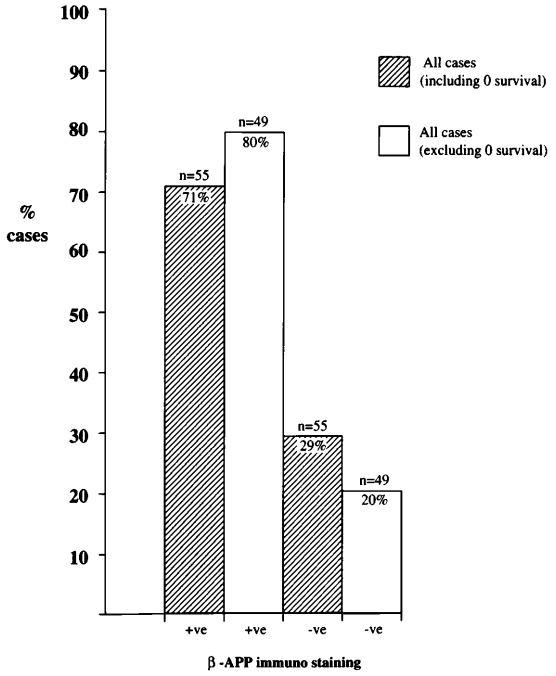

Fig. 2 Bar histogram illustrating the overall frequency of axonal damage, and bulb formation

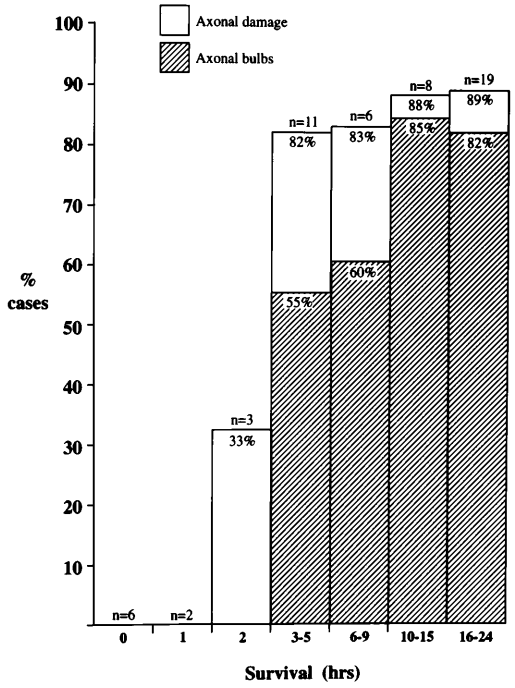

Fig. 3 Bar histogram illustrating the time-course of axonal damage, and bulb formation

The amount of axonal injury, determined by the average rating score (0–3) also increased with increasing survival time. At 2 h survival, when axonal injury was first detectable, the degree of axonal injury was mild. There was then a marked increase in axonal injury at 3–5 h followed by a more gradual increase in axonal injury reaching a peak by 10–15 h, after which there was

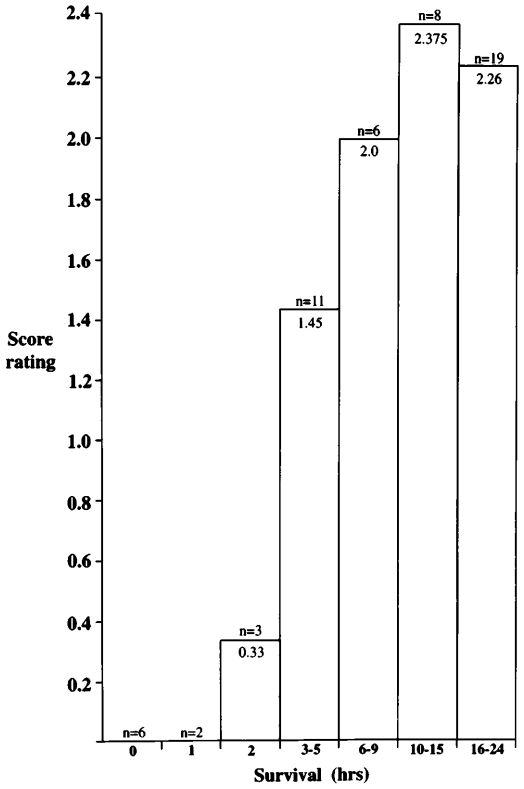

Fig. 4 Bar histogram illustrating the average rating score of axonal damage (scale 0–3) with increasing survival

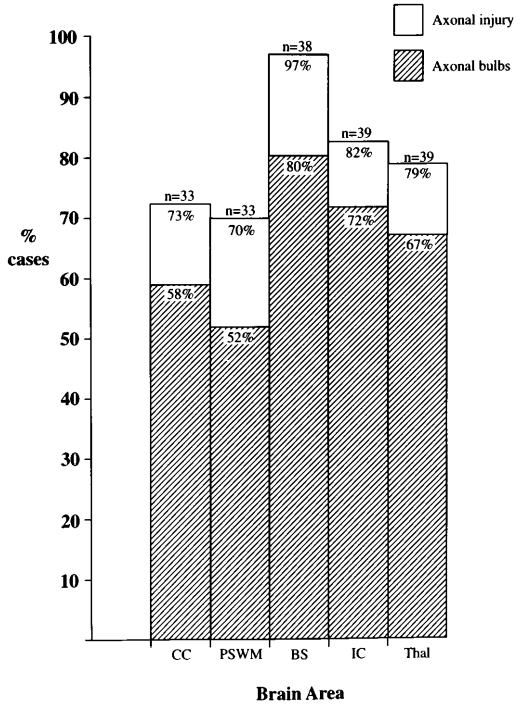

Fig. 5 Bar histogram illustrating the frequency of axonal damage and axonal bulb formation in different brain areas (*CC* corpus callosum, *PSWM* parasagittal white matter, *BS* brain stem, *IC* internal capsule *Thal* thalamic)

no further increase in the degree of axonal injury (Fig. 4).

The distribution of axonal injury in cases that were positive showed that of the various areas examined, evidence of axonal injury was found most frequently in the brain stem (97%). This was followed by the internal capsule (82%), thalamus (79%), corpus callosum (73%) and parasagittal white matter (70%) (Fig. 5). The frequency of axonal bulbs in each of these areas was: brain stem (80%), internal capsule (72%), thalamus (67%), corpus callosum (58%) and parasagittal white matter (52%)

Discussion

β-APP is a membrane-spanning glycoprotein which is a normal constituent of neurons. The function of β-APP is ill-defined but is thought to include cell adhesion, growth and response to injury [8, 9]. It is conveyed by fast axonal transport and accumulates after disruption of the cytoskeleton [10] when it reaches detectable levels. It has been detected by immunocytochemistry at sites of axonal injury in the brains of experimental animals [10–12] and humans [3, 13, 14], and has been found to be a useful marker for injured axons in patients who survive for only 3 h after head injury [5].

The results of our study confirm that β-APP is a sensitive marker of axonal damage in cases of fatal head injury. Evidence of axonal damage was first seen in the form of accumulation of β-APP in slightly dilated axons, in swollen and tortuous axons and in axonal bulbs, as previously described [6]. Axonal damage was first detected at 2 h survival and was then seen in over 80% of patients surviving for 3 h or more.

These figures for the detection of axonal damage using β-APP immunocytochemistry are in contrast to the classic silver techniques in which axonal bulbs are only detected after 15 h survival, and even then are only identified in approximately 30% of cases. Therefore, β-APP immunocytochemstry allows earlier detection of axonal damage and in a greater number of cases.

Our figures for the detection of axonal damage and axonal bulbs using β-APP immunocytochemistry compare with those of a previous study using the same technique [6], in which all 25 cases showed evidence of axonal damage and 23 of the cases showed axonal bulb formation. However, the survival periods in that study were different (2 h – 26 days), and the two cases which did not show axonal bulb formation were of less than 12 h survival.

Some of these changes may reflect temporary alterations in axoplasmic transport in axons which may or may not have the potential for recovery. The time-course for potential recovery is unclear, but β-APP accumulation has been demonstrated in patients surviving as long as 99 days after mild head injury [15]. Therefore, perhaps it is the pattern of axonal bulb formation which is important clinically, as this indicates the evolving pattern of irreversible brain damage.

Axonal bulbs were first identified at 3 h survival and were seen in up to 60% of cases within the first 10 h. The frequency of bulb formation then appeared to plateau at over 80% of cases, between 10–24 h survival. The present findings are therefore consistent with previous studies which have shown a time-course for the development of axonal damage with bulb formation increasing during the first 12–24 h of survival [2, 16], after which it is seen to plateau and then tail off. The time it takes for bulb formation to occur has also been shown to be variable [17], giving rise to the suggestion that individual cases respond differently after injury.

It has previously been shown that the appearance of immunostained axons changes with increasing survival, in that at 3 h, the axons appear only slightly swollen, whereas at 24 h they are grossly swollen and resemble axonal bulbs [5]. In general, a similar pattern was seen in our cases, but it is important to note that even at survival periods as short as 3 h, some cases in our study showed intense positivity, with swollen and tortuous axons and prominent bulb formation.

Comparisons of the time-course of axonal injury and the time-course of axonal bulb formation indicate that whilst over 80% of patients surviving between 3 and 5 h show evidence of axonal damage, as demonstrated by the occurrence of axonal swelling, only 55% show axonal bulb formation, providing further support for the suggestion that there is a spectrum of axonal responses during the first few hours after head injury. Only a proportion of the axons damaged in any one case undergo irreversible injury, and the remainder may have the potential for recovery. However, by 10–15 h survival, the frequency of axonal injury increases only slightly to 88% of cases, but axonal bulbs are now identified in 85% of cases, suggesting that most of the axons have undergone irreversilbe damage. Therefore, it would appear that there is a potential for recovery from axonal injury which is time-dependent, in that within the first 10 h it may be possible in just under 35% of the axons, whereas from 10 h onwards there is potential for recovery in less than 10% of the axons.

There are a number of experimental models that attempt to replicate human diffuse axonal injury [18, 19]. Most of the descriptive work has been obtained from models using fluid percussion supplemented more recently by models of nerve stretch [20, 21]. These studies have shown that except in perhaps the most severe forms of experimental head injury axons are not severed at the time of injury – primary axotomy – by rather undergo a series of changes [22] which may result in secondary axotomy within 24 h of the injury [23, 24]. These findings suggest that there is likely to be a spectrum of axonal pathology which ranges from a population of injured axons that are morphologically normal but are nevertheless functionally impaired [21] to ones showing complex “reactive” changes [25]. Although the temporal course of trauma-induced changes in axons is likely to be different in various parts of the brain and in different species there is increasing belief that delayed or secondary axotomy in both experimental models and in humans might represent a window of opportunity for therapeutic intervention.

The distribution of β-APP immunoreactivity in the different brain areas examined showed that the most common site of axonal injury (as defined by β-APP accumulation in slightly dilated, or swollen and tortuous axons and including axonal bulbs) was the brain stem, with over 95% of cases showing axonal injury in this area. The internal capsule and thalamus were positive in approximately 80% of cases and the least common sites were the corpus callosum and parasagittal white matter, which, however, still showed evidence of axonal injury in approximately 70% of cases. The distribution and amount of axonal damage was always greater than the amount of associated haemorrhage in any of these sites. Therefore, the presence of β-APP immunoreactivity could not be only attributed to the presence of haemorrhage. Moreover, the incidence of β-APP immunoreactivity within the brain stem was not affected by the presence (49% of positive cases) or absence (57% of positive cases) of raised intracranial pressure. This highlights the importance of selecting certain brain areas for histological assessment of cases of head injury. More importantly, it also emphasises that one of the most important regions of the brain, i.e. the brain stem, is also the area most commonly affected in head injury. None of the cases showed β-APP immunoreactivity in the corpus callosum or parasagittal white matter without immunoreactivity to the brain stem. However, two cases showed β-APP immunoreactivity in the internal capsule or thalamus without immunoreactivity in the brain stem. These two cases also lacked immunoreactivity in the corpus callosum and parasagittal white matter. Therefore, it remains important to examine not just the brain stem, but a variety of brain areas, for evidence of axonal injury in cases of head injury. The distribution of axonal bulbs showed a similar pattern to that of axonal injury, but the overall frequency in each brain area was less. The distribution of axonal bulb formation in our study was similar to that of a previous study [6] but with an overall reduced frequency in each brain area in particular in the corpus callosum and parasagittal white matter. These differences may reflect the varying survival periods noted in the previous study (2 h – 26 days) and it is conceivable that the present cases showed a different pattern of brain injury, for example a greater incidence of primary axotomy/axonal shearing because death occurred earlier as a consequence of the severity of the injury.

In summary, β-APP immunocytochemistry is a useful marker of axonal injury in formalin-fixed, paraffin-embedded human brain. It labels injured axons [4] and can reveal axonal injury after 2–3 h survival. However, it must be pointed out that β-APP accumulation is not unique to patients with head injury and it therefore should not be regarded as a specific marker for trauma. For example, accumulation of β-APP has also been seen in a variety of other situations, e.g. cerebral infarcts, intracerebral haemorrhage, neoplasms, etc. [5, 6], where the distribution and appearance of β-APP-immunoreactive axons did not allow distinction between head injury and non-head injury cases. Moreover, in cases of head injury associated with other lesions, e.g. infarcts or haematomas, β-APP immunoreactivity may be seen either as a result of axonal injury or associated with these lesions. However, this problem is not unique to β-APP immunoreactivity but also applies to classic silver impregnation techniques. Increasing experience will establish the potential use of immunocytochemistry in the evaluation of axonal damage in neuropathological practice and, in particular, in material obtained after trauma.

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