Stratification of Spinal Ependyma in Rats in Response to Injury.

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ABSTRACT

The ependymal lining of the central canal in response to injury was investigated in young rats. A unilateral incision on the dorsolateral aspect of the spinal cord (thoraco-lumbar region) was made, after laminectomy, without injuring the ependyma after laminectomy. The sham operated controlled and lesioned spinal cords at different periods, from day 1 to 21 (post-operative) were processed for light microscopy. It was observed that the lumen of the central canal of the lesioned spinal cords was collapsed, irregular and lined by stratified layer (3 to 4 layers) of ependyma. The ependymal cells in the lesioned spinal cords proliferated in response to injury. The ependyma lining the central canal of the spinal cord in rats retains regenerative capacity - a phylogenetic feature of lower forms which can be evoked by a local injury.

INTRODUCTION

The lining of central canal of the spinal cord with a few exceptions is similar to most epithelial membranes, which under pathological conditions exhibit considerable regenerative ability [1]. Cell division is a feature of ependyma during embryonic and early postnatal development [2]. The proliferative activity ceases after early postnatal life and in the adulthood. Ependymal cells of the rat central canal were examined by Bruni and K Reddy with a view to identify features that distinguish them regionally and from their counterparts elsewhere in the ventricular system. The results revealed that the lining consisted for the most part of a pseudostratified layer of uniformly organized cuboidal to columnar ependymal cells present in largest numbers in lumbar and sacral segments and in the conus. Ependymal cells of the spinal cord were not sufficiently dissimilar morphologically from their counterparts in the ventricles to account for differences in proliferative capacity in response to localized injury. Tanyocyte ependymal cells in the two locations were thought to be the reactive elements that proliferate in response to injury [3].

The aim of the present study was to investigate the response of ependymal cells to injury of the spinal cord.

MATERIALS AND METHODS

Young adult male rats (Sprague-Dawley) of about 5-6 weeks are used in this investigation. Animals were anaesthetized with sodium pentobarbital (50mg/kg body weight) intraperitoneally. Laminectomy at the thoracolumbar level of the spinal cord was performed under full aseptic measures. A small superficial, vertical cut was made on the dorso-lateral aspect of the spinal cord without injuring the central canal of the spinal cord. The sham operated controls were identically treated except that the spinal cord was not injured.

All care was taken to maintain the health of the animals. The animals were given free access to food and water. The lesioned rats were divided into five groups.

One group of three animals (lesioned) and one sham operated control were sacrificed by an overdose of ether anaesthesia, at post-operative intervals of 1, 3, 5, 7, and 21 days. The animals were perfused intracardially with formol ammonium bromide. The spinal cords were dissected out carefully and fixed in formol ammonium bromide for three days. The fixed tissues processed for paraffin sectioning. Serial sections of 20 micrometers were cut and stained with Haematoxylin & Eosin and Luxol fast blue & Neutral red. The stained sections were observed under light microscope.
RESULTS

The central canal of the control spinal cord (thoraco-lumbar) was more or less rounded or oval (dorso-ventrally elongated). The ependymal lining consisted of a single layer of cuboidal to low columnar cells with large, rounded nuclei at the base. The cells were arranged regularly around the lumen. The median region of the central canal (especially in the ventral medial aspect) appeared to be less cellular, or lined by low cuboidal to squamous cells with tanycyte-like cytoplasmic processes (Figure 1). The lumen of the central canal in the lesioned spinal cord varied at different post-operative intervals. From day one to day three - post-operative, the lumen was almost collapsed and irregular. The luminal surface of ependymal lining was irregular. The epithelium was stratified (three to four layers). The appearance of ependymal cells was not like that of controls. The cells are smaller in size, and arranged irregularly around the lumen. The ependymal cells were lacking polarization. Proliferation of cells around the ependymal cells into the surrounding neuropil was observed suggesting that the cells divide and migrate towards the site of injury (Figure 2).

Figure 1. CONTROL Central canal is almost circular, lined by single layer of ependyma. Arrow indicates the site of tanycytes. x40

Figure 2: 3rd day post-operative: Central canal has collapsed and ependyma shows stratification (proliferation)x40

On the 5th post-operative day, the lumen of central canal appeared to be regaining the original shape. But the stratification of the ependymal cells still persisted (three to four layers). The ventral median aspect of the central canal was lacking cellular lining. It appeared as though the contents of the canal extruded out and pushed the cells apart. It may be the recovery stage of ependyma (Figure 3).

Figure 3: 7th day post-operative: Central canal regaining its original shape. Ependymal stratification is still visible.x40
From the 7th post-operative day onwards the central canal regained its original shape and the stratification reduced to two layers and by 21st (post-operative) day, the proliferation of the cells was absent and the ependyma became single layered, almost resembling that of the controls (Figure 4).

Figure 4: 21st day post-operative: Central canal regained its original shape. Ependyma became single layered. (Luxol fast blue and neutral red) x10.

**Review of literature and discussion**

Spinal cord injuries (SCI) due to traumatic accidents are quite a common occurrence and often resulting in devastating results. Attar A et al working on SD female rats induced SCI by applying clips to the spinal cord and found electron microscopically that the ependymal cells started to proliferate and migrate away from the central canal, three days after a mild SCI and there was an active proliferation in response to injury. They have also concluded that this proliferation of ependyma may replace the lost neuronal tissue and help in axonal regeneration [4].

Bruni JE, Del Bigio MR, Clattenburg RE observed that the ependymal turn over declines significantly in normal adults post natally and only residual activity persisted in adulthood. Proliferative activity was observed in SCI. In the infra mammalian vertebrates, the ependyma plays a significant role in the regenerative processes in spinal cord. In hydrocephaly also there had been a similar response though it could be a part of overall tissue response[5].

Roales-Bujan et al working on hydrocephalic hyh mutant mice reported a programmed loss of neuroepithelium/ependyma followed by periventricular astrocyte reaction forming a new cell layer over the denuded ventricular surface. They also analysed human foetal hydrocephaly and found that similar phenomenon would occur in humans and suggested that this new layer formed by astrocytes could function as new CSF-brain involved in water and solute transport thus contributing to re-establish lost functions at the brain parenchyma-CSF interphase[6].

Neural stem cells and progenitor cells suggest existing in the ependyma of the central canal of the spinal cord. Inducing SCI in the thoracic part, Takahashi et al found that ependymal cells proliferate and this process depended on the severity of a mild SCI and there was an active proliferation in response to injury. The kinetics of the proliferating ependymal cells in response to injury were analysed by proliferating cell nuclear antigen (PCNA), revealed increasing number of PCNA which was related to the lower limb motor function recovery. It has been indicated that the ependymal cells are themselves multipotent and can divide and proliferate according to the severity of the injury[7].

Vagueru et al and Vallace et al, observed the regenerative activity of ependyma following compression injury to the spinal cord [8,9]. An abnormal proliferation of ependyma following spinal cord transection was shown by Mathews et al. (1978) and (1979)[10,11].

Kasantikul et al. postulated the mechanism for normal occlusion of the central canal in man. The normal occlusion of the central canal in man may be represented by the ependymal proliferation and astrocytes[12].

In response to various disruptive factors like injury, compression etc. the normally mature ependyma may revert to a primitive state and exhibit proliferative activity in comparison with the abnormal neuroepithelium [10]. In the lower vertebrates ependyma plays a very important role in initiation and maintenance of regenerative process [13].
CONCLUSION

The present study is carried out to study the proliferative changes in the spinal ependyma in rats from a normal simple epithelium to stratified epithelium in response to induced SCI and its regenerative capacity—a feature of the phylogeny of lower vertebrates.

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REFERENCES