EXPERIMENTAL RESEARCH

Gamma Hydroxybutyrate Improves Early Ultrastructural Findings After Experimental Spinal Cord Injury

Deneysel Omurilik Travmasını İzleyen Erken Ultrastrüktürel Bulgular Gamma Hidroksibutiratla Düzelmektedir

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Abstract: The ultrastructural effects of gamma hydroxybutyrate after spinal cord injury were studied in rats. In the study 25 rats were divided into 3 groups. First group (group 1) consisted of 5 rats who underwent laminectomy only to serve as sham-operated group. Remaining 20 rats were randomly assigned to the control (group 2) or treatment (group 3) groups, consisting of 10 rats each. Animals were injured at C7-T1 by acute compression of the spinal cord with a clip exerting a pressure of 192 g. Gamma hydroxybutyrate treated and untreated rats were compared according to the ultrastructural and light microscopic findings. The main differences between the two groups were, 1) absence of vasoconstriction, 2) less edema, 3) absence of vessel wall necrosis, and 4) less myelin sheath degeneration in the gamma hydroxybutyrate treated group. The underlying mechanism is discussed. We have concluded that gamma hydroxybutyrate is an encouraging drug in experimental spinal cord injury in rats which need to be further investigated.

Key Words: Gamma hydroxybutyrate, spinal cord injury, ultrastrucutral study

Özet: Sıçanlarda omurilik travmasından 24 saat sonra gamma hidroksibutiratın ultrastrüktürel etkileri incelendi. Çalışmada kullanılan 25 dişi Sprague-Dawley cinsi sıçan 3 gruba ayrıldı. Birinci gruptaki (grup 1) 5 sıçana sadece laminektomi uygulandı. Diğer 20 sıçan gelişigüzel olarak herbiri 10 sıçan içeren kontrol (grup 2) veya tedavi (grup 3) grubuna dahil edildi. Hayvanlara, 192 gramlık basınç uygulayan bir klip kullanılarak C7-T1 seviyesinde omurilik travması yapıldı. Gamma hidroksibutirat verilen ve verilmeyen hayvanlar, ultrastrüktürel ve ışık mikroskobu bulguları eşliğinde karşılaştırıldı. İki grup arasındaki belli başlı farklılıklar gamma hidroksibutirat verilen grupta, 1) vazokonstrüksiyon olmayışı, 2) daha az ödem, 3) damar duvarı nekrozu olmayışı, ve 4) daha az miyelin kılıfı dejenerasyonu oldu. Bu farklılıkların mekanizması tartışıldı. Gamma hidroksibutiratın sıçanlarda deneysel omurilik travmasında olumlu sonuçlar verdiği ve başka çalışmalar ile desteklenmesi gerektiği sonucuna varıldı.

Anahtar Sözcükler: Gamma hidroksibutirat, omurilik travması, ultrastrüktürel çalışma

INTRODUCTION

Blunt trauma to the spinal cord causes immediate physical injury directly to nerve cells and blood vessels which is largely irreversible, and a secondary injury which is a cascade of pathochemical events that are initiated by the primary injury (2,3,12,23). Spinal cord injuries result in severe focal spinal cord ischemia (1,20,22,23,24) and loss of autoregulation (7,19,24,25). Measurements of spinal cord blood flow (SCBF) after spinal cord injury have shown ischemia at the injury site and extending to considerable distances (4,5,10,18,20,22). Gamma hydroxybutyrate (GHB), an analog of gamma

aminobutyric acid (GABA), has previously shown to alter the ischemic response to trauma (24) by increasing SCBF in both normal and injured cats after spinal cord injury. It has been demonstrated in vitro that cerebral blood vessels possess specific binding sites for GABA (14) and that isolated cerebral arteries relax following the administration of GABA and GABA agonists (8,11). The present study was performed to investigate the effect of GHB on ultrastructural findings 24 hours after experimental spinal cord injury.

MATERIALS AND METHODS

Twenty five female Sprague-Dawley rats with weights ranging from 230-310 g were used. Rats were anesthetized with intraperitoneal injection of thiopentone sodium BP (penthothal sodium, Abbott) 30 mg/kg, and laminectomy was performed at C7-T1 by using an operating microscope. The clip (Yaşargil aneurysm clip, force of closure 192 g, curved arms) was applied extradurally to the spinal cord, and remained compressing the cord for 30 seconds. Immediately after injury, each animal was randomly assigned to the treatment or control group, consisting of 10 rats each. Five uninjured animals underwent only laminectomy. In the treatment group, a single dose of GHB (300 mg/kg)(Gamma-OH, Clintec Nutrition Clinic) was administered intraperitoneally immediately after the injury.

Rats were sacrificed 24 hours after the injury. The injured spinal cord segment was excised under the microscope. The tissues for electron microscopic examination were immediately placed in 3 % glutaraldehyde buffered at pH 7,4 with phosphate buffer for 3 hours. The tissue pieces were subsequently fixed in 1 % osmic acid for 2 hours. Tissue samples were then dehydrated in graded ethanols, embedded in araldite, and processed for electron microscopy using conventional methods.

For light microscopic examination, all spinal cords were cut transversely 0,2-0,3 mm in diameter. The tissue was fixed in 10 % buffered formol saline and processed for paraffin embedding. Five μm thick sections were stained with routine methods.

RESULTS

Group 1 (sham operated rats): Normal ultrastructure of the spinal cord. Myelinated and unmyelinated axons were observed. Neuronal cells were normal.

Group 2 (trauma without treatment): Light microscopic examination: Pathologic examination in all traumatic spinal cord showed moderate to severe contusion. There was extensive haemorrhage especially in traumatic side subarachnoidal space and in parenchyma around the central canal. Severe pallor of myelin and edema were revealed. Fibrinoid necrosis in vessel wall (especially capillary wall) was prominent finding in severe haemorrhage and contusion. Extravascular inflammatory cells were seen in some specimens (Figure 1).

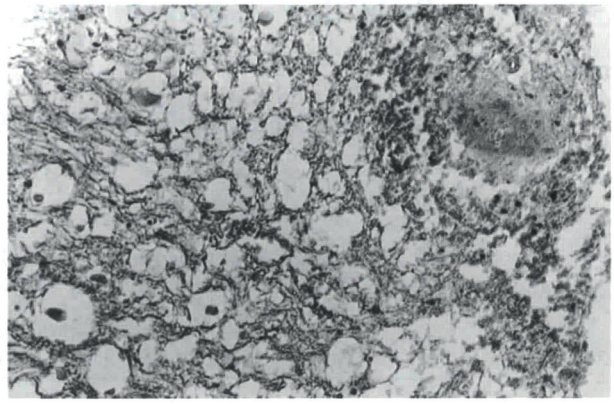

Figure 1: Light microscopic findings in trauma group: necrosis of vessel wall and petechial haemorrhages around the vessel, severe pallor of myelin. (H & E X200)

Electron microscopic examination: In specimens of animals submitted to spinal cord injury, a very loose tissue consistency was observed, due to infiltration of extracellular fluid, associated with this increase of extracellular fluid, dispersed edematous areas were seen, especially located in the pericapillary zones. Constrictive blood vessel sections with shrinked endothelial cell cytoplasm were also major findings in this group. Sparse haemorrhagic fields were noted. The cross-sections of myelinated nerve fibers showed a degenerative aspect, myelin-sheath membranes were fragmented or detachment between membranes was observed. The axonal section showed degenerative features, presence of a vacuolar degeneration was very prominent in axons and also intracellularly (Figure 2).

Group 3 (gamma hydroxybutyrate treatment): Light microscopic examination: Spinal cord contusion areas were smaller than the control group. Haemorrhage was seen in both subpial and parenchymal areas but less prominent. No vessel wall

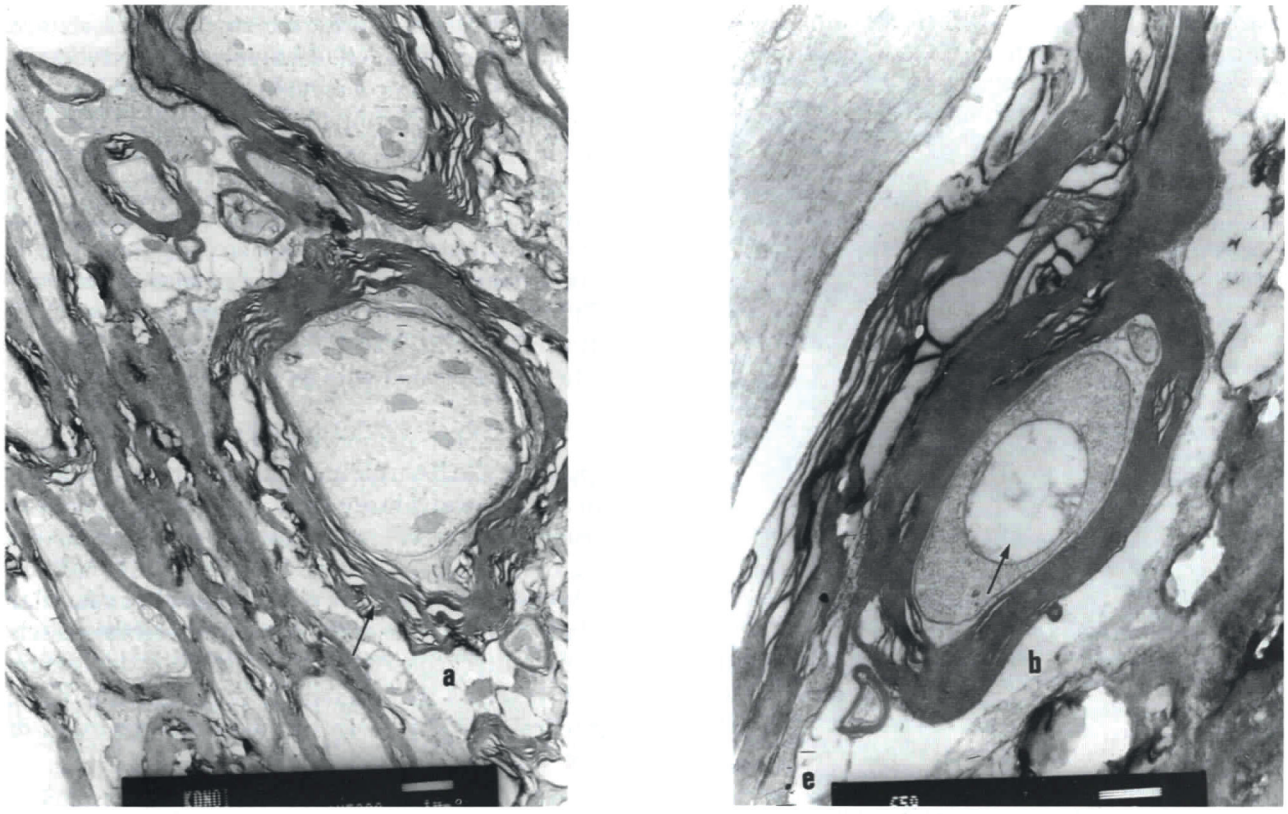

Figure 2: Electron microscopic findings in trauma group; a) disorganized myelin sheaths (arrow) and extracellular degenerative and loose, edematous areas. Axonal degenerative zones with myelin membrane splitting and fragmentation (X5000), b) high magnification of myelin membrane splitting and axonal degeneration (arrow) (X12000).

necrosis was seen. Myelin pallor and edema were mild to moderate in white matter according to the control group (Figure 3).

Electron microscopic examination: GHB administered animal specimens were clearly in a better state than the traumatic ones. The consistency of the spinal cord was dense but in normal limits, haemorrhagic zones were also noted. The vascular components were in better stages. No vasoconstriction, lesser or no edematous areas were the most important findings. The degeneration of the myelin-sheaths were in lower extend (Figure 4).

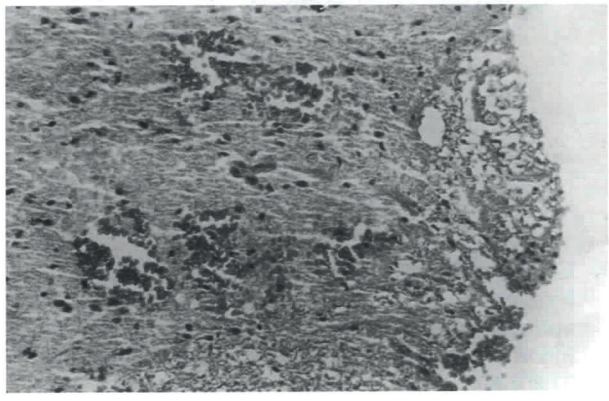

Figure 3: Light microscopic findings in GHB treated group: petechial haemorrhages in traumatic side of the spinal cord. (H & E X200)

DISCUSSION

The present study has shown that GHB improves ultrastructural findings at 24 hours after spinal cord injury in rats. The main differences between GHB treated and non-treated groups were 1) absence of vasoconstriction, 2) less edema, 3) no vessel wall necrosis, 4) less degenerative features in the GHB treated group.

Glutamic acid decarboxylase (GAD) and GABA transaminase, which have been employed extensively as biochemical markers for GABAergic mechanisms (21) have been identified in association with cerebral blood vessels (15,26). These enzymes

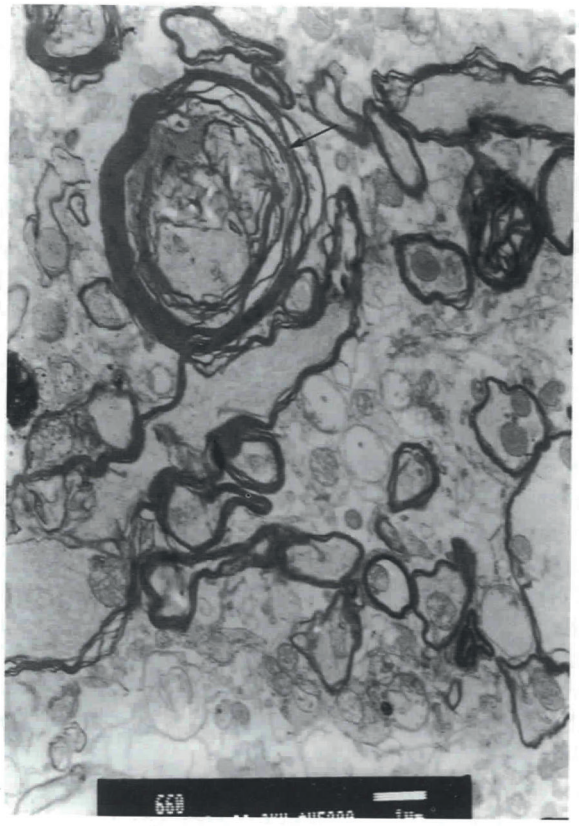

Figure 4: Electron microscopic findings in GHB treated group: dense aspect of the tissue with some myelin degeneration (arrow) among normal neuronal structures. (X5000)

are absent or present in much smaller quantities in peripheral blood vessels (15,26). It has been demonstrated in vitro that cerebral blood vessels possess specific binding sites for GABA (14) and that isolated cerebral arteries relax following the administration of GABA and GABA receptor agonists (8,11). McCulloch et al. (17) have shown in a cat pial arteriolar study that GABA and its analogues can dilate cerebral resistance vessels, presumably by interacting with specific receptor sites present in cerebral blood vessels (14).

Senter et al. (23,24) have determined the effect of GHB on spinal cord blood flow after experimental spinal cord injury in cats and reported that a single dose of GHB produces a significant increase in posttraumatic SCBF that lasts 2 to 3 hours. Dolan and Tator (6) found that GHB had no significant effect on SCBF after injury in rats.

Studies in man have suggested that GHB decreases the cerebral blood flow, cerebral metabolic requirement of oxygen, and glucose utilization rate (9). The mechanisms by which these effects are mediated are unknown. Escuret et al. (9) advocated to use GHB to control elevated ICP in head injury. Leggate et al. (16) compared the effects of GHB and sodium thiopentone on elevated ICP and systemic blood pressure in comatose head injured patients and found that the times to maximum fall of ICP and return to pretreatment levels were longer after GHB therapy and that mean cerebral perfusion pressure (CPP) was preserved with both drugs, however GHB administration was more frequently (60 % of patients) associated with an improvement in CPP.

It has been demonstrated that two potentially opposing GABAergic mechanisms may influence local cerebral blood flow following systemic administration of muscimol (a putative GABA ergic agonist), a direct vascular action of the GABAergic agonist evoking cerebrovascular dilation, and the vasoconstriction of cerebral vessels as an indirect consequence of metabolic depression (13). It seems that metabolic requirements of the cerebral tissue remain the main determinant of cerebral blood flow.

The present study showed that GHB has beneficial effects on the injured spinal cords of rats 24 hours after injury by preventing vasoconstriction which probably due to interaction of GHB with specific receptor sites. Other findings we have seen are less edema, preserved vessel wall and less myelin sheath degeneration may be consequences of vasodilatation and decreased energy consumption. Whatever the underlying mechanism is, these objective findings demonstrated that GHB is an encouraging drug which should be further investigated in spinal cord injury.

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