The Effects Of Nimodipine, Tyrotyrope Releasing (TRH) And Ginkgo Biloba Extract (GBE) in Experimental Spinal Cord Injuries

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✓ There have been interest in the role of some drugs in the pathophysiology of spinal cord injury. In the present study we have evaluated the effects of treatment with nimodipine, TRH, GBE on functional recovery and histopathological changes when these drugs are administered after spinal cord trauma in the anesthetized rabbits. 50 rabbits were divided in five treatment groups. Group 1 (10 rabbits) received nimodipine (500 µg/kg/day intraperitoneally) during first 7 days. Group 2 (10 rabbits) received TRH (2.5 µg/kg/day intraperitoneally) during first 7 day. Group 3 (10 rabbits) received GBE (1 mg/kg/day intraperitoneally) during first 7 day. Group 4 (10 rabbits) received nimodipine, TRH, GBE at the same time, during the first 7 days. Group 5 (10 rabbits) received no drug after spinal cord injury. There was no significant clinical and histopathological differences scores among the three treatment groups (Group 1, 2, 3) whereas Group 4 had better clinical scores than other groups.

Key Words: Spinal cord injury, TRH, GBE, nimodipine.

DeneySEL Spinal Kord Yaralanmalarında Nimodipine, TRH ve Gingko Biloba Ekstrasonun Etkileri

✓ Spinal kord yaralanmalarının fizyopatolojisinde birçok ilaç önemli rolü vardır. Bu çalışmada, anestezide altıncı tavanlara spinal kord travması yapıtılmış sonra Nimodipine, TRH ve Gingko Biloba Ekstrason (GBE) verilerek, bu ilaçların fonksiyonel illeştirmeye ve histopatolojik değişiklikler üzerine etkileri araştırıldı. Toplam 50 tavan beş gruba ayrıldı. 1. Gruba (10 tavan) 500 µg/kg/7 gün süreyle Nimodipine intraperitoneal olarak verildi. 2. Gruba (10 tavan) 2,5 µg/kg/gün TRH intraperitoneal olarak verildi. 3. Gruba (10 tavan) 1 mg/kg/7 gün intraperitoneal olarak GBE verildi. 4. Gruba (10 tavan) Nimodipine, TRH ve GBE aynı anda verildi. 5. Gruba (10 tavan) hiçbir ilaç verilmedi. İlk üç grup arasında önemli bir klinik ve histopatolojik fark yoktu. 4. Grup klinik olarak diğerlerinden daha iyi idi.

Anahtar Kelimeler: Spinal kord yaralanması, TRH, GBE, Nimodipine.

Spinal cord injury (SCI) is a physically, psychologically, socially, and economically devastating disease for the patient and relatives. Paralysis resulting from central nervous system (CNS) injury has been recognized for more than 2000 years. We do not know its incidence in Türkiye, but it is 40 per million population or about 10,000 new cases per year in USA.

Since Allen (1908), many surgical experiments have been done on SCI and various treatments including hypothermia, steroids, hyperosmotic agents and myelotomy to reduce the spinal cord damage[1,2,3,4,5].

Calcium antagonists have been shown to have a variety of physiological effects that theoretically should be beneficial in treating ischemic CNS injury and these include ability to: 1) block post ischemic perfusion; 2) inhibit cerebral vessel contractions induced by serotonin, blood or thromboxane; 3) dilate pial vessels; 4) increase cerebral blood flow[6,7,8,9,10,11]. In 1985 Faden and co-workers demonstrated that treatment with the neuropeptide TRH enhanced neurological recovery from SCI in cats.

The mechanism by which TRH exerts its therapeutic action in spinal injury is uncer-
tain. A free-radical scavenger, Ginkgo biloba extract (GBE) prepared from dry leaves of maiden haus tree (Ginkgo biloba) leads to a multiplicity of effects. The therapeutic benefit of GBE is supported by hemodynamic properties leading to the equilibrium of blood flow in small vessels normalization of metabolic parameters in ischemic and hypoxic models and restoration of muscarinic receptors in old animals GBE has been shown in vitro and in vivo to be a free-radical scavenger; thus it is postulated that at least part of its peculiar qualities are due to a "membrane effect", some others being related to enzymatic regulation and neuromediators [12,13,14].

MATERIALS AND METHODS

The experiments were carried out on 50 male rabbits, weighing 1.5–2 kg. They were anesthetized with ketamine hydrochloride (50 mg/kg intramuscularily) and sodium pentobarbital (40 mg/kg intravenously) and spinal cords were exposed with thoracic (Th8–9) total laminectomy.

The spinal cord was traumatized utilizing a modification of the Allen method by dropping a 10 gr weight from a distance of 30 cm through a guide tube onto a 10 mm² impact plate. All rabbits were traumatized and divided in five groups. The first group (10 rabbits) was treated with 500 µg/kg/day nimodipine (Nimolop 50 ml bottles containing 10 mg Nimodipin; Bayer–Germany), the second group was treated with 2.5 µg/kg/day of TRH (2 ml ampoules each containing 200 micrograms of protein Tyrotropin Releasing Hormone; Roche Products Limited–England), intraperitoneally. The third group (10 rabbits) was treated with 1 mg/kg/day GBE (Tebonin p.i. ampoules containing 50 mg Ginkgo–biloba–Blattern (50:1) equivalent of 12 mg ginkgo glycoside; Dr. Willmar Schwabe Karlsruhe–Germany) intraperitoneally, during the first 7 days. The fourth group (10 rabbits) was treated with nimodipine, TRH, GBE intraperitoneally at the same time. The last group (10 rabbits) was kept as a control group and received only saline. All the rabbits were admini-

tonic NaCl for 24 hours and a catheter was inserted into the bladder until bladder control become automatic.

The modified Tarlov score was used to assess post injury recovery everyday The Modified Tarlov Score is based on a recovery scale of 0 through 5 with respect to the hind limbs: 0, no movement; 1, perceptible movement; 2, active movement; 3, able to support weight; 4, walks with deficit; or 5, normal gait [16,17,18]. 21 days following compression injury all animals were killed by an IV infusion of overdose sodium pentobarbital. Spinal cord segments to include 1 cm above and below the traumatic area were removed for pathological examination. The fixed cord segments were sectioned serially and examined by light microscopy. Serial paraffin sections stained with H.E. were evaluated. Sections with the most extensive and severe lesions were then graded according to the following criteria (Figures 1–3).

Grade 0: no histological evidence of injury.

Grade 1: mild injury minor signs of injury are present such as focal accumulation of hemosiderin–laden macrophages, foamy macrophages, or extracellular pigment; loss of nerve cells with vacuolisation of white matter, primarily involving the base of the posterior columns.

Grade 2: moderate injury; loss of gray matter with central cord cavitation, the cystic lesions may contain foamy macrophages.

Gared 3: severe injury: extensive cystic necrosis involving both gray and white matter. The residual spinal cord if present is represented by a thin peripheral rim of gliotic white matter.

RESULTS

Neurological function of the hind limbs was graded with the modified Tarlov score. There were no significant differences among neurological scores of the groups (Group 1,2,3) but Group 4 was better than others (Table 1). Similarly histoatological scores did not differ significantly among the five treatment groups (Table 2).
Figure-I: Garade I histopatalogical changes: slight edema and localized vacualisation in the gray matter (Hematoxylin and Eosinx25).

Figure-II: Garade II changes with cystic destruction of the gray matter (H&Ex25).

Figure-III: Garade III changes widespread destruction and fibrosis effecting both the gray and white matter (H&Ex25).
Table-I: The Distribution of The Cases according to Tarlov Score and Treatment Type

<table>
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<th>Tarlov score</th>
<th>Nimodipine</th>
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<th>GBE</th>
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Table-II: The Distribution of The Histological Evidence according to Treatment Type

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DISCUSSION

Some models for studying spinal cord trauma have been described. The neurophysiological parameters that can be monitored in these models include blood pressure, spinal cord blood flow, somatosensory-evoked potentials, electroencephalogram, blood gases and vital signs. In addition, histological preparations for histochemical fluorescence and neuronal tissue stains can be made from cryostat sections of the cord tissue\(^{[19,20]}\). Trauma to spinal cord triggers a progressive series of auto-destructive events that lead to varying degrees of tissue necrosis and paralysis, depending on the severity of the injury. Pathological changes include petechial hemorrhage progressing to hemorrhagic necrosis, lipid hyphalysis with subsequent prostaglandin and leukotriene (eicosanoid) formation, loss of Ca\(^{2+}\) from the extracellular space and loss of K\(^{+}\) from the intracellular space, ischemia with consequent decline in tissue oxygen tension and energy metabolites, and development of lactic acidosis, edema, inflammation, and neu-}

ronophagia by polymorphonuclear leukocytes (PMN).

Calcium channel blockers have been studied extensively in the brain and have shown promise in promoting dilation of cerebral vessels and an increase in cerebral blood flow in normal and pathological states\(^{[19]}\). The current study was undertaken to determine if a calcium channel blocker produces similar changes in the spinal cord and compare effects of other agents (TRH and GBE) used in the treatment of CNS injury.

TRH have beneficial effects on spinal cord blood flow in spinal cord injury. Ginkgo biloba extract (GBE) in animals has shown positive influence of the extract on brain edema, brain electrolyte balance, cerebral hipoxia tolerance, cerebral perfusion, and metabolic turn over, but so far, this extract was not used in spinal cord injury.

The purpose of our this study was to determine the most effective drug (nimodipine, TRH, GBE) in neurological recovery in
this study. There was no significant differences among groups 1, 2, and 3 and it was found that better results were observed when all three drugs (Group 4) were used in combination (Mann Whitney U test p<0.05). Since all three agents have different modes of action their synergistic effect was not unexpected.

KAYNAKLAR


