EFFECT OF EGb 761 IN EXPERIMENTAL ACUTE SPINAL CORD INJURIES

ÜMIT KEPOĞLU,HAYATI ORTAESKINAZI

BAKIRKÖY STATE HOSPITAL OF PSYCHIATRIC AND NEUROLOGICAL DISORDERS
NEUROSURGERY DEPARTMENT
ISTANBUL/TURKEY

SUMMARY:

The treatment of spinal cord injuries is still a challenging field of Neurosurgery. To determine the effect of EGb 761 on experimental acute spinal cord injuries 26 wistar albino rats were used. The spinal cords were clipped at C9 level with Yasargil Aneurysm clips (Aesculap FE 752K) in all rats. Soon after the operation,a dose of 100 mg/kg EGb 761 was given intraperitoneally to the experiment group. The motor performances were evaluated and compared. The rats were sacrificed on the first day after the operation. The lesioned spinal cords were removed including three upper and lower levels and placed into 10 % formalin for light microscopic evaluations. After the fixation the spinal cords were embedded in paraffin. For electron microscopic evaluations the proximal and distal parts of lesions were fixed in milania phosphate buffer at 7,4 pH. The samples were refixed in 1 % osmic acid and embedded in amadit. Motor performance measurements revealed no statistically significant differences between the EGb 761 group and the control group. Although the spinal cord injury was found to be less severe in EGb 761 group than the control group in histopathologic and light microscopic examinations, there were no significant differences in ultrastructural comparisons.

Material and methods:

26 Wistar Albino young rats were used in this study. The average body weight of rats were 289.56 +/- 43.89 before trauma. Sixteen rats were male and 10 were female.
Anesthesia; The operative procedures were performed under general anesthesia. 2.5% solution containing 10mg/g. sodium thiopental was applied intraperitoneally.
Trauma; the Yaşargil anevrizma clip AEUSCULAP FE 752 was used to produce standard trauma. The closing force of clip was 192g, with 9mm length and 6.8 mm opening.
Procedure: After 20 minute general anesthesia, rats were replaced on a platform in decubitis ventralis position. The area (2x2 cm2) between lower cervical and upper dorsal was shaved and cleaned with an antiseptic. The incision was performed in subcutan tissue at the C9 level, the laminas were revealed by dissection of the paraspinal muscles. The clipper was applied extradurally for 30 second. After application of clipper, tonic spasm was observed at lower extremities and sacral section. The

impacts produced flask paraplegia only after application of clipper. When the clipper removed, the circular hemorrhagical contusion fields (1mm) were observed at this area. The rats with ruptured dura were sacrificed during procedure. Later the paravertebral muscles were sutured with 3/0 silk suture.

Application of Drugs; Soon after operation, a dose of 100mg/kg Egb 761 was given intraperitonally to the experimental group.

Postoperative Care; The rats were replaced to the cages containing four rats at 24 degrees. The dose of 3cc, 9 % NaCL solution was given intraperitonally to prevent hypertension caused by pentotalin.

Evaluation of motor functions; The motor performance was evaluated by means of "Sloppy Field Method" . The platform which is covered with rubber (with 2mm width, 2mm length 90 gradient) was prepared for evaluation. The rats were left on this platform for following . In every 5 seconds , the gradient of platform decreased for 5 degrees until the rats could stay on the platform on the maximum gradient. The measurements were completed by reducing the gradient of platform for 2.5 degrees when the failure to stay on platform was observed. These measurements were performed for both groups before operation and one day after operation. The comparison of pretrauma and post trauma measurements for both groups were accomplished with Student t Test.

Preparation of Samples; The rats were sacrificed one day after operation by injection of high level sodium tiopenthal. The spinal cords between three level upper and lower of C9 level were removed by laminectomy and replaced in 10% formalin for light microscopic evaluations. After the fixation the spinal cords were embedded in paraffin. For electron microscopic evaluations, the proximal and distal parts of lesions were fixed in milonig phosphate buffer at 7.4 pH. The samples were refixed in 1% osmic acid and embedded in aradit.

Histophatologic Evaluations; The samples were prepared from electron microscopic blocks and dyed with toluidin. After the examination of the samples under light microscopy and determination of areas for electron microscopic examination, the section with 200- 400 A width were prepared from the same blocks. These samples were dyed with uranil acetate and pb citrate and examined under electron microscopy. The140 samples (3-4 micron) were prepared from paraffin blocks by means of rotary microtome.

FINDINGS EVALUATION OF MOTOR PERFORMANCE

The motor performance measurement which was obtained before trauma and 24 hours after trauma were evaluated statistically with Student t test.

The global motor performance for control group was 34,75+/- 3,054 degree. The global motor performance for EGb 761 group was 39,5+/- 3,84 degree. The variation for control group was 9, 3125. After comparison of control and EGb, the differences were 0,68471 (p). In the evaluation of motor performances for control and EGb group, the differences between these groups were not statistically significant (p= 0,68).

HISTOPATHOLOGIC EVALUATION RESULTS

The rats were sacrificed soon after the evaluation of motor performance. In the microscopic evaluation of the samples which obtained from dura matter and spinal cord of these rats, a lesion with 3mm thickness were observed. The lesion were more evident in

control group compared to EGb group. It was observed that the spinal cord without lesion preserved its normal color. In the control group, the central canal destruction, cystic hemorrhage in central canal and gray matter were observed in the proximal of the lesion under light microscopy. There were petechiae in white matter, especially in the dorsal area. In the control group lesions, the destructive central canal and fresh hemorrhage in gray matter were observed. There were petechiae and cystic degeneration in white matter. In neural cells of anterior horn, the border of nuclei were disappeared and exhibited amfofilic view.

There were neural cells destruction. In the distal area, the central hemorrahagic areas and central canal destructions were more evident compared to proximal area. In white matter, there were cystic occurrence in different sizes. The number of petechiae were less compared to lesion area. In EGb group, the proximal area had better view compared to the lesion and the distal areas

almost in every sample. In the EGb group lesions, hemorrhage were observed in the dorsal area.

ELECTRON MICROSCOPIC EVALUATION

In electron microscope evaluation of control and EGb group, the samples were obtained from the proximal area, the distal and lesion area were examined. It is reported that trauma influence the similar structure, but in the proximal and distal area had less damage compared to lesion areas. In both group, the proximal area were effected less than the distal and lesion areas. In the control group, in proximal lesion areas, there were loss of myelin sheath in axons and constriction in axon and vacuole formation between axon and myelin sheath. Although, any change was not seen in capillary endothelia, the capillary stase, extravasate fluid and erythrocytes were rarely observed. An evident vazospasm was not seen in capillary. Vacuolization, partial lipid accumulation and change in astrocyt and oligodentrocyt structures were observed in the capillary. In lesion area, the loss of myelin sheath, axon constriction, vacuole formation between myelin sheath and axon, loss of myelin sheath in axons were observed. Extravasate fluid and erythrocytes were commonly observed. There was destruction in asrocyts and oligodentrocyts.

In EGb group, evaluation results of the proximal, the distal and lesion areas were similar to control group. However, in the distal area there was decrease in vacuole formation between axon and myelin sheath and stase formation. In conclusion, it was found that the trauma effected less the proximal part of lesion and the similar type injury was observed in the distal area and the lesion under electron microscopy.

DISCUSSION

Acute spinal cord injuries has been investigated in two phase. First phase contains neural and vesicular injury in medulla spinal compression areas with mechanical effect. In this phases, injury could be reduced with prevention, but it could not be treated. Secondary phase contains injury which occurs in trauma area, adjacent tissue with phisyopathologic mechanism. In spinal injury studies, the basic target is to reduce neural injury by interfering the secondary phase. In experimental studies related to spinal cord injuries, dogs, cats and rhesus monkeys have been used. In present study , we used Albino rats because of their advantages features. Moreover, We used one year old rats because medulla spinal injury is

seen in 16 -25age group human. In our study, two rats died in operative period, three rats died in postoperative period because of respiratory arrest and one is excluded form studies due to its damaged dour. In the acute spinal cord injury studies, it was concluded that ischemia in white matter in secondary phase could be reduced with three pharmacologic methods;

1. Calcium canal blockage
2. To prevent the formation of arachidonic acid vasoactive prostanoic metabolites
3. To prevent lipid prexidation which is activated by free oxygen radicals

In spite of other mechanisms which effect ischemia formation, to prevent the progressive ischemia with these three mechanisms was the basic target. Hypothesis of Hall and Wolf about pathogenesis of ischemia after trauma is still valid. In this hypothesis, it is thought that the basic mediator which reduce blood flow is microvascular lipid peroxidation. The blood flow rate in the medulla spinal reduce because of two reason. One of them is petechial hemorrhage in gray matter. The other is accumulation of Ca+2 in intracellular area. The phospholipase activation begin in this phase. An increase in arachidonic acid which is an membrane phospholipid was determined in cat medulla spinal 5 minute after trauma. Prostanoids PG F2 alfa releasing from vessel wall and TxA2 releasing from thrombocyts cause local ischemia by means of vasoconstriction and thrombocyte aggression. The damage occurs due to free radical formation and lipid peroxidation activated by local ischemia . Free radical formation during prostanoid biosynthesis was reported by several investigators . Iron (hemoglobin), copper complex derived from petechia cause oxidative tissue damage by catalyzing the free radical product and lipid peroxidation. Early and dramatic prostanoid formation increase PGF2 and thrombin formation with positive feedback effect by accelerating phospolipase A2 activation. All these occurrences come into being in gray matter of central segment of medulla spinal after trauma. In this area, petechia cause formation of PGF2 alfa and TXA2, hemoglobin containing iron and free radical and lipid peroxidatiton.

Lipid peroxidations spread from gray matter to white matter. The damage on axon and myelin becomes irreversible because of ischemia caused by microvascular destruction in white matter . As a conclusion; in acute spinal cord trauma, the main goal is to prevent formation and increase of free oxygen radicals which lead to neurovascular lipid peroxidation, increase in vasoactive prostanoid formation, ischemia spreading from gray matter to white matter. For this purpose, megadose steroid and high level dose naloxen, vitamin C and E (alpha tocopherol), selenium and enzyme Q is used to prevent lipid peroxidation . We applied EGb 761 which is PAF antagonist and free radical scavenger in acute medulla spinal injury for this purpose. Although EGb 761 has been used in Chinese medicine for 5000 years, it has been used in modern medicine since 1965. The compound is consisted of 5 different subgroup with the main group. Flavonoids, which are low molecular compounds, have free radical scavenger, membrane stabilization and enzymatic features. Terpenids are the second main group in the compound. Gincogolids has PAF antagonist features. The rest of these groups composed of bilobalids and organic acids. EGb is applied on rats retina after lipid peroxidation formed by tissue damage. Pharmacologic measurements revealed its primer free radical (superoxideAnion O2, and hydroxyl radical OH) scavenger effects. It was confirmed that EGb has same effect on seconder free radicals (lipoperoxiden ROO) but in less degree. Later this effects was

exhibited on rats whose central nervous system is damaged by trauma and TET (triethyltin) toxicification. In this study, it is reported that the extre inhibits neuro toxic in antihypoxic edema. This effect occurs because of its PAF antagonist, free radical scavenger and prostacycline synthesis activation features . In experimental SAH model which is performed on rabbits, it is found that EGb application is effective against to vasospasm in acute period. Its PAF antagonist effect and interactions with EDRF (endogen vascular releasing hormone), prostacycline, Comt (catechol-o-methyle transferase, MAO (monoamineo oxidase) is showed in this model. In several studies, the free radical scavenger features of EGb and its antihypo toxic effects is revealed . In the present study EGb was applied systematically, and the effective doses were chosen in the literature. The motor performance measurement which is obtained before trauma and 24 hours after trauma were evaluated statistically with Student t test. In the evaluation of motor performance for control and EGb group, the differences between these groups were not statistically significant (p= 0,68). In microscopic scanning performed 24 hours after trauma, it was found that the application of clipper originate damage in all groups, but the damages in EGb group were less than damages in control group significantly. Although, any significant differences between these groups were observed under light microscope, in EGb group there was positive differences in central hemorrhage sizes and density of the cell with nucleus. In electron microscope evaluation of control and EGb group, any ultra structural differences between these groups. However, there were an decrease in vacuole formation between axon and myelin sheath and intervascular stase formation especially in distal part of lesion in EGb Group. The histopathologic findings of our studies agree with other studies findings. It was found that the trauma effects less the proximal part of lesion and the similar type injury was observed in the distal area and the lesion under electron and light microscopy.

CONCLUSION

After motor performance measurement accomplished by means of sloppy field method in rats that underwent acute medulla spinal injury, no significant differences were found statistically between EGb 761 group and the control group. Although the medulla spinal injury was observed less in EGb 761 group compared to control group in histo-pathological and microscopic measurements, it was found that between these groups, there was no significant difference in ultra structural comparisons. In the light and electron microscopic measurements, it was determined that the trauma effects the proximal medulla spinalis more significantly compared to distal medulla spinalis in both these groups.

READ CAREFULLY THE INSTRUCTIONS ON REVERSE SIDE
DEADLINE: 15 NOVEMBER 1996

ÉFFECT OF EGb 761 IN EXPERIMENTAL ACUTE SPINAL CORD INJURIES

ÜMIT KEPOĞLU,HAYATI ORTAESKINAZI
BAKIRKÖY MENTAL HOSPITAL
ISTANBUL/TURKEY

The treatment of spinal cord injuries is still a challenging field of Neurosurgery.
To determine the effect of EGb 761 on experimental acute spinal cord injuries 26 wistar albino rats were used.The spinal cords were clipped at C9 level with Yasargil Aneurysm clips (Aesculap FE 752K) in all rats. Soon after the operation,a dose of 100 mg/kg EGb 761 was given intraperitoneally to the experiment group.The motor performances were evaluated and compared .The rats were sacrificed on the first day after the operation. The lesioned spinal cords were removed including three upper and lower levels and placed into 10 % formalin for light microscopic evaluations.After the fixation the spinal cords were embedded in paraffin. For electron microscopic evaluations the proximal and distal parts of lesions were fixed in milania phosphate buffer at 7,4 pH. The samples were refixed in 1 % osmic acid and embedded in amadit. Motor performance measurements revealed no statistically significant differences between the EGb 761 group and the control group. Although the spinal cord injury was found to be less severe in EGb 761 group than the control group in histopathologic and light microscopic examinations,there were no significant differences in ultrastructural comparisons.

Indicate here what kind of presentation you prefer (see also page 24):

- [x] oral
- [ ] poster
- [ ] video VHS
  - [ ] PAL
  - [ ] SECAM
  - [ ] NTSC

SUBJECT AREA
(please check one)

- [ ] 1. cerebrovascular
- [ ] 2. tumour
- [ ] 3. basics
- [ ] 4. stereotactic and functional
- [ ] 5. pediatric
- [ ] 6. trauma and critical care
- [x] 7. spine
- [ ] 8. peripheral nerves
- [ ] 9. infection and parasitic
- [ ] 10. hydrocephalus
- [ ] 11. technology
- [ ] 12. imaging
- [ ] 13. miscellaneous