Lazaroid Attenuates Edema by Stabilizing ATPase in the Traumatized Rat Brain

Ramazan Durmaz, Güngör Kanbak, Fahrettin Akyüz, Serap Isiksoy, Ferruh Yücel, Mine Inal, Esref Tel

ABSTRACT: ***Objective:*** The aim of the present study was to determine the potential therapeutic value of the lazaroid U-83836E on blood brain barrier (BBB) breakdown and edema with respect to the changes in the synaptosomal Na^+/K^+ and Mg^{2+}/Ca^{2+}- adenosinetriphosphatase (ATPase) activities, tissue malondialdehyde levels and the neuronal viability in the rat brain subjected to cerebral trauma. ***Methods:*** Traumatic brain injury (TBI) was introduced by applying a 75 gm. cm force to the right parietal cortex using the weight-drop method. The first set of animals was used for determining time course changes of the synaptosomal Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase and the malondialdehyde levels and were sacrificed 2, 6 and 24h after lesion production. A group of the animals was treated with U-83836E proir to TBI and sacrificed 24h after cerebral injury. A second set of animals was used for evaluating the alterations in BBB disruption and tissue water content and were sacrificed 2, 6 and 24h after lesion production. Two groups of animals were treated with U-83836E and sacrificed after 2 and 24h following TBI. U-83836E was given intraperitoneally thirty minutes before trauma at a dose of 10 mg/kg. Neuronal necrosis was also evaluated in the groups of U-83836E and physiological saline-treated animals. ***Results:*** Extravasation of Evans blue into the traumatized hemisphere was maximum at 2h ($p<0.001$) and returned close to the control levels at 24h after TBI ($p>0.05$). Edema had developed progressively over time and reached the maximum degree of 2.1 % ($p<0.001$) at 24 h. U-83836E showed no effect on the BBB breakdown and the tissue water content at 2h and still had no effect on the BBB breakdown after 24h following the trauma ($p>0.05$), although it reduced edema after 24h ($p<0.01$). The losses of Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase activities were found as 39.5 % ($p<0.001$) and 29.4 % ($p<0.01$) of the control value, respectively, and remained at the decreased levels throughout the experiment. Malondialdehyde level continued to increase over time reaching up to 209 % ($p<0.001$) of the control value 24h after TBI. Both ATPase activities were improved to near control values ($p>0.05$) by the effect of U-83836E. U-83836E inhibited the increase of lipid peroxidation ($p<0.001$) and also salvaged neuronal necrosis ($p<0.05$). ***Conclusion:*** U-83836E given prophylactically after cerebral trauma appears to reduce edema, possibly by inhibiting increases in lipid peroxidation and by stabilizing ATPase. Further studies are recommended to verify the similar effects of the brain penetrating lazaroids when they are given after trauma.

RÉSUMÉ: Le lazaroïd (U-83836E) atténue l'oedème en stabilisant l'activité Na^+/K^+ et Mg^{2+}/Ca^{2+}-ATPase dans le cerveau de rat traumatisé. ***Objectif***: Le but de cette étude était de déterminer la valeur thérapeutique potentielle du lazaroïd U-83836E sur l'effondrement de la barrière hémato-encéphalique et l'œdème par l'évaluation des changements dans l'activité Na^+/K^+ et Mg^{2+}/Ca^{2+}-ATPase synaptosomale, les niveaux de malondialdéhyde tissulaire et la viabilité neuronale dans le cerveau de rats soumis à un traumatisme cérébral. ***Méthodes***: La lésion traumatique était induite par la méthode de chute d'un poids appliquant une force de 75 g cm au niveau du cortex pariétal droit. Le premier groupe d'animaux a servi à déterminer les changements dans le temps au niveau des taux synaptosomaux de Na^+/K^+ et Mg^{2+}/Ca^{2+}-ATPase et de malondialdéhyde tissulaire et ils ont été sacrifiés 2, 6 et 24 heures après avoir subi le traumatisme. Un premier groupe d'animaux a été traité par le U-83836E et sacrifié 24 heures après le traumatisme. Un second groupe d'animaux a servi à évaluer les altérations de la barrière hémato-encéphalique et le contenu tissulaire aqueux. Ils ont été sacrifiés 2, 6 et 24 heures après le traumatisme. Deux groupes d'animaux ont été traités par le U-83836E et sacrifiés soit 2 heures ou 24 heures après la lésion. Le U-83836E a été administré par voie intrapéritonéale trente minutes avant le traumatisme à la dose de 10 mg/k. La nécrose neuronale a été évaluée chez le groupe d'animaux ayant reçu le U-83836E et chez le groupe ayant reçu du soluté physiologique. ***Résultats***: L'extravasation du Bleu Evans dans l'hémisphère traumatisé était à son maximum 2 heures après le traumatisme ($p<0,001$) et rejoignait celle des témoins après 24 heures ($p<0,05$). L'œdème apparaissait progressivement et était à son maximum de 2,1% ($p<0,001$) après 24 heures. Le U-83836E n'avait aucun effet sur l'altération de la barrière hémato-encéphalique et le contenu tissulaire aqueux après 2 heures et n'avait toujours pas d'effet sur l'altération de la barrière hémato-encéphalique après 24 heures ($p<0,05$), bien que l'œdème était diminué chez ces animaux après 24 heures ($p<0,01$). Les pertes d'activité Na^+/K^+ et Mg^{2+}/Ca^{2+}-ATPase étaient de l'ordre de 39,5% ($p<0,001$) et 29,4% ($p<0,01$) de la valeur témoin respectivement et sont demeurées à des niveaux abaissés pendant toute la durée d'observation. Le niveau de malondialdéhyde a continué à augmenter, atteignant 209% ($p<0,001$) de la valeur témoin 24 heures après le traumatisme. Les deux activités ATPase étaient améliorées par l'effet du U-83836E, à des niveaux voisins de ceux des témoins ($p<0,05$). Le U-83836E a inhibé l'augmentation de la peroxydation lipidique ($p<0,001$) et protégé de la nécroses neuronale ($p<0,05$). ***Conclusion***: Le U-83836E administré de façon prophylactique après un traumatisme cérébral semble diminuer l'œdème. Il est possible que ce soit dû à l'inhibition de l'augmentation de la peroxydation lipidique et à la stabilisation des ATPases. Des études plus poussées devraient être entreprises afin de vérifier les effets des lazaroïds qui pénètrent dans le cerveau, quand ils sont administrés après un traumatisme.

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Brain edema associated with the traumatic brain injury (TBI) is a clinical phenomenon that exacerbates delayed cell death and may threaten life for some patients. Edema formation following a primary cerebral insult is seen as a sequela of secondary

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molecular and physiological events, including N-methyl-D-receptor activation, release of oxygen free radicals and fatty acids, lipid peroxidation, acidosis, disturbance of ionic gradients and the breakdown of the blood-brain barrier (BBB).[1-4] Na^+/K^+ and Mg^{2+}/Ca^{2+}-adenosinetriphosphatase (ATPase) are membrane-bound enzymes, which regulate intracellular Na^+, Mg^{2+} and Ca^{2+} concentrations. In presynaptic vesicles of the nerve terminals there is a high activity of Ca^{2+}-ATPase and the phospholipid content of the synaptosomal membrane is essential for the activity of Na^+/K^+-ATPase.[5,6]

Klatzo[7] has classified brain edema into two main forms; vasogenic and cytotoxic. Vasogenic edema is defined as the extracellular space enlargement induced by BBB breakdown. Cytotoxic edema is characterized by intracellular water accumulation resulting from the cessation of ionic pumps, in turn caused by an interruption of energy supply. However, severe cellular injury is required for persistent cytotoxic swelling of the cells.[8] The 21-aminosteroids (lazaroids) are potent lipid peroxidation inhibitors that have been demonstrated to protect neuronal tissue and the BBB from the peroxidative damage in a number of experimental models.[9-14] They are effective in lowering post-ischemic and post-traumatic tissue-water content,[15,16] possibly by attenuating BBB breakdown, since they are not expected to cross the BBB.[14] U-83836E [(-) 2-((2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl)methyl)-3,4dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol dihydrochloride] is a second generation lazaroid that combines the amino functionalities of the 21-aminosteroids with the antioxidant ring of α-tocopherol and has access into the brain parenchyma.[12] We aimed to assess the potential therapeutic value of U-83836E on BBB disruption and the edema following traumatic brain injury. In particular, we examined changes in synaptosomal Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase activities, lipid peroxidation and neuronal viability.

Materials and methods

Experimental protocol

A total of 104 adult male Spraque-Dawley rats weighing 250-320 g were studied. The animals were anesthetized by intraperitoneal injection of a mixture of ketamine hydrochloride (60 mg/kg) and xylasine (12 mg/kg). Atropine sulfate (10 mg/kg) was also given in order to prevent pulmonary secretions. The trachea was intubated with a 14-gauge angiocatheter and the lungs were mechanically ventilated with a mixture of oxygen and room air using a small rodent ventilator (Ugo Basile Biological Research Apparatus). Throughout the experiment the tidal volume was 1.8 ml and the ventilation setting was adjusted to maintain arterial CO_2 and O_2 pressure within the desired limits. Arterial blood samples were taken from the left ventricle by a tuberculin syringe. The right femoral artery was catheterized for continuous blood pressure recording. The experiment was performed under normotensive conditions and the rectal temperature was maintained by an automated heatlamp at approximately 37 ºC. Blood gases are shown in the Table.

We designed a new device to semi-automate the experimental trauma method of Feeney.[17] After a craniotomy had been made, brain injury was introduced using an electrical circuit switch which caused a 10 g brass rod to drop from a distance of 7.5 cm, through a plexiglass guide tube, onto the exposed dura over the right parietal cortex.

The first set of animals was used to determine the time-course of tissue changes of the Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase activities and malondialdehyde (MDA) in the traumatized hemisphere. A group of the animals (n=6) undergoing craniotomy operation alone was used as a control and sacrificed after 24 h. Three groups of animals (each n=9) were killed at various time points; 2, 6 and 24h after cerebral injury. U-83836E was dissolved in 0.9 % saline solution with a final concentration of 12.5 mg/ml. Vehicle was saline alone. Thirty minutes prior to trauma, a group of animals was administered a single intraperitoneal dose of 10 mg/kg of U-83836E (n=9) or vehicle (n=7) and sacrificed 24h after cerebral injury.

A second set of animals was used for determining the time-course of BBB breakdown and tissue water-content changes in the traumatized hemisphere. Sham-operated animals (n=6) were sacrificed after 24h and served as a control. Three groups of animals (each n=7) were sacrificed at various time points; 2, 6 and 24h after cerebral injury. Two groups of animals (each n=7) were treated with U-83836E at a dose of 10 mg/kg or vehicle (n=6) 30 minutes prior to trauma and sacrificed 2 and 24h after

Table: Data for blood pH and Gas levels, A) in the first, and B) in the second set of animals. Values are given as mean ± SEM.

A						
Groups	**Sham**	**2 h**	**6 h**	**24 h**	**U83836E-treated**	**Saline-treated**
pH	7.47±0.02	7.41±0.02	7.44±0.03	7.46±0.02	7.45±0.02	7.49±0.06
P_aCO_2 mm Hg	35.3±1.4	33.5±1.2	37.4±1.2	34.7±1.2	35.9±2.0	35.5±1.1
P_aO_2 mm Hg	113±9.7	119±12.0	123±11.0	157±18.5	140±13.2	142±13.5

B							
Groups	**Sham**	**2 h**	**6 h**	**24 h**	**U83836E-treated/2 h**	**U83836E-treated/24 h**	**Saline-treated/24 h**
pH	7.34±0.10	7.48±0.02	7.54±0.03	7.40±0.14	7.46±0.15	7.39±0.02	7.58±0.02
P_aCO_2 mm Hg	33.0±1.1	36.5±1.2	38.6±2.1	35.2±1.2	36.1±1.2	36.9±1.0	37.5±1.3
P_aO_2 mm Hg	164±14.5	124±15.1	118±11.0	139±17.3	152±20.1	125±12.2	112±10.4

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cerebral injury. After animals had been reanesthetized, Evans blue dye (2%, 5 ml/kg) was given intravenously an hour before sacrifice. Approval for the study was granted by The Committee on Animal Experiments of the Medical Faculty of Osmangazi University and all experimental procedures were performed in accordance with the National Institute of Health's Principles of Laboratory Animal Care.

Rats of set 1 and 2 were perfused with 200 ml physiological saline through the left ventricle using an infusion pump. Animals were killed by an overdose of the mixture of anesthetic drugs described above or an additional dose in the second set of animals. After decapitation, the brains were immediately removed, frozen in liquid nitrogen and stored at -80°C.

Histological examination was performed on a third set of animals. One group (n=4) was treated with physiological saline, the other (n=4) with U-83836E 30 minutes prior to trauma. Before sacrifice at 24h, the brains were fixed with 200 ml buffered formalin (2%), after 50 ml physiological saline perfusion through the left ventricle. After the removal of the brains, coronal sections were made and tissue blocks 2 mm in thickness were excised and fixed in 10% buffered formalin. The sections were stained with hematoxylin and eosin, and acid fuchsine.

Morphometry

In the traumatized hemisphere, a coronal section was made through the center of the lesion. The volume fraction of the dead neurons of the cortical tissue was determined by "point count" analysis at the light microscopic level, as previously applied.[18,19] Counts were made from the surface to the base of the neocortex, using three adjacent frames in which a lattice (quadratic test frame) was superimposed randomly on a microscopical field sampled within the section, with the other frames evenly spaced. To calculate the volume fraction of acid-fuchsine positive neurons which are presumed to be moribund cells,[20,21] the number of points of acidophilic neurons throughout the entire cortical layer was divided by the number of points on the lattice.

Evaluation of edema and BBB breakdown

The water content in the injured hemisphere was calculated using wet weight to dry weight ratios as described previously.[22] Subsequently, Evans blue content of the dried tissue of the traumatized hemisphere was measured using a spectrophotometer (Schimadzu UV-1201) at the wavelength of 660 nm.[23] Results were presented as μg / g dried weight.

Preparation of brain synaptosomes and assay of membrane-bound ATPase activities

After thawing the brain at 4°C, two blocks of samples of 3 mm thickness were taken by coronal section from the center of the area of trauma and used for the determination of the Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase activities and MDA levels. Preparation of synaptosomes was according to the method of Braughler and Hall.[24] In these preparations, the Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase activities were assayed as previously described[25] and the results were expressed as U/mg protein.

Lipid peroxide assay

Tissue peroxide was measured by the colorimetric reaction of thiobarbituric acid-reactive substances in the presence of MDA at 532 nm, according to the method of Ohkawa et al.[26] The results were expressed as nM/mg protein. Proteins were determined by using a commercial protein detection kit based on the method of Lowry et al.[27]

Statistical analysis

The data were normally distributed and the data analysis was performed using statistical analysis software.[28] The significance of the differences between the groups was ascertained by one-way analysis of variance, followed by the Bonferroni test for multiple comparisons. In order to compare the neuronal loss between the vehicle- and the U-83836E-treated groups, a one-tailed t test was used. Statistical significance was defined by a p value <0.05.

Results

When compared to the control, extravasation of Evans blue into the traumatized hemisphere was maximum at 2h ($p<0.001$), started to decline at 6h ($p<0.01$) and returned close to control levels at 24h after TBI ($p>0.05$, Figure 1). No significant difference in the dye concentration was observed between 6 and 24h ($p>0.05$), but there was a difference, which was observed between 2 and 6h ($p<0.01$). The water content of the traumatized hemisphere increased in a time-dependent manner and reached maximal levels at 24h (Figure 2A). Compared to the control, TBI caused an increase in the water content of the injured hemisphere; 0.5% ($p>0.05$) at 2, 1.3% at 6h ($p<0.001$), and 2.1% ($p<0.001$) at 24h, respectively. The increases in the edema at 2 to 6 and 6 to 24 hours were also significant ($p<0.01$). Pre-treatment of animals with the lazaroid U-83836E did not influence BBB breakdown at 2 and 24h or edema at 2h after TBI. On the other hand, the agent attenuated edema at 24h compared to the results of saline-treated animals ($p<0.01$, Figure 2B).

Loss in synaptosomal Na^+/K^+-ATPase activity in the rat brain homogenate of the traumatized hemisphere occurred in the early period following the trauma and remained close to this level at 6 and 24h after TBI; 39.5% ($p<0.001$) at 2h, 37.9% ($p<0.01$) at 6h, and 37.8% of the control ($p<0.01$) at 24h post-trauma (Figure 3A). Similarly, a decrease in the Mg^{2+}/Ca^{2+}-ATPase activity was observed during the early hours after TBI, with a temporary elevation at 6h and a maximum decrease at 24 h. Losses in the activity of Mg^{2+}/Ca^{2+}-ATPase were 29.4% ($p<0.01$) of control at 2h, 16.7% ($p>0.05$) at 6h, and 42.5% ($p<0.001$) at 24h post-trauma (Figure 3A). The decrease in both the Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase activities was restored to control values by U-83836E given prophylactically (Figure 3B).

Malodialdehyde content of the injured tissue increased, reaching maximal levels at 24h post-trauma (Figure 4A). Levels were 122% ($p>0.05$) of the control at 2h, 136% ($p<0.01$) at 6h, and 209% ($p<0.001$) at 24h post-injury. The U-83836E-treated animals had a significantly lower MDA content in the injured tissue than those of the saline-treated animals ($p<0.001$, Figure 4B).

Twenty-four hours after trauma, the TBI region contained hemorrhage and some cavitations. Microscopically, there was edema, congestion, hemorrhage and infarction in the areas of the impact side, including the thalamus and subthalamus. The infarcted area was pale and edematous including disintegrated cells (Figure 5A). There were injured neurons in the cortical

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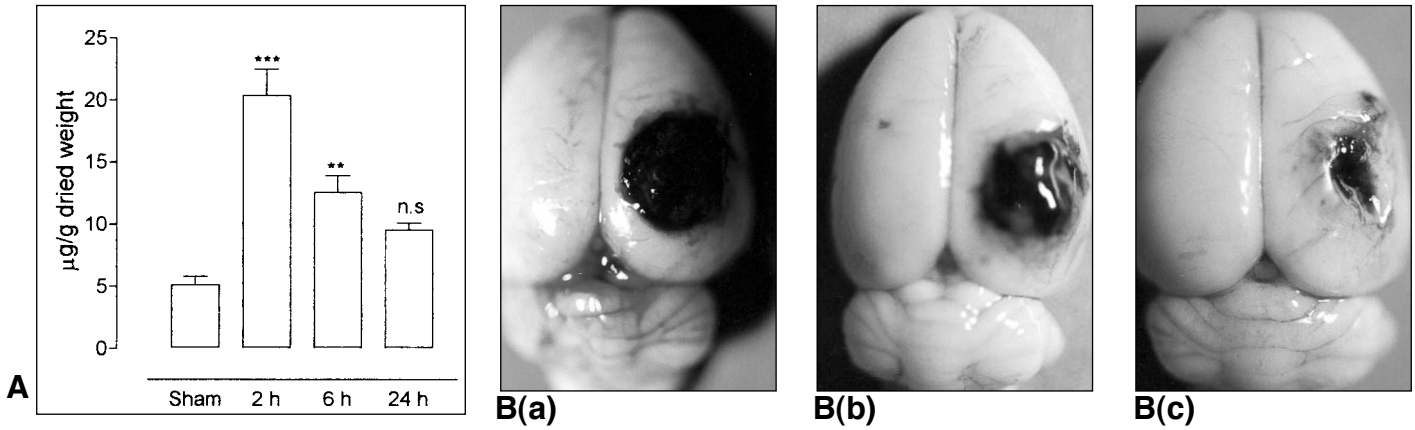

Figure 1: *A) The extravasation of Evans blue dye into the traumatized hemisphere. Each bar shows the mean Evans blue content of traumatized hemisphere ± SEM. Asterix denotes the significant difference from control. n.s: not significant, ** Significant at p<0.01 and *** p<0.001.* ***B)*** *Gross appearance of Evans blue extravasation into injured brain a) at 2h, b) 6h and c) 24h after trauma.*

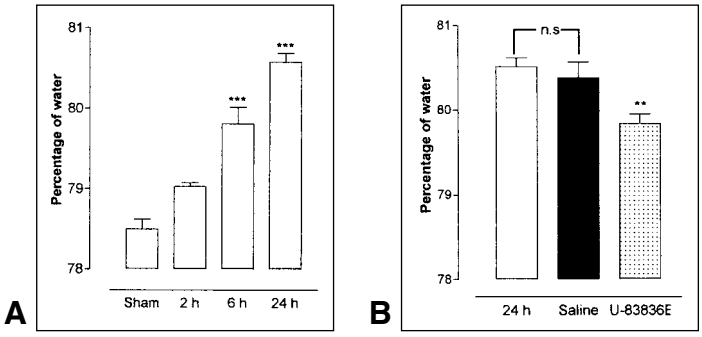

Figure 2: *Graph shows* ***A)*** *an increase in the water content of the traumatized hemisphere when compared to control and,* ***B)*** *attenuation of 24h post-traumatic edema by the effect of U-83836E when compared to saline-treated group and also untreated group of animals (which were those sacrificed 24h after trauma). Values are given as mean ± SEM. Asterix denotes the significant difference. n.s: not significant, ** Significant at p<0.01 and *** p<0.001.*

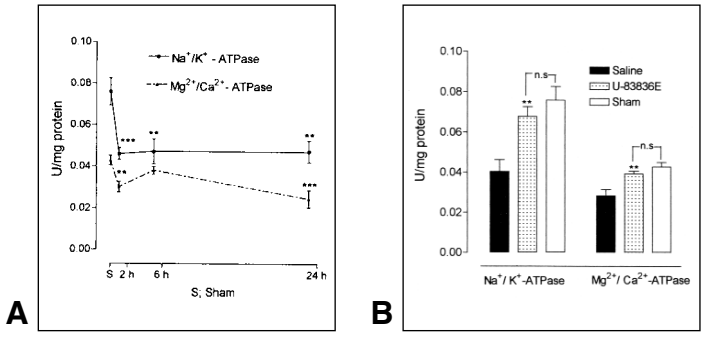

Figure 3: *Graph shows A) the changes of synaptosomal Na^+/K^+ and Mg^{2+}/Ca^{2+}ATPase activities over time in the traumatized hemisphere when compared to control and, B) enhancement of the activities of Na^+/K^+ and Mg^{2+}/Ca^{2+}ATPase by U-83836E when compared to the sham-operated group of animals (p>0.05); there was significant difference between the groups of animals treated with U-83836E and saline (p<0.01). Values are given as mean ± SEM. Asterix denotes the significant difference. n.s: not significant, ** significant at p<0.01 and *** p<0.001.*

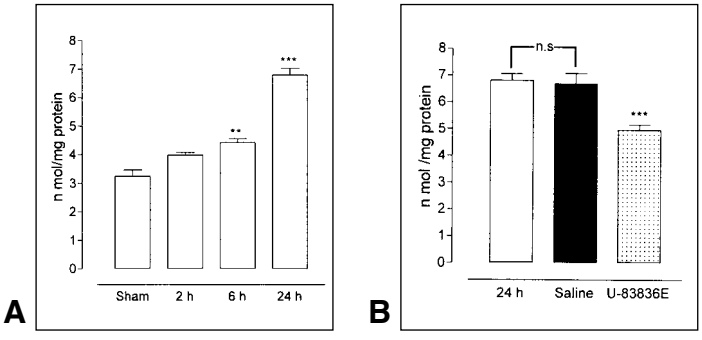

Figure 4: *Graph shows A) the tissue changes of MDA levels over time in the injured hemisphere when compared to control and, B) the reduction of tissue MDA content by the effect of U-83836E when compared to saline-treated group and also untreated group of animals (which were those sacrificed 24h after trauma). Values are given as mean ± SEM. Asterix denotes the significant difference. n.s: not significant, ** Significant at p<0.01 and *** p<0.001.*

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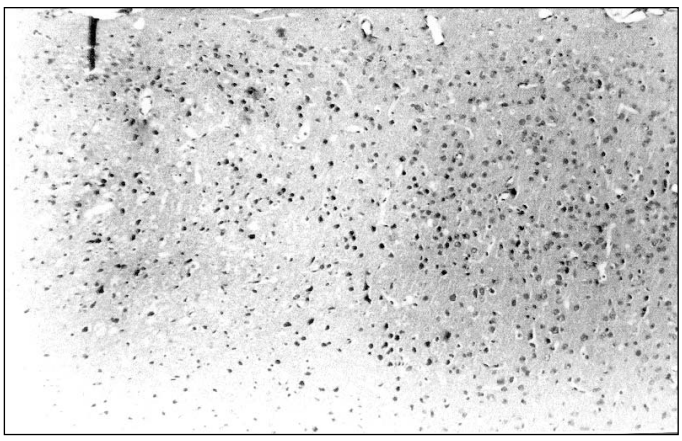
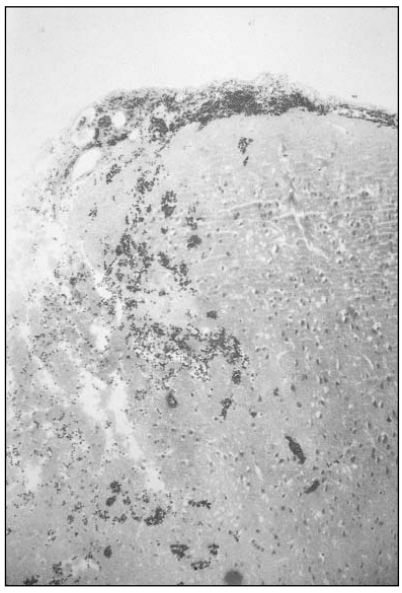


A



B

Figure 5: A) *The infarcted region is pale and edematous 24h after cerebral injury (on the left side). There are vacuolizations within neuroglial cells, in perineural locations and the interstitium, which are compatible with edema. In the cortical areas adjacent to the infarct side there are injured dark neurons (Hematoxylin and eosin X 40).* ***B)*** *Severely damaged neurons stained positively with acid fuchsine can be seen in the cortical areas (acid fuchsine X 20).*

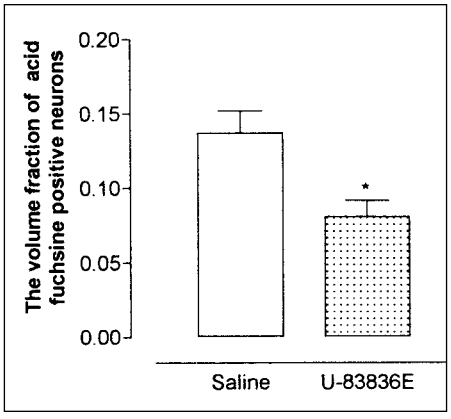


Figure 6: *Bar graph of attenuation of 24h post-traumatic cortical neuronal necrosis by U-83836E (one-tailed "t" test). Values are given as mean ± SEM. Asterix denotes the significant difference from control. * Significant at p<0.05.*

regions of the brain adjacent to the impact and also in the contra-lateral hemisphere. The injured neurons had shrunken cell bodies with condensed cytoplasm and pyknotic nuclei, which were stained with acid fuchsine (Figure 5B). The volume fraction of dying neurons in the cortical layer was found to be significantly lower ($p<0.05$) in the U-83836E-treated rats than in those of saline-treated animals (Figure 6).

Discussion

In the present study, BBB breakdown in the injured hemisphere after TBI was maximum at 2h following the trauma, showed a decline at 6h and returned close to the control at 24 h. This suggests that maximal BBB breakdown occurs during the early hours following trauma and returns to normal values at 24h, as previously shown.[29,30] Blood-brain barrier breakdown has been found to be greater after focal injury than after diffuse injury,[29] although BBB disruption is transient in both models.[31,32] Edema in the injured hemisphere showed a progressive development, although no significant increase occurred until 6h after the trauma. Our finding supports the study of Barzo et al;[33] widespread post-trauma edema formation occurred later, due predominantly to cellular swelling.

In the present study, inhibition of Na^+/K^+-ATPase activity was observed in the early phases following the trauma and remained at a decreased level throughout the first 24 h. A similar result was obtained in the studies of Ildan et al[34] and Jamme et al,[35] in which Na^+/K^+-ATPase activity diminished within a short period following the focal cerebral ischemia model in rats and remained below the control level over 120 minutes. On the other hand, the inhibition in the Mg^{2+}/Ca^{2+}-ATPase activity occurred soon after the trauma and became particularly marked at 24 h. This result is in accordance with the study of Parson et al[36] in which the inhibition of microsomal Mg^{2+}/Ca^{2+}-ATPase activity in rat brain subjected to global ischemia had increased to the maximum level after 60 minutes. In our study, the losses in the activities of Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase were not time-dependent, while the increase in MDA and the tissue water content was time-dependent, suggesting that the mechanisms for maintenance of ionic homeostasis of the cells was not attributable to membrane fluidity alone. This finding supports observations that stress-induced oxidation and ischemia in the rat brain causes inhibition of Na^+/K^+-ATPase and a rise in intracellular Na^+ and Ca^{2+} concentrations, without changes in lipid peroxidation.[37,38] Experimental evidence suggests that the plasma membrane enzymes are a target site for neuroactive steroid action; methylprednisolone has been demonstrated to enhance Na^+/K^+ and Mg^{2+}-activated ATPase in the focal cerebral ischemia and

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spinal cord trauma animal models.[24,34] The present study provides evidence that U-83836E given prophylactically, enhances Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase activities, possibly by scavenging iron-catalyzed free radical formation, and inhibiting lipid peroxidation,[9] the latter of which was shown in our previous study.[39]

21-aminosteroids have been shown to reduce edema in ischemic and traumatic brain injury,[15,16,40] possibly protecting the BBB from peroxidative damage.[10-13] However, they appear to act mainly on microvascular endothelium and have been demonstrated to ameliorate BBB breakdown more potently than brain penetrable lazaroids.[14,41] A brain penetrating lazaroid, U-83836E, demonstrated no effect on the BBB breakdown or the early edema development in the present study. However, Schoettle et al[42] have concluded that an increase in the tissue water content of a damaged hemisphere in the early phase after trauma most likely represents the combination of hemorrhage, vasodilation, and edema in focal brain injury models. In the present study, tissue water content and MDA levels increased progressively, reaching a maximum level at 24h, which may suggest a close relationship between edema formation and lipid peroxidation after TBI. It seems that in later hours edema may be mostly of the cytotoxic type, although the BBB breakdown contributes to edema development in the early phase after trauma. When given prophylactically, U-83836E reduced edema at 24h, possibly inhibiting the increase of lipid peroxidation and enhancing the activities of Na^+/K^+ and Mg^{2+}/Ca^{2+}-ATPase that cause intracellular Ca^{2+} and Na^+ overload, cellular edema and cell death.[43]

In the present study, the traumatic area had a spongy appearance, involving necrosis and dying neurons. Acidophilic neurons were most prominent in the contused cortex at 6 and 24h after TBI,[21] with concomitant accumulation of calcium in the cells.[44] Pretreatment with U-83836E salvaged neuronal necrosis. This outcome was consistent with previous observations that the brain penetrating lazaroid U-78517F (U-83836E is the minus enantiomer of the racemic compound of U-78517F), amelioration of post-ischemic neuron necrosis is more potent than 21-aminosteroid.[11,12]

In conclusion, these results suggest that the brain penetrating lazaroids, when given prophylactically, may be beneficial in the treatment of TBI. Further studies are recommended to determine if similar effects of the second generation of lazaroids are observed when they are given after the trauma.

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