The Effects Of Nimodipine, Mannitol And Their Combination On Experimental Cerebral Ischaemia

AHMET SELÇUKLU, R. KEMAL KOÇ, HATİCE PAŞAOĞLU, OLCAY KANDEMİR, AYDIN PAŞAOĞLU

Erciyes University, Medical Faculty Neurosurgery (AS, RKK, AP), Biochemistry (HP) and Pathology (OK) Departments Kayseri, Türkiye

Abstract : Recent studies suggest that Nimodipine, a potent calcium-channel antagonist that causes significant cerebrovascular dilatation, may improve neurological outcome after acute experimental permanent focal cerebral ischaemia in animals when given before or immediately after occlusion of the middle cerebral artery (MCA).

In this study, the effect of Nimodipine and Mannitol, singly or in combination was investigated on focal cerebral ischaemia induced by MCA occlusion using the transorbital approach in the rabbit model. At 1 hour after occlusion, the rabbits were treated for 6 and 24 hours duration in a double-blind technique with either, saline, nimodipine, mannitol and nimodipine + mannitol. Neurological outcome was better in rabbits treated with 6 and 24 hours nimodipine + mannitol at 1 hour after occlusion, and the size of areas of infarction was statistically the smaller in the nimodipine + mannitol treated group when compared with the controls. The degree of ischemic injury in the nimodipine+mannitol group was statistically reduced compared with the other groups. These results suggest that the possible mechanism of action of nimodipine + mannitol is on the "penumbra" of the ischaemic area.

Key Words: Mannitol, Nimodipine, cerebral ischaemia

INTRODUCTION

A few minutes after the onset of severe ischaemia, depolarization of cell membranes causes increased permeability resulting in an influx of Ca^{++}, H_2O and Na^+ from the extracellular to the intracellular space and an increase of extracellular K^+. Increased intracellular Ca^{++} induces arachidonic acid cascade which consequently produces thromboxane A_2, leukotriene and free radicals. Thromboxane A_2 and leukotriene are the most important vasoconstrictors. In addition,leukotriene causes brain aedema by changing the membrane permeability. Free redicals are directly responsible for cell injury. As a consequence of these physiological and biochemical events, it has been shown that Ca^{++} is the most important factor in cell death (7,15,25,34,36,42,43).

The aim of the therapy of cerebral ischaemia is to increase ischemic tolerance by increasing cerebral blood flow (CBF) and improve biochemical changes caused by cell injury (18). Blood viscosity has an important role for the CBF in ischaemic brain injury. In several experimental and clinical studies of cerebral ischaemia it has been shown that hypervolemic haemodulition and mannitol decrease blood viscosity (4,22), and nimodipine increases CBF, thus both prevent vasospasm due to vasoactive agents and decrease intracranial pressure (ICP) (3,8,12,16,41).

This study was carried out to determine the effects of nimodipine, mannitol and their combination on cerebral ischaemia.

MATERIAL AND METHODS

To produce cerebral ischaemia in rabbits the technique introduced by Sundt and Waltz (39) was modifed. The experiments were carried out in the Clinical and Experimental Research Centre of Erciyes University Medical School, Kayseri.

In the study, 37 New Zealand Rabbits, 2.5-3.8 kg

in weight, were sedated with 6 mg/kg IM ketamine then anaesthetized with 15 mg/kg IV Thiopentone. Paralysis and analgesia was maintained with 0.2 mg/kg IV Pancroniumbromide and 0.1 mg/kg IV fentanyl. Trachaostomy was performed. The animals were ventilated with air-room following the arterial canula was inserted into the femoral artery for continuous recording of blood pressure and blood gas samples.The animals were placed in the right lateral position with the head stabilised. A skin incision was made 2-3 cm. along the superior lateral rim of the orbit and the soft tissue was dissected to the rim of the orbital bone. The bone was removed with rongeur. The remaining orbital content was dissected subperiosteally and removed at the apex of the intraorbital muscular cone and the optic nerve. Using a surgical microscope, craniectomy (6x10 mm^2) was carried out just above the optic foramen with a high speed drill. After 5x6 mm^2 excision of dura and dissection of the underlying arachnoid membrane, the intracranial portion of the middle cerebral artery (MCA), the internal carotid artery (ICA) and the anterior cerebral artery (ACA) were identified. The ICA was closed 2-3 mm distal of the birfucation with bipolar electrocoagulation. The middle of the closed artery was cut prevent recanalization. The craniectomy defect was covered with gelfoam and the wound was closed with 3/0 sutures. 100mg/kg IM Seftriakson was given prophylactically. 8 rabbits were used to improve the technique of MCA occlusion. Sham operations were performed on 5 rabbits. There was no neurologicil deficit or infarct in any rabbit at the 7^{th} day. Thus it was demonstrated that the rabbit did not die and had no neurological deficit do to anaesthesia and the surgical process.

Treatment group: The rabbits were divided into four groups: control, nimodipine, mannitol, and nimodipine + mannitol. Three rabbits in each group were treated for 6 hours and another three for 24 hours. Treatment was begun in the first hour of MCA occlusion. Nimodipine at a dose of 1ug/kg/dk was given via intravenous infusion.

Mannitol was given at a dose of 0.2 g/kg/10 min twice in the 6-hour treatment group and six times in the 24-hour treatment group. The total liquid given to all animals was 4.2 ml/kg/h.

Physiological measurement: Physiological parameters were recorded one hour before, during and one and three hours after occlusion (temperature, pulse, respiration rate, hct, MABP, pH, pCO_2, pO_2). Body temperature was monitored by a rectal thermometer and animals were maintained normothermic (37°C) by external heating when required. Blood gas analysis was measured by CIBA CORNING 278 Blood gas system. Arterial pH, pO_2 and pCO_2 were measured periodically and kept constant.

Neurological examination: Blind neurological examinations were made by two observers. Results were classified as a normal, abnormal and died (24). Abnormal findings included generalized weakness, markedly reduced motor activity, decreased feeding ,grooming or obtundation. The findings of the observers were in agreement. Neurological examination was made before surgical procedure, postoperative 6 hours on the first day and the 7^{th} day.

Neuropathological examination: On the seventh day of MCA occlusion, following neurological determination, the rabbits were anaesthetized with ketamine and killed with an intravenous injection of Potassium chloride. The brains were removed. Coronal sections were made at the level of 5mm anterior to the optic chiasm and 5mm brain slices were obtained for biochemical examination. The infarct area was measured at the left hemisphere and the right hemisphere was used as a control. The remaining brain was fixed with 10% formalin. After 7^{th} days coronal sections were obtained at the middle of the optic chiasm 5mm anteriorly and posteriorly. The slices were stained with Hematoxylin and Eosin. The volume of infarction at the same hemisphere, and the total of infarction at the cortex and basal ganglia were calculated. The intensity of ischaemia was calculated as a ratio of the affected neurons to total neurons at 8 areas measured 1 mm^2 from the cortex, subcortex and basal ganglia. The infarct area was measured by planimetry of tracings from projected photographic slides of the section.

Biochemical examination: The volume of cortical aedema was measured as mentioned previously. The samples were dried at 110°C for two days and the percentage water content.

Na^+ and K^+ values: For determining Na^+ and K^+ rates at left ischaemic and right nonischemic regions the equal amounts of Nitric acid (HNO_3) and Percloric acid (HCO_3) solution were hydrolised by

boiling up to clear. Then Na^+ and K^+ amounts were measured.

Statistical analysis: Mann Whitney U and Kruskall Wallis tests were used. All results were presented as mean + standard deviation.

RESULTS

During the surgical procedure, temperature, respiration rate, pH, pCO_2 and pO_2 kept constant. Minimal metabolic acidosis was observed following ischaemia in all groups.

The mean arterial blood pressure (MABP) was kept between 90-118 mmHg permanently. A slight decrease of MABP was observed in the nimodipine treated animals but did not reach statistical significance (p>005). The blood lost was between 5-12 ml in the cour se of the surgical procedure.

The rabbits hct was 38.79+3.59 % before surgical procedure and 35.04+3.65 % after procedure hct changes are presented (Table I).

Table I: Haematocrit levels of experimental groups

Groups		−1	+1	+3
Control	06 h	37.67±2.52	32.67±2.52	32.00±1.73
	24 h	38.33±3.51	35.67±3.79	34.67±3.06
Nimodipine	06 h	36.33±4.16	33.00±3.61	32.00±3.46
	24 h	40.00±8.49	36.00±6.93	35.00±4.36
Total		38.08±3.94	34.33±4.16	33.42±3.18
Mannitol	06 h	40.67±7.02	37.00±6.24	33.00±4.36
	24 h	39.33±0.58	34.67±1.15	30.67±1.53
Nimodipine+	06 h	38.33±0.58	34.67±0.58	31.00±1.00
Mannitol	24 h	39.67±1.53	36.67±2.08	32.00±1.00
Total		39.50±3.07	35.72±3.08	31.68±2.27

−1: one h. before occlusion, +1:one h. after occlusion,
+3: three h. after occlusion

Electrolyts and cerebral water content changes: The only significant changes were found in the nimodipine treated group in which the water content and sodium amount at 6 hours were found to be decreased. Other data is presented in table II.

Neurological outcome: At clinical examination on the first day,there were signs of cerebral infarct in all rabbits of which MCA were occluded. Five of 8 animals with severe neurological deficits died on the 4th day postoperatively, 2 at the 5th day, 1 at day 6. In the postoperative 7th day, there were no neurological deficit in the 8 animals but abnormal finding in the 8 animals.

Table II: Water content, sodium and potassium levels of ischaemic and non-ischaemic regions

Groups		Water content (%)		Sodium levels (mEq/kg)		Potassium levels (mEq/kg)	
		non-ischaemic	ischaemic	non-ischaemic	ischaemic	non-ischaemic	ischaemic
C	06 h	81.17±0.56	84.19±1.01	52.03±4.19	60.30±5.15	79.17±07.36	73.70±05.15
	24 h	82.34±1.77	84.42±1.64	51.00±3.53	58.57±5.67	85.47±01.42	83.63±02.44
N 06	h	80.69±0.55	81.40±0.49*	47.79±3.76	51.40±4.28**	74.03±14.22	67.97±15.38
	24 h	82.21±2.29	83.32±2.16*	49.40±2.19	53.87±0.23	82.10±04.83	70.53±06.71
M	06 h	80.43±0.50	82.16±1.27	50.70±6.01	56.63±7.09	74.33±11.50	67.00±05.57
	24 h	81.24±1.52	83.83±0.94	50.43±8.65	56.43±1.75	88.80±10.79	79.93±11.64
N+	06 h	80.26±0.39	84.12±0.90	48.83±2.75	57.17±1.72	81.47±11.49	79.83±11.12
M	24 h	80.63±1.05	83.31±1.82	46.67±9.17	51.80±8.52	81.97±02.00	79.13±00.91

C:control, N: nimodipin, M: mannitol, N+M: nimodipine + mannitol
* Significantly different from control and other groups, P ÷0.05 (Kruskal Wallis Variance analysis)
** Significantly different from control, P ÷0.05 (Mann Whitney U test)

Infarct area: The size and location of infarct was slightly different between groups. The infarct area was at deep middle line and near the basal ganglia in some animals but extending superficially to cortex in most. The area of infarction of the affected hemisphere following MCA occlusion was 45.97+18.58 % in controls, 24.48+11.81% in the nimodipine, 22.85+17.52 % in mannitol, and 5.7+5.8 % in the nimodipine + mannitol group. The size of infarct at the cortex and basal ganglia and its relation to outcome is presented in Table III. Generally, the degree of neurological deficit showed close relation to its location rather than size. However,

Table III: Infarct distribution at the cortex and basal ganglia and its relation to the clinical outcome

Groups		T.I. (%)	Cortex (%T.I.)	Bas.gang. (%T.I.)	Normal	Abnormal	Death
Control	06h	39.23±11.79	52.70±18.18	47.30±18.18	0	2	1
	24h	52.70±24.25	80.70±16.79	19.30±16.79	0	1	2
Nimodipine	06 h	27.27±13.11	59.87±36.52	40.13±36.52	2	0	1
	24 h	21.20±12.03*	76.53±25.14	23.47±25.14	0	2	1
Mannitol	06 h	34.20±16.76	70.57±27.94	29.43±27.94	2	0	1
	24 h	11.50±10.12*	80.20±23.05	19.80±23.05	1	1	1
Nimodipine+	06 h	05.10±04.52**	97.00±40.41	03.00±04.24	1	1	1
Mannitol	24 h	05.03±06.64**	76.67±40.41	23.33±40.41	2	1	0

(T.I: total infarct area)
* Significantly different from control, P ÷0.05 (Mann Whitney U test)
** Significantly different from control ard other groups, P ÷0.05 (Kruskal Wallis Variance analysis)

wakefulness of animals was in accordance with the size of the infarct. There was severe infarct histopathologic ally with a mean infarct size of 28.01+23.24 % in the 8 animals had severe neurological deficit at neurological examination which died (3 control, 2 mannitol, 2 nimodipine and 1 nimodipine+mannitol). In the 8 neurologically abnormal animals (3 control, 2 nimodipine, 1 mannitol, 2 nimodipine+mannitol) mean infarct size was 26.45+16.90 %. In the 8 neurologically normal animals (2 nimodipine, 3 mannitol, 3 nimodipine + mannitol) the mean infarct size was 20.81+19.54 % with a less severe infarct histologically. The best effect was found in the nimodipine + mannitol treated group though nimodipine or mannitol per se were both effective at the 24th hour.

Histopathological examination:

Microscopic examination: Distribution of the ischaemic changes was similar but the intensity was different in all groups. Ischaemic damage was more severe in the basal ganglia region.

6-hour control group: The cortex, subcortex and basal ganglia were entirely affected. As a result of ischaemic change, perikaryon showed dark eosinophil colour and the nucleus was either absent or hardly seen. Nissl bodies were lost and the size of cell was usually small. Histiocytes were more prominent in every region of the cortex and subcortex. Congestion and aedema were severe.

24-hour control group:The aedema was intensive and necrosis was prominent. There were particles of necrotic cells in the ground. Histiocytes, eosinophils and neutrophils were prominent. In addition to ischaemic changes, neuronal necrosis was seen.

6-hour nimodipine group: Necrosis was seen in the subcortex and basal ganglia. Polymorphonuclear leucocytes, particles of cells and histiocytes were seen. Severe aedema and ischaemic neurons were determined at the cortex.

24-hour nimodipine group: Intensive histiocytes, lymphocytes, ischaemic neurons, particles of cells and polymorphonuclear leucocytes were determined but the aedema was less severe.

6-hour mannitol group: The ischaemic changes were prominent at the cortex, subcortex and basal ganglia. Aedema was intensive. Perikaryons were small and dark. Nissl bodies were lost. Polymorph and cell particles were found at the subcortex. There was histiocytosis in all regions

24-hour mannitol group: There was minimal aedema at the cortex, subcortex and basal ganglia. The neurons showed ischaemic changes. Polymorphonuclear leucocytes, histiocytosis and dense mononuclear leucocytes around the vessels were observed. These changes were more prominent in the cortex.

6-hour nimodipine+mannitol group; Histiocytosis was observed at the cortex and subcortex. There was minimal aedema in the basal ganglia. The number of ischaemic neurons was less than in other groups. Normal neurons were also seen.

24-hour nimodipine+mannitol group; Neuronal ischaemic changes showed marked improvement and aedema was minimal.

Quantitative findings: The total number of cells in the cortex, subcortex and basal ganglia are shown in Table IV. Minimal differences were observed between areas. This could be attributed to difficulty in determining the exact borders of areas rather than cell phagocytosis.

Table IV: Total neurons

	Control	Nimodipine	Mannitol	Nimod+Mannitol
Cortex	241.67±38.42	260.00±61.78	318.67±85.78	360.67±139.59
Subcortex	264.33±58.71	264.00±54.96	289.60±68.19	344.67±103.46
Basal ganglia	290.60±50.42	324.33±64.53	317.33±70.41	330.67± 62.08

Cortex: İschaemic neuronal damage was relatively less severe than other area. However 24 hour nimodipine and nimodipine + mannitol treated groups showed significantly less neuronal damage ($p<0.005$). (Table V).

Tablo V: Quantitative results of cortex with ischaemic neuronal damage

Groups		Total neurons	Ischaemic neurons	%(isc neur/tot neur)
Control	06 h	238.00±17.32	156.33±70.32	64.82±26.01
	24 h	245.33±57.87	194.67±30.29	83.76±28.13
Nimodipine	06 h	268.00±90.42	117.33±101.93	54.30±47.03
	24 h	234.67±23.09	69.33±76.87	27.96±31.00
Mannitol	06 h	264.00±52.46	152.00±52.46	60.73±26.69
	24 h	373.33±81.71	170.67±95.44	44.44±17.88
Nimodipine+	06 h	314.67±66.61	34.67±60.04	14.44±25.02
Mannitol	24 h	418.67±190.38	0.00± 0.00*	0.00± 0.00

* Significantly different from control and mannitol groups. $P<0.05$ (Mann Whitney U test)

Subcortex: İschemic neuronal damage was more severe than in the cortex. The damage in the nimodipine + mannitol treated group was significantly slight. ($p<0.005$), (Table VI).

Table VI: Quantitative results of subcortex with ischaemic neuronal damage

Groups		Total neurons	Ischaemic neurons	% (isc neur/tot neur)
Control	06 h	267.33±91.88	141.67±33.47	60.14± 7.46
	24 h	261.33±12.22	242.67± 9.24	92.88± 1.49
Nimodipine	06 h	226.67± 4.62	109.33±67.09	41.07±29.40
	24 h	301.33±57.87	98.67±58.97	31.79±17.21
Mannitol	06 h	293.33±72.59	156.00±106.66	50.51±31.08
	24 h	258.67±87.76	160.00±83.52	59.60±10.68
Nimodipine+	06 h	270.67±33.94	21.33±36.95*	07.84±13.58
Mannitol	24 h	418.67±88.48	10.67±18.48*	03.17±05.50

* Significantly different from control ard other grups. $P<0.05$ (Kruskal Wallis Varyans analysis)

Basal ganglia: Ischaemic damage was similar to the subcortex. The 24-hour nimodipine and nimodipine + mannitol treated groups demonstrated sligth damage ($p<0.005$), (Table VII).

Table VII: Quantitative results of basal ganglia with ischaemic neuronal damage

Groups		Total neurons	Ischaemic neurons	% (isc neur/tot neur)
Control	06 h	268.33±65.61	124.00±77.87	43.32±22.29
	24 h	309.33±28.10	269.33±56.76	86.42±10.86
Nimodipine	06 h	344.00±56.00	170.67±12.22	50.12±05.09
	24 h	306.67±78.93	42.67±48.88	13.82±13.36
Mannitol	06 h	322.67±48.22	93.33±88.48	30.63±27.62
	24 h	312.00±99.92	141.33±140.93	44.88±38.69
Nimodipine+	06 h	293.33±68.04	24.00±41.57*	08.11±14.04
Mannitol	24 h	368.00±52.46	29.33±50.81*	07.80±13.51

* Significantly different from control and 6 h. nimodipine groups. $P<0.05$ (Mann Whitney U test)

DISCUSSION

Kato et al. (20) studied the ratio of Na^+, Ca^{++} and K+ in the ischaemic zone induced by occlusion of the right common carotid artery in gerbils. In their study, while the water content of the non-ischaemic region of the cerebral cortex was 79.0+03, it was 82.0+4 and 80.7+04 all the periphery and the centre of the ischaemic regions, respectively. Na^+ content was increased and K^+ content was decreased most prominently in the periphery of the ischaemic region. K^+ depletion and exogenous Ca^{++} accumulation in the peripheral region were visualized by K^+ staining and Ca^{++} autoradiography respectively.

The experimental study of Betz et al.(1) confirmed the study of Kato et al. (20) that the water and electrolyte content in the ischaemic zone undergoes important changes. It has been noted that Na^+ transport across the blood brain barrier is the most important factor for the development of ischaemic brain aedema and this can be limited by using drugs which prevent Na^+ transport across the brain capillary.

In several studies, the ratio of aedema accumulation is related to the increase in tissue of Na^+ content (9,35,44).

Borzeix and Chan (2) showed experimentally that an increase in cerebral water, Ca^{++} and freefatty acids content is associated with a decrease in cerebral potassium content.

In our study, decreased K^+ content was significantly less in the nimodipine+mannitol group than other groups ($p<0.05$), probably due to milder cerebral ischaemic injury in this group. The increase of Na^+ and water content in nimodipine treated group wa s statistically significant compared to the other groups ($p<0.05$) when mannitol was added to nimodipine, the water and Na^+ content showed some increase probably related to increased CBF in the ischaemic region.

The effect of nimodipine on CBF, MABP, and cerebral perfusion pressure is dose-dependent (12,14,21). McCalden et al. (26) experimentally showed that 1ug/kg/min of nimodipine caused a modest increase in basal CBF without significant alteration in cerebral metabolism and systemic blood pressure.

Hadley et al. (13) in their experimental study, found a 18-20 % decrease in MABP with nimodipine (2ug/kg/min loading and 1ug/kg/min maintenance dose) but Steen et al. (38) and Germano et al. (8) suggested that nimodipine caused no significant chan ge in MABP

In the study of Hadley et al.(13) nimodipine treated animals tended to have smaller areas of infarction but it was not statistically significant. However, Germano et al. (8) showed that the size of area of infarction was statistically smaller in nimodipine treated groups. The most important conclusion which can be drawn from these studies is that the neurological outcome was better in the nimodipine treated groups compared to controls.

In our study, we observed some decrease in MABP with nimodipine (1ug/kg/min IV infusion) but this effect was not important. Despite the minimal decrease of MABP, the neurological outcome was better and the areas of infarct were small in the nimodipine treated animals compared to the controls at 7 days postoperatively.

Following ischaemia, systemic blood pressure decreases after a few days in humans and after several hours in mammals and then again increases to a normal level (11).

There are different opinions regarding the timing of nimodipine administration to prevent cell death due to cerebral ischaemia Gotoh et al. (10) claimed that treatment with nimodipine started within as short a time as 5 min after the production of the focal ischaemic lesion was ineffective. Steen et al. (37,38) previously reported that nimodipine improves the outcome only when given before 10 min of complete cerebral ischaemia but their next study, showed that nimodipine dose improved the outcome even after complete ischaemia.

Germano et al. (8) found the neurological outcome was better in rats treated with nimodipine 1,4,6, hours after occlusion and the size of areas of infarction was statistically smaller in nimodipine treated groups.

Mohamed et al. (31) found profound reductions in CBF in the neocortical areas and the caudat nucleus 30 min after MCA occlusion and in animals pretreated with nimodipine (30 min before and 30 min after MCA occlusion) the decrease in CBF was significantly less than in control animals.

Meyer et al. (30) investigated the effects of nimodipine on intracellular pH, cortical CBF and EEG. The results showed that in nimodipine treated animals, the increase of CBF was associated with the increase in intracellular pH.

Haws et al. (17) concluded that nimodipine caused an increase in CBF with no change in cerebral O_2 consumption. This increase in blood flow was thought to be a result of the direct vasodilatating effect of the drug and not secondary to increased cerebral metabolism.

It has been reported that intracellular Ca^{++} concentration decreases after reperfusion in cerebral ischaemia(27). The restoration of CBF and/or preventing influx of Ca^{++} into the cell can prolong the viability of the cell. As a consequence of this finding, it has been thought that the area of importance in ischaemia is the "ischaemic penumbra" the zone of nonfunctioning but structurally preserved brain tissue at the periphery of the infarcted area(8,37).

In our study, neurological outcome was better, the size areas of infarct was smaller and the number of ischaemic neurons was less than in controls in the nimodipine treated animals (1,6,24 h of postocclusion) but its effect was not superior to mannitol. The effect of nimodipine is probably dependent on the restoration of CBF and/or the blockage of calcium ions entering the cell and therefore prolongs the viability and restores the activity of the cell.

Meyer et al. (29) examined the values of intracellular brain pH, cortical CBF and EEG in severe and moderate ischaemic areas by giving 1g/kg mannitol after permanent occlusion of MCA in rabbits. While mannitol moderately improved CBF in the severe ischaemic area, it was not able improve the metabolic consequences determined by brain pH. On the other hand, mannitol markedly improved CBF in the moderate ischaemic areas and thus would have a useful effect on moderate ischaemic areas but not severe isch a emic areas.

In a recent study of Meyer et al. (28) on the same model similar results were obtained. Mannitol improved the microcirculation flow in moderately affected areas by reducing the progressive aedema which prevents the residual circulation and thus restores the basal metabolism.

Little (23) showed that neurological results improved and the infarct area decreased by giving mannitol after the closure of MCA in cats. He believes that this effect is a result of decreased blood viscosity, increasing tissue oxygenation by preventing deformation of erythrocytes and enlarging intravascular volume(5,32,33). In addition, it has been shown that mannitol had a positive inotropic effect on the myocardium and also increased cardiac output (6).

In our study, the slight decrease of haematocrit in the first hour of occlusion might be related to loss of blood during the surgical procedure, the decreased haematocrit at the 3th hour of occlusion in animals receiving only mannitol could be attributed to increased the plasma volume due to the osmolar effect of mannitol. The neurological outcome of the

animals treated with mannitol was relatively better in comparison to controls but not significant statistically.

The infarct size in animals treated with mannitol for 24 hours was significantly less in comparison with controls ($P<0.05$). It was also less on 6 hours treatment but not statistically significant. The number of ischaemic neurons at 6 and 24 hours was less in comparison to the controls but not statistically significant. This useful effect of mannitol is probably the result of pulling intracellular liquid to the extracellular compartment, as a consequence of increasing plasma osmolarity, the decrease of viscosity and haematocrit concentration and improvement of the microcirculation of blood.

Sutherland et al. (40) examined the effects of mannitol (0.25g/kg over 60 min), nimodipine (10ug/kg 5 min before ischaemia, additionally two 5ug/kg doses with 10 min intervals), indomethasin and their combinations on temporary global cerebral ischaemia in rats. By counting the ischaemic neurons directly definite improvement of ischaemic injury (ischaemic neurons/ total neurons) was obtained with mannitol. The useful effect of nimodipine was only in the hippocampal area, and indomethasin had no useful effect. When compared with the controls, nimodipine + mannitol combination showed a significant useful effect on neuronal damage. However, this effect was no better than that achieved with mannitol only.

In our study, the neurological symptoms of most rabbits treated with nimodipine-mannitol combination one hour after the permanent focal ischaemia had improved though not important statistically and the infarct area was statistically smaller than controls and other groups ($P<0.05$).

The differentiation of our results from Sutherland et al.(40) may be related to the different experimental model and infarct type and to the dose of drugs.

In conclusion, the useful effects of this combination is probably due to the sum of the effects of nimodipine and mannitol. This combination may increase CBF over the ischaemic threshold and prevent the insufficiency of energy and may put the ion flow in order. Thus optimal diffusion may be continued both for oxygen and for materials

Correspondence: Ahmet Selçuklu M.D.
Erciyes Üniversitesi Tıp Fakültesi
Nöroşirürji ABD
38039 KAYSERİ
Tel 374937/1770

REFERENCES

1. Betz AL, Ennis SR, Schielke GP: Blood-brain barrier sodium transport limits development of brain edema during partial ischemia in gerbils. Stroke 20:1253-1259,1989
2. Borzeix MG, Cahn J: Experimental approach of treatment of cerebral ischemia by a combination of raubasine and almitrine. Arzneim-Forsch/Drug Res 37:491-494,1987
3. Brandt L, Andersson KE, Edvinsson L, Ljunggren B: Effects of extracellular calcium and of calcium antagonists on the cantractile responses of isolated human pial and mesenteric arteries. J Cereb Blood Flow Meteb 1:339-347,1981
4. Brown FD, Johns L, Jafar JJ, et al: Detailed monitoring of the effects of mannitol following experimental head injury. J Neurosurg 50:423-432,1979
5. Cote CJ, Greenbow DE, Marshal BE: The hypotensiv response to rapid intravenous administration of hypertonic solutions in man and in the rabbit. Anesthesiology 50:30-35,1979
6. Dennis RC, Harlow C, Egdahl RH, et al: Enhancement of Myocardial function with glucose, insulin and potassium. Surg Gynecol Obstet 151:185-190,1980
7. Gelmers HJ: Nimodipine in ischemic stroke. Clin Neuropharmacology 10:412-422,1987
8. Germano IM, Bartkowski HM, Cassel ME, Pitts LH: The therapeutic value of nimodipine in experimental focal cerebral ischemia. J Neurosurg 67:81-87,1987
9. Gotoh O, Asano T, Koide T, Takakura K: Ischemic brain edema following occlusion of the middle cerebral artery in the rat. I: The time courses of the brain water, sodium and potassium contents and blood-brain barrier permeability to 125I-albumin. Stroke 16: 101-109,1985
10. Gotoh O, Mohamed AA, McCulloch J, et al: Nimodipine and the haemodynamic and histopathological consequences of middle cerebral artery occlusion in the rat. J Cereb Blood Flow Metab 6:321-331, 1986
11. Guyton AC: Text of Medical Physiology, ed 6. WB Saunders, Philadelphia 1981, pp 255
12. Hadley MN, Spetzler RF, Fifield MS, et al: The effect of nimodipine on intracranial pressure. Volume-pressure studies in a primate model. J Neurosurg 66:387-393,1987
13. Hadley MN, Major MC, Zabramski JM, et al: The efficacy of intravenous nimodipine in the treatment of focal cerebral ischemia in a primate model. Neurosurgery 25:63-70,1989
14. Harper AM, Craigen L, Kazda S: Effect of the calcium antagonist, nimodipine, on cerebral blood flow and metobolism in the primate. J Cereb Blood Flow Metab 1: 349-356,1981
15. Harris RJ, Symon L, Branston NM, et al: Changes in extracellular calcium activity in cerebral ischemia. J Cereb Blood Flow Metab 1:203-209,1981
16. Hayashi S, Nehls DG, Kieck CF, et al:Beneficial effects of induced hypertension on experimental stroke in awake monkeys. J Neurosurg 60:151-157,1984
17. Haws CW, Gourley JK, Heistad DD: Effects of nimodipine on cerebral blood flow. J Pharmacol Exp Ther 225:24-28,1983

18. Heiss WD: Flow thresholds of functional and morphological damage of brain tissue. Stroke 14:329-331,1983
19. Ikeda Y, Long MD: The molecular basis of brain injury and brain edema: The role of oxygen free radicals. Neurosurgery 27:1-11,1990
20. Kato H, Kogure K, Sakamoto N, Watanabe T: Greater disturbance of Water and ion homeostasis in the periphery of experimental focal cerebral ischaemia. Exp Neurol 96:118-126,1987
21. Kazda S, and Towart R: Nimodipine: A New calcium antogonistic drug with a preferential cerebrovascular action. Acta Neurochir (Wien) 63:259-265,1982
22. Little JR: Modification of acute focal ischemia by threatment with mannitol. Stroke 9:4-9,1978
23. Little JR: Modification of acute focal ischemia by threatment with mannitol and high-dose dexamethasone. J Neurosurg 49:517-524,1978
24. Lyden PD, Zivin JA, Kochhar A, Mazzarella V: Effects of calcium channel blokers on neurologic outcome after focal ischemia in rabbits. Stroke 19:1020-1026,1988
25. Mabe H, Nagai H, Takagi T, et al: Effect of Nimodipine on cerebral functional and metabolic recovery following ischemia in the rat brain. Stroke 17:501-505,1986
26. McCalden TA, Nath RG, Thiela K: The effects of a calcium antagonist (nimodipine) on basal cerebral bood flow and reactivity to various agonists. Stroke 15:527-530, 1984
27. Meldrum B, Evans M, Griffiths T, et al: Ischemic brain damage: the role of excilatory activity and of calcium entry. Br J Anasth 57:44-46,1985
28. Meyer FB, Anderson RE, Sundt TM Jr: Intracelluler brain pH, indicator tissue perfusion, electroencephalography, and histology in severe and moderate focal cortical ischemia in the rabbit. J Cereb Blood Flow Metab 6:71-78,1986
29. Meyer FB, Anderson RE, Sundt TM, et al: Treatment of experimental focal cerebral ischemia with mannitol. J Neurosurg 66:109-115,1987
30. Meyer FB, Anderson RE, Yaksh TL, Sundt TM Jr: Effect of nimodipine on intracellular brain pH, cortical blood flow, and EEG in experimental focal cerebral ischemia. J Neurosurg 64:617-626,1986
31. Mohamed AA, Gotoh O, Graham DI, et al: Effect of pretreatment with the calcium antagonist nimodipine on local cerebral blood flow and histopathology after middle cerebral artery occlusion. Ann Neurol 18:705-711,1985
32. Obrist WD, Thompson HK Jr, Wang HS, et al: Regional cerebral blood flow estimated by 133xenon inhalation. Stroke 6:245-256,1975
33. Rosner JM, Coley I: Cerebral perfusion pressure: A hemodynamic mechanism of mannitol and the postmannitol hemogram. Neurosurgery 21:147-156,1987
34. Schanne FAX, Kane AB, Young EE, Faber JL: Calcium dependence of toxic cell death: A final common pathway. Science 206:700-702,1979
35. Schuier FJ, Hossmann KA: Experimental brain infarcts in cats. Stroke 11: 593-601,1980
36. Siesjo BK: Cerebral circulation and metabolism. J Neurosurg 60:883-908,1984
37. Steen PA, Gisvold SE, Milde JH, et al: Nimodipine improves outcome when given after complete cerebral ischemia in primates. Anesthesiology 62:406-414,1985
38. Steen PA, Newberg LA, Milde JH, et al: Nimodipine improves cerebral blood flow and neurologic recovery after complete cerebral ischemia in the dog. J Cereb Blood Flow Metab 3:38-43,1983
39. Sundt TM Jr, Waltz AG: Experimental cerebral infarction: Retro-orbital, extradural approach for occluding the middle cerebral artery. Mayo Clin Proc 41:159-168,1966
40. Sutherland G, Lesiuk H, Bose R, Sima A: Effect of mannitol, nimodipin, and indomethacin singly or in combination on cerebral ischemia in rats. Stroke 19:571-578,1988
41. Symon L, Harris RJ, Branston NM: Calcium and calcium antagonists in ischaemia. Acta Neurochir (Wien) 63:267-275,1982
42. Wolfe LS: Eicosanoids: Prostoglandins, Thromboxane, Leucotrienes and other derivatives of carbon-20 unsaturated fatty acids. J Neurochem 28:1-14,1982
43. Yoshida S, Inoh S, Asano T, et al: Effect of transient ischaemia on free fatty acids and phospholipids in the gerbil brain. Lipid peroxidation as a possible cause of postischemic injury. J Neurosurg 53:323-331,1980
44. Young W, Rappaport ZH, Chalif DJ, Flamm ES: Regional brain sodium, potassium, and water changes in the rat middle cerebral artery occlusion model of ischemia. Stroke 18:751-759,1987