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COMPARISON OF THE EFFECTS OF MANNITOL AND DEXAMETHASONE ON THE HYPOXIC-ISCHEMIC BRAIN EDEMA* (Part - I)

S. ÇOBANOĞLU**

SUMMARY:

In a modified LEVINE preparation, chemical alterations of Na, K and H_2O contents were investigated with the effects of mannitol and dexamethasone on these alterations, on 72 rats. In conclusion, chemical alterations were not compatible with the neuronal alterations which were observed in the rats of a similar preparation and therefore changes in electrolyte and water contents were within the narrow limits. In conclusion, mannitol-dexamethasone combination appeared to be the choice of treatment to effect H_2O contents in moderate hypoxic-ishemic brain insults.

INTRODUCTION

As a part of long–range study of various aspects of cerebral edema, the effect of hypoxic–ischemia on the distrubition of water, sodium and potassium ions in brain tissue and upon those changes, the effect of mannitol and dexamethasone were investigated in rats at different time intervals using a modified Levine preperation (16, 33).

Ischemia and hypoxia are insults of brain tissue that commonly acompanied head injury, stroke, hydrocephalus, increased intracranial pressure and mass effect caused by vairious neoplasms. Experimentally and clinically, there is abundant evidence that hypoxia and / or ischemia accompanies a type of brain swelling, namely ISCHEMIC, CYTOTOXIC cerebral edema in which the accumulation of edema fluid confined to cells, particularly to astrocytes in the perivascular regions in both gray and white matter leaving the blood-brain barrier (BBB) intact depending on the duration and degree of the insult (11, 14, 15).

The minimum duration of ischemia necessary to trigger edema formation in human is not yet known, but experimentally it was suggested to be as early as 5 minutes and upto 15 hours (5, 23).

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** Trakya University Medical Faculty, Department of Neurosurgery (Yard. Doç. Dr.) EDİRNE

Key words: Hypoxia, Ischemia, Rat, Brain chemistry, Na^+ and K^+ ions.

In this experiment, water and electrolytes changes in the rats subjected to hemispheric ischemia through a common carotid artery ligation for 3 or 6 hours at the end of which being followed by hypoxia and postischemic reperfusion (modified preperation) and also on those changes, the effects of mannitol, dexamethasone alone and in combination, were investigated to see whether these agents are any good in using in ischemic conditions. As well known, the optimal management of an acute ischemic insult to the brain still remains problematic and protection the already insulted brain with steroids and /or mannitol are also still contraversial (11, 32). The results are reported with pertinent literature.

MATERIALS AND METHODS

This experiment was carried out in Istanbul DETAM on the adult Wistar type male rats weighing 200–300 g and divided into 3 and 6 hours experimental groups on the basis of duration of unilateral common carotid artery ligation for hemispheric ischemia, prior to, hypoxia to induce the effect of ischemia and unligating the carotid artery for postischemic reperfusion. Each experimental group was also further subdivided to HYPOXIC ISCHEMIC–REPERFUSED to serve as CONTROL and MANNITOL, DEXAMETHASONE and MANNITOL–DEXAMETHASONE TREATED groups.

GROUP–A: In this experimental group, left common carotid artery was ligated in 45 rats for 3 hours at the end of which all rats were placed in a low pressure camera to expose them to hypoxia at 320 torr for 48 hours in the Department of Physiology, Medical Faculty of Cerrahpaşa. During hypoxia procedure 9 rats died, therefore 36 rats were to be employed for the experiment 9 of 36 rats, received no treatment but they were exposed to postischemic reperfusion for 60 minutes prior to decapitation, served in the HYPOXIC–ISCHEMIC–REPERFUSED CONTROL group. The rest 27 rats were employed in the MANNITOL, DEXAMETHASONE and MANNITOL–DEXAMETHASONE TREATED groups, having 9 rats in each respective treated groups.

Following hypoxia: the rats in MANNITOL treated group were exposed to postischemic reperfusion for 60 minutes and received mannitol % 20 2g /kg as an IV bolus through the penis vein, immediately after the unligation of carotid artery for reperfusion. 60 minutes following reperfusion and mannitol infusion, they were decapitated; the rats in DEXAMETHASONE treated group were treated with dexamethasone 0.14 mg /kg as a

bolus given through the penis vein and then treatment continued with dexamethasone 0.075 mg/kg intraperitoneally q.i.d for 48 hours by the time all rats were exposed to postischemic reperfusion for 60 minutes before decapitation; the rats in MANNITOL–DEXAMETHASONE treated group were treated with dexamethasone as in dexamethasone treated group but at the end of 48 hours treatment with dexamethasone, they received mannitol %20 2 g/kg through the penis vein immediately following the unligation for 60 minutes before decapitation

GROUP–B: In this experimental group left common carotid artery was ligated for 6 hours in 40 rats, at the end of which all rats were placed in a low pressure camera to expose them to hypoxia at 320 torr for 48 hours. 4 rats died during hypoxia procedure, therefore 36 rats were to be employed in the experiment, having 9 rats in each HYPOXIC–ISCHEMIC–REPERFUSED CONTROL and MANNITOL, DEXAMETHASONE, MANNITOL–DEXAMETHASONE TREATED groups respectively. The rest of the experimental procedure applied in this group was the same as in GROUP–A.

After completing all the procedure applied for CONTROL and TREATED groups of both experimental groups of A and B, all rats were decapitated, being followed by brain removal which was carried out as quickly as possible in less than 5 minutes. Gross inspection was made on the cerebral hemispeheres which were both swollen, being more marked in the left side but not stained with Evans blue, injected intravenously prior to decapitation using 5 ml/kg. Both cerebral hemispheres were dissected to the CORTEX and W. MATTER from each of which samples were taken. Samples were placed in the known weighed pots which were then reweighed for wet tissue weight of the brain parts. All pots were placed in an oven for 48 ∓ 2 hours to dry brain parts at 105 ∓ 5°C. The water content of each sample was calculated from the difference in the wet and dry weights.

Dried brain parts were digested with 5ml 0.75 M HNO3 for 24 hours. After centrifugation of the mixture, the supernatant was taken and Na^+ and K^+ ion concentrations were measured with a flame photometer. Ion concentrations were calculated in mEq/kg wet tissue, using–Dilution factor x the figure read on the flame photometer for each ion; Dilution factor being calculated as 0.75M HNO3 volume/wet weight of the brain part–(in Ref: 25).

During the experiment, all procedures, i.e, ligation and unligation of the carotid arteries, IV and intraperitoneal treatments, decapitations were carried out under ether anesthesia and all rats inspired room air spontaneusly, being except in the low pressure camera.

Following anesthesia and ligation of the carotid artery, the observed neurological abnormalities in all rats were permanent left sided Horner syndrome and right sided weakness for short duration, which persisted longer in the rats survived hypoxia, being about 2 hours. Following hypoxia, the rats were also lethargic for some time as well. No seizure any total loss of consciousness were observed during the experiment. The other point to be mentioned is that after hypoxia the rats became much more sensitive to anesthesia, especially in longer treated groups, which was put down to the developing ischemic edema.

RESULTS

Na^+ concentrations: Na^+ values in the both rats with no ligation but hypoxic and the rats with left common carotid arteries ligated for 3 and 6 hours and also hypoxic have showed no significant differences. Kruskall–Wallis' analysis of Na^+ concentrations of w. matter and cortex with 3 and 6 hours carotid artery ligation suggested that mannitol, dexamethasone and mannitol–dexamethasone showed significantly different effect from each other (Table-1, Figure–1).

Table 1. Mean values of Na^+ concentrations and Kruskal-Wallis' variance analysis amongst three treated groups. (concentrations in mEq / kg wet tissue) (n: number of rats)

TREATED GROUPS		DURATION OF CAROTID ARTERY LIGATION			
		3 hours		6 hours	
		W. Matter	Cortex	W. Matter	Cortex
Dexamethasone n:9		53.4∓ 4.30	53.4∓14.1	44.5∓ 4.2	48.0∓6.1
Mannitol n:9		49.5∓ 7.0	58.2∓ 4.4	57.1∓17.0	49.9∓3.1
Dexamethasone / Mannitol n:9		43.1∓ 3.3	45.3∓ 2.5	40.5∓ 3.9	46.3∓3.0
Kruskal-Wallis' Variance Analysis N:		KW=10.416 P< 0.01	KW=12.436 P< 0.01	KW=11.503 P < 0.01	KW=3.792 P < 0.05
Control	Left n:9	46.7∓ 8.0	52.6∓10.0	54.1∓ 6.9	55.2 ∓6.2
	Right n:9	47.3∓ 4.7	47.9∓ 6.2	50.1∓ 5.2	52.5 ∓6.8

Na^+ concentrations (in HYPOXIC, not ligated rats, n: 9):

Right-W. MATTER: 45.4 ∓ 4.2 CORTEX: 47.0 ∓ 2.4

Left-W. MATTER: 47.9 ∓ 3.4 CORTEX: 48.0 ∓ 5.0

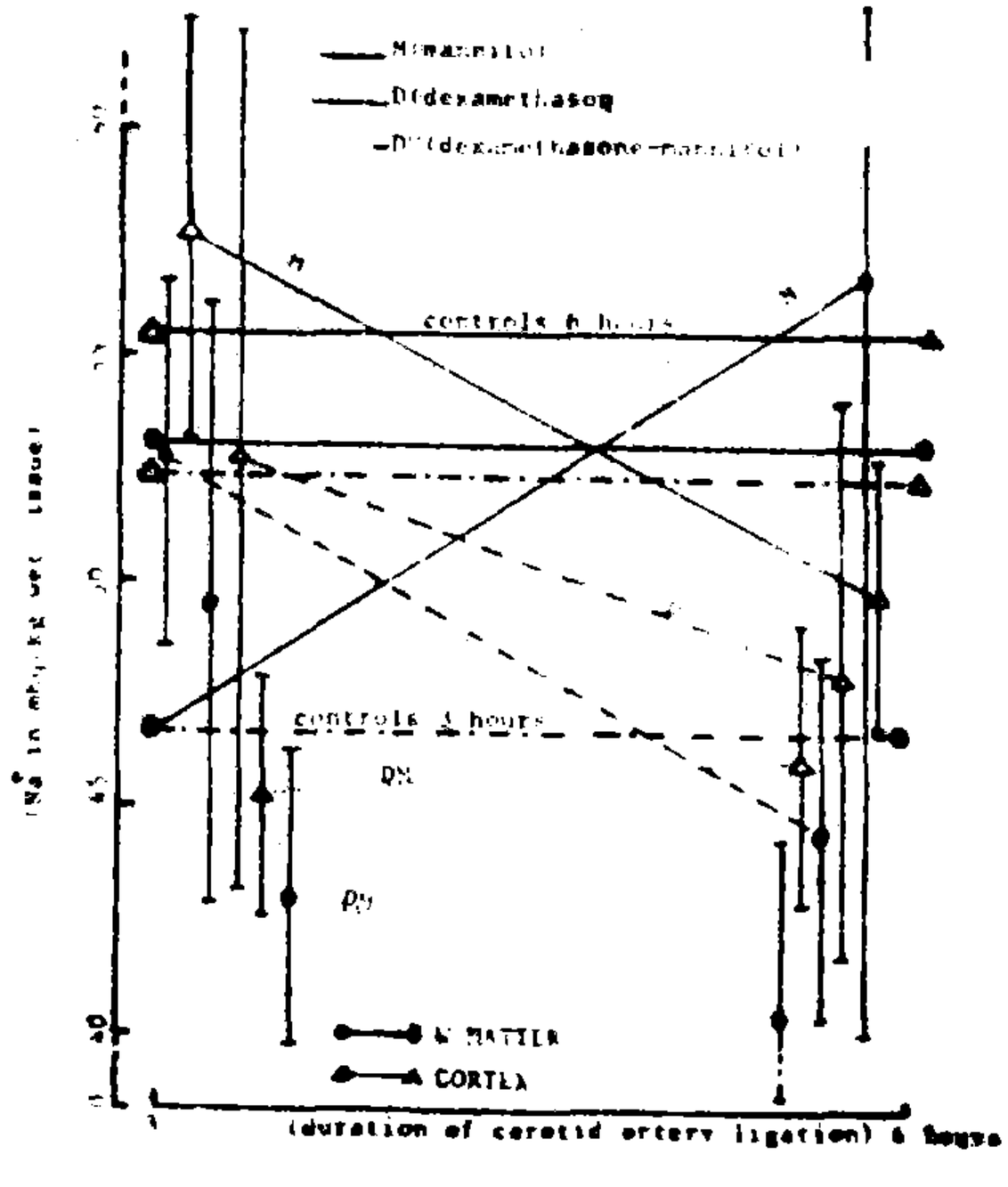

Figure 1. Na^+ concentrations of W. Matter / Cortex in hypoxic-ischemic edema.

In the 3 hours ligated group, Na^+ concentrations of w.matter and cortex were increased by dexamethasone and mannitol in comparison to the control group, mannitol-dexamethasone creating an opposite effect. But in the 6 hours group, both dexamethasone and mannitol–dexamethasone decreased Na^+ concentrations of w. matter and cortex in comparison with the control group. In mannitol treated group Na^+ concentrations were found to be above the control group's level for w. matter but it was opposite for the cortex. In table–2 and 3, comparison of the mean Na^+ values in the combined subdivided groups are displayed.

K^+ concentrations: The effects of mannitol, dexamethasone and mannitol–dexamethasone on K^+ ion levels in the left cerebral hemisphere were found to be statistically different, being except in the cortex of 6 hours ligated group. (Table–4). Essentially K^+ levels in the left and right cerebral hemispheres were more or less near to each other, except in the cortex of 3 hours ligated group (Table– 4, 5, 6) K^+ levels, in the dexamethasone treated groups, of w. matter and cortex with both 3 and 6 hours ligation of left common carotid arteries were always found to be lower than the control values of the left side. While combination treatment with mannitol–dexamethasone showed an unstable trend, but in mannitol treated group K^+ values were

Table 2: Comparison of the mean values of Na^+ concentrations of Cortex and M. Matter in the subdivided experimental groups.

Compared Subdivided Groups	Duration of Carotid Artery Ligation and Hemispheric Regions			
	3 Hours		6 Hours	
	Cortex	W. Matter	Cortex	W. Matter
Dexamethasone / Mannitol	$t = 0.975$ $P > 0.05$	$t = 1.424$ $P > 0.05$	$t = 0.833$ $P > 0.05$	$t = 2.158$ $P < 0.05$
Dexamethasone / Dexamethasone-Mannitol	$t = 1.697$ $P > 0.05$	$t = 5.700$ $P < 0.01$	$t = 0.750$ $P > 0.05$	$t = 2.094$ $P > 0.05$
Mannitol / Dexamethasone-Mannitol	$t = 7.651$ $P < 0.01$	$t = 2.481$ $P < 0.05$	$t = 2.503$ $P < 0.05$	$t = 2.855$ $P < 0.02$
Control (Left / Right Side)	$t = 1.198$ $P > 0.05$	$t = 0.194$ $P > 0.05$	$t = 0.880$ $P > 0.05$	$t = 1.389$ $P > 0.05$
Dexamethasone / Life side control	$t = 0.139$ $P > 0.05$	$t = 2.213$ $P < 0.05$	$t = 2.484$ $P < 0.05$	$t = 3.566$ $P < 0.01$
Mannitol / Left side control	$t = 1.538$ $P > 0.05$	$t = 0.790$ $P > 0.05$	$t = 2.293$ $P < 0.05$	$t = 0.490$ $P > 0.05$
Dexamethasone-Mannitol / Left side control	$t = 2.124$ $P < 0.05$	$t = 1.248$ $P > 0.05$	$t = 3.876$ $P < 0.01$	$t = 3.246$ $P < 0.01$
Dexamethasone / Right side control	$t = 1.071$ $P > 0.05$	$t = 2.873$ $P < 0.02$	$t = 1.478$ $P > 0.05$	$t = 2.513$ $P < 0.05$
Mannitol / Right side control	$t = 4.065$ $P < 0.01$	$t = 0.783$ $P > 0.05$	$t = 1.044$ $P > 0.05$	$t = 1.181$ $P > 0.05$
Dexamethasone-Mannitol / Right side control	$t = 1.167$ $P > 0.05$	$t = 2.194$ $P < 0.05$	$t = 1.010$ $P > 0.05$	$t = 4.430$ $P < 0.01$

Table 3: Comparison of the mean values of Na^+ concentrations of W.Matter and Cortex in the treated and control groups.

Treated Groups		Compared Groups			
		3 hours ligation (W.Matter / Cortex)	6 hours ligation (W.Matter / Cortex)	W.Matter / W.Matter (3 Hours / 6 hours)	Cortex / Cortex (3 hours / 6 hours
Dexamethasone		$t = 0.000$ $P > 0.05$	$t = 1.417$ $P > 0.05$	$t = 4.441$ $P < 0.01$	$t = 1.054$ $P > 0.05$
Mannitol		$t = 3.157$ $P < 0.01$	$t = 1.25$ $P > 0.05$	$t = 1.240$ $P > 0.05$	$t = 4.637$ $P < 0.01$
Dexamethasone / Mannitol		$t = 1.594$ $P > 0.05$	$t = 3.536$ $P < 0.01$	$t = 1.527$ $P > 0.05$	$t = 0.768$ $P > 0.05$
Control	Left	$t = 1.382$ $P > 0.05$	$t = 0.356$ $P > 0.05$	$t = 2.102$ $P > 0.05$	$t = 0.663$ $P > 0.05$
	Right	$t = 0.231$ $P > 0.05$	$t = 0.841$ $P > 0.05$	$t = 1.199$ $P > 0.05$	$t = 1.500$ $P > 0.05$

Table 4. Mean values of K^+ concentrations and Kruskal-Wallis' variance analysis amongst three treated groups. (concentrations in mEq / kg wett tissue) (n: number of rats)

TREATED GROUPS		DURATION OF CAROTID AFTERY LIGATION			
		3 hours		6 hours	
		W. Matter	Cortex	W. Matter	Cortex
Dexamethasone n:9		70.1∓ 7.7	75.7∓ 5.5	79.8∓ 3.8	80.9∓ 2.9
Mannitol n:9		82.6∓ 9.0	93.7∓ 7.8	100.7∓25.3	84.3∓12.5
Dexamethasone / Mannitol n:9		70.5∓ 4.1	81.4∓ 4.5	70.6∓11.7	80.3∓5.1
Kruskal Wallis Variance Analysis N:27		KW=20.987 P< 0.01	KW=17.086 .P<0.01	KW=13.922 P< 0.01	KW=1.340 P > 0.05
Control	Left n:9	75.9∓14.9	79.6∓ 8.3	82.6∓16.0	87.6∓10.1
	Right n:9	78.0∓12.3	87.8∓14.9	82.8∓ 9.0	86.4∓10.4

K^+ concentrations (in HYPOXIC, not ligated rats, n:9): Right-W. MATTER: 78.2 ∓ 4.6, CORTEX: 81.0 ∓ 9.5; Left-W. MATTER: 83.8 ∓ 7.8 COTREX: 86.1 ∓ 8.8

always found to be higher than the control levels, being except in the cortex of 6 hours ligated group.

In comparison to the control values, the minimum alteration of K^+ appeared to be in the dexamethasone treated group (Figure–2). In table–5, 6 detailed comparisons of different combined treated and control groups are seen.

% H^2O *concentrations:* Values were not found to be statistically different in the both left and right cerrebral hemispheres of control group with 3 and 6 hours ligation of the left common carotid arteries. (Table–7, 8, 9).

The effects of mannitol, dexamethasone and mannitol–dexamethasone on % H^2O concentrations were however significantly different from each other, being expect in the cortex of 3 hours ligated group. (Table–7, Figure–3) The minimum alteration of % H^2O concentrations was observed in the mannitol–dexamethasone treated group on comparison to the values in the control groups (of ligated rats). In table–8, 9, comparisons of the mean % H^2O values amongst various combined groups are presented.

Tablo 5: Comparison of the mean values of K concentrations of W.Matter and Cortex in the treated and control groups.

Treated Groups		Compared Groups			
		3 hours ligation (W.Matter / Cortex)	6 hours ligations (W.Matter / Cortex)	W.Matter / W.Matter (3 hours / 6 hours)	Cortex / Cortex (3 hours / 6 hours)
Dexamethasone		$t = 1.775$ $P > 0.05$	$t = 0.433$ $P > 0.05$	$t = 3.389$ $P < 0.01$	$t = 2.510$ $P < 0.05$
Mannitol		$t = 2.796$ $P < 0.02$	$t = 1.743$ $P > 0.05$	$t = 2.022$ $P > 0.05$	$t = 1.914$ $P > 0.05$
Dexamethasone / Mannitol		$t = 5.372$ $P < 0.01$	$t = 2.280$ $P < 0.05$	$t = 0.024$ $P > 0.05$	$t = 0.485$ $P > 0.05$
Control	Left	$t = 0.651$ $P > 0.05$	$t = 0.793$ $P > 0.05$	$t = 0.919$ $P > 0.05$	$t = 1.836$ $P > 0.05$
	Right	$t = 0.236$ $P > 0.05$	$t = 0.785$ $P > 0.05$	$t = 0.945$ $P > 0.05$	$t = 0.231$ $P > 0.05$

Table 6: Comparison of the mean values of K^+ concentrations of Cortex and W.Matter in the subdivided experimental groups.

Compared Subdivided Groups	Duration of Carotid Artery Ligation and Hemispheric Regions			
	3 Hours		6 Hours	
	Cortex	W. Matter	Cortex	W. Matter
Dexamethasone / Mannitol	$t = 4.895$ $P < 0.01$	$t = 3.166$ $P < 0.01$	$t = 0.795$ $P > 0.05$	$t = 2.45$ $P < 0.05$
Dexamethasone / Dexamethasone-Mannitol	$t = 2.406$ $P < 0.05$	$t = 0.137$ $P > 0.05$	$t = 0.307$ $P > 0.005$	$t = 2.244$ $P < 0.05$
Mannitol / Dexamethasone-Mannitol	$t = 4.037$ $P < 0.001$	$t = 3.670$ $P < 0.01$	$t = 0.889$ $P > 0.05$	$t = 3.240$ $P < 0.01$
Control (Left / Right Side)	$t = 1.442$ $P > 0.05$	$t = 0.051$ $P > 0.05$	$t = 0.248$ $P > 0.05$	$t = 0.033$ $P > 0.05$
Dexamethasone / Left side control	$t = 0.354$ $P > 0.05$	$t = 0.185$ $P > 0.05$	$t = 1.913$ $P > 0.05$	$t = 0.511$ $P > 0.05$
Mannitol / Left side control	$t = 3.714$ $P < 0.01$	$t = 0.199$ $P > 0.05$	$t = 0.616$ $P > 0.05$	$t = 1.814$ $P > 0.05$
Dexamethasone-Mannitol / Left side control	$t = 0.182$ $P > 0.05$	$t = 0.203$ $P > 0.05$	$t = 1.936$ $P > 0.05$	$t = 1.816$ $P > 0.05$
Dexamethasone / Right side control	$t = 2.286$ $P < 0.02$	$t = 0.338$ $P > 0.05$	$t = 1.528$ $P > 0.05$	$t = 0.921$ $P > 0.05$
Mannitol / Right side control	$t = 0.188$ $P > 0.05$	$t = 0.905$ $P > 0.05$	$t = 0.387$ $P > 0.05$	$t = 2.000$ $P > 0.05$
Dexamethasone-Mannitol / Right side control	$t = 0.238$ $P > 0.05$	$t = 1.735$ $P > 0.05$	$t = 1.580$ $P > 0.05$	$t = 2.480$ $P < 0.05$

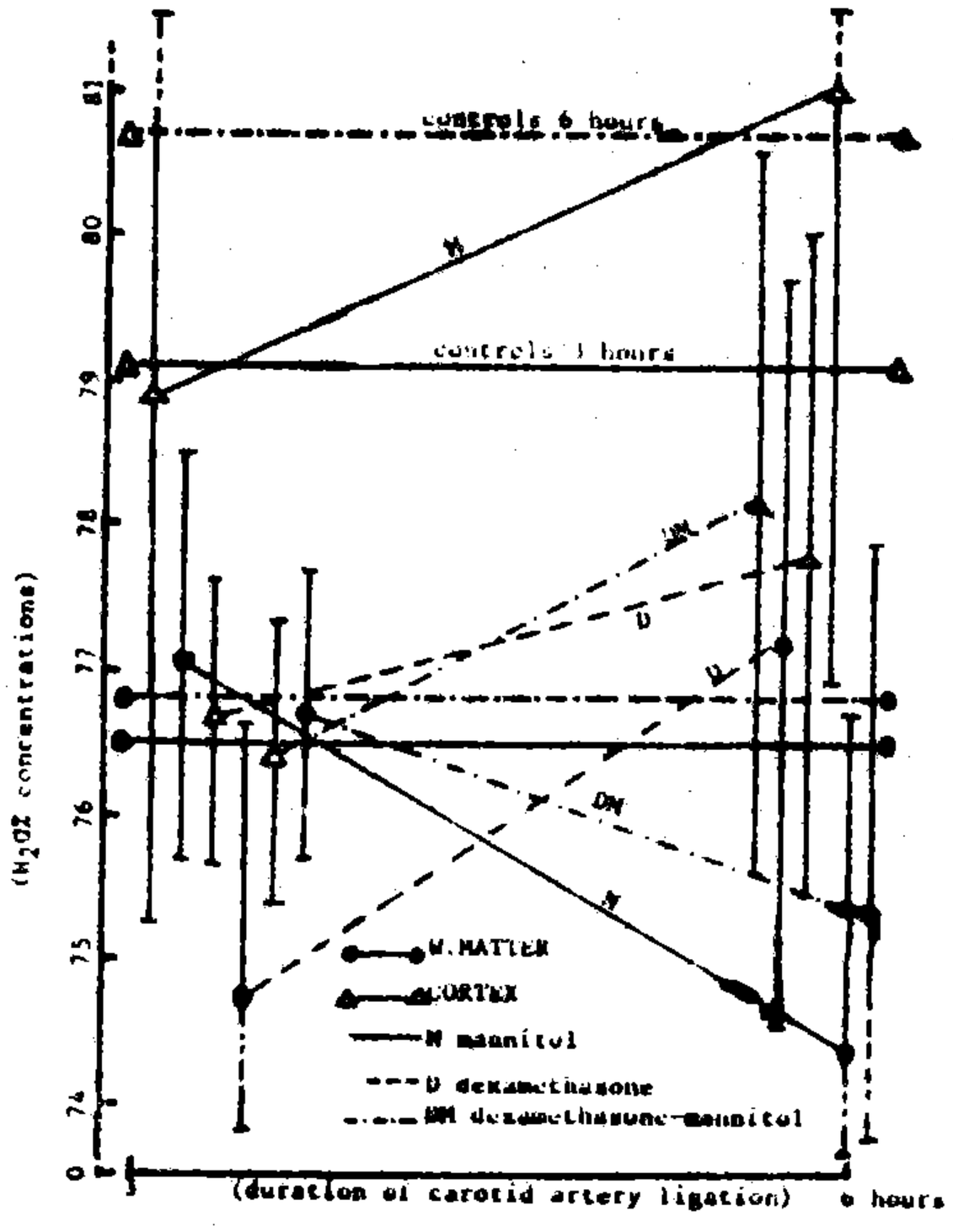

Figure 2. K^+ concentrations of W. Matter / Cortex in hypoxic-ischemic edema.

Table 7. Mean values of H_2O % concentrations and Kruskal-Wallis' varience analysis amongst three treated groups. (n: number of rats)

TREATED GROUPS		DURATION OF CAROTID ARTERY LIGATION			
		3 hours		6 hours	
		W. Matter	Cortex	W. Matter	Cortex
Dexamethasone n:9		74.7∓ 1.9	76.7∓ 1.0	77.2∓ 2.4	77.8∓2.3
Mannitol n:9		77.0∓ 1.4	78.8∓ 3.6	74.4∓ 2.3	81 ∓4.1
Dexamethasone / Mannitol n:9		76.7∓ 1.0	76.4∓ 1.0	75.3∓ 2.6	78.2∓2.5
Kruskal-Wallis' Variance Analysis N:27		K=10,747 P < 0.01	K= 1.320 P > 0.05	K= 4.924 P < 0.05	K=6.066 P <0.05
Control	Left n:9	76.5∓ 2.3	73.1∓ 3.0	76.3∓ 3.0	80.7∓ 6.1
	Right n:9	74.1∓ 2.3	76.5∓ 1.3	74.8∓ 4.3	77.1∓3.1

% H_2O concentrations (in HYPOXIC, not ligated rats, n:9): Right-W. MATTER: 78.2∓2.0, CORTEX: 75.7 ∓ 2.7 Left- W. MATTER: 79.2 ∓ 4.2, CORTEX: 78.1 ∓ 4.0

Table 8: Comparison of the mean values of H_2O % concentrations of W.Matter and Cortex in the treated and control groups.

Treated Groups		Compared Groups			
		3 hours ligation (W.Matter / Cortex)	6 hours ligation (W.Matter / Cortex)	W.Matter / W.Matter (3 hours / 6 hours)	Cortex / Cortex (3 hours / 6 hours)
Dexamethasone		$t = 2.793$ $P < 0.02$	$t = 0.541$ $P > 0.05$	$t = 2.451$ $P < 0.05$	$t = 1.574$ $P > 0.05$
Mannitol		$t = 1.399$ $P > 0.05$	$t = 4.212$ $P < 0.01$	$t = 2.898$ $P < 0.02$	$t = 0\ 665$ $P > 0.05$
Dexamethasone / Mannitol		$t = 0.637$ $P > 0.05$	$t = 2.413$ $P < 0.05$	$t = 0.509$ $P > 0.05$	$t = 2.236$ $P < 0.05$
Control	Left	$t = 2.698$ $P < 0.02$	$t = 0.857$ $P > 0.05$	$t = 0.159$ $P > 0.05$	$t = 1.026$ $P > 0.05$
	Right	$t = 2.727$ $P < 0.02$	$t = 1.302$ $P > 0\ 05$	$t = 0.431$ $P > 0.05$	$t = 0.536$ $P > 0.05$

Table 9: Comparison of the mean values of $H_2O\%$ concentrations of Cortex and W.Matter in the subdivided experimental groups.

Compared Subdivided Groups	Duration of Carotid Artery Ligation and Hemispheric Regions			
	3 Hours		6 Hours	
	Cortex	W. Matter	Cortex	W. Matter
Dexamethasone / Mannitol	$t = 1.687$ $P > 0.05$	$t = 2.922$ $P < 0.01$	$t = 2.042$ $P > 0.05$	$t = 2.527$ $P < 0.05$
Dexamethasone / Dexamethasone-Mannitol	$t = 0.637$ $P > 0.05$	$t = 2.793$ $P < 0.02$	$t = 0.353$ $P > 0.05$	$t = 1.611$ $P > 0.05$
Mannitol / Dexamethasone-Mannitol	$t = 1.928$ $P > 0.05$	$t = 0.523$ $P > 0.05$	$t = 1.749$ $P > 0.05$	$t = 0.778$ $P > 0.01$
Control (Left / Right Side)	$t = 3.120$ $P < 0.01$	$t = 2.214$ $P < 0.05$	$t = 1.578$ $P > 0.05$	$t = 0.858$ $P > 0.05$
Dexamethasone / Left side control	$t = 3.415$ $P < 0.01$	$t = 1.811$ $P > 0.05$	$t = 1.334$ $P > 0.05$	$t = 0.72$ $P > 0.05$
Mannitol / Left side control	$t = 3.649$ $P < 0.01$	$t = 0.557$ $P > 0.05$	$t = 0.122$ $P > 0.05$	$t = 1.508$ $P > 0.05$
Dexamethasone-Mannitol / Left side control	$t = 3.130$ $P < 0.01$	$t = 0.239$ $P > 0.035$	$t = 1.138$ $P > 0.05$	$t = 0.756$ $P > 0.05$
Dexamethasone / Right side control	$t = 0.366$ $P > 0.05$	$t = 0.604$ $P > 0.05$	$t = 0.544$ $P > 0.05$	$t = 1.462$ $P > 0.05$
Mannitol / Right side control	$t = 1.802$ $P > 0.05$	$t = 3.233$ $P < 0.01$	$t = 2.277$ $P < 0.05$	$t = 0.246$ $P > 0.05$
Dexamethasone-Mannitol / Right side control	$t = 0.183$ $P > 0.05$	$t = 3.110$ $P < 0.01$	$t = 0.829$ $P > 0.05$	$t = 0.298$ $P > 0.05$

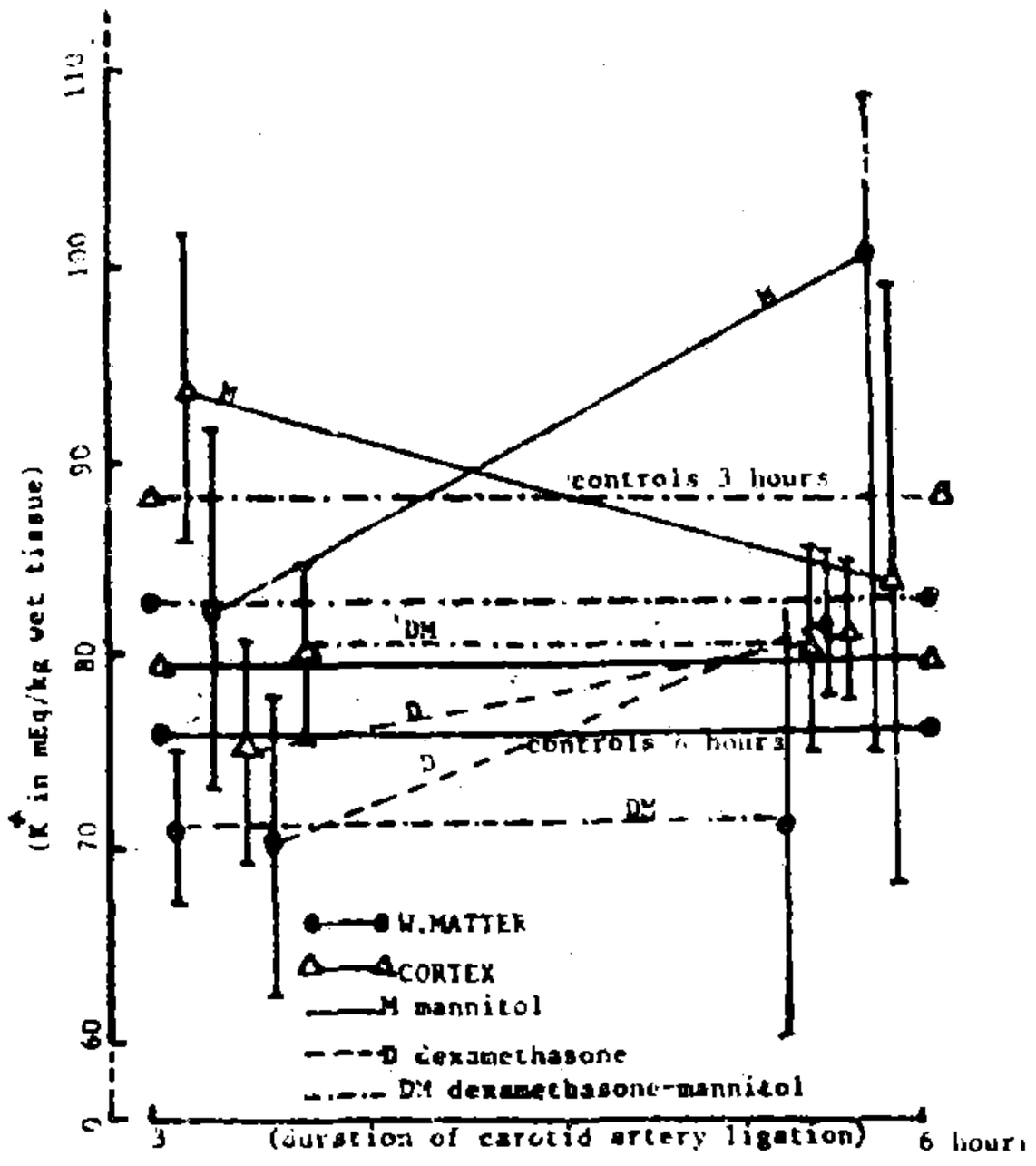

Figure 3. H_2O % concentrations of W. Matter / Cortex in hypoxic-ischemic edema.

DISCUSSION

Clinico–pathologic studies indicate that hemispheric edema and brain swelling with subsequent tentorial herniation are the direct reason for fatal outcome of supratentorial ischemic stroke in at least 10 % of the cases (20). Ischemic brain edema causes progressive microcirculatory compression and thereby aggravates the primary ischemic insult (12, 31). Edema commences shortly after the onset of ischemia and reaches a peak after about 2 days. The most significant amount and rate of edema accumulation occurs days after the vascular occlusion. It is accompanied by a significant brekdown of the blood brain barrier (BBB) to macromolecules, first observed at 4–6 hours and having a maximum at 2–4 days. It is therefore referred to as "vasogenic". Early water uptake without significant permeability changes of the BBB is thought to be "cytotoxic" type of brain edema (14, 15, 23, 24, 33).

In this experiment, edema was mainly of cytotoxic type which was confirmed by the absence of Evans blue staining. In hypoxic–ischemic cerebral edema, cytotoxic phase (early phase) is ussually accompanied by chemical changes with rising Na^+, H^2O % concentrations and a fall in K^+ concentration; but some evidences have been shown by HOSSMAN and SCHUIER that the threshold of blood flow for edema formation and biochemical chan-

ges is not the same (12). In this experiment, biochemical alterations did not show parallelism to our electron microscopic (EM) observations of the ischemic edema (9). Also the same observations were made by SHIBATA and et al. in dogs with a middle cerebral artery clipped , that if no infarct occured during the experiment, water and electrolyte contents were not also obviously changed from the controls' level (33). PLUM and et al. also in their (similar to this) experiment on the rats which were exposed to severe hypoxia correlated the chemical changes closely with histological changes (28).

In this experiment, chemical alterations in Na^+, K^+ and H^2O % concentrations fluctuated within the narrow limits and teherefore major changes were not observerd, while EM observations showed obvious histological neuronal alterations (9). The fluctuations of chemical contents (Na^+, K^+, H^2O) in the rats of this study were most likely secondary to the hypoxia which was thought to have had a moderate effect on ion homeostasis(30).

For the effect of mannitol, a combination of many factors are most likely responsible, including improvement of cerebral blood flow (CBF) to the ischemic area by reduction in blood viscosity induced by mannitol (19), maintenance of microcirculatory patency (7, 8, 17), reduction of edema (7, 8, 17, 34). On the other hand, in a study, it is suggested that mannitol could act like free radical scavengers, if it is given in high concentration in ischemic insult (4). Early administration of mannitol is particularly important as its dehydrating action in ischemic tissue depends upon the functioning brain capillary, that is, preserved BBB (10).

In this experiment, mannitol was infused in post-insult phase but BBB integrity was tested with Evans blue by observing that effected hemisphere was not stained and also by EM observation in which no necrosis was present of endothelial cells (9).

The results of both clinical and laboratory investigations on mannitol effect are contradictory (3, 7, 8, 10, 17, 18, 19, 27, 34). None of these studies related to the chemical contents (Na, K, H^2O %), they rather relate to the observations in reduction of the brain swelling and neurological improvement or CBF.

In cerebral ischemia, steroids may have a beneficial effect when given prior to the onset of ischemia by inhibiting the release of arachidonic acid from ischemic cells but the evaluation of its therapy by both clinical and experimental was also conflicting (1, 2, 6, 13, 21, 22, 26, 29).

In conclusion, although chemical alterations were not compatible with the neuronal alterations in a similar preperation, mannitol–dexamethasone

combination appeared to be the choice of treatment to effect H_2O contents in moderate hypoxic–ischemic brain insults.

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