Effects Of Endothelin On Experimental Intracerebroventricular Haematoma

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Abstract : The effects of endothelin administered intravenously and intracerebroventricularly (i.c.v.) into the haematoma on arterial pressure, heart rate, other physiological parameters and intracerebroventricular haematoma were studied in the conscious rat. Control and intravenous (i.v.) endothelin-treated animals received 0.05 ml of normal saline injected into the clot, and the i.c.v. endothelin-treated group received an equal volume endothelin solution (10 nanogram) and i.v. endothelin-treated group received 2 ml endothelin solution (720 nanogram) intravenously. Intravenous injection of endothelin (720nanogram/2 ml, 12 nanogram/minute i.v.) increased mean arterial pressure (MAP) and heart rate. But i.c.v. injection of endothelin (10 nanogram) produced tachycardia ($p>0.05$). By 24 hours, clot was lysed in 16.7 % of controls, in 42 .8% of i.v. endothelin-treated animals and in 62.8 of i.c.v. endothelin-treated animals. There was no significant difference in results of all groups for the lytic efficacy of both I.V. and I.C.V. endothelin injection.

Key Words : Intracerebroventricular haematoma, Endothelin.

INTRODUCTION

Large intracerebral haemorrhages are generally associated with high morbidity and mortality rates. Authors have reported several treatment modalities for the treatment of large intracerebral haematomas, including conservative, surgical and stereotaxic aspiration methods. Several authors have suggested that the stereotaxic evacuation of these haematomas may be preferable to conventional surgical evacuation because of limited invasion of the overlying normal brain (14,17). Nevertheless, the adequate evacuation of clotted blood remains a problem (17,29). For this reason, a number of investigators have conducted experimental (4,16,28,32) and clinical (29) studies, using urokinase and tissue plasminogen activator to liquefy clot and facilitate its removal. Because of the risk of haemorrhagic complications of fibrinolytic agents (27), we therefore carried out this experiment to study the effectiveness for clot lysis and safety of endothelin, using its effects on the serum level of the endogen tissue plasminogen activator and prostoglandin 12.

MATERIALS AND METHODS

In this study, 21 adult rat (each weighing between 260-320 gram) were used. We separated these animals three groups; 6 animal in control group, 8 animals in I. experimental group (intrahaematomal Endothelin-treated) and 7, II. experimental group (intravenous Endothelin-treated).

Animals were anaesthetized with intraperitoneal pentobarbital (40 mg/kg, Nembutal, Abbott Lab.) and were then placed in the stereotaxic frame. Femoral artery and vein were cannulated via No. 24 Intracat; artery for arterial blood pressure monitorisation, and vein in order to get blood samples and to give necessary fluid and drugs.

Parasagittal incision 2 centimetre in length was made. Then a hole, about 0.5 millimetre in diameter, was created 3 millimetre lateral to the midline and 2 millimetre posterior to the coronal suture on the left. Through this hole, 0.05 ml clotted blood was injected stereotaxically by means of doubleject syringe

via a No. 22 needle at a depth of 4 millimetre from the cranial surface.

For the haematoma preparation, 5 ml banked human blood (O Rh (-)) was treated with human thrombin (20 units) to initiate clotting. Fifteen minutes later, the clot was separated from the plasma by centrifugation at 3000 G for 15 minutes.

Control and IV Endothelin-treated group animals received an injection of 0.05 ml normal saline and I. experimental group animals received an equal volume of Endothelin Solution (10 nanogram Endothelin/0.05 ml) intracerebroventricularly. The needle was allowed to remain in place for 3 minutes and was then withdrawn. The incision was closed. Then control and I. experimental group animals received 2 ml Ringer Lactate Solution and II. experimental group animals 2 ml Endothelin Solution (720 nanogram/2 ml, 12 nanogram/minute) for one hour period. Then the animals were allowed to wake up in another room.

At 24 hours after haematoma formation, all animals were then anaesthetized with intraperitoneal sodium pentobarbital (40 mg/kg). Blood samples were collected via femoral vein. Then 1 ml (50 mg/ml) ketamin hydrochloride was injected via femoral vein in order to sacrifice the animals.

Pathological Analysis :

The rat brains were removed and fixed 10 % neutral buffered formolin for 15 days prior to sectioning. Coronal sections of the fixed brains were made at 1 or 2 millimetre intervals. The presence or absence of an intracerebral or Intraventricular haematoma was noted. Photographic documentation was obtained. These sections were studied by the pathologist.

Haematologic and Biochemical Analysis :

Blood samples were drawn from the femoral catheter, prior to and at one hour after the haematoma injection into the brain.

Statistics :

All values are expected as means and standard error of means. The means were compared using Student's t test. We also used Fisher's Exact Test for the evaluation of clot lysis.

RESULTS

Hemodynamic Effects of Endothelin :

Two animals in Control, three animals in I. Experimental Group and one animal in II. Experimental Group were died within 2 minutes after the intracerebroventricular injection of the haematoma. One animal in Control Group has focal convulsions after haematoma injection and then died.

The pressor and tachycardic responses were followed by cardiovascular collapse and death; 2 animals in I. Experimental Group died 25 minutes after intracerebroventricular injection of Endothelin and 2 animals after the intravenous infusions of Endothelin. These animals were excluded from the study which's results presented below.

Physiological Variables:

No significant differences were found among the physiologic, Haematologic, biochemical and blood-gas variables in Control Group. In II. Experimental Group, endothelin produced a marked and transient elevation in blood pressure and cardiac rates. These changes in blood pressure and cardiac rates were statistically significant ($p>0.05$). In addition, in I. Experimental Group, there was statistically significant increases in cardiac rates, appeared within 5 minutes after Endothelin injection into the haematoma ($p>0.05$).

Changes in the physiologic, Haematologic, biochemical and blood-gas variables are summarized in (Table-I.)

Clot Lysis:

A total 21 animals with 24 hour therapy were studied. All of them were sacrificed at 24 hours after haematoma injection. Of the 8 intrahaematomal Endothelin-treated animals, 5 (62.9 %) demonstrated clot lysis macroscopically. But only 3 (42.8 %) of 7 animals in intravenous Endothelin-treated animals, and 1 (16.7 %) of 6 control animals exhibited clot lysis (Fig.1a,b). In the statistical analysis, using Fisher's Exact Test, there was no significant efficacy of intrahaematomal and intravenously injection of Endothelin on the haematoma lysis ($p>0.05$).

Histopathologic Results:

There was some perihaematomal mononuclear cell and, less commonly, polymorphonuclear cell infiltration, especially in the intrahaematomal

Table I : Physiological variables in the there groups

	Control G.		Experimental G.I		Experimental G.II	
			Haematoma Injection			
	Pre	Post	Pre	Post	Pre	Post
MAP	10.8 ±2.85 t=0.724	108.4 ±1.85	106.8 ±2.03 t=0.177	108 ±2.36	106 ±4.74 t=2.308*	114.2 ±3.48
Heart rate	362.2 ±6.82 t=1.114	370.6 ±5.42	363.2 ±10.87 t=2.303*	382 ±8.12	370.4 ±11.48 t=2.154*	388.4 ±6.97
pH	7.289 ±0.04 t=0.039	7.296 ±0.03	7.319 ±0.06 t=0.005	7.31 ±0.11	7.322 ±0.066 t=0.009	7.310 ±0.11
pCO_2	34.42 ±2.233 t=0.039	34.9 ±1.128	33.62 ±1.95 t=0.013	33.9 ±1.78	33.42 ±1.93 t=0.074	32.5 ±2.576
Hb	10.78 ±0.962 t=0.056	9.54 0.538	9.666 ±1.191 t=0.105	10.24 ±1.55	9.826 ±1.395 t=0.016	10.065 ±1.527
Hct	32.8 ±2.501 t=0.391	31.104 ±1.48	27.22 ±7.63 t=0.011	28.26 ±8.52	26.82 ±7.58 t=0.0165	28.16 ±8.52
Sodium	138.4 ±3.92 t=0.409	135.2 ±3.815	137.4 ±4.8 t=0.167	134.6 ±5.748	136.8 ±4.069 t=0.0596	135.2 ±5.912
Potassium	3.46 ±0.795 t=0.322	3.7 ±0.209	3.475 ±0.807 t=0.021	3.4 ±0.273	3.525 ±0.035 t=0.221	3.45 ±0.335

*=significant difference statistically ($p>0.05$).

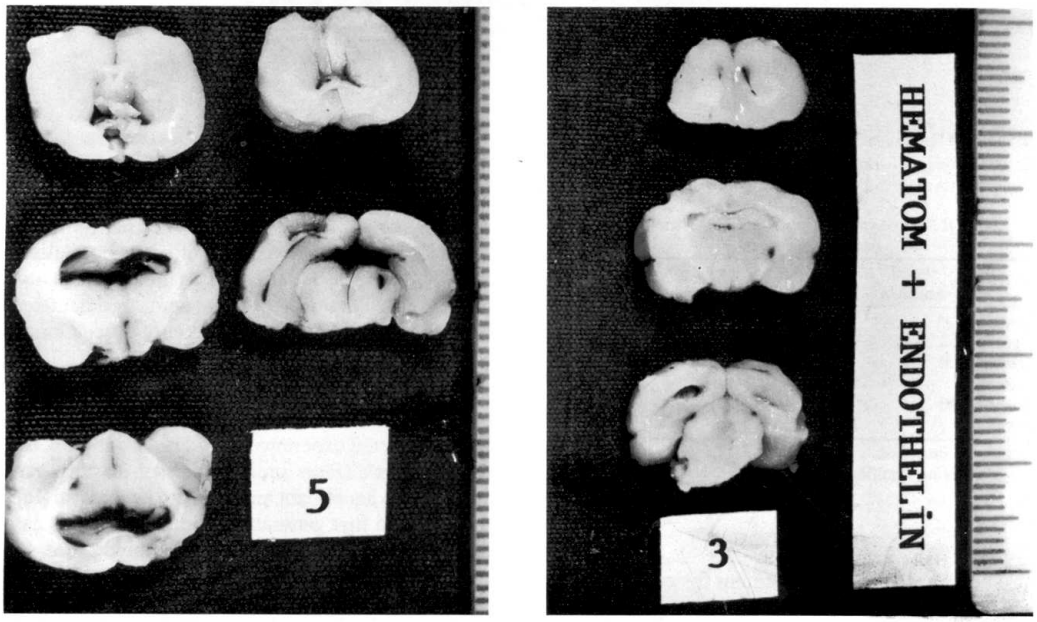

Fig. 1 : Photography of coronal section of control (a) and intracerebroventricular endothelin-treated brains. In a, the areas of the intracerebral-ventricular haematoma were shown, but the haematoma has effectively lysed shown in b.

Endothelin-treated animals. Also brain edema was noted, predominantly at the adjacent ependymal areas. In addition to these findings, lytic areas were seen in the intracerebral and Intraventricular clots. Clot lysis was demonstrated macroscopically. However, in some cases the clot lysis could not be demonstrated macroscopically, but there were lytic areas on microscopic evaluation (Fig.2a,b).

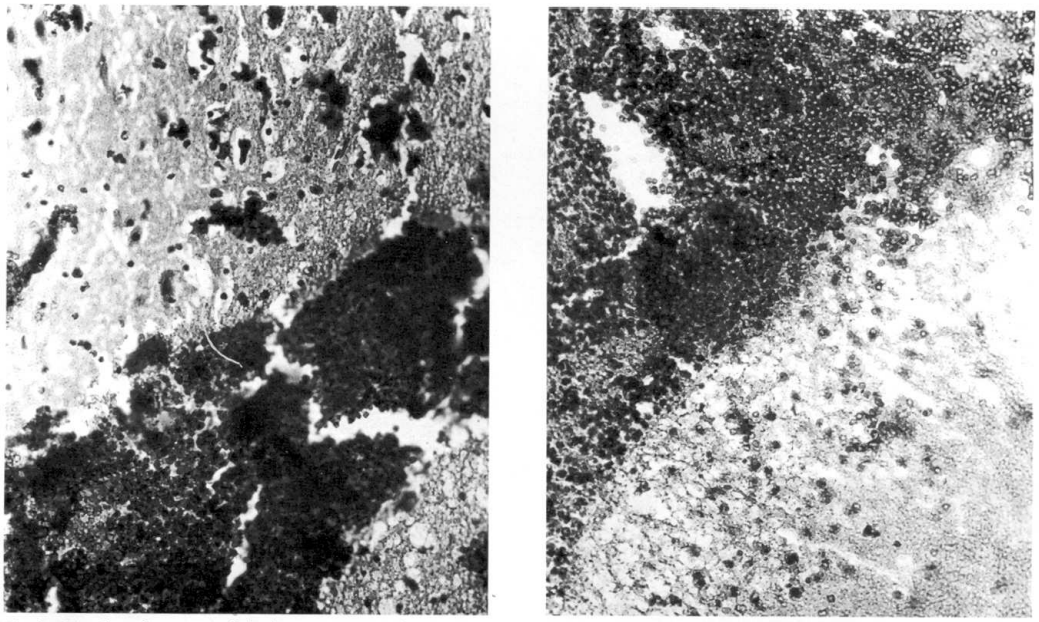

Fig. 2 : Histological sections of the brains. a: Control animal. Note the dens haematoma and diffuse edema (H&Ex25). b: Intracerebral-ventricular endothelin-treated animal brain section. Note the lytic areas and edema (H&Ex25).

Table II : Clot Lysis in the Groups at 24 hours after haematoma injection

	Total Animals	Haematoma present	Lysis absent	significance
Control group	6	1(%16,7)	5(%83,3)	
Experimental group	8	5(%62,5)	3(%37,5)	± (p=0.121)
Experimental group	7	3(%42,8)	4(%57,2)	± (p=0.343)

* = Fisher's exact test
± = There is no significant difference.

DISCUSSION

The optimal form of treatment for patients who suffered the spontaneous onset of a supratentorial intracerebral-ventricular haemorrhage remains undetermined. Surgical treatment of spontaneous intracerebral haemorrhages has improved during the past four decades. But many important problems still remain to be resolved. The postoperative mortality rate has varied greatly in different reports, ranging from 20 % to 80 %, with some patients, such as those in deep coma or those with a medial (thalamic) haemorrhage, having mortality rate as high as 90 % (15,20,30). It is known that, a few hours after the onset of symptoms, a haematoma consists of liquid blood (about 20% of its volume) and dens clots (about 80%). For this reason, attempts to remove haematomas through a cannula, even one with large diameter, have often been unsuccessful because evacuation of the dense clots was practically impossible (15).

Because the clot may be so solid that only a portion of it can be aspirated, a number of investigators have conducted experimental and clinical studies, using fibrinolytic agents (urokinase. tissue plasminogen activator) to liquefy clot and facilitate its removal (16). Urokinase, a first generation fibrinolytic agent, has the disadvantage of being fibrinogenolytic. The second generation fibrinolytic agents (tissue plasminogen activator), which is potentially only fibrinolytic has been said to be much more effective than the first generation agents (1,2,4,6,12,16,21).

Although there were findings that the fibrinolytic agents were effective to liquefy clot, exogenous injection of fibrinolytic agents has rebleeding risk for intracerebral vascular events (27). For this reason, some authors don't recommend to use these agents for the treatment of intracerebral events (34).

Endothelin is a polypeptide isolated from cultured porcine aortic endothelial cells (3,5,7,8,9,10,18,23). Binding sites for this peptide (Endothelin) have been shown to exist in the central nervous system (10,11,13,23). Intracerebroventricular Endothelin-1 (25 picomol/kg) provoked a prompt increase in arterial pressure within 5 minutes, and peak values were obtained at twenty minutes (24,31). Makino et al. reported that intracerebroventricular administration of Endothelin (5,20 or 40 picomol/2 microliter) was associated with dose dependently increased arterial pressure (23).

In this study, although intracerebroventricular injection of Endothelin in dose of 10 nanogram was associated with tachycardia, appeared at the first ten minutes, there was no significant blood pressure elevation. This finding is explained with dose dependent pressor effect of Endothelin.

Siren et al. reported that administration of Endothelin in dose of 30 picomol/kg via intracerebroventricular route has produced a profound pressor response and increase in total peripheral resistance and this pressor and tachycardic responses were followed by cardiovascular collapse and death (in 7 out of 8 animals) (35). Our study reports 33.3% mortality (in 3 of 12 animals) due to cardiovascular collapse after intracerebroventricular injection of 10 picomol dose of Endothelin. Because intracerebroventricular Endothelin injection was associated with haematoma formation, mortality is not merely bound to Endothelin.

Our results are in accordance with recent reports demonstrating pressor and tachycardic responses to intravenous endothelin use in rats (25,26).

Systemic administration of Endothelin-1 inhibits platelet aggregation due to the release of prostacyclin (PGI2). Prostacyclin has also been implicated in the mediation of the anti-aggregatory and fibrinolytic properties. Lidbury et al. reported that Endothelin-1 (0.1-1 nmol/kg), Endothelin-2 (0.1-1 nmol/kg) or Endothelin-3(0.3-3 nmol/kg) dose dependently inhibited platelet aggregation induced by adenosine diphosphate ex-vivo. In the same study, there was enhancement of plasma fibrinolytic activity. They reported that this Endothelin-induced enhancement of plasma fibrinolytic activity was associated with a release of tissue plasminogen activator into the circulation (19,22). Pruis at al. also reported that stimulation of the isolated perfused rat hindleg vascular bed with various concentration of Endothelin resulted in the acute release of tissue-type plasminogen activator and of von Willebrand factor (33). In our study, although there is no definitive explanation, clot lysis in the intracere-broventricular Endothelin-treated group might be secondary to the enhancement of fibrinolytic activity in and around the haematoma.

In summary, we suggest that other experimental studies might be prepared to discuss relationships between intracerebral haematoma and endothelin.

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