

LYSIS OF INTRACRANIAL HAEMATOMAS WITH TISSUE PLASMINOGEN ACTIVATOR IN A CAT MODEL

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SUMMARY :

In an attempt to investigate the effect of tissue plasminogen activator in the resolution of intracerebral haematoma an experimental model of intracerebral haematoma was designed in the cat. A total of 24 adult cats were studied. Intracerebral hematomas were provoked by stereotaxically injecting 0.2 ml of clotted human blood into the left frontal lobe. Control animals received 0.2 ml of normal saline injected into the clot and the experimental group received 0.2 ml of tissue plasminogen activator solution (3750 unite/ml). Six hours after injection, clot lysis was demonstrated in 11 (84.6%) of 13 tissue plasminogen activator-treated animals and 1 (5.5%) of 11 controls ($p < 0.001$). Tissue plasminogen activator did not cause inflammation or bleeding in the ependyma or parenchyma except in the perihematoma parenchyma.

KEY WORDS :

Intracerebral Haematoma, Tissue Plasminogen Activator, Cat.

INTRODUCTION

Intracerebral haematomas (ICH) may be caused by head injury or arise as a complication of hypertension, rupture of an aneurysm or arteriovenous malformation, anticoagulation therapy, bleeding disorders, or intracranial tumours (3,9,10,28,31). The advent of computed tomographic scanning (CT) has made the diagnosis, localization and treatment of such lesions much easier (6,9,10,11,14,20,31). Several authors have suggested that the stereotaxic evacuation of large deep-seated ICH's may be preferable to conventional surgical evacuation because of limited invasion of the overlying normal brain. Nevertheless, the adequate evacuation of clotted blood remains a problem. The clot may be so solid that only a portion of it can be aspirated (21). For this reason, thrombolytic agents have been proposed for use intracranially to facilitate more complete drainage of ICH's (15,21).

This study was designed to investigate the efficacy and safety of tissue plasminogen activator (tPA) in a cat-ICH model.

MATERIALS AND METHODS

The twenty-four-adult cats (each weighing between 2.8-5.3 kg) were studied, 13 animals in the experimental group and 11 the control group. Animals were anesthetized with ketamin hydrochloride (10 mg/kg, intramuscularly). Blood was drawn from a peripheral vein for baseline coagulation analysis. Animals were then placed in a stereotaxic frame.

20 ml banked human whole blood was mixed with human thrombin (80 units) to initiate clotting. The clot was separated from the plasma by centrifugation at 3000G for 15 minutes.

On the left parietal bone, a sagittal incision 3 cm in length was made and a hole, about 0.5 mm in diameter drilled, 2 mm posterior to the coronal suture and 3 mm lateral to the midline. 0.2 ml of clotted blood was injected stereotactically by means of a doubleject injector via a no. 22 needle at a depth of 5 mm from the external surface of the skull. At the same time, control animals received an injection of 0.2 ml normal saline, whereas the experimental group received an 0.2 ml of tPA solution (3500 IU/ml), injected into the hematoma by means of the same injector. After 5 minutes, the needle was withdrawn. The incision was closed and the animal was allowed to wake up. The neurological status of the animals was graded according to a standard neurological chart (24) and the animals were also examined for evidence of systemic bleeding involving venipuncture sites, the sclera and conjunctiva before death. Control blood samples were drawn from a peripheral vein for haematological evaluation and then the animals were sacrificed with high-dose intravenous ketamin hydrochloride.

The cat brains were removed and fixed in 10% neutral buffered formalin for 2 weeks prior to sectioning. Coronal section of fixed brains were made at 2-4 mm intervals. At the gross examination of the

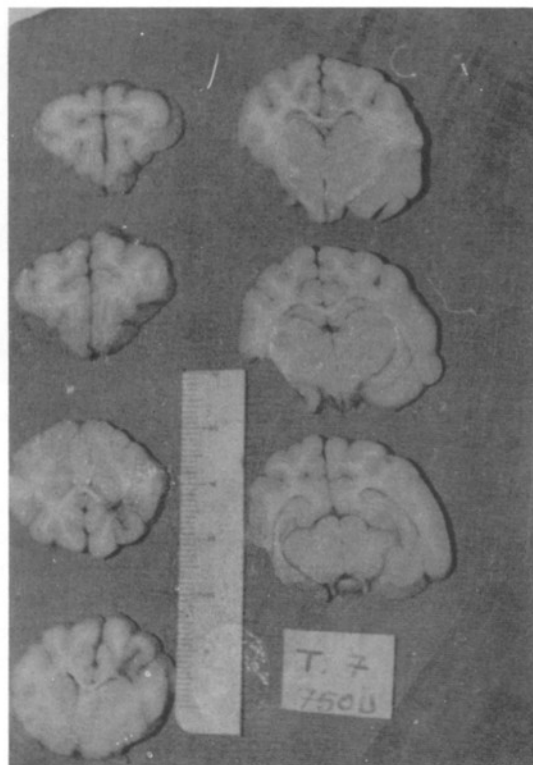
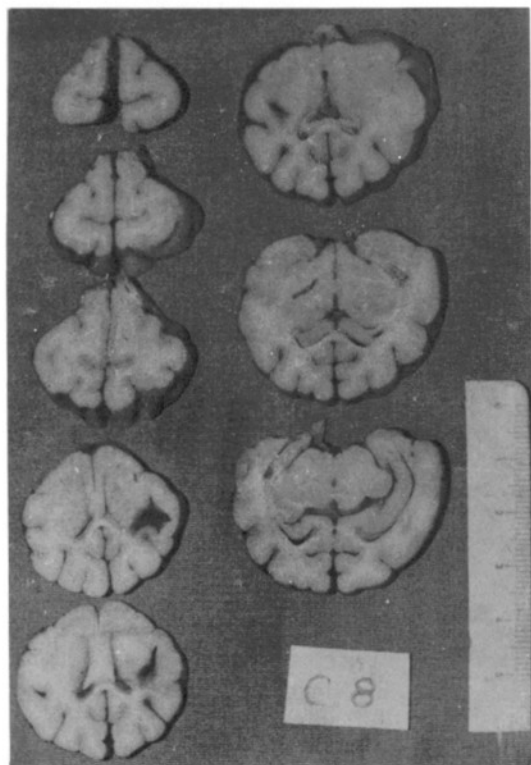


Fig. 1 : Photography of coronal sections of control (A) and tPA-treated (B) cat brains. The intracerebral haematoma seen in A has effectively lysed in the 6-hour tPA-treated animal shown in B.

haematoma lysis was noted. Paraffin sections were made and were stained with hematoxylen and eosin.

RESULTS

Clot Lysis

Two (5.5%) of eleven control animals exhibited clot lysis. In contrast, eleven (84.6%) of the 13 tPA-treated animals exhibited clot lysis (Table 1). (Figures. II, III). This difference in both groups was statistically significant ($p < 0.001$).

Table I : Clot lysis in control and tPA-treated animals at 6 hours.

	Total Animals	Clot Lysis	Significance *
Control Group	11	2(18%)	Not significant
tPA-treated Group	13	11(84.6%)	$p < 0.001$

* Fisher's exact test

Table II : Neurological grading in animals of the control and tPA-treated group *

	Grades									
	Control					tPA-treated Group				
	I	II	III	IV	V	I	II	III	IV	V
Consciousness	3	5	1	2	-	5	4	2	2	-
Motor Strength	1	4	1	4	1	2	5	4	2	-
Standing-Gait	1	1	4	5	-	1	7	3	2	-
Feeding	1	-	4	3	3	1	2	6	2	2

* Neurological grading according to the system of Pang, et al. (24)

CLINICAL STATUS

No evidence of systemic bleeding diathesis was noted in any of the animals. In one tPA-treated animal, there was a small haemorrhagic area at the site of injection. In one control animal, the second injection of ketamin hydrochloride caused respiratory difficulty that was relieved by the administration of oxygen and manually assisted respiration.

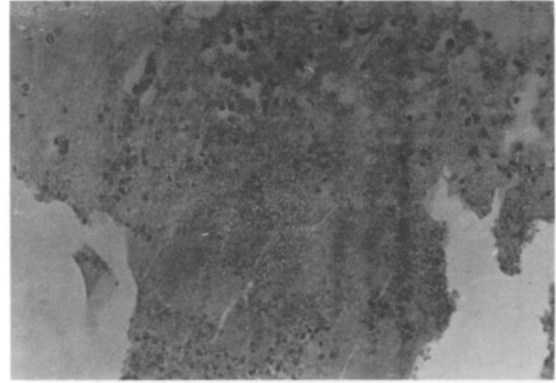
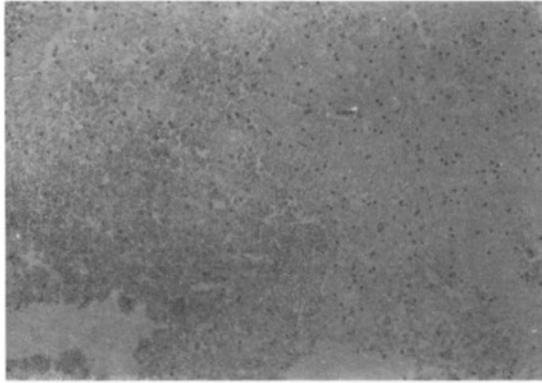


Fig. II : Histological sections. A: Control animal. Note the per sistent haematoma (H&E x 25). B: tPA-treated animal. Note the haematoma lysis (H&E x 25).

The neurological grades are summarized in Table II, Figures IV, V. At the sixth hour, in the tPA-treated group, the animals were in a good grade for consciousness, motor strength and standing compared with the animals in the control group. But no important differences were present, probably secondary to early evaluation time.

COAGULATION STUDIES

The partial thromboplastin time (PTT) was between 15 and 41 seconds in the control group (average 27.7 sec) and between 22 and 42 sec (average 30.1 sec) in the tPA-treated group. The prothrombin time (PT) was between 9 and 22 sec (average 13.2 sec) in the controls and between 10 and 18 sec (average 12.6 sec) in the tPA-treated group. The PTT and PT data were analysed and no significant differences were found among these groups ($p < 0.005$) (Table VI).

HISTOPATHOLOGICAL STUDIES

In both groups, there was a mild perihæmatomal oedema and some small hæmorrhagical areas. In one tPA-treated animal, there was gliosis not related to the treatment. In one control and one tPA-treated animal, leptomeningeal lymphocyte infiltration appeared. In contrast, there was leptomeningeal polymorphonuclear infiltration in one tPA-treated animal. In addition to these findings, there was hæmatoma lysis in two of the controls and eleven of the tPA-treated animals.

DISCUSSION

Spontaneous ICH constitutes between 6.3% to 12% of all cerebrovascular accidents (21). Although surgical treatment of spontaneous intracerebral hæmorrhage has improved during recent decades, the postoperative mortality rate has varied greatly in different reports, ranging from 20% to 80% to (13).

The principle of stereotaxic evacuation of ICH's proposed by Backlund and von Holst in 1978, is original and imaginative (13). Since then, stereotaxic aspiration of these lesions has been proposed by several authors (12,16,20,33). However, this technique allows for only partial removal of the hæmatoma, even with the use of special needless. It is known that, a few hours after the onset of symptoms, a hæmatoma consists of liquid blood (about 20% of its volume) and dense clots (about 80%). (13). For this reason, a number of investigators have conducted experimental (15,21,24,26,27,30) and clinical studies (20,22,23), using thrombolytic agents.

Blood normally contains a fibrinolytic system which is capable of dissolving blood clots. The inactive proenzyme, plasminogen, is converted in to an active enzyme, plasmin, which in turn lyses fibrin clots (1,2,4,5,17,21). Of the exogenous activators, the best known are urokinase (UK), which is synthesized by the kidney and normally found in human urine, and streptokinase, which is produced by beta-hæmolytic streptococci (1,7,8,28). tPA is a recently available thrombolytic agent with many advantages compared to streptokinase and urokinase. The affinity for circulating plasminogen is low relative to the affinity of tPA for fibrin-bound plasminogen (7,8,15,17,18,25,32,33). In addition, the reaction rate of conversion of plasminogen to plasmin is increased several fold in the presence of fibrin (2,5). Once generated, fibrin-bound plasmin is relatively protected from degradation by alpha-2 antiplasmin. This property results in "clot-selective" thrombolysis (1,25,30). Because of the its short biological half-life (approximately 5 min) and because it does not induce a systemic lytic state, the thrombolytic effect of tPA is faster then conventional activators (25, 30).

Several preliminary experiments were performed in order to develop the ICH model described in



Figure VI: Graph indicating prothrombin and partial thromboplastin time in the two groups. Comparisons of the results in the two groups for PT and PTT were not significant ($p=0.05$).

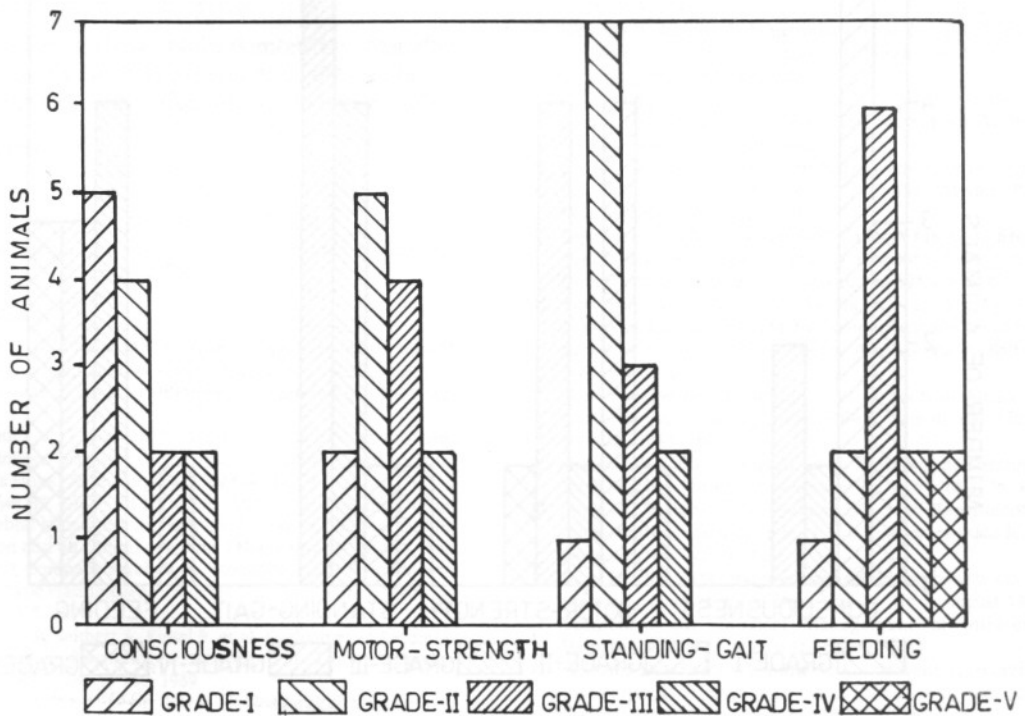


Figure V: Bar graph depicting a comparison of the neurological changes in the TPA-treated group.

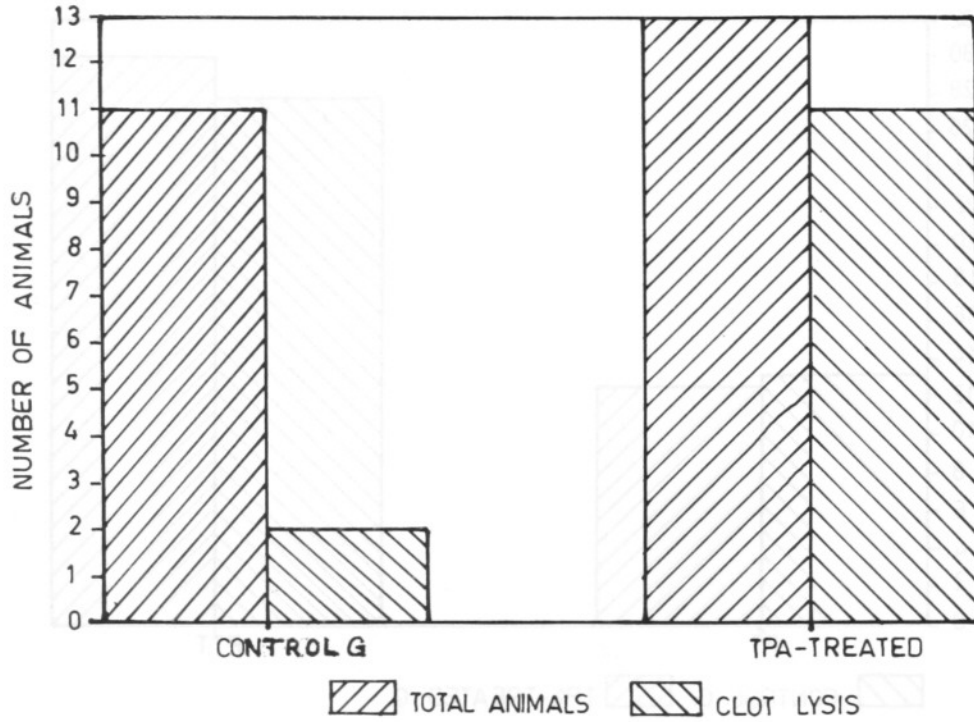


Figure III: Graphic presentation of the number of animals showing clot lysis in both groups.

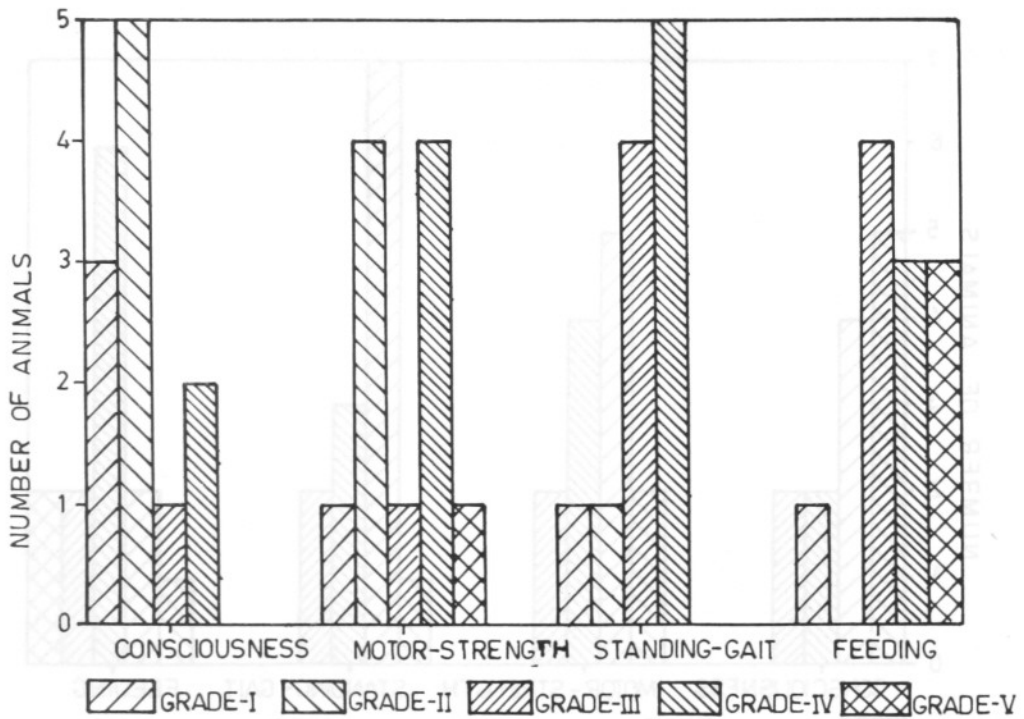


Figure IV: Bar graph showing a comparison of the neurological changes in the control group.

this report. To produce ICH, animal blood was usually used, but in some experiments, human blood was used (21). We also used human blood to produce ICH in this experiment, since our intention was to reproduce a model as close to the human situation as possible.

Segal et al. demonstrated the efficacy of urokinase in accelerated lysis of ICH in a monkey model (19,21,27,29). After defining the size and location of hypertensive ICH's with a CT scan in 51 cases, Matsumoto and Hondo directed the catheter toward the haematoma centre via a burr-hole with the patients under local anesthesia. The haematomas were then aspirated as completely as possible after urokinase infusion into the haematoma (19). Narayan et al. reported on a rabbit model in which urokinase injected into the ICH accelerated haematoma lysis (19, 21).

In 1987, Kaufman et al carried out a series of experiments to study the effectiveness and safety of tPA for clot lysis. They injected one-tenth of milliliter of various solutions stereotactically into the thalamus. The solutions were placebo, single dose (3750 units/ml), double dose (7500 units/2ml) tPA with normal saline or blood. In their studies, they reported some small haemorrhages and some necrosis, and very little inflammation in the tPA-injected animals (15).

As a result of this study, our observations in a cat model demonstrate that tPA, in situ, can effectively and safely lyse intracranial haematomas without any apparent adverse effects, such as parenchymal necrosis and bleeding or systemic effects.

In conclusion, these results demonstrate that after other supportive animal experiments, tPA can be used clinically to liquefy a clot and facilitate its removal.

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