The effects of tissue plasminogen activators on experimental cerebral ischemic infarcts

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In this study, the effects of tissue plasminogen activators (TPA) on experimental cerebral embolic ischemia and/or infarcts in rats were investigated. By administrating TPA IV bolus fortyfive minutes after embolus material that will cause experimental cerebral embolic ischemia and/or infarcts had been given into the internal carotid artery; a significant improvement in neorological deficits was observed ($p < 0.05$). In the histopathological evaluation; it was observed that TPA caused a significant decrease in the size of ischemia and/or infarct areas ($p < 0.05$) but did not cause any bleeding in them. In this study it was also concluded that, application of TPA as intra-venously bolus "which is different from the classical way" is useful in embolic strokes and does not cause a significant complication. [Turk J Med Res 1994; 12(1): 5-10]

Key Words: Embolism, Infarct, Ischemia, Tissue Plasminogen Activator (TPA), Rat

The most important part of the cerebral ischemic infarcts are results of thromboembolies (1,2).

If regional ischemia which is due to the regional cut off of the cerebral blood flow (cbf) by thromboembolies is not abolished immediately, that will result in irreversible tissue necrosis and cerebral infarct. It is known that, in recent years the importance of fibrinolytic (thrombolytic) treatment methods are increasing (3,17). If there is thrombus, TPA turns, inactive plasminogen to active plasmine by provocating the fibrinolytic enzymes. This property is specific to clot (5,7,11). The lysis of the clot occurs by this way.

TPA's selectivity to clot inhibits systemic activation of the fibrinolytic system and this causes lesser damage in hemostatic system. In addition to that when it is compared to the other fibrinolytic agents its complications are rare (3,15,18,31).

MATERIALS AND METHODS

In the study, 26 Wistar rats weighing 200-300 g were used. The rats didn't take anything by mouth for 6 hours before operation and their body temperatures were kept. For anesthesia intraperitoneal ketamin and 0.1 ml IM atropine were used. After the anesthesia, the manipulation was begun while the rat was respirating spontaneously and fixated at the supine position. The muscles were passed by skin incision which was made just left of the midline between the xyphoid and crichoid cartilages. Under the operation microscope the carotis sheath was dissected ten minutes after the local anesthetic was dropped on it. Then, the internal and external carotid arteries were exposed. Later left yoid bone was extracted partially and the pterygopalatinal branches of external carotid artery were electrocauterised. Embolus material was prepared by the modification of the technic suggested by Kaneko et al (9). Blood which was taken 24 hours before from a healthy rat by cardiac puncture was kept at 37 °C. 0.25-0.50 mm^3 of it was mixed with 0.3 ml saline and injected slowly and caudally into the external carotid artery which was held up at the distal end through the common carotid bifurcation with 27 G catheter, while the catheter was taken out; external carotid artery held up from the suture proximal of it and the hole of the catheter was cauterised.

After internal carotid artery was seen intact, one layer closure was made.

In the study group, 45 minutes after the embolus material had been injected, 200 IU TPA/ml was given in 5 minutes from the canulised femoral vein (32). Rats in both were observed for ten minutes after the operation for their vital signs in the recovery cage in the semifowler position. All rats were evaluated in 6

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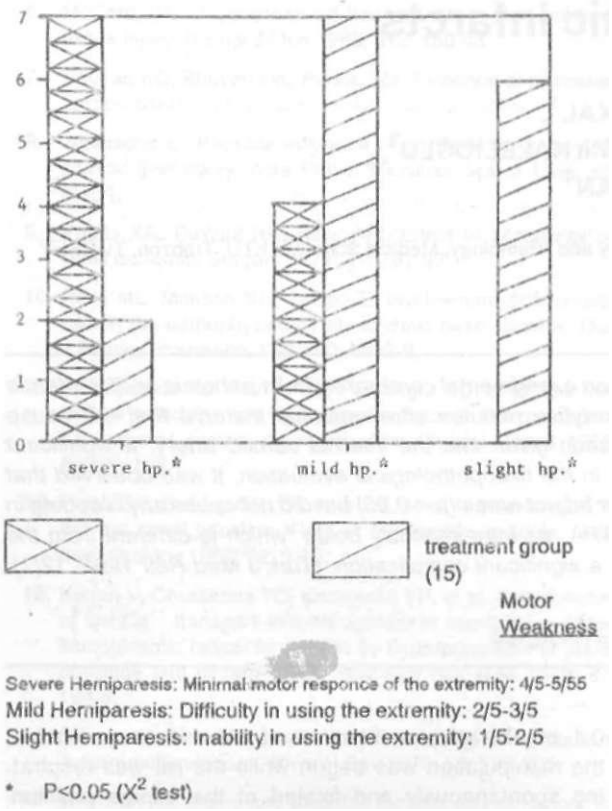
table 1. Neurologic grading of the rais at the control and treatment groups.



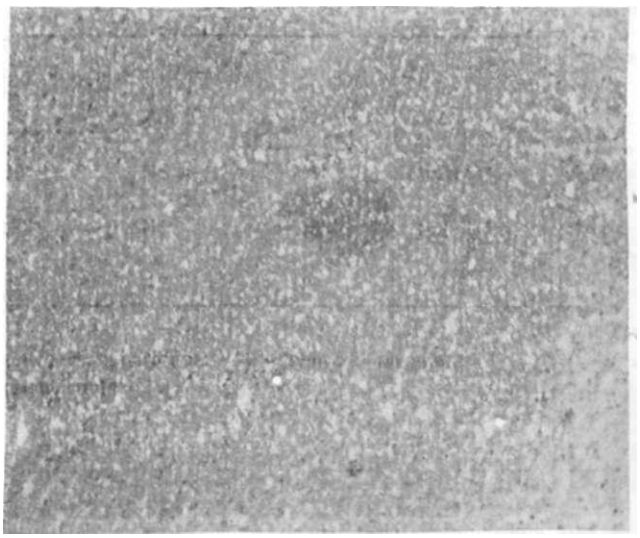
hours intervals with the modifications of the neurological scales used by Zvin et al, Threepoint (16) and Pang group. Rats which had neurological deficits at the end of the 24 houis were included to the study and normal ones were excluded. The rats in the study group were sacrified 24 hours later under ether anesthesia. Their brains were extracted by craniectomi. The extracted brains were fixated in %10 phosphate tamponade solution for 15 days.

After the fixation procedures, the brains were sliced in coronal plane from front to back (middle fronlal-parietal-cerebellar) and the tissue samples were stained with hematoxylen-eosln (HE) for histopathological evaluation after usual processing.

RESULTS

There were ill rats in the control group. 7 (%64) had severe, 4 (%36) had mild herniparesis. There were 15 rats in the study group. TPA was administered to these rats, 2 (%14) of them had severe, 7 (%46) of them had mild, 6 (%40) of them had slight herniparesis (Table I).

The rats in the control group had a mean PTT value of 28.63 sec. In the control mean PT value was 23.89 sec. and the mean PTT VALUE WAS 28.63 sec. In the histopathological examination of the rat (brains) in the control group; ischemic infarct zones which were emerging from softening of the supratentorial cerebral parenchyma, pale neurophilies; edema and necrotic remnants had been observed (Figure 1). In ipsilateral supratentorial legions 6 rats had 3, 5 rats had 2 different infarct zonos (Figure 2). However, in



Figuie 1. Supratentorial brain sections of the control gioup. Ischemic Infartc area occured by softening in the white matter, pale neuro^hillies, edema and necrosis (HE x 12.5).

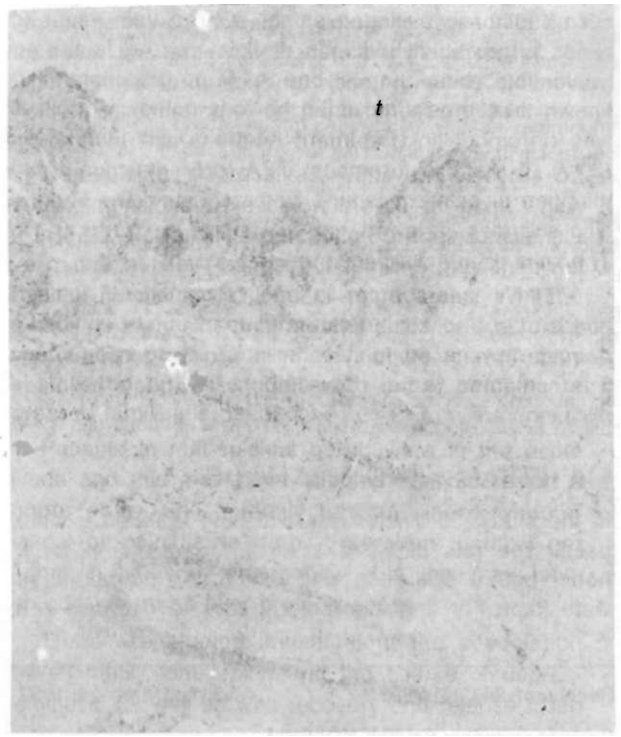


Figure 2. Multiple ischemic infarct areas at the supratentorial brain sections of the control group (HE x 3.2).

Table 2 MiCrometdc sizes ci the cerebral infarcts at the controi and treatment groups*

	Unit scala	Micrometric Scala	
1	35.35	1319.	1319
	20.23	1055,6.	867.
	10.18	377.	378,6.
2	13.22	490.	029.4.
	10.20	377.	754.
	5.15	188,5.	565.5.
3	12,27	452,4.	1017.S.
	5.9	188,5	339.
	14.1 i	527,8	414,7.
4	10.20	377.	1055,6
	15 23	565,5.	867.
	18.20	678,6	754.
5	20.15	754.	565,5.
	13.15	490	565,5.
	6.l0	226.	377.
6~	23.20	867	754.
	14.22	527,8.	829,4.
7	28.30	1055,6.	1131.
	20.15	754.	565.5.
tT	20.15	754.	565,5.
	¡8.10	6/8,6.	377.
	12.22	452,4.	829,4.
		678,6.	754.
10	20.22	754.	629.
	17.26	640.	980.
11	13.15	490.	565.5
	10.15	377.	565.5.
	18.8	670,6.	301.6.

* One distance is equal to 37.7 Micron (x10,4)

the treatment group 10 rats had 3, 5 rats had 2 dif ferent ipsilateral supratentoriai iniarcis (Table ll). The Infarct zones which were observed in the study group had widespread inflamatory cell infiltration beside the infarct signs in the control group (Figure 3,4). The dimension of the ischemic infarcts which were ex^ amined micrometrioally in the control group were; the biggest 1319;1319 micron, the least I88,5;113 micron (Table ll, lll). Neither microscopically nor macroscopically hemorrhage haven't been observed in both groups.

DISCUSSION

There are two handicaps in the evaluation and classification of the experimental cerebral ernbolies.

First is the determination of the observation period after the embolic material had been given. Thus, in many cerebral infarcts which were obtained by clipping or ligating the main cerebral arteries or injecting the embolus material directly into the cerebral artery. It was observed or declared that neurological deficits could recover or improve within a few days spontaneously. It is known that this is due to" the

Treatment Groups

	Unit Scaia	Micrometric Scala	
1	55	188,5.	188,5
	3.5	11.3	188,5.
	8.i4	301,6	527,8.
2	10.5	377.	^88,5.
	3.5	113.	138,5.
	3.3	113.	188,5
3	15.12	565,5	'.52,4.
	3.;o	301,6.	377.
4	8.8	301,6.	377.
	10.12	377.	452,4.
	8.11	301,6	414,7.
5	11.12	414,7.	452.4.
	4.5	160,8.	188,5
	5.5	188,5.	183,5.
6	$.17 "	301,6.	640,9.
	3.3	113.	113.
	5.4	188,5.	150,8.
1	3.4	113.	150,8.
	33	113.	113.
	610	301,6	377.
8	13.3	490.	400.
	3.4	113.	150,8.
9	8.8	301,6.	301,6.
	5.6	188,5 .	226.
	4.8	150,8.	301,6.
10	10.10	377\-	377.
	5.3	188,5.	113.
	710	263,2.	377.
11	18.12	678,6.	452,4.
	6.6	226.	226.
12	5.9	188,5.	389.
	9.11	339.	414,7.
	3.5	113.	188.5.
13	8.15	301,6	565,5.
	3.4	113.	150,3.
	10.6	377.	226.
14	11.11	414,7.	414,7.
	8.8	301,6.	301,6.
15	8.8	301,6.	301,6.
	4.9	150,8.	339.

decrement of the ischemic brain edema ai postoc elusive 3-4 days or controlateral hemisphere undertakes the functions of the ipsilateral hemisphere (33-34).

The second handicap is the timing of ihe incubation period of the embolus material. This period is important, because this can lead us to false results in fibrinolytic treatment (13).

In the light of these two factors; In our study rats were sacrified 24h after embolus material had been given. In addition to that by incubating embolus material for 24 hours, the fibrinolytic activity in the fresh clot was tried to be minimized.

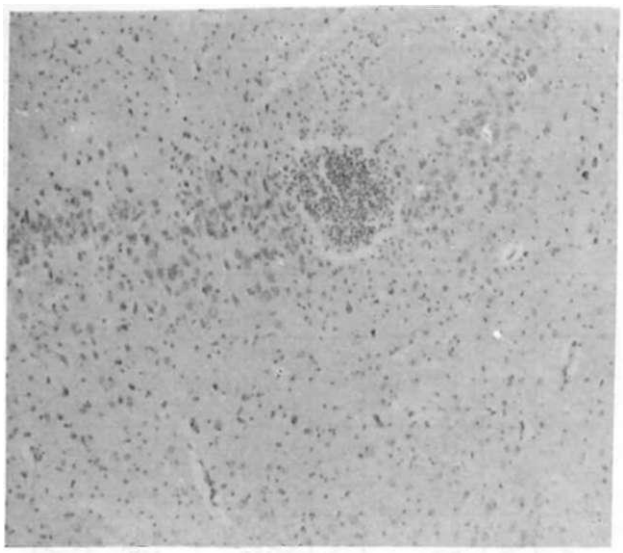


Figure 3. Supratentorial brain sections of the treatment group. Significant inflammatory cell infiltration at the infarct areas (HE x 12.5).

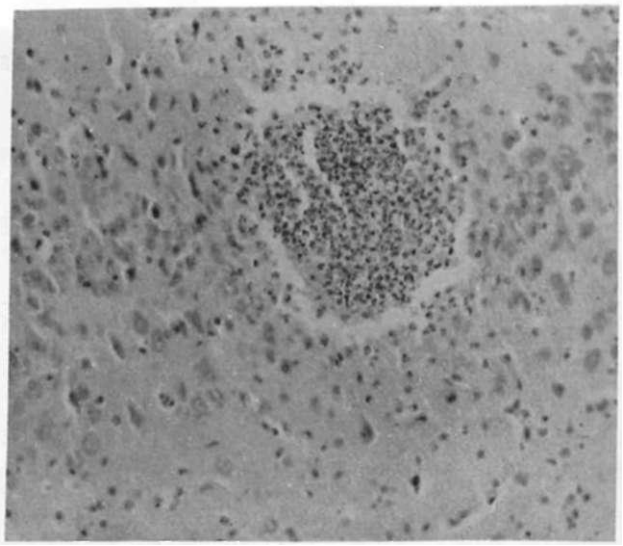
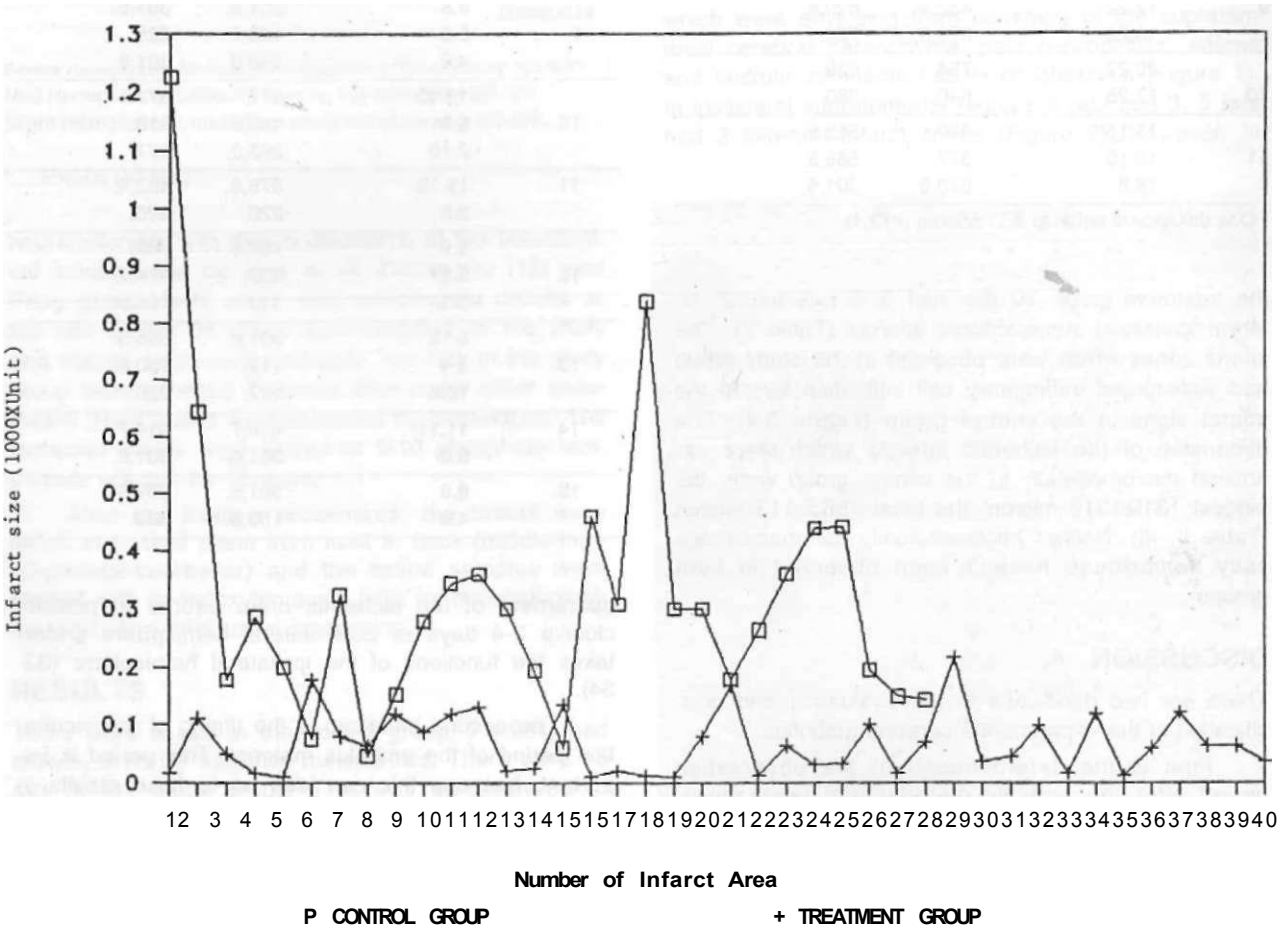


Figure 4. Magnified view of the ischemic infarct zones (HEx25).

Tablo 3. Comparison of the infarct sizes.



In the fibrinolytic treatment total dose of TPA was administered gradually. Sixty percent of total dose was given as quick intravenous infusion to obtain therapeutic dose that establishes reperfusion and remaining %40 was infused with 30-90 minutes intervals as continuous perfusion to decrease the reocclusion incidence. Although the perfusion treatment is suggested. It is also known that it affects the coagulation system negatively (38).

In our study by giving TPA's total dose IV in 5 minutes, we tried to overcome coagulation defect and have maximal lytic effect of the fibrinolytic agent.

In the presence of fibrin TPA turns plasminogen to plasmine and provides an effective thrombolysis. By this way it opens %60 of occluded cerebral vessel and increases the regional ischemic flow (39).

Papadopoulos (40), Kissel (36), Zivin (16) and other researchers showed in their experimental cerebrovasculer studies that TPA increases the blood flow in embolic vessels (23).

When both groups in our study were compared, we observed that improvement in the neurological deficits of the rats in the treatment group were statistically significant ($p<0.05$) (Table I). When both groups in our study were compared, we observed that improvement in the neurological deficits of the rats in the treatment group were statistcally significant ($p<0.05$). This histopathological improvement was parallel to the clinical improvement. It is possible that TPA can cause these effects by lysing the intravascular embolus and restoring the blood flow in the ischemic zone (36,39,40). Therefore, as idling neurons in penumembrana gain function, improvement both histopathologically and clinically ensues.

On the other hand none of the infarcts in the study group neither showed hemorrhage nor turned to hemorrhagic infarcts. It is known that, although continuous infusion of TPA minimizes reocclusion risk, it affects the coagulation system negatively (38). In our study by giving TPA rapidly in six hours we provided a maximal lytic effect on the embolus but didn't have a negative effect on the coagulation system and this correlates well with the reports saying that, TPA does not provocate changing of ischemic infarcts to hemorrhagic infarcts (13,24,28,41,42). By giving TPA as bolus, TPA did not change the nature of the ischemic infarcts in the study group.

According to these data, we can say that; under the general indications of fibrinolytic treatment, giving, the total TPA dosage IV in a short time will improve the neurological deficits in the cerebral ischemic infarcts and won't cause any important complications.

Doku plazminojen aktivatörlerinin deneysel serebral infarktlar üzerine etkisi

Bu çalışmada, doku plazminojen aktivatörlerinin (TPA) ratlarda oluşturulan deneysel serebral embolik iskemi ve/veya infarktlara etkisi araştırıldı. Internal karatosiarterine araştırıldı. Embolus materyali verildikten 45 dk. sonra intravenöz bolus tarzında TPA verilerek nörolojik desifitlerde anlamlı bir iyileşme belirlendi ($P<0.05$). Histopatolojik değerlendirmede TPA'nın iskemi ve/veya infarkt alanlarında anlamlı derecede azalmaya (ancak kanama yapmadığı) yol açtığı görüldü ($p<0.05$).

Bu çalışmada TPA'nın klasik yoldan farklı olarak intravenöz bolus tarzında uygulamasının embolik inmelerde yararlı olacağı ve önemli bir komplikasyona yol açmayacağı soucuna ulaşılmıştır. [TurkJMedRes 1994; 12(1): 5-10]

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