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Defining the macroscopic and microscopic findings of experimental focal brain ischemia in rats from a forensic scientist's point of view

[Adli tıp uzmanı'nın görüş açısından sıçanlardaki deneysel fokal beyin iskemisinin makroskobik ve mikroskobik bulgularının tanımlanması]

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Abstract

Stroke is the third leading cause of death in the Western world and approximately 10 % of all deaths occur as a result of stroke in the world. Determination of the time schedule of the occurrence of pathologic events in a stroke patient is invaluable for a forensic specialist. The aim of this study was to define the schedule of macroscopic and microscopic changes occurred in an experimental animal model of permanent focal ischemia. Male Wistar rats weighing 250- 350 g were used in this study. Animals were anesthetized with ketamine and medetomidine. Permanent focal ischemia was applied by the suture occlusion method. The animals were divided in to 7 experimental groups (n= 4) with time schedules including 1.5, 3, 6, 12, 24 and 72 hours and the sham. Brains were harvested at the end of the determined time schedule. Harvested brains which had visible well-demarcated infarcts were used for the study. Images of the brains were recorded. Later, brain samples were fixed in formalin 10 % solutions for 24 hours and routine paraffin procedure was applied. 5 µm sections of frontoparietal cortex were prepared and stained with hematoxilen-eosin (H.E.) and evaluated under light microscope. Lesions were observed as pink areas in frontoparietal cortex. The impression of the authors by the evaluation of the brains with naked-eye was the enlargement and the darkening of the infarct area until 24 hour period but there were great variations among the animals for proposing an accurate definition of time from the area and colour of the infarct. At 72 hour the infact was seen pale. Edema couldn't be observed in 1.5 hour and 72 hour periods with naked eye. It was apparent at 6, 12 and 24 hours. Maximum edema was at 24 hour according to the authors' impression.

Özet

Stroke batı dünyasındaki ölüm sebepleri arasında 3. sırada yer almakta olup, dünyadaki tüm ölümlerin %10 u stroke sonucu meydana gelmektedir. Adli tıp uzmanları için stroke hastalarında patolojik olayların meydana geliş zamanının tanımlanması paha biçilmez değerdedir. Bu çalışmanın amacı deneysel kalıcı fokal iskemili hayvan modellerinde meydana gelen makroskobik ve mikroskobik değişikliklerin şemasını tanımlamaktır. Bu çalışmada 250-350 g ağırlığında erkek Wistar sıçanlar kullanıldı. Hayvanlar ketamine ve medetomidine ile anesteziye tabi tutuldu. Sütür tıkama yöntemi ile kalıcı fokal iskemi sağlandı. Hayvanlar 1.5, 3, 6, 12, 24, 72 saat zaman dilimlik ve sham olmak üzere 7 araştırma grubuna (n=4) ayrıldı. Beyinler zaman dilimi tanımlaması sonunda çıkarıldı. Çıkarılan beyinlerden belirli lezyon gözlenenler çalışmada kullanıldı. Beyinlerin resimleri kaydedildi. Daha sonra beyin örnekleri %10 luk formalinde 24 saad fiske edildi ve rutin parafin yöntemi uygulandı. Fronto parietal korteksten 5 µm lik kesitler alınarak hematoxilen-eosin (H.E.) ile boyandı ve ışık mikroskobu altında değerlendirildi. Lezyonlar frontoparietal kortekste pembe alanlar olarak gözlendi. Beyinlerin çıplak gözle araştırmasında araştırıcıların gözlemlerine göre 24 saatlik periyoda kadar infarkt alanı genişlemiş ve koyulaşmışken hayvanlar arasında bir çok varyasyon bulunması nedeniyle 72 saatte infarkt soluk olarak görüldü. Çıplak gözle 1.5 saat ve 72 saat periyotlarında ödem gözlenemedi. Ödem 6, 12 ve 24. saatlerde izlendi. Araştırıcıların izlemine göre maksimum ödem 24. saatte idi. Mikroskobik olarak infarkt çekirdeği kortekte değerlendirildi. Tüm hayvanlarda III den V e kadar olan tabakalar belirgin olarak etkilenmişti.

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Microscopically, the infarct core was evaluated in the cortex. Layers III to V were affected prominently in all animals. Two types of neurons with swelling of the cytoplasm and shrinkage of the cytoplasm and nucleus were observed together at 1.5 hour. Swelling of glial cells and astrocytic processes were other observations in this time point. Triangulations of neurons were observed at 3 hour in addition to the previous findings. Pyknosis and karyorhexis of the nucleus of some neurons were discriminated at 6 hour. There was a diffuse pallor of the eosinophilic background. A few polymorphonuclear leukocytes (PNL) infiltrating the area was present. Neurons with eosinophilic cytoplasm and pyknotic nucleus (red neurons) appeared at 12 hour. Axonal swelling and the prominent increase in the the number of PNLs was observed. The number of neurons was decreased and red neurons were increased prominently at 24 hour. Vacuolation of dendrites were observed. PNLs were more abundant in the region. Astrocytes were increased. Pannecrosis was observed at 72 hour. Red neurons were still present. The number of astrocytes was prominently increased. Histology of the brain tissues of sham operated animals were evaluated as normal. The results of this study provide clues which can be used by a forensic scientist for the determination of the time of death of stroke.

Keywords:

Forensic pathology, experimental focal brain ischemia, rats.

1.5 saatte bir kısmında stoplazması şişmiş, diğer kısmında ise çekirdek ve stoplazması büzüşmüş iki tip nöron gözlendi. Glial hücrelerde şişme ve astrositik proçesler bu zaman diliminde görülen diğer değişikliklerdi. 3 saatte bir önceki bulgulara ek olarak nöronlarda triangulasyonlar izlendi. Bazı nöronlarda nükleuslarında piknoz ve karyoreksis 6. saatte ayırd edilen değişikliklerdi. Eozinofilik arka planda yaygın solgunluk vardı. Alanda birkaç polimorfonüklear lökosit (PNL) infiltrasyonu mevcuttu. 12 saatte eosinofilik stoplazmalı ve piknotik nukleuslu nöronlar (kırmızı nöronlar) görüldü. Aksonal şişme ve PNL lerin sayısında belirgin artış izlendi. 24 saatte nöronların sayısı azalırken kırmızı nöron sayısı hızla artmıştı.. Dendritlerde vakuolleşme izlendi. PNL ler çoğunlukla bu bölgeyi terk etmişti. Astrositler artmıştı. Pannekroz 72 saatte gözlendi. Kırmızı nöronlar halen mevcuttu.. Astrositlerin sayısı belirgin olarak artmıştı. Sham operasyonuna tabi tutulan hayvanlarda beyin histolojisi normal olarak izlendi. Bu çalışmanın sonuçları, stroke ölümlerinin zamanlarının tayininde adli araştırıcılar tarafından kullanılabilecek önemli ipuçları sunmaktadır.

Anahtar Kelimeler:

Adli patoloji, deneysel fokal beyin iskemisi, sıçanlar.

1. Introduction

Stroke is the third leading cause of death in the Western world and approximately 10 % of all deaths occur as a result of stroke in the world.

Therefore, a forensic scientist has to evaluate the death body of a stroke patient very often during his professional life.

The term '*stroke*' encompasses three different disorders which are:

- ischemic stroke,
- intracerebral hemorrhage and
- subarachnoid hemorrhage.

The vast majority (75 %) of strokes are of the ischemic type.

An ischemic stroke is the clinical manifestation of the event develops as a result of the occlusion of a large intracranial artery.

The middle cerebral artery or one of its main branches are the vessels most commonly involved by these occlusive events.

The occlusion of an intracranial artery results in a parenchymal lesion of the brain.

Stroke ultimately involves neurological deficits which reflect the location and size of the compromised brain area and even the death.

Two areas can be discriminated in the ischemic territory:

The 'core' region with severe reduction of blood flow and the peripheral zone of mild to moderate ischemia known as 'penumbra' adjacent to the core region.

Determination of the age of the lesion in a stroke patient is invaluable for a forensic scientist but it is very difficult to provide useful clues directly from human subjects and the number of such studies are limited.

Time course of the pathologic events can be determined in an animal model and experimental model of focal ischemia of the rat is a widely accepted model of human stroke.

2. Aim

To define the schedule of macroscopic and microscopic changes occurred in an experimental focal brain ischemia in the rat in a way suitable for the practical purposes of forensic specialist by using naked- eye examination and a conventional staining technique.

3. Materials and methods

Forty- two male adult Wistar rats weighing 250- 350 g were used.

They were anaesthesized with ketamine and medetomidine.

Permanent focal ischemia of the right MCA was induced by the suture occlusion model.

Experiments were terminated at predetermined time intervals:

1.5, 3, 6, 12, 24 and 72 hours after MCA occlusion and 24 hours after the sham operation.

Brains were harvested after cardiovascular perfusion with ice- cold 0.9 % saline and 10 % formalin solution.

Only the brains which had well defined lesions were

used for the study.

All brains were evaluated with naked- eye examination and images were recorded.

Hematoxylin-Eosin stained sections of the ischemic core region were observed under a light microscope.

4. Results

All evaluations were done qualitatively but not quantitatively.

Although there was an impression that a time-dependent change occurred in size and colour, because of the great variability and subjectivity of the findings, they couldn't be used to predict the age of the lesion.

There was a doubtful enlargement probably related to edema at the right ischemic hemisphere at 6 hour.

Enlargement was apparent at 12 hour.

Prominent enlargement was observed at 24 hour.

Enlargement was doubtful again at 72 hour.

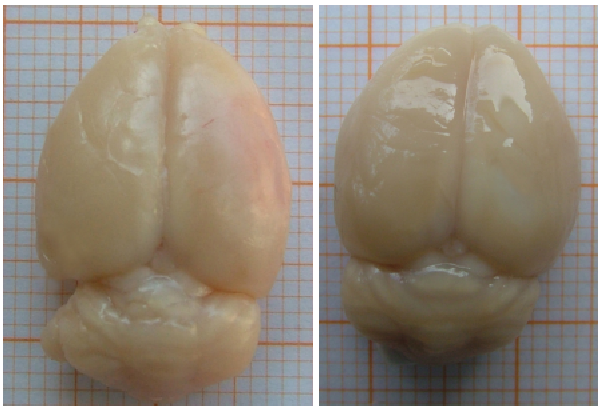

Figure-1. *Gross appearances of 12 h ischemia and sham*

Microscopically, layers III to V of the frontoparietal cortex were affected prominently in all animals in the ipsilateral hemisphere.

1.5 h. : Swelling or shrinkage of the perikarya and nucleus of the neurons and swelling of the glial cells and their processes

1.6 3 h. : More neurons were affected and in addition to the previous findings triangulation of the neurons and vacuolation of neuropils were observed.

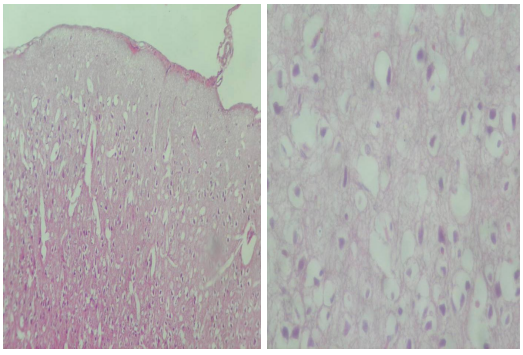

Figure-2. *1.5 h ischemia*

6 h. : Neurons with pyknotik nucleus and karyorrhexis were observed. PMNLs appeared around the blood vessels.

12 h. : Cells with pyknotik nucleus and eosinophilic cytoplasm (eosinophilic cells) appeared (first step of the late ischemic damage). PMNLs observed in the parenchyma. Swollen axons were present.

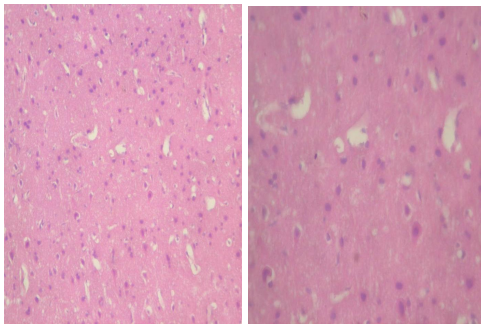

Figure-3. *12 h ischemia*

The brains of the sham-operated animals were macroscopically and microscopically normal.

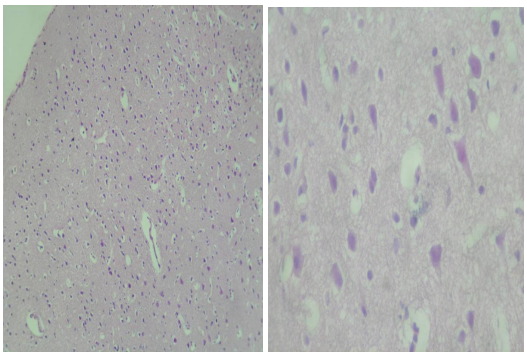

Figure-4. *Sham*

5. Discussion

The histopathologic changes occurred following MCA occlusion in rats were confined to the region including the ipsilateral preoptic area, caudoputamen and frontoparietal cortex.

Among these three regions, lesion in the frontoparietal cortex was easily definable and visible externally.

Ischemic core was preferred as the study region by authors because of the easiness of the discrimination than penumbra.

Conventional histological procedures as hematoxylin eosin staining are less sensitive for detecting axonal and oligodendrocyte damage than neuronal damage.

Therefore, ischemic core region in the gray matter of the frontoparietal cortex was preferred and proposed to the forensic scientists as the study region.

Analyses of the lesions and evaluation of the histologic changes allows prediction of the approximate age of the ischemic lesion.

1) Grossly no edema and histologically early ischemic

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damage: Less than 6 hours

2) Grossly doubtful edema and histologically more numerous early ischemic damage and PMNLs around the blood vessels: Between 6- 12 hours

3) Grossly apparent edema and histologicaly early ischemic changes accompanies eosinophilic cells with PMNLs in the parenchyma: 12 hours to less than 72 hours

4) Grossly doubtful edema and histologicaly pannecrosis, abundant PMNL infiltration: Around 72 hours

6. Conclusion

The rat model of focal brain ischemia can be used to develope basic concepts of an approach for age determination of human brain infarct.

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