行政院國家科學委員會專題研究計畫成果報告

實驗性局部缺血模式下腦中風的組織型態學及磁共振造影像之相關性（1/2）

The Correlation between the Histomorphology and Magnetic Resonance Images in Experimental Focal Cerebral Ischemia

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中文摘要

本研究之目的在於透過實驗性局部腦缺血的動物模式與組織型態化學及磁振造影技術，來探討缺血性腦中風的可能機制。驗證缺血性腦梗塞的理論 腦缺血半影區的假說。

第一年計劃，以定時間動脈結紮與重灌流的實驗性局部腦缺血動物模式來蒐集各類磁振造影脈衝序列(T_1WI, T_2WI, PDWI, DWI)下各種影像參數。以及研究這些參數下的造影訊號強度與動物的缺血性腦中風其不同組織型態化學(H/E, S100, GFAP)所得的各種腦組織缺血性變化之定量相關性。

結果顯示，在本研究觀察之急性缺血性腦中風的情況下只有 T_2WI 與擴散造影技術，具偵測之價值。組織化學的分析方面，H/E 染色觀察的是神經死亡及膠質細胞之增生所代表的是較慢性及完整之缺血變化，如預期的其產生變化之面積較小。而 GFAP 則可反應出不同嚴重度之缺血反應。其染色之喪失，代表了完整嚴重之缺血性變化。染色程度增加之部分，代表了部分或較輕度之缺血性變化。此一區域可能代表了腦缺血半影區。然而相對於組織型態化學中的 GFAP 的發現，即使以對急性缺血性腦梗塞較敏感之擴散影像技術也無法完全代表其範圍
。至於更早期的缺血性變化及半影區的磁振影像與組織型態學的變化則是目前尚在進行之計劃所將解答的。

關鍵詞：磁振影像，動物模式，實驗性局部腦缺血，缺血半影區，組織型態學。

Abstract

The purpose of this study is to investigate the possible mechanism in the penubrum of ischemic infarct through the correlation study of magnetic resonance imaging (MRI) and histomorphological changes in a focal ischemic model of rat.

In this first-year project, focal cerebral ischemia is produced through ligation and reperfusion of middle cerebral artery with predetermined duration. MR imaging is carried out with pulse sequences of T1WI,

T2WI, PDWI and DWI. Histo-morphological study included histo-chemical stains with H/E, S100, and GFAP.

The results showed in the acute stage of stroke, only T_2WI and DWI are of great value in the detection of ischemic infarction. In histomorphological study, the H/E represented an observation of neuronal damage, rarefaction and gliosis in a more complete or later-stage change of ischemic infarction. On the other hand, GFAP revealed ischemic change of different severity and might indicate incomplete or earlier ischemic change.
However, the quantitative study revealed that even the most sensitive DWI was not able to show up all area with partial and complete ischemic change as it can be demonstrated in the histochmical stain of GFAP.

As for the earlier ischemic change in MR image and histomorphology are currently under investigation in our second year project.

Introduction

In the region where brain tissue suffers from ischemic insult, three zones of differing hemodynamics and metabolic functions can be identified. The central zone destined to infarction, the border zone has flow and metabolism fluctuated between conditions adverse and favorable for tissue viability, and the collateral zone is frequently a site of hyperemia in which tissue retains its viability,. In the border zone, known as ischemic penumbra, critical flow and metabolic alteration determine cell damage, which in turns decide the prognosis of ischemic infarction. Therefore, it is important that we understand more about the hemodynamics in the region of penumbra in order to develop therapeutic methods at limiting the boundaries of this region.

With the MR technique, the acute findings of ischemic infarct can be demonstrated as either indirect signs, such as loss of gray matter—white matter interface, perifocal (vasogenic) edema represented by increased intensity on T_2WI, breakdown of BBB by Gd-DTPA enhancement, etc. In the later stage, the change in tissue characterization such as loss of neurons, encephalomalacia indicated by decreased intensity on T_1WI. Diffusion weighted imaging theoretically can outline ischemic lesions within minutes of onset. The concept underlying DWI involves brownian motion of water molecules, which occurs within all tissue. Rapid failure of high-energy metabolism and associated ionic pumps dysfunction in ischemic regions leading to sodium and calcium influx and consequent rapid cytotoxic edema. The restricted movement of water molecules intra-cellularly results in more coherent MR signal and increased intensity on MR image.

The histomorpholgical methods for the infarct brain are H/E stain and GFAP stains. The former is for identification of neuronal damage and the later is for classifying the severity of the ischemic infarct through the

amount of the glial fber protein.

Material and method

Twelve Long Evans male rats weighted from 250 to 350 gm were used in this study. Ligation of the right middle cerebral artery was performed using the procedure as in the subprogrm 2.

The images were implemented on a Bruker 4.7 T machine of 40 cm bore quipped with small animal coils at the institute of biomedical science in Academia Sinica. The parameters for the imaging are as follow, T_1WI TR 500 / TE 17 /64 X 128 / NEX = 4; T_2WI TR 2000 / TE 80 / NEX = 2; PDWI TR 2000 / TE 20; and the DWI TR 2000 / TE 70 / big delta 40 / duration 20 ms / Z-axis and multiple b values at 0, 750 s/mm^2, 1500 s/mm^2.

The measurement of the MR image area of infarction is identified by histogram segmentation accumulated in terms of pixel number.

All the brain specimens taken from the sacrified rat were fixed in 10% neutral-buffered formalin. The brain tissue was cut coronally into slices of 3 mm in thickness. After dehydration and infiltration in the sequence of alcohol, xyline, and paraffin, the tissue pieces were embedded in paraffin. Five mm-thick unstained sections were cut from the paraffin blocks and mounted on the silanized slides for Hematoxylin and Eosin stain and immunohistochemical study of glial fibillary acidic protein (GFAP). The procedure for immuno- histochemical stain is as following.

The deparaffinized and rehydrated unstained sections were immersed in citric buffer and heated in microwave oven. Endogenous peroxidase activity was quenched with a 10-min incubation in 3% H_2O_2 in methanol. The nonspecific binding sites of IgG were blocked by incubating the slides in normal goat serum for 10 min. Primary antibody GFAP was applied for 1 hour at room temperature. This was followed by a biotinylated broad spectrum secondary antibody for 10-20 min and then by Horse-radish peroxidase complex for 10 min. Careful rinses was done with three changes of PBS buffer between each stage of the procedure. The color was developed with deamino-benzidine whereafter the sections were lightly counterstained with hematoxylin. The sections were dehydrated in graded alcohol. The negative control was obtained by substituting the primary antibody with nonspecific mouse Ig G.

The morphometric measurement of the infarct brain tissue is based on H/E and GFAP stains. Three level of GFAP stain can be identified i.e., normal increased and decreased. Regions with different stain character are delineated and measured for area. The result was expressed in percentage of the total area for comparision.

Result and Conclusion

We assessed neuronal damage by H/E sections under light microscope (Fig. 1). The infarct severity was graded according to the amount of GFAP staining i.e., decreased, normal, or increased stain on the astrocytes. In

GFAP stain (Fig. 2), total loss of glial cell activity in the area of complete loss of stain indicates total infarction. Reactive gliosis results in increased stain level, which indicates a tissue reaction to hypoperfusion or partial ischemic infarction.

Fig. 1

Fig. 1 H/E stain of the ischemic brain tissue showing cytoplasmic eosinophilia, pyknosis, karyorrhexis, karyolysis and ghost neurons (light microscope 40X 3.3).

Fig. 2A 2B

Fig. 2 GFAP stain showing different level of stain A) low power field (4 X 3.3) in which complete loss of stain in right upper quadrant, increased stain in the middle and normal level in the left half; B) high power field (20 X 3.3) showing linear or curvy linear threads of the glia fibrillary protein.

MR images showed significant changes in the ischemic area only with T_2WI (Fig. 3A) and DWI (Fig. 3B). The morpho-metric measurement was then done on MR images with the aforementioned pulse sequences and on the histochemical stain with GFAP (Fig. 3C).

Fig. 3A 3B 3C

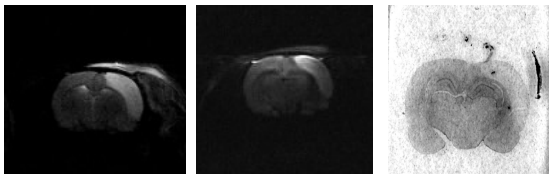

Fig 3. A) MR image T_2WI, B) DWI, and C) hitochemical stain of GFAP of the whole rat brain available for measurement of areas.

Table 1 shows averaged relative areas from different image modalities & histochemical stains (n=12).

T2WI	DWI	H/E	GFAP (-)	GFAP (+)	GFAP_ALL
17.35%	18.03%	13.65%	15.12%	7.54%	22.66%

GFAP (-): decreased level or loss of stain;
GFAP (+): increased level of stain.

The results of the measurement were summarized in Table 1. Although the area obtained form DWI is greater than from T_2WI but the difference does not reach a statistic significant level ($p > 0.05$). This is also true if we compare the area from T_2WI and that of the GFAP (-). However, the DWI does show significant greater area than that of the GFAP (-). Finally, the area of GFAP_ALL is greater than any other categories at a very significant level ($p < 0.001$) which includes, of course, the DWI.

Therefore, we conclude that although DWI theoretically sensitive to cytotoxic edema of the early ischemic change. It does not show an outstanding effect at 24 to 48 hours after stroke. The GFAP demonstrates far greater area

with ischemic insult than the MR images can show. Further study on the earlier effect of ischemia on MR images and histo-chemical morphology are now being investigated in our 2nd year of this two-year project.

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