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

[illegible] NSC 87~88-2314-B-002-033-M08
[illegible] 86 [illegible] 8 [illegible] 1 [illegible] 88 [illegible] 7 [illegible] 31 [illegible]
[illegible]

[illegible]

[illegible] (T_1WI, T_2WI, PDWI, DWI) [illegible] (H/E, S100, GFAP) [illegible]

[illegible] (24-72 [illegible]) [illegible] T_2WI [illegible] H/E [illegible] GFAP [illegible]

[illegible] GFAP [illegible] (2-6 [illegible]) [illegible] H/E [illegible] GFAP [illegible] GFAP [illegible] (2-6 [illegible]) [illegible] (57.1%, n=14) [illegible] T_2WI (21.4%, n=14) [illegible]

Abstract

The purpose of this two-year study is to investigate the correlation of magnetic resonance imaging (MRI) and histomorphological changes in a focal brain ischemia model of rat.

In the first-year project, focal cerebral ischemia was produced with a predetermined duration. MR imaging is carried out with different pulse sequences of T1WI, T2WI, PDWI and DWI. Histo-morphological study included histo-chemical stains with H/E, and GFAP. In the second year, we emphasized the effect of different post-stroke duration. The “early” changes of the brain images as well as histochemistry were studied. We also performed artificial neural net analsysis on the images obtained in these two years. The purpose was to improve the power of the imaging technique in differentiating severity and stage of stroke.

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 a more complete or later-stage change of ischemic infarction. 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 in the histochmical stain of GFAP.

As for the earlier ischemic changes in histomorphology and MR image, the study revealed less satisfactory resutls. Neither H/E nor GFAP can reflect different severity of stroke. This is because neuroanl damage, reactive gliosis or loss of GFAP has not yet completed in such a short duration after an ischemic stroke. The performance of the MR images in such early change of stroke is also poor. Although artificial neural net did help with further analysis of MR images of stroke, it depends on the knowledge of the original images, histomorphology and histochemistry of the ischemic lesions.

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 à µwhite 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

Long Evans male rat weighted around 250 gm was used as experiment animal. The rat was anestized with Ketamine (100 mg/Kg, IP) and Xylazine (5mg/Kg, IM). The rat was prepared by temporary clamping of the bilateral ICA and released in 1 hour.

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, T1WI TR 500 / TE 17 /64 X 128 / NEX = 4; T2WI 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. Hematoxylin and Eosin stain and immunohistochemical study of glial fibillary acidic protein (GFAP) stain were prepared. Please refer to our first-year report for the detail of the histochemical stain.

A combined algorithm of Self-organized artificial neural net of Kohonen feature map fuzzy C means was applied in the further analysis of the MR images. The input of the artificial neural net is the image acquired from T1WI, T2WI and DWI. DC condition of the time domain was computed and removed pixel by pixel. The output of the Kohonen feature map was assigned as an N×N matrix. These N^2 nodes were again normalized by removing their DC condition. Afterwards, the output of the Kohonen feature map became the

input of the fuzzy C means and resulted in the final output in M categories. The numbers of the M and N were adjusted empirically in either increment or decrement in order to achieve the optimal performance of this design.

Result and Conclusion

The early ischemic changes investigated through histomorphology and MR image in this study revealed less satisfactory resutls than their counterpart, which were completed in the first year. Neither H/E nor GFAP can reflect different severity of stroke. This is because neuronal damage, reactive gliosis or loss of GFAP has not yet completed in such a short duration after an ischemic stroke. Furthermore, the performance of the MR images in such early change of stroke was also poor. The early detection rates were low even with DWI (57.1 %, n=14) and T2WI (21.4 %, n=14) in which the ischemic lesion can be confirmed by direct examination of the brain tissue or follow up image study. The artificial neural net analysis was indeed capable of separating tissues with different severity of ischemia (Fig. 1 A-D). However, the clinical and pathological significance of each area awaits the assignment by the researcher basing on the knowledge from the original MR images as well as the findings from histomorphology and histochemistry.

In conclusion, in this two-year project we confirmed that the DWI is the most sensitive image-acquisition technique in terms of area of ischemia in the acute stroke. However, it still can not reflect the complete changes occurred in an ischemic event of the brain revealed by histochemical stain. On the other hand, its performance in the very early stage, such as within a few hours of stroke, is not yet a reliable method for one to rule out an ischemic lesion in the brain. Finally, the artificial neural net, in spite of their robustness and effectiveness in the aspect of computation, its value of application is indeed rely on the clinical and pathological significance of such areas segregated from MR images.

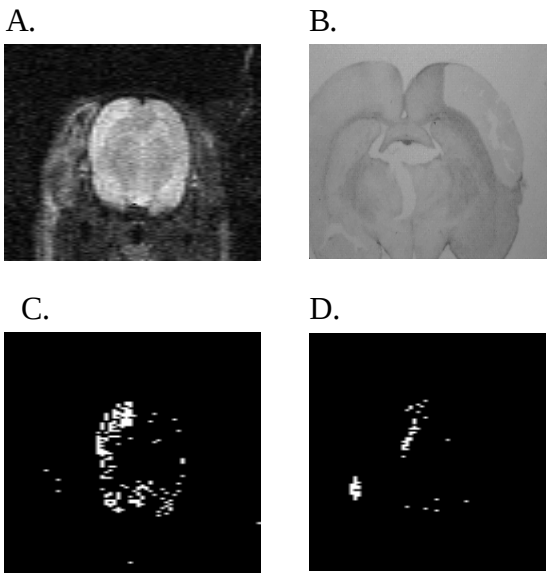

Fig. 1A) T2WI of brain lesion 48 hours after stroke; 1B) GFAP stain demonstrating areas with different severity of ischemic infarcts; 1C) and 1D) Areas separated and identified by analysis with artificial neural net indicating areas with different severity of ischemia;