Evaluation of the Applicability of Resovist in DSC-MR Perfusion-Weighted Imaging of Rat Hyperacute Cerebral Infarction

ABSTRACT

AIM: The aim of this study was to evaluate the use of Resovist in perfusion-weighted imaging (PWI) of rat hyperacute cerebral infarction.

MATERIAL and METHODS: 30 Wistar rats were randomly divided into 2 groups. Group A was intravenously injected with 8 μmol Fe/kg body weight of Resovist, whilst Group B was injected with 0.2 mmol/kg body weight of Gd-DTPA. These dosages were chosen to provide comparable maximum signal changes in normally perfused brain. CBV, CBF and MTT parameter profiles were obtained for the core diseased region and the penumbra of brain ischemia and compared between the two groups. These results were then correlated with pathological findings and TTC staining.

RESULTS: In our rat stroke model, signal-time curves were similar between Gd-DTPA and Resovist, both in the core area with severe ischemia and in the penumbra area with moderate ischemia. The CBV, MTT, and TTP values of PWI for ischemic penumbra in Groups A and B showed no statistical disparity.

CONCLUSION: The efficacy of Resovist in MR PWI is similar to Gd-DTPA in the diagnosis of perfusion reduction in the rat stroke model.

KEYWORDS: Resovist, Hyperacute cerebral infarction, MRI, Perfusion weighted imaging, Rat

INTRODUCTION

In recent years, with the development of MRI scanning technology, there has been an increased use of MR perfusion imaging studies to observe cerebral blood flow. MR perfusion imaging using Gd-DTPA as the main contrast agent makes it possible to obtain a fast and accurate diagnosis and to acquire hemodynamic parameters and metabolic change information through imaging studies of cerebral infarction (5, 9). However, high-dose intravenous injections of Gd-DTPA inevitably bring about side effects (1) such as anaphylactic shock, blood pressure drop, skin hives, and even systemic fibrosis (NSF). Pharmacokinetic studies of Resovist have confirmed human intravenous bolus injection of Resovist to be safe (12). Resovist is one type of SPIO, which are iron oxide particles wrapped in carboxydextran that act as a negative enhancer of MRIs in clinical settings. Resovist can significantly shorten T2 reducing signal intensity of target organs, which leads to a cleaner appearance of lesioned parts. This characteristic makes Resovist a possible contrast agent for use in MR perfusion (3, 7, 9, 12-14).

In previous studies on MR perfusion-weighted imaging (PWI) (19), we found that differences in both the magnetic...
susceptibility of the contrast agent and the dosage affect the results. Therefore, the appropriate selection of contrast agent dosage was very important. When replicating or comparing tests performed on different individuals, the comparability of the administered doses should be a point of attention. Therefore, for an accurate and objective analysis, the dosage choice for two different types of contrast agent was based on obtaining the same maximum signal intensity changes in MR PWI of normal brain tissue. Our previous findings (19) showed that similar signal attenuation values could be obtained using 0.2 mmol/kg body weight (BW) Gd-DTPA and 8 umol Fe/kg BW Resovist in MR PWI of normal rabbit brain. The purpose of this study is to evaluate applicability of Resovist in MR PWI of artery embolization ischemia in a rat brain model by comparing it with Gd-DTPA.

MATERIAL and METHODS

Animals

30 male Wistar rats (BW 350 – 400 g) were provided by Sun Yat-sen University Animal Experimental Breeding Center. The rats were given free access to food and water and subjected to a 12 h circadian rhythm feeding. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Guangdong General Hospital. Rats were randomly divided into 2 groups (n = 15 per group): Group A was given Resovist and Group B was given Gd-DTPA. After occluding the left middle cerebral artery with thread for 1 hr in all rats, two dynamic MR perfusion imaging series were acquired after intravenous injections of Resovist or Gd-DTPA,.

Preparation of Line Embolism

Line embolism was prepared with imported nylon line 0.2 mm in diameter and 40 mm in length. Under the microscope, quick-drying lacquer was applied on 5 mm of one end of the nylon line, forming a round and smooth head end with a diameter of 0.26 - 0.28 mm. The line was dried and placed in a solution of 1% heparin for further use after UV disinfection.

Establishing the Hyperacute Cerebral Infarction Model

The hyperacute cerebral infarction model was established using the line embolism method, where a line is used to occlude the brain artery. Rats were anesthetized with an intraperitoneal injection of 10% chloral hydrosol, 0.03 mg/100 g BW, fixed, disinfected, and towel-spread. The thigh was laid supine with their head in the center of the coil and normal breathing was permitted. All the animals were given coronary-status scanning, with a thickness of 2 mm and with the optic chiasm as the center. The order and sequence of MRI examination were as follows: FSE T2WI: TR 3000 ms, TE 95 ms, Matrix 256 × 192, NEX = 2; SE T1WI: TR 400 ms, TE 14 ms, Matrix 256 × 192, NEX = 3; PWI: T2*GE-EPI sequence, 2 mm thick, axial scan of 4 consecutive layers, field view 14 × 14 cm. Fifty phases were collected in total, and the scan parameters were TR = 800 ms, TE = 40 ms and NEX = 1. Contrast agents were injected after collection of the first seven phase images.

Pathological Examination

Three rats from each group (randomly selected from Groups A and B) underwent pathological examination. After MRI examination, the rats were decapitated under anesthesia, the brains were removed and the level of brain corresponding to MRI imaging was cut into a 2 mm sheet. Basal ganglia and adjacent slices were taken, placed in 2% TTC solution at 37°C water bath for 30 min, and the remaining brain tissue was fixed with 10% formalin for immunohistochemistry and HE staining.
Image Processing and Data Analysis

Original images from MR PWI were transferred to an AW4.2 workstation for post-processing. Four regions of interest (ROI) of the same size were selected for analysis: one was in the central area of the lesion, one was at the edge of the lesion (i.e., half dark band in the image), and the two others were located at the corresponding anatomical positions in the contralateral brain hemispheres. The selected ROIs were expressed as ROI1, ROI2, ROI3, ROI4, respectively. The corresponding negative enhancement signal intensity-time curve was obtained for each ROI, and the data obtained were entered into the appropriate software program to calculate the cerebral blood volume (CBV), cerebral blood flow (CBF), and mean transition time (MTT) values. The formulas used are as follows:

\[
\Delta R_{2*} = -\ln(SI_t/SI_0)/TE
\]

In the above formulas, \( \Delta R_{2*} \) represents the change in T2*, \( R_{2*} \) is the change rate of 1/T2*, \( SI_t \) is the signal intensity at any time point during the contrast agent's first passing, and \( SI_0 \) is the signal intensity of baseline level. Before injection of Gd-DTPA, the rats in both groups all experienced a small and transient increase in blood pressure, but none of the rats developed hypertension.

Four ROI signal intensity-time curves are shown in Figure 1 (A,B) along with the cerebral ischemia function of the rats. The signal intensity-time curves of ROI1, ROI2, ROI3, ROI4 were almost identical between Groups A and B. The curves of the central area of the lesion (ROI1, ROI2, ROI3) indicated a delayed peak and a prolonged steady state recovery time, while the curves corresponding to the contralateral normal hemispheres (ROI4a, ROI4b) all showed normal morphology.

The CBV, MTT and TTP values of Groups A and B were calculated and are shown in Table I.

In both groups the brain tissue from the middle cerebral artery area with TTC embolism appeared with no staining. Changes due to infarction were observed under light microscopy (Figure 1), and we observed under the electron microscope that the swelling part of the nerve cells in the central area of the lesions had partially disintegrated, the number of organelles was reduced, the nucleus was shriveled and the nucleoli were no longer visible, the mitochondria swelled markedly, the Golgi showed expansion with vacuolation, the perivascular space expanded, and the internal membranes were damaged. The TTC around lesions was mildly stained in pink, and while we observed no obvious brain tissue abnormalities under light microscopy, nerve cells were swollen under electron microscopy, the nuclei were slightly deformed, while the mitochondrial cristae, rough endoplasmic reticulum and Golgi were enlarged.

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DISCUSSION

Villringer (17) derived the following formulas: \( \Delta R_{2*} = k\cdot C_v \), \( SI = SI_0 e^{-k\cdot 1/TE} \), and \( C_v = -\ln(SI_t/SI_0) / k\cdot TE \), in which \( \Delta R_{2*} \) is the change rate of 1/T2*, \( C_v \) is the concentration of the contrast agent within the tissue, \( SI \) is the signal intensity at t time point, and \( SI_0 \) is the signal intensity of baseline level. Therefore, in studying MR PWI, the dosage of contrast agent can affect the results and influence the comparative study of different contrast agents. In order to compare two different contrast agents, we selected our dosage based on generating the same maximum signal changes of MR PWI in normal brain tissue. Our previous findings (7) showed that using the same scan sequence and parameters, the rate of signal decrease in
Figure 1: T1WI, T2WI, functional diagram a, functional diagram b, STC figure and TTC staining of ROI (from left to right and from top to bottom).
The original image of MR PWI showed relatively hyperintense and constant range of abnormal signal. The signal intensity-time curves corresponding to ROI1, ROI2, ROI3, ROI4 of Groups A and B were almost identical, whereas the curves of the central area of the lesion did not show an obvious peak, indicating the formation of perfusion defects because of reduced perfusion of contrast agent. The curves of the edge area of the lesion indicated the peak was delayed and the time of recovery to a steady state was prolonged, while the curves corresponding to the contralateral normal hemispheres of ROI 3 and ROI 4 all maintained normal morphology. Our data show that not only is the rat model used in this study a successful model of focal cerebral ischemia, but that the signal strength-time curves of Resovist in acute cerebral ischemia are stable and reliable. The rCBV of ROI2 of Groups A and B were 54.51 ± 7.20 and 51.10 ± 6.16, which were significantly smaller than the rCBV of ROI4, which was 89.93 ± 11.02 and 84.34 ± 8.48 for Groups A and B, respectively. This observation further indicates that similar results can be obtained using a comparable dose of Resovist and Gd-DTPA for MR brain perfusion in the diagnosis of cerebral ischemic penumbra. There was no obvious difference in rrCBV (i.e., the ratio between the affected side and uninjured side), consistent with the ischemic region shown by TTC staining.

These results show that Resovist is very useful in MR PWI of acute cerebral ischemia and there is no significant difference in diagnostic sensitivity compared with a comparable dose of Gd-DTPA. For ROI 2, the rMTT and TTP values of Group B were both slightly greater than the values of Group A, but without a statistically significant difference.

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<th>Table 1: rCBV, rMTT, TTP Values and the Corresponding Ratios (n = 15)</th>
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<td><strong>Group A (Resovist)</strong></td>
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Similarly, there was no significant difference between the two sets of rMTT and rTTP, which further confirms the feasibility of using Resovist. At the same time, the negative enhancement functions and the measurement methods of the parameter values generally used in MR PWI were equally applicable to Resovist in MR of brain perfusion samples. In addition, rCBV, rMTT and TTP values of ROI, and ROI, were not significantly different between Groups A and B, which further supports our conclusions.

When Resovist is used in MR PWI, another potential issue is its side effects. Previously, AMI-25 and Feridex could only be used for slow intravenous infusion because of significant toxicity caused by rapid injection. As a kind of early SPIO, it was found that rapid intravenous injection of AMI-25 can cause significant toxicity and side effects in the cardiovascular system, as well as shoulder and back pain, dizziness and other symptoms in clinical trials (16). During clinical use, 10 to 15% of patients experience various degrees of side effects when AMI-25 is administered by intravenous infusion. These side effects include facial flushing, rash, dyspnea and lower back pain (2). Resovist can be administrated by intravenous bolus injection due to its small particle size, good solubility in water, and stability conferred by the carboxyldextran coating. In our studies, after all experimental animals were injected with contrast agent, only a slight and transient rise in blood pressure was observed and not in one instance was there any obvious hypertensive response. Phase II clinical trials of Resovist also showed that no adverse reactions were caused by rapid intravenous injection of 4 umol Fe/kg, 8 umol Fe/kg and 16 umol Fe/kg (11). Another study confirmed these results with intravenous bolus injections of Resovist (12). Therefore, Resovist is a good candidate for use in brain perfusion and functional MRI.

This study demonstrates that rapid intravenous bolus injection of Resovist is safe and that more histological and hemodynamic information that cannot be obtained in conventional MR can be provided in clinical diagnosis and follow-up of acute cerebral infarction by its use in perfusion MR imaging. In this study of the applicability of MR PWI in acute cerebral infarction, Resovist and Gd-DTPA proved to be equally effective. However, because Resovist requires a smaller dose and does not cause renal toxicity, it has a broader application potential in brain perfusion analyses.

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REFERENCES


