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Imaging of rat cerebral ischemia-reperfusion injury using ^{99m}Tc-labeled duramycin

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Abstract

Objectives—Prompt identification of necrosis and apoptosis in the infarct core and penumbra region is critical in acute stroke for delineating the underlying ischemic/reperfusion molecular pathologic events and defining therapeutic alternatives. The objective of this study was to investigate the capability of ^{99m}Tc-labeled duramycin in detecting ischemia-reperfusion injury in rat brain after middle cerebral artery (MCA) occlusion.

Methods—Ischemic cerebral injury was induced in ten rats by vascular insertion of a nylon suture in the left MCA for 3 hr followed by 21–24 hr reperfusion. After i.v. injection of ^{99m}Tc-duramycin (1.0–3.5 mCi), dynamic cerebral images were acquired for 1 hr in six rats using a small-animal SPECT imager. Four other rats were imaged at 2 hr post-injection. *Ex vivo* images were obtained by autoradiography after sacrifice. Histologic analyses were performed to assess cerebral infarction and apoptosis.

Results—SPECT images showed that ^{99m}Tc-duramycin uptake in the left cerebral hemisphere was significantly higher than that in the right at 1 and 2 hr post-injection. The level of radioactive uptake in the ischemic brain varied based on ischemic severity. The average ratio of left cerebral hot-spot uptake to right hemisphere radioactivity, as determined by computerized ROI analysis, was 4.92±0.79. Fractional washout at 1 hr was 38.2±4.5% of peak activity for left cerebral hot-spot areas and 80.9±2.0% for remote control areas (*P*<0.001). Based on triphenyltetrazolium chloride staining and autoradiograph image data, the hotspot uptake may be associated primarily with the ischemic penumbra, in which high apoptotic activity was observed by cleaved caspase-3 immunocytochemical staining.

Conclusions—^{99m}Tc-duramycin SPECT imaging may be useful for detecting and quantifying ongoing apoptotic neuronal cell loss induced by ischemia-reperfusion injury.

Keywords

^{99m}Tc; Duramycin; SPECT; cerebral ischemia; stroke

1. Introduction

Ischemic stroke is a consequence of total or partial interruption of cerebral arterial blood supply [1]. Stroke is a leading cause of death and disability in the United States and

throughout the world. Pathophysiological findings after stroke indicate a complex cascade of events including loss of plasma-membrane integrity, apoptosis and neuronal cell death. Regions suffering the most severe degrees of blood-flow reduction rapidly progress to irreversible damage, representing an ischemic core [2]. Typically, the ischemic core is surrounded by a region of brain tissue known as the ischemic penumbra, in which cerebral cells are potentially salvageable, but the extent of the penumbra decreases over time by gradual recruitment of cells into the core. Prompt identification of cell death in the ischemic core and apoptosis in the penumbra region is critical for delineating the molecular pathologic events underlying ischemia/reperfusion and defining therapeutic alternatives.

Neuronal apoptosis after cerebral ischemic insult is characterized by specific changes such as cell shrinking, chromatin condensation, nucleus fragmentation, and morphological changes of the cell membrane [3–5]. Cell membranes, including those of neurons and neuroglia, are composed of proteins and a lipid bilayer. Phospholipids including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomyelin (SM) are the dominant lipid components of cell membranes. PE is the second most abundant mammalian membrane phospholipid and constitutes 20–50% of total phospholipids. In the brain, PE accounts for at least 45% of total phospholipids. Accumulating evidence indicates that cell-surface exposure of PE on apoptotic cells is well correlated with PS exposure in the early phase of apoptosis [6].

The presence of PE on apoptotic cell surface may play a regulatory role. Blebbing and the formation of apoptotic bodies are essential processes in which intracellular components are discretely packaged and designated to be engulfed. Like PS, PE is a constituent of the inner leaflet of the plasma membrane, with little presence on the surface of typical viable cells [7]. In apoptosis, PE is exposed onto the cell surface as the redistribution of phospholipids across the bilayer is facilitated [8]. In necrosis, PE becomes accessible to the extracellular milieu due to compromised plasma membrane integrity. In contrast to PS, which when externalized serves as a signaling mechanism for scavenging of dying cells, the externalization of PE is likely to contribute to reorganization of membrane structure and cytoskeletal components. The molecular diversity of phospholipids is essential for their structural and signaling functions in cell membranes. Many individual phospholipid constituents are known to be involved in specific signaling functions necessary for cells to respond to external stimuli [8–10].

Duramycin, a 19-amino-acid peptide produced by *Streptovercillium cinnamoneus*, has been found to be reactive with PE and is a potential molecular probe candidate. It binds the head group of PE with high affinity at a molar ratio of 1:1. Duramycin is the smallest known polypeptide that has a defined 3-dimensional binding site. The biologic activities of ^{99m}Tc-labeled duramycin have been preliminarily characterized, and its PE-binding capability has been shown to be useful for imaging of cardiac cell death [11]. Given its low molecular weight, high stability, high binding affinity, and specificity, ^{99m}Tc-duramycin may have favorable properties as a potential molecular probe for cellular apoptosis after cerebral ischemia-reperfusion injury. Recent research has revealed that many neurons in the ischemic penumbra or peri-infarct zone may undergo apoptosis after several hours or days, and thus they are potentially recoverable for some time after the onset of stroke [4,12]. Direct visualization of the apoptotic ischemic penumbra with ^{99m}Tc-duramycin may represent an opportunity for early therapeutic intervention.

The objective of this study was to investigate the capability of ^{99m}Tc-duramycin in detecting ischemia-reperfusion injury and visualizing the ischemic penumbra in rat brain after middle cerebral artery occlusion (MCAO) using a high-resolution small animal SPECT imager.

2. Materials and methods

2.1. Radiolabeling duramycin with ^{99m}Tc

For ^{99m}Tc -labeling, duramycin was conjugated with succinimidyl 6-hydrazinonicotinate acetone hydrazone (HYNIC). The detailed procedure has been described previously [11]. The reagent in the vial was a lyophilized composition of HYNIC-conjugated duramycin, 30 mg of tricine, 9.5 mg of trisodium triphenylphosphine-3,3',3''-trisulfonate (TPPTS), and 6.25 μg of SnCl_2 . The vial of duramycin was reconstituted by addition of 500 μL of ^{99m}Tc as sodium pertechnetate ($\sim 30\text{ mCi}$) in saline. The solution in the vial was incubated at 80°C for 20 minutes. The radiochemical purity was determined with HPLC analysis.

2.2. Establishment of rat middle cerebral artery occlusion model

The rat model of middle cerebral artery occlusion (MCAO) was developed using the technique described previously [13]. Ten male Sprague–Dawley rats (250 to 300 g) were used for these studies. Briefly, the rats were intubated using a 14 G catheter and anesthetized with 1.5% isoflurane throughout surgery. An incision was made in the neck exposing the left common carotid artery. The external carotid artery was isolated and ligated using 5-0 silk (Ethicon, Somerville, NJ, USA). Middle cerebral artery occlusion was achieved by inserting a 4-0 nylon suture (Doccol Corporation, Redlands, CA) via the stump of the external carotid artery into the internal carotid artery a distance of 1.5 cm from the bifurcation of the common and internal carotid arteries. Once the suture was successfully placed, the occlusion of left cerebral artery was maintained for 3 hours. Subsequently, the wound was closed using sutures and the rat was recovered from anesthesia. After the scheduled duration of temporary ischemia was reached, the rat was re-anesthetized using 1.5% isoflurane and the suture was removed to initiate cerebral reperfusion. All animals were allowed to recover and underwent cerebral reperfusion overnight (21–24 hours), followed by SPECT imaging. For all experiments, the contralateral side of the brain (right hemisphere) was used as an intrinsic negative control.

2.3. High-resolution SPECT imaging

Scintigraphic brain images were obtained using a small-animal stationary SPECT imager, FastSPECT II, which was designed and built in the Radiology Research Laboratory of The University of Arizona. FastSPECT II consists of 16 modular cameras and a cylindrical aperture with one interchangeable pinhole per camera. It is capable of acquiring projection data without any motion of the object or detection system. The spatial resolution is about 1 mm with the 1-mm-diameter pinholes used in this study.

After overnight cerebral reperfusion, four rats were anesthetized with 1.0–1.5% isoflurane and intravenously injected with ^{99m}Tc -duramycin (1.0–3.5 mCi, 0.3 mL). The rats were allowed to recover for 2 hours and then imaged using FastSPECT II. Anesthetized rats were placed inside the aperture using a translation stage for 15-minute imaging data acquisition. Sixteen projections were obtained, one from each camera, to generate the data set for tomographic reconstruction. The animals were positioned so that the imaging field of view covered the entire brain. In order to determine the cerebral radiopharmaceutical kinetic profile, ^{99m}Tc -duramycin (1.0–3.5 mCi, 0.3 mL) was intravenously injected in six other rats via a pre-installed jugular vein catheter using a Harvard PHD2000 syringe pump (Harvard Apparatus, Holliston, MA) followed by a 0.1-mL saline flush. Immediately after injection, dynamic cerebral images were recorded over a 60-minute period using FastSPECT II. Specifically, one-minute images were acquired every minute for 10 minutes, followed by 5-minute acquisitions at 15, 20, 30, and 45 minutes post-injection. To enhance signal intensity for better visualization and region-of-interest (ROI) establishment, images at 60 minutes post-injection were acquired for 10 minutes. At the end of the imaging session, the rats were

sacrificed and brains were excised and sectioned for histological, immunohistochemical and autoradiographic analysis.

2.4. Image processing

Tomographic reconstructions of FastSPECT II data were processed using 50 iterations of the OS-EM algorithm. Using AMIDE 0.9.2 software, 3D images were generated in an $81 \times 121 \times 121$ voxel format, and tomographic transverse, coronal, and sagittal slices with one-voxel thickness (0.5 mm) were produced.

For each rat imaged dynamically, a 3D ROI analysis was applied for semi-quantitative analysis of the cerebral uptake of ^{99m}Tc -duramycin. ROIs around the left hemispheres with increased radioactive uptake were created on all transverse slices of the 60-minute post-injection images; the ROIs could be viewed simultaneously on the coronal and sagittal slices. The 60-minute ROIs were applied to all of the dynamic images from 1 to 60 minutes for determining averaged cerebral time-activity curves (TACs), which were corrected for ROI size (pixels), radioactive decay, and acquisition time. The radioactivity (counts/pixel) and TACs of control right hemispheres were generated using similar ROI analysis. Care was taken to draw ROIs on both hemispheres to avoid potential calvarial bone-marrow uptake [13,14]. To avoid effects from variations of injected dose, individual TACs were normalized at each time point for initial peak counts, which typically appeared at 2 minutes post-injection in remote control areas and 10 minutes in ischemic cerebral zones. The counts per pixel represent the average of counts within the left and right cerebral hemispheres, midbrain, and posterior fossa.

2.5. Infarct volume determination and autoradiograph analysis

Lesion volume was assessed on postmortem analysis by 2,3,5-triphenyl-tetrazolium chloride (TTC) (Sigma, St Louis, MO) staining. The isolated rat brains were sliced into 1.0–1.5 mm coronal sections. Representative sections were immersed in 3% TTC buffer for 20 minutes at 37° C. The sections were then transferred to 10% formalin and photographed immediately using a digital camera. Infarct volume was measured with SigmaScan Pro 5.0 software (SPSS Science, Chicago, IL) in trace-measurement mode. The percentage infarct volume was calculated by dividing infarct volume by contralateral hemisphere volume.

After TTC staining, the *ex vivo* spatial distribution of radioactivity in the cerebral sections was examined using autoradiography. The tissue slices were covered with 1 layer of plastic wrap and exposed to FujiFilm phosphor imaging plates for 5–15 minutes. A FujiFilm BAS5000 Bio-Imaging Analysis System (Stamford, CT) was used to scan the plates for digital autoradiograph acquisition. The average intensity of autoradiograph images at gray-scale display in normal and ischemic hemispheres was determined using SigmaScan Pro 5.0 software.

2.6. Histology and immunohistochemistry assay

For histology and immunohistochemistry assay, coronally sliced brain blocks were collected and fixed in 4% paraformaldehyde in 0.1 M PBS for 48 hours. The fixative was replaced once at 24 hours. The tissue blocks were processed with sequential dehydration in 20% and 30% sucrose solution. After dehydration, the samples were sectioned with a microtome at 20- μm thickness and mounted onto poly-L-lysine-coated slides. Hematoxylin and eosin (H&E) staining was performed to obtain morphological evidence of cell death.

Detection of apoptotic cells in cerebral sections was based on immunohistochemical staining of cleaved caspase-3 with rabbit anti-cleaved caspase-3 monoclonal antibody (mAb) (Cell Signaling Technology, Beverly, MA). All procedures, including staining of the tissue

sections, deparaffinization, and cell conditioning (antigen retrieval with a borate-EDTA buffer) were performed on a Discovery XT Automated Immunostainer (Ventana Medical Systems, Inc, Tucson, AZ), using Ventana-validated reagents. Rabbit anti-cleaved caspase-3 mAb was detected using an anti-rabbit biotinylated secondary mAb followed by biotinylated-streptavidin-horseradish peroxidase and 3,3'-diaminobenzidine system (DAPMap). Primary antibody staining was detected by hematoxylin counterstaining. Immunohistochemistry was also performed without incubation with the primary antibody as an experimental negative control. This allowed us to determine the experimental signal to noise ratio. Following staining, slides were dehydrated through graded alcohols, cleared in xylene and cover-slipped with mounting medium. Images were captured using a Nikon microscope with digital image software.

2.7. Statistical analysis

Significant differences between infarct and noninfarct regions for single measurement parameters were assessed using unpaired Student's t-test. Significant differences for infarct volume measurements were determined via repeated one-way analysis of variance with Bonferroni post hoc analysis. For all statistical tests, $P < 0.05$ was considered significant.

2.8. Ethics

The animal experiments were performed in accordance with the Principles of Laboratory Animal Care from the National Institutes of Health (NIH Publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Arizona.

3. Results

3.1. Cerebral radioactive distribution and infarct determination

The FastSPECT II cerebral images showed sufficient resolution and radioactive intensity to define the individual hemispheres for comparison, even though only about 1.0 mCi ^{99m}Tc -duramycin was injected in some animals. The radioactive distribution in the control right cerebral hemisphere was barely detectable and at the level of soft-tissue background. The *in vivo* focal distributions of ^{99m}Tc -duramycin in the left cerebral hemisphere with MCAO could be visualized on tomographic images at 60 and 120 minutes post-injection. FastSPECT II images showed that ^{99m}Tc -duramycin uptake in the left cerebral hemisphere was significantly higher than that in the right hemisphere. Left cerebral hemispheres in the rats with MCAO typically showed distinctly increased focal radioactive accumulations (hotspots), which were identifiable on multiple transverse, coronal, and sagittal slices, as shown in Fig. 1. However, the increased radioactivity levels and localizations varied within the left cerebral hemispheres. In several rats, the ^{99m}Tc -duramycin uptake appeared to be quite inhomogeneous in a large area of the left hemisphere. As shown in Fig. 2, the SPECT images sometimes also revealed doughnut-shaped areas of radioactive uptake in the ischemic hemisphere, in which an area of lower radioactivity was surrounded by higher radioactive accumulation. The radioactivity levels in the inhomogeneous or doughnut distribution patterns were lower overall compared to the radioactivity of small focal hotspots.

Postmortem analysis demonstrated that the volume of cerebral infarction (TTC-unstained) in the ischemic hemisphere ranged from about 1.5 cm³ to almost the whole left hemisphere (see Fig. 3). When TTC staining revealed a large cerebral infarction, the results of autoradiograph imaging indicated that the dominant TTC-unstained areas were mismatched with ^{99m}Tc -duramycin hotspots on most cerebral slices. The cerebral hotspots were predominantly associated with the less-TTC-stained areas, potentially representing ischemic

penumbral tissues. Representative TTC staining and autoradiograph images from a rat with a large brain infarction are shown in Fig. 3 B–C. The *in vivo* ^{99m}Tc -duramycin SPECT images from the same animal are shown in Fig. 2). On those cerebral slices, the infarcted areas were partially surrounded or mixed with tissues that were less-TTC-stained, and most of the hotspot activity was in this less-TTC-stained region. In other animals, when the cerebral infarct was small, the hotspots of ^{99m}Tc -duramycin on autoradiograph images were well matched with the infarcted areas in size and location, as demonstrated in Fig. 4. Accordingly, *in vivo* SPECT imaging of small infarcts showed clear lesions with focal radioactive uptake (Fig. 5).

Results of quantitative analysis of autoradiograph images are shown in Fig. 6. The focal intensity of ^{99m}Tc -duramycin uptake in the ischemic areas was significantly higher than that in the control right hemisphere. However, the focal areas of ^{99m}Tc -duramycin uptake on autoradiograph images were significantly smaller than the unstained cerebral areas on TTC images (see Fig. 7).

3.2. Kinetic analysis of dynamic images

Cerebral time-activity curves of ^{99m}Tc -duramycin are shown in Fig. 8. After reaching an initial peak at about 2 minutes, ^{99m}Tc -duramycin in the right hemisphere exhibited rapid clearance over the observation period of 60 minutes post-injection. In contrast, ^{99m}Tc -duramycin in the left cerebral ischemic area showed increasing uptake until 10 minutes, after which there was slow washout. A significant difference was observed at each time point from 3 minutes to 60 minutes between the left cerebral ischemic area and non-ischemic right hemisphere. At 60 minutes post-injection, the average ratio of left cerebral hot spot activity to right hemisphere radioactivity was 4.92 ± 0.79 . Fractional washout was $80.9 \pm 2.0\%$ of peak activity for remote control areas and $38.2 \pm 4.5\%$ for left cerebral hotspot areas ($P < 0.001$).

3.3. Histopathology and immunohistochemical assay

H&E staining showed morphological evidence of cerebral damage in the left hemisphere of all rats. Representative images are shown in Fig. 9 A–B. H&E staining clearly demonstrated the infarct border and pathological features of cerebral infarction, characterized by cell body shrinkage, darkly stained pyknotic nuclei, and intensely stained red eosinophilic cytoplasm. High apoptotic activity was observed by cleaved caspase-3 immunocytochemical staining (Fig. 9 C). High numbers of neuronal cells with positive caspase-3 staining were found in the ischemic penumbra. Nuclear morphology of most of the caspase-3-stained cells was consistent with apoptosis, with signs of cell shrinkage, nuclear fragmentation, and chromatin condensation. No staining could be detected in negative controls (Fig. 9 D).

4. Discussion

If cerebral arterial blood flow is not restored within a short period in acute stroke, the ultimate result of the ischemic cascade is irreversible loss of neuronal function [2,6,15]. Prompt identification of apoptosis in the infarct core and penumbra region is critical for delineating the underlying ischemic area and defining therapeutic alternatives. Current thrombolytic therapeutic protocols have a short therapeutic window following vessel occlusion and remain suboptimal due to the lack of available diagnostic modalities to reflect dynamic *in vivo* molecular pathology processes [16]. Clinically, there is a need to develop new imaging approaches to define the role of apoptosis in the ischemic penumbra region and assess therapeutic interventions aimed at decreasing neuronal damage [5,17,18]. Unlike necrosis, which is an essentially irreversible process, apoptosis may be amenable to therapeutic intervention.

Although neurons are large cells that can reach in all parts of the body through axons, the vast majority of nerve cell bodies are found only in the central nervous system. These nerve cell bodies are extremely sensitive to ischemia. Visualization of cell death after stroke has until recently been restricted to the histological investigation of postmortem brains [19]. Using modern molecular imaging methods, it has become possible to monitor neuronal dying in the human brain in real time. For example, apoptosis can be imaged indirectly with radiolabeled annexin V in animal models and humans using SPECT and PET. Annexin V is a human protein that has high affinity for PS, which is selectively exposed after activation of caspase-3 [5,13,17,20]. It has been shown that ^{99m}Tc -labeled annexin V can identify hypoxic/ischemic cerebral reperfusion injury in rat MCAO models. As early as 2 hours following reperfusion, radiolabelled annexin V images revealed a two to three fold increase in annexin V uptake in the involved hemisphere compared with the normal contralateral hemisphere [14]. In addition, cell death induced by cerebral ischemia in a mouse model of stroke can be well visualized using near-infrared fluorescence imaging of annexin V [21]. Furthermore, ^{99m}Tc -HYNIC-annexin V imaging can identify neuronal apoptosis within 2 hours after reversal of hypoxia in an animal model of global cerebral ischemia [17]. It is also possible to investigate the apoptosis mechanism in the brains of patients with Alzheimer's disease using ^{99m}Tc -HYNIC-annexin V SPECT imaging [22].

The results of this study demonstrate for the first time that intravenous administration of ^{99m}Tc -duramycin resulted in a rapid targeting of brain infarct areas induced by MCAO. In this study with a limited number of animals, multiple focal hot spots were observed in the ischemic hemispheres. We also found in some animals that small hotspots were often visualized along with irregular areas that had low radioactivity. This phenomenon is likely attributable to a mixture of small foci of necrotic, apoptotic, and viable cells. Overall, the ischemic core was not clearly separable from the ischemic penumbra on most *in vivo* images. In one animal with left cerebral infarction, however, a large doughnut pattern of radioactive uptake was found in the ischemic hemisphere, suggesting uptake of ^{99m}Tc -duramycin in an ischemic penumbra surrounding a central nonviable core. SPECT imaging may permit separation of such ischemic peripheral areas from nonviable central regions.

Postmortem analyses in this study showed that the protocol of transient focal ischemia produced variable sized ischemic infarcts in the ipsilateral hemisphere, as visualized by both TTC staining and H&E staining. Based on comparison of TTC staining and autoradiographs, the pattern of radioactive uptake varied considerably in areas of infarction. ^{99m}Tc -duramycin showed good localization in small infarcts areas of ischemia. In large infarctions, however, ^{99m}Tc -duramycin uptake tended to underestimate the extent of infarction and be associated primarily with the ischemic penumbral areas, where TTC staining was reduced but not completely absent. Presumably, the avid accumulation of ^{99m}Tc -duramycin in the region of infarction is because of high levels of accessible PE. ^{99m}Tc -duramycin uptake may occur not only in the penumbra involving high apoptotic activity, but also in ischemic core with significant necrosis because PE becomes accessible to the extracellular milieu in necrotic cells. On the other hand, the low or absent uptake of ^{99m}Tc -duramycin in large ischemic cores most likely is due to poor tissue perfusion and impaired microvascular function. Currently, the exact roles and percentages of each mode of cell death after ischemia-reperfusion in the rat stroke model are not completely clear. More *in vivo* studies on this agent at different ischemia-reperfusion times are warranted.

We examined the appearance of caspase-3 activation to determine evidence for apoptosis in the ischemic brain tissues. We found high apoptotic activity in the ischemic penumbra on immunohistochemical staining. In a prior study, caspase-3 activity was reported as early as 8 hours following 50 minutes of transient MCAO in rats, with a peak at 24 hours after reperfusion, and was still present 14 days later [19]. Thus, the high level of apoptotic

activity in the ischemic penumbra region was apparently related to the cerebral hotspot uptake of ^{99m}Tc -duramycin observed in our MCAO rat models.

^{99m}Tc -duramycin exhibits higher affinity with PE than annexin V, and accordingly may be more useful as a biomarker of early apoptotic processes. ^{99m}Tc -duramycin has excellent radiopharmaceutical kinetics, with fast blood clearance and extremely low distribution in soft tissues and major organs except kidneys. A potential practical application of ^{99m}Tc -duramycin, as shown in this study, is the capability to delineate injured cells in an area of cerebral ischemia. Depending on the time course and severity of ischemia-reperfusion injury, the highest uptake of ^{99m}Tc -duramycin may not necessarily be in the infarct core, but rather in the penumbra, where tissues are less severely damaged but are undergoing apoptosis. To this end, ^{99m}Tc -duramycin may be useful for detecting tissues with ischemic damage that are potentially salvageable with treatment.

There are limitations that need to be considered in this study. First, because cerebral ischemia leads to disturbances in the blood–brain barrier, non-specific radiotracer influx might contribute to the increased uptake in the ischemic hemisphere. The pathophysiological changes in the ischemic-reperfused brain increase vascular permeability and also increase interstitial space. As a result, the radioactivity detected in the ischemic hemisphere might be a combination of specific ^{99m}Tc -duramycin uptake and nonspecific leakage of the tracer. The contribution of nonspecific binding and leakage needs to be quantitatively clarified in further studies using a nonspecific radiotracer similar in molecular weight to ^{99m}Tc -duramycin. During the period of our data acquisition, a micro-CT system was not available to obtain co-registration of rat brain CT to ^{99m}Tc -duramycin SPECT images. It is difficult to visualize the brain based on hotspot images and identify the difference between core and penumbra radioactivity due to lack of co-registered CT images. Moreover, the autoradiographic images in this study were collected on thick (1.0–1.5 mm) cerebral sections, not on micro sections, so that direct correlation of ^{99m}Tc -duramycin uptake on autoradiographic images with histologic demonstration of ischemic core and penumbra was not possible.

Conclusions

We have demonstrated that ^{99m}Tc -duramycin SPECT imaging can detect sites of injury in a rat model with cerebral ischemia-reperfusion injury. The uptake of ^{99m}Tc -duramycin may be associated primarily with the ischemic penumbra, in which high apoptotic activity exists, rather than in the infarct core. However, the level of radioactive uptake in the ischemic area may vary widely, depending on the time course of cerebral artery occlusion-reperfusion and severity of injury, so that the ischemic core may not always be distinguishable from the ischemic penumbra. The cerebral infarct size may also be underestimated by ^{99m}Tc -duramycin SPECT imaging. The pharmacokinetics of ^{99m}Tc -duramycin is suitable for early imaging following injection. Thus, it is possible to assess ongoing apoptotic neuronal cell loss in patients with ischemic stroke in real time using dynamic ^{99m}Tc -duramycin SPECT imaging.

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Fig. 1. FastSPECT II tomographic transverse (A), sagittal (B), and coronal (C) slices (0.5 mm thick) of ^{99m}Tc -duramycin (3.0 mCi) 2 hr post-injection in a rat with 3-hr cerebral ischemia followed by 21-hr reperfusion. Projection data were acquired for 15 minutes. The images show areas of abnormally increased radiotracer uptake, indicated by the yellow dashed line, within the left hemisphere.

Fig. 2. FastSPECT II tomographic transverse slices (0.5 mm thick) of ^{99m}Tc -duramycin (1.8 mCi) 60 minutes post-injection in a rat with 3-hr cerebral ischemia followed by 24-hr reperfusion. Projection data were acquired for 10 minutes. The white dashed line indicates the margin of the brain. The yellow dashed line indicates a doughnut-shaped area of increased radiotracer uptake localized in the left middle cerebral artery region.

Fig. 3.

A: Harvested whole rat brain from the animal in Fig. 2. A large white or pale pink portion (yellow dashed line) is present in the left hemisphere and corresponds to stroke-related ischemia. B: Cerebral slices with TTC staining from the rat brain in A, but oriented differently, in which viable cerebral tissue is stained red. Large TTC-unstained segments consistent with infarction are present. The white dashed line indicates the area of radiotracer uptake from autoradiograph images. C: Autoradiograph images from the cerebral slices in B. The blue dashed line indicates the brain margin. The prominent radioactive uptake (dark black) was associated with less-TTC-stained areas or mixed areas of stained and unstained, as shown in B.

Fig. 4.

A: Representative cerebral slices with TTC staining from a rat with a small infarct. The white dashed line indicates the area of radioactivity from autoradiograph images. B: Autoradiograph images from the slices in A. The blue dashed line indicates the margin of the brain. Hotspot radioactive uptake (dark black) on autoradiograph images approximately matches the small TTC-unstained areas in A. On far left slice, the hotspot size is larger than TTC-unstained area.

Fig. 5. FastSPECT II images (representative transverse slices) with focal ^{99m}Tc radioactive accumulation (yellow dashed line) acquired 60 minutes after injection of 2.1 mCi ^{99m}Tc -duramycin from a rat subjected to 3-hr MCAO and 23-hr reperfusion. Postmortem slices from this rat are shown in Fig. 4. The white dashed line indicates the brain outline. Projection data were acquired for 10 minutes.

Fig. 6. Results of quantitative analysis of autoradiograph images. Focal uptake of ^{99m}Tc -duramycin in the ischemic area was significantly higher than that in the remote right hemisphere. * $P < 0.01$.

Fig. 7. Comparison of infarct measurement on TTC staining and hotspot accumulation of ^{99m}Tc -duramycin on autoradiograph imaging. Infarct size measured by TTC staining was significantly larger than the area of ^{99m}Tc -duramycin accumulation on autoradiographs. * $P < 0.01$ compared to autoradiograph imaging.

Fig. 8. Cerebral time-activity curves of ^{99m}Tc -duramycin from FastSPECT II quantitative imaging in rats with cerebral ischemia-reperfusion. Starting at 3 minutes, the radioactivity in the ischemic area was significantly higher than in the remote viable zone ($P < 0.05$).

Fig. 9. Photomicrographs of a rat cerebral slice following 3-hr MCAO and 24-hr reperfusion. A: H&E staining (1X) demonstrates an infarction (arrows) in the left MCA region. B: H&E staining (20X) shows a mixed area with healthy and injured neuronal cells. C: Representative photomicrograph of immunostaining demonstrates caspase-3 staining predominantly in the ischemic penumbra. D: Negative control immunostaining without primary antibody shows no neuronal staining in ischemic penumbra.