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^{99m}Tc-Annexin-V Uptake in a Rat Model of Variable Ischemic Severity and Reperfusion Time

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Background To determine whether mild to moderate ischemia that is not severe enough to induce myocardial infarction will cause myocardial cell damage or apoptosis, the ^{99m}Tc-Annexin-V (Tc-A) uptake was studied in groups of rats with various intervals of coronary occlusion and reperfusion times.

Methods and Results After left coronary artery occlusion for 15 min (n=23), 10 min (n=23), or 5 min (n=12), Tc-A (80–150 MBq) was injected at 0.5, 1.5, 6, or 24 h after reperfusion. One hour later, to verify the area at risk, ^{201}Tl (0.74 MBq) was injected just after left coronary artery re-occlusion and the rats were killed 1 min later. Dual tracer autoradiography was performed to assess Tc-A uptake and area at risk. In all 5-min occlusion and reperfusion models, no significant Tc-A uptake was observed in the area at risk. Tc-A uptake ratios in the 15-min and 10-min ischemia models were 4.46±3.16 and 2.02±0.47 (p=0.078) at 0.5 h after reperfusion, 3.49±1.78 and 1.47±0.11 (p<0.05) at 1.5 h after reperfusion, 1.60±0.43 and 1.34±0.23 (p=0.24) at 6 h after reperfusion, 1.50±0.33 and 1.28±0.33 (p=0.099) at 24 h after reperfusion, respectively. With 15-min ischemia, in 3 of the 5 rats there were a few micro-foci of myocardial cell degeneration and cell infiltration in less than 1% of the ischemic area at 24 h after reperfusion. No significant histological change was observed in rats with 10-min or 5-min ischemia.

Conclusion The data indicate that Tc-A binding depends on the severity of ischemia even without a significant amount of histological change or infarction. (*Circ J* 2007; **71:** 1141 – 1146)

Key Words: Apoptosis imaging; Myocardial ischemia; Reperfusion; ^{99m}Tc-Annexin-V

Technetium-99m labeled annexin-V (^{99m}Tc-annexin-V) has enabled noninvasive imaging of apoptosis.[1–7] In healthy cells, phosphatidylserine (PS) is actively transported from the outer to the inner leaflet of the cell membrane by an aminophospholipid translocase. Once cells activate their cell death program, PS is externalized from the inner leaflet of the membrane and the PS expression is an early sign that the cell death program has been activated.[8,9] Detection of PS exposure on the outer leaflet of the cell membrane can be easily achieved with annexin-V, a 36-kD physiologic protein, because it binds with nanomolar affinity to cell-membrane-bound PS in a calcium-dependent manner.[10] It has been demonstrated that ^{99m}Tc-annexin-V imaging is feasible in animal models of acute myocardial ischemia and reperfusion, myocarditis, or heart transplant rejection.[11–15] In addition, investigation of the biodistribution and dosimetry of various forms of radiolabeled annexin in human subjects has demonstrated the safety of this agent, as well as the efficacy of imaging for the detection of acute myocardial infarction (MI) and cardiac transplant rejection.[16–20]

In patients with MI, there is intense localization of ^{99m}Tc-Annexin-V in the infarct region, both in patients with and those without reperfusion,[18,21] which suggests that a considerable number of cells in the infarct zone die by apoptosis.[22,23] Although the most effective method of limiting the zone of injury in areas of markedly decreased perfusion is restoration of blood flow, experimental studies have demonstrated that reperfusion is a major stimulus for apoptosis in previously ischemic tissue, especially in nonsalvageable cells.[24,25] Our previous study demonstrated that ^{99m}Tc-annexin-V accumulates intensely in the ischemic area of a 20-min coronary artery occlusion and reperfusion model, which causes infarction.[11] However, how its uptake depends on the severity of the ischemia/reperfusion is still unspecified. Therefore, the aim of the present study was to determine the intensity, distribution, and time course of ^{99m}Tc-annexin-V uptake in mild-to-moderate ischemia, which would not produce significant infarction, and various reperfusion times. To accomplish this, we performed an autoradiographic study on a series of rats after various coronary artery occlusion and reperfusion times.

Methods

Animal Model of Acute Ischemia and Reperfusion

All experimental procedures involving animals were conducted in accordance with the guidelines set by the Institute for Experimental Animals, Kanazawa University

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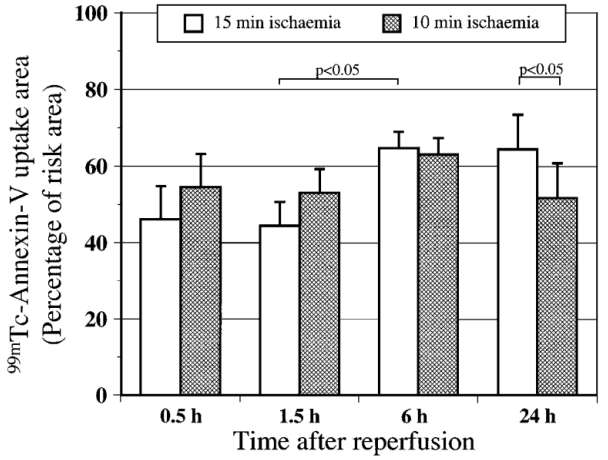

Fig 1. Percentage of ^{99m}Tc-Annexin-V uptake area for various ischemic severities and reperfusion times.

Advanced Science Research Center. Eight to eleven-week-old male Wistar rats (n=58) were anesthetized with intraperitoneal administration of 40 mg/kg pentobarbital and ventilated mechanically with room air. After left thoracotomy and exposure of the heart, a 7-0 polypropylene suture on a small curved needle was passed through the myocardium beneath the proximal portion of the left coronary artery (LCA), and both ends of the suture were passed through a small vinyl tube to create a snare. The suture material was pulled tight against the vinyl tube to occlude the LCA. Myocardial ischemia was confirmed by ST-segment elevation on the ECG and regional cyanosis of the myocardial surface. To determine the ^{99m}Tc-annexin-V uptake following various severities of ischemia the LCA was occluded for 15, 10, or 5 min. Reperfusion occurred with release of the snare and was confirmed by a myocardial blush over the risk area. The snare was left loose on the surface of the heart for reocclusion of the LCA just before killing the study animals at 0.5 and 1.5 h, to identify the area at risk. In the remaining animals, the snare was also left loose on the surface of the heart until repeat thoracotomy. The groups of animals with 15-min occlusion were administered ^{99m}Tc-annexin-V at 0.5 h (n=6), 1.5 h (n=6), 6 h (n=6), 24 h (n=5) after reperfusion; those with 10-min occlusion were administered it at 0.5 h (n=7), 1.5 h (n=5), 6 h (n=5), 24 h (n=6) after reperfusion; and those with 5-min occlusion were administered it at 0.5 h (n=6) and 1.5 h (n=6) after reperfusion. In each group, 80–150 MBq of ^{99m}Tc-annexin-V was injected via a tail vein and 1 h later, 0.74 MBq of ^{201}Tl was injected just after reocclusion of the proximal portion of the LCA for delineation of the area at risk. One minute later the rat was killed and the heart was removed, rinsed in saline, frozen in isopentane, cooled in dry ice, and embedded in methyl cellulose. Serial short-axis sections 20-μm thick were obtained by sectioning the heart on a cryostat to create a series of rings for autoradiography.

Radiolabelling of Annexin-V

Mutant annexin-V (annexin-V-117 mutant, a form of recombinant human annexin engineered to include a binding site for technetium) was prepared through expression in Escherichia coli as previously described[5] This material retains PS binding activity equivalent to that of native annexin-V. A specific activity of 3.7–7.4 MBq (100–200 μCi)/μg protein, with a radiopurity >90%, was achieved using the previously described radiolabeling protocol[5]

Dual-Tracer Autoradiography

Dual-tracer autoradiography of the left ventricular short-axis slices was performed for the assessment of ^{99m}Tc-annexin-V uptake and the ischemic area (^{201}Tl uptake). The first autoradiographic exposure on an imaging plate (BAS-MS, Fuji Film) was performed for 15–20 min to visualize ^{99m}Tc-annexin-V distribution 1 to 2 h after sacrifice. Three days later (12 half-lives of ^{99m}Tc) the second exposure was made for 24 h to image the area at risk as expressed by ^{201}Tl distribution.

Data Analysis

^{99m}Tc-annexin-V accumulation was evaluated in 3 myocardial slices spaced 1 mm apart at the mid ventricular level. The distribution of the tracers was determined by analysis of the digitized autoradiographs. The photostimulated luminescence in each pixel (100×100 μm) was determined using a bioimaging analyzer (BAS-5000, Fuji Film). For quantitative analysis, the uptake values (UV) of each region of interest (ROI) were expressed as the background corrected photostimulated luminescence per unit area (1 mm^2). A background ROI was set adjacent to the left ventricle. Ischemic and normally perfused areas were defined from the ^{201}Tl image and these ROIs were applied to the ^{99m}Tc-annexin-V images to evaluate the uptake of ^{99m}Tc-annexin-V. Significant ^{99m}Tc-annexin-V uptake area was also defined manually as a ROI. The ^{99m}Tc-annexin-V uptake ratio was calculated by dividing the UV in the ^{99m}Tc-annexin-V uptake region by that of the normally perfused area. The ratio of ^{99m}Tc-annexin-V uptake ROI area to the ischemic ROI area was defined as a percentage of the ^{99m}Tc-annexin-V uptake area. All parameters in each rat were expressed as an average value obtained from analysis of 3 representative slices. In the 15-min occlusion and 30-min reperfusion model, the ischemic myocardial area was divided into 3 transmural stratified layers of equal thickness (endocardial, middle, and epicardial) and the ^{99m}Tc-annexin-V uptake ratio was calculated.

In Situ Detection of Nuclear DNA Fragmentation (TUNEL)

In all reperfusion models, short-axis frozen sections adjacent to the slices used for autoradiography were mounted on slides for processing using TUNEL staining. The stains were performed with the in situ cell death detection kit, POD, according to the manufacturer's protocol (Roche Diagnostics GmbH, Mannheim, Germany). The number of TUNEL-positive cardiomyocytes was divided by the total number of cardiomyocytes, to determine the ratio of TUNEL-positive myocytes within both the area at risk and normally perfused area. More than 50 different fields for each section were analyzed. As a positive control, we used rat intestine. Several epithelial cells in the villous tip showed positive staining. In the 15-min occlusion and 30-min reperfusion model, the ratio of TUNEL-positive myocytes in each of the 3 myocardial layers was also calculated.

Histopathologic Examination With Light Microscope

Light microscopic histopathologic examinations (×400) were performed with hematoxylin-eosin stained slices adjacent to the slices used for autoradiography.

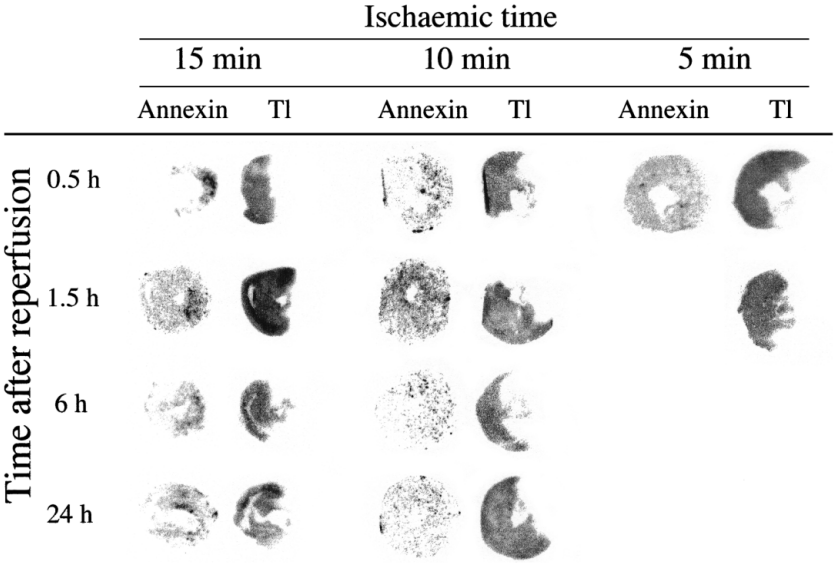

Fig 2. Autoradiography using of ^{99m}Tc-Annexin-V and ^{201}Tl. After 15, 10, and 5 min of ischemia ^{99m}Tc-Annexin-V was injected at 0.5, 1.5, 6 and 24 h after reperfusion. Single mid-ventricular slices are shown from representative animals from each group. The ^{201}Tl images demonstrate the area at risk, while the annexin images reflect the area of and intensity of apoptosis. Significant ^{99m}Tc-annexin-V uptake is observed in the area at risk and the uptake is higher with longer ischemia and decreased when reperfusion time elapsed.

Statistical Analysis

All results are expressed as mean ± 1 SD. Statistical analyses were performed using a Macintosh computer with StatView 5.0 software. Group comparisons were performed using analysis of variance, followed by Scheffe's test to identify differences among groups. A value of $p<0.05$ was considered statistically significant.

Results

Size of Area With ^{99m}Tc-Annexin-V Uptake Against the Area at Risk

In the animals with 15-min and 10-min occlusions, the percentages of ^{99m}Tc-annexin-V uptake area against area at risk at each time point are shown in Fig 1. In both groups the percentages were similar at 0.5 h after reperfusion (46.2±12.1% and 54.5±8.6%, respectively), at 1.5 h after reperfusion (44.5±12.8%, and 53.1±6.2%, respectively), and at 6 h after reperfusion (64.7±11.0%, and 63±4.3%). However, at 24 h after reperfusion, the percentage of ^{99m}Tc-annexin-V uptake area was smaller in the 10-min ischemia group (51.8±9.0%, $p<0.05$ vs 15-min ischemia) than that in the 15-min group (64.4±4.4%).

In the groups with 5-min occlusion and 0.5 h and 1.5 h reperfusion, no significant ^{99m}Tc-annexin-V uptake was observed visually, so the percentage of the ^{99m}Tc-annexin-V uptake areas was not able to be calculated. However, the annexin uptake ratio was calculated by setting the ROI arbitrarily on the area at risk that was represented by the ^{201}Tl image.

^{99m}Tc-Annexin-V Uptake

An irregular area of ^{99m}Tc-annexin-V uptake was observed in the area at risk in rats with every degree of ischemic severity and at every time point after reperfusion except for the 5-min occlusion models. Significant uptake was observed predominantly in the mid-myocardium (central uptake pattern) in rats with 15-min occlusion at 0.5 and 1.5 h after reperfusion (Fig 2). The mid-myocardial ^{99m}Tc-annexin-V uptake ratio in rats with 15-min occlusion at 0.5 h after reperfusion was significantly higher than the ratios in the endocardial ($p<0.05$) and epicardial ($p<0.05$) layers (4.29±2.10, 2.07±0.84, and 2.11±0.62, respectively). At 6 h after reperfusion, the uptake was still predominately in the mid-myocardium in the 15-min ischemic group, with mild uptake expanded to the epicardial and/or endocardial layers. After 24 h of reperfusion, annexin-V uptake demonstrated inhomogeneous uptake throughout the endo- and epicardial layers in half of the cases in the 15-min occlusion group, with the remainder showing a central uptake pattern with mild expansion. In rats with 10-min occlusion, weak central uptake pattern was observed at 0.5 and 1.5 h after reperfusion, followed by faint inhomogeneous expanded uptake at 6 h and 24 h after reperfusion.

Comparison between the intensity of the ^{99m}Tc-annexin-V uptake in the area of increased ^{99m}Tc-annexin-V accumulation and that in the normal myocardium revealed that the longer the ischemia, the higher the ^{99m}Tc-annexin-V uptake when reperfusion time was 0.5 h or 1.5 h (Fig 3). At 0.5 h after reperfusion, ^{99m}Tc-annexin-V uptake tended to be higher in the rats with 15-min occlusion (4.46±3.16) than 10-min occlusion (2.02±0.47, $p=0.078$), and higher than 5-min occlusion (0.97±0.08, $p<0.05$). At 1.5 h after reperfusion, the highest uptake was observed in the animals with 15-min occlusion (3.49±1.78, $p<0.05$ vs 10 min occlusion, $p=0.001$ vs 5-min occlusion), compared with animals with 10-min occlusion (1.47±0.11) or 5-min occlusion (0.87±0.06). At 6 h after reperfusion, higher uptake was also observed in the rats with 15-min occlusion (1.60±0.43) than with 10-min occlusion (1.34±0.23); however, it did not reach statistical significance ($p=0.24$). At 24 h after reperfusion, uptake intensity was also higher in the rats with 15-min occlusion (1.50±0.33) than 10-min occlusion (1.28±0.33), but it also did not reach statistical significance ($p=0.099$).

TUNEL-Positive Cardiomyocytes

TUNEL-positive cells in the ischemic area were more frequently observed in rats with longer ischemia for every reperfusion time. At any reperfusion time, the percentage of TUNEL-positive cells was significantly higher in rats with 15-min ischemia than in rats with 10-min ischemia. The respective percentages of TUNEL-positive cells in the rats with 15-min ischemia and 10-min ischemia were 1.01±0.37% and 0.25±0.12% ($p<0.001$) at 0.5 h after reperfusion, 1.50±0.32% and 0.39±0.14% ($p<0.001$) at 1.5 h

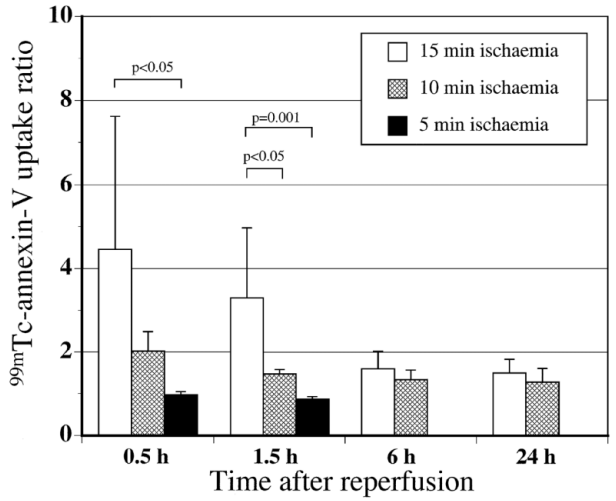

Fig 3. ^{99m}Tc-annexin-V uptake ratio with various ischemic severities and time points after reperfusion. The ^{99m}Tc-Annexin-V uptake ratio was calculated by dividing the ^{99m}Tc-annexin-V count density in the significant annexin uptake area by that of non-ischemic area. Reperfusion time indicates the time of ^{99m}Tc-Annexin-V injection after reperfusion. The longer the coronary artery occlusion time, the higher the ^{99m}Tc-Annexin-V uptake ratio.

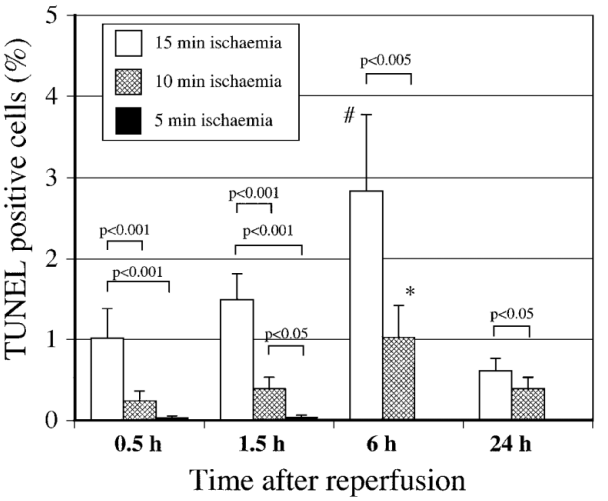

Fig 4. Percentage of TUNEL-positive cardiomyocytes in the rats with 15-min, 10-min and 5-min ischemia at 0.5 h, 1.5 h, 6 h and 24 h after reperfusion. Myocardial %TUNEL-positive staining is highest in the 15-min occlusion model than in the rats with 10-min and 5-min ischemia at every reperfusion time point. With both 15-min and 10-min ischemia, the %TUNEL-positive cells peaked at 6 h after reperfusion. #Significant differences in the rats with 15-min ischemia between those at 6 h after reperfusion and those at 0.5 h, 1.5 h, and 24 h after reperfusion ($p<0.001$). *Significant differences in the rats with 10-min ischemia between thsoe at 6 h after reperfusion and those at 0.5 h, 1.5 h, and 24 h after reperfusion ($p<0.001$).

after reperfusion, 2.84±0.94% and 1.03±0.40% ($p<0.005$) at 6 h after reperfusion, and 0.61±0.16% and 0.39±0.14% ($p<0.05$) at 24 h after reperfusion. The %TUNEL-positive cells in the rats with 5-min ischemia was minimal (0.040±0.015% at 0.5 h after reperfusion and 0.046±0.019% at 1.5 h after reperfusion).

In both the rats with 15-min or 10-min ischemia, the %TUNEL-positive cells increased up to 6 h after reperfusion and declined at 24 h after reperfusion (Fig 4). In the 15-min occlusion and 0.5 h reperfusion model, the %TUNEL positive cells in the mid-myocardial layer (1.49±0.52%) tended to be higher than that of endocardial layer (0.88±0.42%, $p=0.09$) and significantly higher than that of the epicardial layer (0.55±0.22, $p<0.05$).

Cardiomyocytes in the remote area demonstrated minimal TUNEL staining in any group within the range of 0.10–0.038%.

Representative TUNEL stainings at 6 h after reperfusion with 15-min and 10-min ischemia are shown in Fig 5.

Histopathologic Findings

In the rats with a 15-min occlusion, there was no observable myocardial degeneration, necrosis or inflammatory cell infiltrates at 0.5 h or 1.5 h after reperfusion. An imperceptible change of a few micro foci of minimal inflammatory cell infiltrations was observed in some of the slices of the specimens from 2 of 6 rats at 6 h after reperfusion. At 24 h after reperfusion, only a few micro foci of myocardial cell degeneration and cell infiltration were observed in some slices in 3 of 5 rats. However, the area was less than 1% of each ischemic area.

Among the 10-min occlusion rats, none of the group with 0.5–24 h of reperfusion had evidence of myocardial degeneration, necrosis or inflammatory cell infiltrates.

No significant histological change was also confirmed in all 5-min occlusion rats.

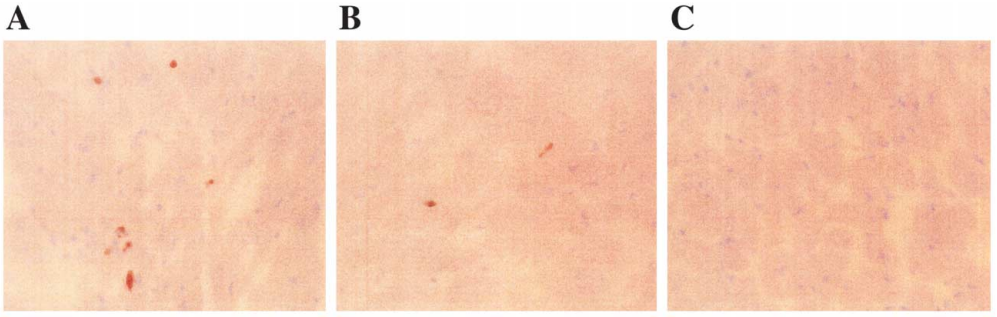

Fig 5. Representative TUNEL-stained slice from a frozen specimen of a rat with (A) 15-min occlusion and 6-h reperfusion demonstrates several TUNEL-positive (brown staining of nucleus) nuclei of cardiomyocytes, and (B) with 10 min occlusion and 6 h after reperfusion shows minimal TUNEL-positive nuclei of cardiomyocytes. No TUNEL staining was observed in the non-ischemic area (C) of (A).

Discussion

The present study demonstrates that ^{99m}Tc-annexin-V can accumulate even in areas of less severe ischemia that does not induce significant amount of necrosis and histological changes, indicating that ^{99m}Tc-annexin-V is a sensitive marker of ischemic insult to the myocardium. In addition, the intensity of the uptake depends on the ischemic severity and the reperfusion time after ischemia, but the relative uptake area against area at risk is less dependent on these 2 factors. Significant and mild ^{99m}Tc-annexin-V uptake within the ischemic lesion was observed at 0.5 h after reperfusion after 15-min and 10-min occlusion, but no accumulation was observed after 5-min ischemia. The degree of ^{99m}Tc-annexin-V uptake depended on the severity of ischemia, with more intense uptake with more severe ischemia. This uptake dependency on ischemic severity was observed early after reperfusion (0.5 h and 1.5 h). In each ischemic severity group, the uptake intensity decreases when the reperfusion time elapsed, which indicates that the sensitivity of ^{99m}Tc-annexin-V imaging for detecting ischemic insult decreases as the timing of annexin-V administration is delayed after reperfusion.

In the model of 15-min occlusion, considerable uptake of ^{99m}Tc-annexin-V, together with mild to moderately positive TUNEL staining, was demonstrated despite the histologic finding of negligible infarction and necrosis, which comprised less than 1% of the area at risk. In the animals with 10-min occlusion, mild ^{99m}Tc-annexin-V uptake with minimal positive TUNEL staining was observed despite the lack of any abnormal histologic findings. The discrepancy between the significant amount of ^{99m}Tc-annexin-V binding to the ischemic myocardium and the relatively low number of TUNEL-positive cells, coupled with the minimal histological changes, raises questions about the mechanism of the phenomenon. Recent studies suggest that PS externalization can occur without lethal cell injury or irreversible changes, such as DNA fragmentation, and its expression might be reversible upon removal of various apoptotic stimuli.[26–33] Using a temperature-sensitive p53 cell line, Geske et al demonstrated that early apoptotic cells with externalized PS could be rescued and proliferate if the apoptotic stimulus was removed.[29] B-cell lymphoma also demonstrated that signal-induced annexin-V-positive cells are viable and can resume growth and reestablish phospholipid asymmetry once the signal is removed.[26] Similarly, white blood cells show reversible PS exposure by hypotonic shock.[30] In addition, Kenis et al demonstrated that annexin V mediates the internalization of the PS-annexin V complex during the process of apoptosis.[31] More directly, using a rabbit model of myocardial ischemia and reperfusion Narula et al suggested that, once externalized, PS might return to the inner leaflet of the cell membrane. In their experiment, ^{99m}Tc-annexin-V was injected after 10-min ischemia and 30-min of reperfusion and 3 h later the rabbits were killed. A significant amount of ^{99m}Tc-annexin-V had accumulated in the ischemic area without evidence of infarction or apoptosis, and ultracentrifugal isolation of the subcellular components of the once-ischemic myocardium revealed that more than 50% of the radioactivity had been internalized.[32,33] Considering all these findings together, a significant amount of myocardium in the area at risk might express PS to the outside of the cell membrane early after reperfusion and thereafter some of the PS might return to inner side of the cell membrane or reestablish phospholipid asymmetry as time elapses after removal of the ischemic insult and might not execute the cell death process until the irreversible stage. In our 15-min and 10-min ischemia/reperfusion models, such a hypothesis would explain the discrepant finding of low numbers of TUNEL-positive cells with less inflammatory cell infiltrate or no histological change related to infarction and necrosis, despite a significant amount of ^{99m}Tc-annexin-V uptake. In addition, this hypothesis also could explain the reperfusion time-related reduction of ^{99m}Tc-annexin-V uptake because PS externalized in the early stage after reperfusion might gradually return to inner side of the cell membrane during continuous dissolution of the ischemic insult by reperfusion. Another possible mechanism is that the cells with externalized PS execute apoptosis rapidly and had disappeared after phagocytosis by the surrounding myocardium or macrophages. However, histological examination showed only a few micro foci of inflammatory cell infiltration in some of the rats, suggesting that rapid disappearance of cells might be a minor process. To investigate whether the cardiomyocytes that bind the annexin-V have really turned on their apoptotic machinery or not, immunohistochemical analysis with caspase activation might be useful. The lack of these data is a significant limitation of this study.

In both the 15-min and 10-min ischemia groups, ^{99m}Tc-annexin-V uptake was most prominent at 0.5 h after reperfusion and was followed by a gradual reduction until 24 h after reperfusion (Fig 3), whereas the %TUNEL-positive cardiomyocytes peaked at 6 h after reperfusion (Fig 4). This difference in the time courses is in keeping with the known temporal sequence of apoptosis. The externalization of PS, which is one of the earliest events after triggering cell death, should be detected as ^{99m}Tc-annexin-V uptake followed by DNA fragmentation, which can be detected by TUNEL staining. Van den Eijnde et al used an intracardiac injection of biotin-labeled annexin-V in the developing embryo to detect sites of apoptosis during fetal development. Annexin-V positive and TUNEL-negative cells were found in the early execution phase of apoptosis, whereas cells that were positive for both annexin-V and TUNEL staining were in a later phase of apoptosis. The pyknotic cell fragments were often only TUNEL positive.[34]

The percentage of ^{99m}Tc-annexin-V uptake area against area at risk tended to be larger with 10-min ischemia than with 15-min ischemia and there were some significant difference between some groups. This might be partially caused by the difficulty of subjective delineation of significant but weak and inhomogeneous ^{99m}Tc-annexin-V uptake area. Such an area might be recognized as larger because of blurring of the uptake and, in addition, if the uptake was further decreased as to be faint, the ROI might be delineated as smaller because of the difficulty in identifying the significant uptake area. However, there are no clear, objective and quantitative ways of differentiating the significant uptake area from the non-uptake area.

As for hot spot imaging, the time frame of ^{99m}Tc-annexin-V accumulation in jeopardized myocardium is quite different from that of the imaging agent used in the past to detect acute infarction, ^{99m}Tc-pyrophosphate. The uptake of ^{99m}Tc-pyrophosphate is usually observed in acute MI, starting at least 3 h after the onset and becomes increasingly positive in the first 24–74 h, followed by reduction of uptake. The ^{99m}Tc-annexin-V uptake, on the other hand, starts and peaks just after the ischemic insult, depending on its severity, even if the insults does not cause infarction.

Therefore ^{99m}Tc-annexin-V imaging could be used for early diagnosis and evaluation of the ischemic severity of acute coronary syndrome.

Conclusion

Our data demonstrate that significant ^{99m}Tc-annexin-V uptake is observed with less severe ischemia (15-min or 10 min occlusion and reperfusion) that causes only minimal or negligible necrosis or none at all. The uptake of ^{99m}Tc-annexin-V within the ischemic area depends on the ischemic severity and reperfusion time: higher uptake with longer ischemia and higher uptake at an earlier time after reperfusion. These findings suggest that the ^{99m}Tc-annexin-V can potentially be a sensitive marker of ischemic insult even when it does not cause infarction.

Acknowledgments

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