Potential use of ^{99m}Tc-annexin V imaging in ischemically damaged myocardium

"...apoptosis imaging could be a promising noninvasive method to identify patients at risk of heart failure development..."

KEYWORDS: ^{99m}Tc-annexin V ■ acute coronary syndrome ■ apoptosis ■ heart failure ■ left ventricular remodeling ■ myocardial ischemia

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Four decades ago, the term apoptosis was introduced by Kerr *et al.* as a special form of cell death different from necrosis [1]. Necrosis is characterized by irreversible loss of plasma membrane integrity with cell swelling and rupture after sudden severe insults that preclude adequate homeostatic energy-dependent cell functions, leading to the release of intracellular contents and a subsequent inflammatory response. In contrast to necrosis, apoptosis is characterized morphologically by the condensation of nuclear chromatin, cytoplasmic condensation, cell shrinkage, followed by the nuclear and cellular fragmentation and phagocytosis of apoptotic bodies by neighboring cells in the absence of inflammation. Apoptosis is considered to be an active and highly regulated ATP-dependent programmed cell death process, which is governed by two central pathways:

- The death receptor pathway, which is initiated by activation of cell-surface receptors by factors such as the Fas-ligand, TNF-α;
- The mitochondrial pathway, which responds to cellular stress such as ischemia, reperfusion injury, toxin or radiation.

Both pathways result in the activation of caspase-3, the final effector enzyme of apoptosis, followed by externalization of phosphatidylserine (PS) on the cell surface, cleavage of cytosolic proteins and DNA fragmentation.

After the initial description of apoptosis based on the morphological features, several useful biochemical and immunohistochemical detection methods were subsequently introduced based on the understanding of the mechanistic basis of apoptosis. As a histochemical technique for the detection of apoptosis, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) has become a standard technique for *in situ* labeling and localization of DNA breaks in individual nuclei in tissue sections. However, for molecular targets in *in vivo* imaging, it is favorable that the target exists on the cell surface rather than in the cytoplasm or nucleus. Accordingly, to date, the most noninvasive imaging of apoptosis targets PS, a membrane aminophospholipid that is normally located on the inner leaflet of cell membrane but is rapidly translocated to the outer leaflet of cell membrane once the cell becomes apoptotic. Annexin V, a 36-kD physiologic protein, binds with nanomolar affinity to PS in a calcium-dependent manner, therefore, ^{99m}Tc-labeled annexin V permits imaging of apoptosis *in vivo* in its early stage [2–4].

Ischemic heart disease & acute coronary syndrome

It was once widely accepted that the myocardium starts to die via necrosis shortly after the onset of myocardial infarction (MI), where a wave of necrosis spreads from endocardium to epicardium. However, recent experiments in animals with permanent coronary artery occlusion have revealed that the cell death process starts as apoptosis and severe ATP depletion due to ischemia may preclude the execution of apoptosis and lead to plasma membrane permeability barrier breakdown and secondary necrosis. Reperfusion accelerates apoptosis due to reperfusion injury and also induces a shift from necrosis to apoptosis, which may be due to the restoration of ATP [5,6].

Serial autoradiographic imaging with ^{99m}Tc-annexin V in a rat model of 20 min ischemia and reperfusion demonstrated that strong ^{99m}Tc-annexin V uptake starts in midmyocardium in areas at risk 30 min after reperfusion, it then extends in the subendomyocardium and subepicardium at 6 h and declines until 24 h in

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a relatively rapid manner, and then eventually regresses over 3 days [7]. In contrast to the severe ischemia that produced MI, even in rats with brief ischemia (10 and 15 min) and reperfusion, relatively weak but significant ^{99m}Tc-annexin V uptake was observed at 30 min, 90 min and 6 h of reperfusion, whereas no morphological signs of necrosis and apoptosis were observed until 24 h of reperfusion [8]. In addition, several cardioprotective interventions, such as caspase inhibitors, ischemic preconditioning and postconditioning, attenuate ^{99m}Tc-annexin V uptake [9]. Furthermore, Kenis *et al.* demonstrated that brief ischemia externalizes PS for at least 6 h and internalizes after annexin V binding without cell death (reversibility of apoptotic process) [10]. In nine patients with acute MI (AMI), ^{99m}Tc-annexin V uptake was clearly observed in the area at risk (perfusion defect before revascularization) and the size of perfusion defect decreased at 1–3 weeks after MI, suggesting that part of the cardiomyocyte damage is reversible in the area of ^{99m}Tc-annexin V uptake [11]. These findings indicate the potential use of ^{99m}Tc-annexin V imaging in patients with ischemic heart diseases and may have future roles in the:

- Evaluation of the extent and severity of myocardial damage in AMI;
- Detection of myocardial ischemic insult in patients with acute coronary syndrome without ST elevation and troponin leakage;
- Assessment of the effect of revascularization in acute coronary syndrome;
- Evaluation of the effect of postconditioning at reperfusion therapy and antiapoptotic drugs such as caspase inhibitors, if these become available in near future;
- Detection of transient PS externalization due to brief spontaneous or stress-induced ischemia.

However, several questions remain unanswered concerning the clinical application of ^{99m}Tc-annexin V imaging. *In vivo* imaging 1 h after tracer injection is feasible in rats since blood clearance of ^{99m}Tc-annexin V was rather fast, although the earliest optimal time for imaging after tracer injection should be investigated in humans. Speedy imaging after tracer injection is crucial, especially in emergency situations. Important unanswered questions include: are therapeutic interventions beneficial for all annexin-positive myocardium, or only in a specific pathological status or limited time window? How much of the shift from necrosis to apoptosis by therapeutic intervention is cardioprotective? Necrosis is more harmful than apoptosis, because cells are removed without inflammation in apoptosis but cells are removed with inflammation and fibrosis follows in necrosis. Is an annexin V-positive scan in exercise stress related to subsequent prognosis, or an indication of percutaneous coronary intervention? These potential imaging concepts regarding the assessment of myocardial injury, stress and cell death in acute ischemia should be validated in clinical studies.

> *"...several questions remain unanswered concerning the clinical application of ^{99m}Tc-annexin V imaging."*

Left ventricular remodeling following acute myocardial infarction

Despite dramatic improvements in therapies for ACS, post-AMI mortality has reached a plateau and post-AMI heart failure due to ventricular remodeling is on the increase. Approximately 15–25% of AMI patients develop heart failure that remains progressive despite continuous pharmacological therapy. It has been reported that myocardial apoptosis shortly after AMI might be a strong predictor of unfavorable left ventricular (LV) remodeling and early post-AMI symptomatic heart failure in 16 patients dying 10 days or more after AMI [12]. In rats with MI, long-term caspase inhibition ameliorates apoptosis, preserves myocardial contractile proteins, reduces systolic dysfunction and attenuates LV remodeling [13]. If similar effects occur in humans, apoptosis imaging could be a promising noninvasive method to identify patients at risk of heart failure development due to LV remodeling and to monitor the treatment effects, if and when some specific antiapoptotic agents reach the stage of clinical study.

Conclusion

It has become increasingly clear that apoptosis is a major contributor to early cardiomyocyte cell death after AMI and post-MI ventricular dysfunction and adverse remodeling leading to heart failure. These findings emphasize the need for reliable *in vivo* imaging methods for apoptosis that assess the pathological status so that rational preventive therapies can be applied, and the consequence of therapies can be assessed. ^{99m}Tc-annexin V imaging can be used to assess the myocardium at risk of cell

death in acute coronary syndrome and post-MI ventricular remodeling and heart failure by allowing visualization of PS externalization that might precede or underlie changes in pathophysiology, morphology and LV function.

Future perspective

Based on the research achievements to date, we believe that ^{99m}Tc-annexin V imaging is one of the most promising and feasible molecular imaging techniques for clinical application. Following the initial preliminary clinical trials, we should take several concrete actions to apply this imaging modality in order to determine the pathophysiology in specific clinical situations (i.e., to investigate its feasibility as a positive imaging modality in the detection of transient PS externalization owing to exercise stress-induced ischemia, in the detection of previous ischemic insults in patients with ACS without ST elevation and troponin leakage, in the stratification of the patients at risk of LV remodeling and heart failure development after MI and so on). These clinical investigations will allow us to provide more accurate information of the particular patient's pathophysiology and to improve therapeutic decision making.

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