⁶⁸Ga-labeled exendin-4 to image cardiac repair after myocardial infarction: From lizard venom to laboratory and beyond

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Acute myocardial infarction (MI) and ischemic cardiomyopathy are major causes of death and disability worldwide.¹ The inflammatory response after MI (Figure 1) plays a critical role in determining MI size.^{2,3} A persistent pro-inflammatory reaction can contribute significantly to adverse left ventricular remodeling, making inflammation an important target for imaging and therapy.^{2–8}

Glucagon-like peptide-1 (GLP-1) and its G-protein-coupled receptor, the GLP-1 receptor (GLP-1R), have been shown to exhibit cardioprotective effects after myocardial ischemia and reperfusion injury in both animal models and clinical trials.^{9–11} GLP-1 is an intestinal peptide hormone stimulating insulin secretion after a meal. GLP-1Rs are present on pancreatic β -cells and expressed in other tissues such as the brain, kidneys, lungs, the immune system, stomach, and small and large intestines.¹² GLP-1R has also been identified in the cardiovascular system.¹² Cardiac imaging of GLP-1R may be an interesting target because its activation has been shown to induce differentiation of human macrophages into an anti-inflammatory or reparative M2 phenotype via STAT3 activation, increase the amount of

M2 macrophages after MI, and attenuate myocardial inflammation and interstitial fibrosis post MI.¹⁰

Previously, GLP-1 agonists such as exendin-4 have been radiolabeled with positron emitters to image pancreatic tumors.¹³ Exendin-4 is a 39-amino acid peptide that was originally found in the venom of the Gila monster lizard and shares a 53% amino acid identity with GLP-1.¹⁴ Exendins are named as such because they were isolated from an *exocrine* gland and were shown to have *endocrine* actions. Compared to native GLP-1, exendin-4 is a long acting agonist at the GLP-1R but otherwise displays similar properties to GLP-1 in regulating gastric emptying, insulin secretion, inhibition of glucagon secretion, and appetite reduction.¹¹

MULTIMODALITY IMAGING OF MYOCARDIAL REPAIR

In this issue of the *Journal of Nuclear Cardiology*®, Stahle et al¹⁰ evaluated whether dynamic positron emission tomography (PET) imaging of ⁶⁸Ga-NODAGA-exendin-4 can detect up-regulation of GLP-1R expression in the rat heart after MI and whether tracer uptake was associated with histological markers of myocardial repair. Of the 22 rats that underwent permanent ligation of the left coronary artery in their study, 6 were analyzed 3 days post MI, 7 were analyzed 1 week post MI, and 9 were analyzed 12 weeks post MI. Of the 18 rats that underwent sham operation, 9 were analyzed at 1 week post operation and another 9 were analyzed at 12 weeks post operation. Myocardial uptake of ⁶⁸Ga-NODAGA-exendin-4 was measured with PET and anatomical information was provided by contrast-enhanced computed tomography (CT). Digital autoradiography and histology of the left ventricle (LV) tissue sections were also performed. Specificity of tracer uptake was assessed by injection of unlabeled exendin-

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4 ten minutes prior to administration of ⁶⁸Ga-NODAGA-exendin-4.

The authors found focally increased tracer uptake in the infarcted regions peaking at 3 days and continuing at 1 week after MI (although at a lower level) on ⁶⁸Ga-NODAGA-exendin-4 PET/CT images taken at 50–60 minutes post-tracer injection. By contrast, there was low uptake of ⁶⁸Ga-NODAGA-exendin-4 in the myocardium of sham-operated rats or in the remote myocardium of rats with MI on these late averaged PET images (50–60 minutes). Moreover, graphical analysis by Patlak plot supported irreversible ⁶⁸Ga-NODAGA-exendin-4 uptake in the infarcted area. The net influx rate constant (K_i) of ⁶⁸Ga-NODAGA-exendin-4 was significantly higher in the infarct region of the LV than the remote, non-infarcted myocardium at 3 days, 1 week, and 12 weeks post MI. Pre-treatment with an unlabeled exendin-4 peptide significantly reduced ⁶⁸Ga-NODAGA-exendin-4 uptake, indicating that the tracer binding was likely specific.

In support of the PET/CT findings, autoradiography results showed focally increased ⁶⁸Ga-NODAGA-exendin-4 uptake, which was highest at 3 days and then gradually decreased at 1 and 12 weeks post MI. Compared with the myocardium of sham-operated rats, ex vivo myocardial uptake of ⁶⁸Ga-NODAGA-exendin-4 was about 9-fold higher at 1 week and 4.5-fold higher at 12 weeks post MI. In the remote myocardium, ex vivo tracer uptake was shown to be significantly increased at 1 and 12 weeks post MI when compared with the myocardium of sham-operated rats.

Interestingly, the autoradiography data suggest that the remote myocardium may also incur changes after MI.

Furthermore, Stahle et al showed another intriguing result. They found that the level of ⁶⁸Ga-NODAGA-exendin-4 uptake was positively correlated with the number of CD68+ macrophages in the infarcted area both by PET ($r = .56$, $P = .018$) and autoradiography ($r = .71$, $P < .001$) during the healing phase of MI. There were very few CD68+ macrophages in the myocardium of sham-operated rats and the remote myocardium of rats with MI (area percentage < 1%), but they were present in the infarcted area, where the area of CD68 staining decreased gradually from day 3 ($15\% \pm 3.9\%$) to week 1 ($9.5\% \pm 2.6\%$) to week 12 ($4.3\% \pm 1.2\%$) after MI. The ex vivo analyses also showed that there was a negative correlation ($r = -.49$, $P = .021$) of tracer uptake with α -smooth muscle actin (α -SMA) staining in the infarcted area, whereas in the remote myocardium, ⁶⁸Ga-NODAGA-exendin-4 uptake correlated positively ($r = .52$, $P = .040$) with the area of α -SMA+ interstitial cells. Lastly, ⁶⁸Ga-NODAGA-exendin-4 uptake did not correlate with the area of fibrosis in Masson's trichrome staining in the infarcted area or in the remote myocardium.

The work by Stahle et al is related in some ways to a study from Xiaoyuan (Shawn) Chen's group⁹ at the National Institutes of Health (NIH) which followed the dynamic pattern of GLP-1R after myocardial ischemia-reperfusion injury and the following remodeling process with an ¹⁸F-labeled exendin-4 tracer. The authors discuss the NIH study⁹ in their paper, but there is an additional point to be made. While F-18 tracers produce

Figure 1. The pro-inflammatory and healing monocyte/macrophage response after myocardial infarction. Adapted from Frantz et al.⁸

higher spatial resolution PET images due to fluorine-18's lower effective positron range prior to annihilation, there are several practical and economic advantages to using a ⁶⁸Ga tracer.¹⁵ Gallium-68 is a generator-eluted, short-lived radionuclide (half-life = 68 minutes) that enables improved dosimetry and repeat imaging. Moreover, the parent isotope germanium-68 has a very long half-life of 271 days which allows routine manufacture and shipment. Generator-based tracers also have the advantage in that they allow clinical studies to be done without the need for an expensive on-site cyclotron, which would make them favorable for use in developing countries.

LIMITATIONS AND FUTURE DIRECTIONS

Several limitations of the study by Stahle et al must be acknowledged. First, the identity of the two radiometabolites of ⁶⁸Ga-NODAGA-exendin-4 and whether they can cross into the heart tissue from the blood remain unknown. These uncertainties in the tracer characteristics in vivo can affect the quantitative accuracy of the results, as the PET scanner cannot distinguish whether the tissue signal is from the authentic tracer or a combination of the authentic tracer and the contaminating radiometabolites. This will require a mass spectrometry analysis and an examination of the tissue homogenates by radio-high-performance liquid chromatography. Second, while the double immunofluorescence staining for GLP-1R and CD68+ macrophages showed complete co-localization in the infarcted area, the source of ⁶⁸Ga-NODAGA-exendin-4 uptake in the remote myocardium was left unresolved. This needs to be investigated in future studies. Third, a more complete compartmental analysis of the radioligand-receptor kinetics could have been carried out for estimation of the binding affinity of the tracer to the receptor site and the density of the receptors. Lastly, it is unclear whether ⁶⁸Ga-NODAGA-exendin-4 imaging early after MI can predict cardiac function and outcome. This can be a good starting point for future research. However, despite these limitations, the findings by the authors advance our knowledge about the feasibility of imaging GLP-1R expression post MI and its correlation to pathophysiology during healing of MI.

In summary, the work presented by Stahle and colleagues show that ⁶⁸Ga-NODAGA-exendin-4 PET/CT imaging detects up-regulated cardiac GLP-1R expression after MI in rats. Additionally, they report that the level of ⁶⁸Ga-NODAGA-exendin-4 uptake in the infarct region positively correlates with the presence of macrophages during the healing phase of MI. The authors indicate that ⁶⁸Ga-NODAGA-exendin-4 PET/CT may be a useful translational tool for monitoring the

activated repair mechanisms after an ischemic myocardial injury. This is important because direct interrogation of infiltrating immune cells, which contribute to the infarct healing process, could provide insight into the progression of disease and response to therapies that aim to modulate inflammation by enhancing endogenous healing and attenuating adverse remodeling. The authors should be commended for their efforts, but there is still more work to be done.

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Disclosure

The author declares no conflict of interest.

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