Research Highlights

Highlights from the latest articles in imaging

Evaluation of myocardial injury and remodeling by nuclear medicine techniques

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Evaluation of: Higuchi T, Fukushima K, Xia J *et al.*: Radionuclide imaging of angiotensin II type 1 receptor upregulation after myocardial ischemia-reperfusion injury. *J. Nucl. Med.* 51(12), 1956–1961 (2010).

As important as the renin–angiotensin system (RAS) is in maintaining of blood pressure and hydroelectrolytic balance of the organism, it seems that any imbalance of normal homeostatic mechanisms can be further tipped by the unchecked actions of this system. These phenomena have been reunited under the generic name of 'maladaptive mechanisms', and their targeted imaging may provide insights into the evolution of disease, as well as identify possible therapeutic avenues.

The present work introduces a new method of PET imaging of the angiotensin II type I receptors (AT_1R) thought to be responsible for a series of maladaptive responses within the heart after a myocardial infarction (MI) in experimental animals (rats).

The authors have used a specific AT_1R ligand – KR 31173, tagged with 'C' as a specific PET tracer – for determining its potential use in evaluating the density of AT_1R at the myocardial level and the evolution of its upregulation in the days following the MI.

A series of very elaborate protocols have been developed for inducing an ischemia-reperfusion lesion in the myocardium of the rats – identifying and properly documenting the extent of the scar tissue.

These protocols used ^{201}Tl chloride for the delineation of the nonperfused scar area and ^{99m}Tc-tetrofosmin for delineating the risk areas. The *ex viva* distribution of AT_1R was measured by coupling saralasin–isoleucine–angiotensin II (^{125}I-SI-ang II) – an angiotensin analog – to the flash-frozen heart sections.

Region of interest analysis was performed on the Ang II activity, infarction scar (^{201}Tl defect) and areas at risk (defect on ^{99m}Tc-tetrofosmin but not on ^{201}Tl).

After the administration of ^{11}C-KR31173 (37MBq), the animals were sacrificed prior to autoradiography or kept alive for *in vivo* PET studies. The administration of ^{11}C-KR31173 was also made in the presence of a clinically approved angiotensin-converting enzyme (ACE-I) inhibitor – enalapril and of an angiotensin receptor blocker – valsartan.

The *in vivo* PET scan was performed after injection of ^{11}C-KR31173 in study groups and the control group, followed by injection of ^{13}N-ammonia, for visualizing the myocardium and hypoperfused area.

The results demonstrated that binding of ^{125}I-SI was increased at 1 and 3 weeks in the infarcted territories, well matched with the infarction area as demonstrated by the ^{201}Tl studies. The areas at risk, identified by ^{99m}Tc-tetrofosmin, showed no increase in the ^{125}I-SI uptake.

The same uptake pattern was demonstrated by ^{11}C-KR31173, both in autoradiography studies and in the *in vivo* PET studies, with a maximum at 3 weeks after the MI.

Administration of valsartan (angiotensin receptor blocker) partially (51%) blocked the ^{11}C-KR31173 uptake, while enalapril (ACE-I) had no effect on the said parameter.

The results demonstrated a clear increase in the expression of the AT_1R in the infarcted areas, which seems to be responsible for the increased fibrosis and remodeling of the cardiac wall and the hemodynamic

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changes that lead to heart failure. It is known that activated myofibroblasts, which are the first cells to react after a MI, highly express AT_1R and synthesize large amounts of collagen, thus contributing to the localized fibrosis.

The present study also demonstrated that blockage of the AT_1R, with its specific blocker valsartan, reduced the binding of the specific ligand, unlike the inhibition of Ang II synthesis with enalapril, which had no effect on ^{11}C-KR31173 coupling.

In summary, this was a very elegant and well-designed study, which was able to obtain several objectives:

- Finally and definitively demonstrated localized increase of AT_1R in infarcted areas of the myocardium;
- Demonstrated the potential beneficial effect of angiotensin receptor blockers upon myocardial fibrosis and remodeling;
- Validated a new PET-tracer – ^{11}C-KR31173 for evaluating the infarcted area and its evolution;
- Introduced the notion that imaging might be helpful in the optimization of post-MI anti-RAS medication.

Evaluation of stem cells administration in infarction foci using dual imaging techniques

Evaluation of: Zhang H, Qiao H, Bakken A *et al.*: Utility of dual-modality bioluminescence and MRI in monitoring stem cell survival and impact on post myocardial infarct remodeling. *Acad. Radiol.* 18(1), 3–12 (2011).

Over the last decade, the use of stem cells in the regeneration of postmyocardial infarction scar tissue and regeneration of the cardiac muscle has been considered to be one of the best approaches towards true regenerative therapy of the heart. Unfortunately, peripheral or local administration of stem cells did not produce the expected results in laboratory animals and the evolution of those cells has been particularly difficult. It was practically impossible to quantify the degree of delivery of the stem cells to the myocardium, their fixation rate in the tissue and the long-term effects. The present work uses a very sophisticated dual modality monitoring of the stem cells, using both bioluminescence and MRI.

The cells can be tagged with superparamagnetic iron-oxide (SPIO) beads that allow their visualization using ECG-gated MRI. However, this method does not allow for tracking of the survival of the cells or their evolution in time. Thus, the authors decided to label the stem cells using a reporter gene, the firefly luciferase (*Fluc*), using the bioluminescence imaging (BLI) technique. This technique is highly sensitive; however, tomographic localization is impossible. By coupling the two techniques the stem cells can be followed both in time and localization, for periods long enough to demonstrate, or not, their efficiency in treating postmyocardial infarct remodeling.

The murine embryonic stem cells (ESCs) were tagged with *Fluc* using techniques of molecular biology, followed by incubation with labeling media containing 50 µg of iron per ml. Unfortunately, the tagging process with the Fluc enzyme made the ESC immunogenic and were rejected in immunocompetent mice. This problem was resolved by using athymic nude rats, which were not able to reject the tagged ESC.

The adult athymic rats were subjected to surgical procedures (left anterior descending coronary artery ligation for 45 min followed by reperfusion) that induced a myocardial infarction and the subsequent reperfusion injuries. After surgery, the labeled ESCs were injected directly into the infarcted area. After several weeks, imaging studies were carried out. The BLI studies were conducted by injecting intravenously a dose of luciferin (substrate for luciferase) and acquiring a series of bioluminescent images. The MRI studies were performed using specific MRI protocols for ECG-gating, assessment of left ventricular global function, fractional shortening and SPIO-associated hypoenhancement. After imaging, the animals were euthanized and sections were taken from the hearts, for visualizing SPIO particles with Prussian Blue staining and grafted cells with Fluc staining.

The results of this extremely complex series of experiments demonstrated on one hand that both MRI and BLI are necessary to confirm the intramyocardial delivery and the survival of the stem cells, results confirmed by histology and the fact that the administration of ESC-induced changes in the study groups compared with control group. The group treated with ESCs were separated into two groups, one that received and accepted the myocardial delivery of ESCs (engraftment subgroup) and another that did not accept the ESCs (nongraft subgroup).

The ESC injection produced some functional improvement, mainly in reducing the stiffness of the infarcted region; however, as the study group was too small, no statistically significant outcomes could be identified. The study itself was oriented towards the demonstration of the imaging techniques and not towards evaluating the effects of intracardiac delivery of the ESCs. This task has been elegantly and efficiently

resolved, demonstrating that dual modality imaging is necessary to confirm delivery and survival status of the cells. The technique demonstrated a much higher sensitivity than other imaging techniques, as the BLI was able to detect 0.3 million ESCs in the first day, while the PET signal of 5 million cells was not detectable at the same time. Most of the ESCs (60%) died shortly after injection due to inflammatory response. As the dead cells (together with the SPIO) were absorbed by macrophages, these remained at the site of the injury for weeks.

One of the seven animals from the engrafted group developed teratoma, thus raising safety issues even at very small doses (0.3 million cells).

This article is an illustrative study that demonstrates how the vision of fundamental scientists may improve the outcome of clinical approaches.