CT imaging of myocardial infarction in mice using a novel iodine-based contrast agent

S. Sawall[1], A. Kraupner[2], D. Franke[2], J. Kuntz[1], A. Kirchherr[2], J. Maier[1], A. Briel[2], and M. Kachelrieß[1]

[1]Medical Physics in Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[2]nanoPET Pharma GmbH, Berlin, Germany

Correspondence to: stefan.sawall@dkfz.de

GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

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Introduction

Computed tomographic (CT) cardiac imaging of small rodents is a challenging task due to the low contrast between blood and the myocardium as well as the rapid heart and respiratory rates of up to 600 beats per minute and 300 respirations per minute, respectively. Recent advances in contrast media research indicated that iodine-based blood pool agents might accumulate in the myocardium after a prolonged blood circulation time and allow for the visualization of pathophysiological processes [1]. We herein evaluate a novel blood pool agent in a mouse model of myocardial infarction including a correlation of the findings from CT to histological stains.

Materials and Methods

The used polymeric nanoparticular system NPG 4006 was prepared using an interfacial deposition method of a polymer, followed by solvent displacement [2]. The iodine-based contrast moiety was incorporated in the polymeric system and colloidal stability was achieved by steric stabilization. The osmolality and pH value of the formulation were adjusted to physiological conditions and the imaging agent was thereafter sterilized. The nanostructures were found to have a mean hydrodynamic diameter of approx. 250 nm and a high iodine content of 210 mg/mL (determined by *in vitro* phantom measurements). The polymeric nanoparticular system shows long-term colloidal stability in aqueous media for several months.

Five BALB/c nu mice were inflicted with myocardial infarctions by a ligation of the left anterior descending artery (LAD). In particular, the thorax of the animals was carefully opened after deep narcosis was verified by a lack of pupil and paw reflexes. The LAD was exposed and ligated using a single Prolene suture. The thorax was closed afterwards. All animals were administered with a dose of 125 µL of the novel blood pool contrast agent NPG 4006 approx. two weeks after the operation.

The animals were imaged repetitively over 300 min using a flat detector-based computed tomography system (VolumeCT, Siemens Healthcare, Forchheim, Germany, see figure 1) to evaluate the kinetics of the blood pool contrast agent by measuring the time-dependent enhancement in selected regions of interest (see figure 2). All image reconstructions were performed using a low-dose phase-correlated reconstruction method, i.e. all reconstructions show a distinct cardiac and respiratory state of the animal to prevent a degradation of image quality due to motion artifacts [4]. In particular all reconstructions show an exhale respiratory state and a diastolic cardiac state while the reconstructions incorporate 10% of the data in the correspondent motion cycle (see figure 3). To allow for repetitive image acquisitions the scan protocol was optimized to minimize radiation dose. Each of the measurements within the 300 min post injection comprises a radiation dose of 50 mGy accumulating to a total dose of 500 mGy. After 300 min the animals were sacrificed, hearts were excised and fixated in paraffin. Slices with a thickness of 10 µm were cut and underwent histological preparation using Masson's Trichrome staining [5]. Based on the reconstructed CT images, the infarct volumes were measured using a manual segmentation. Infarct sizes in the corresponding histological slices were measured by an automatic segmentation method after all slices corresponding to a single heart were photographed using a high resolution system, stitched and registered (see figure 4).

VolumeCT (VCT)

- VolumeCT (Siemens Healthcare, Forchheim, Germany)
- X-ray source:
  - Focal spot size: 400 µm × 400 µm
  - Tube voltage range: 80 kV – 140 kV
  - Tube current range: 10 mA – 50 mA
- Detector:
  - Varian flat detector
  - 1024×768 pixel (2×2 binning)
  - 1024×192 @ 100 fps
  - 388 µm pixel size
  - Spatial sampling: 238 µm
  - 10 ms integration time
- Protocol:
  - Scan time: 20 s
  - Rotation speed: 18 °/s
  - Number of projections: 2000
  - Estimated dose: 50 mGy

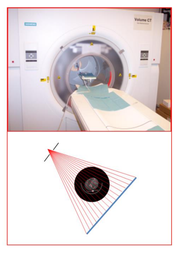

Figure 1: Specification of the used imaging system and scan protocol.

Contrast Enhancement

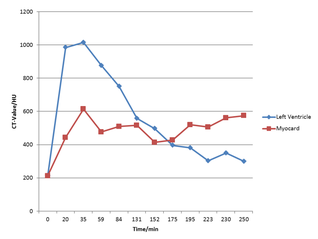

Figure 2: Contrast enhancement after injection of NPG 4006 in one of the mice under investigation.

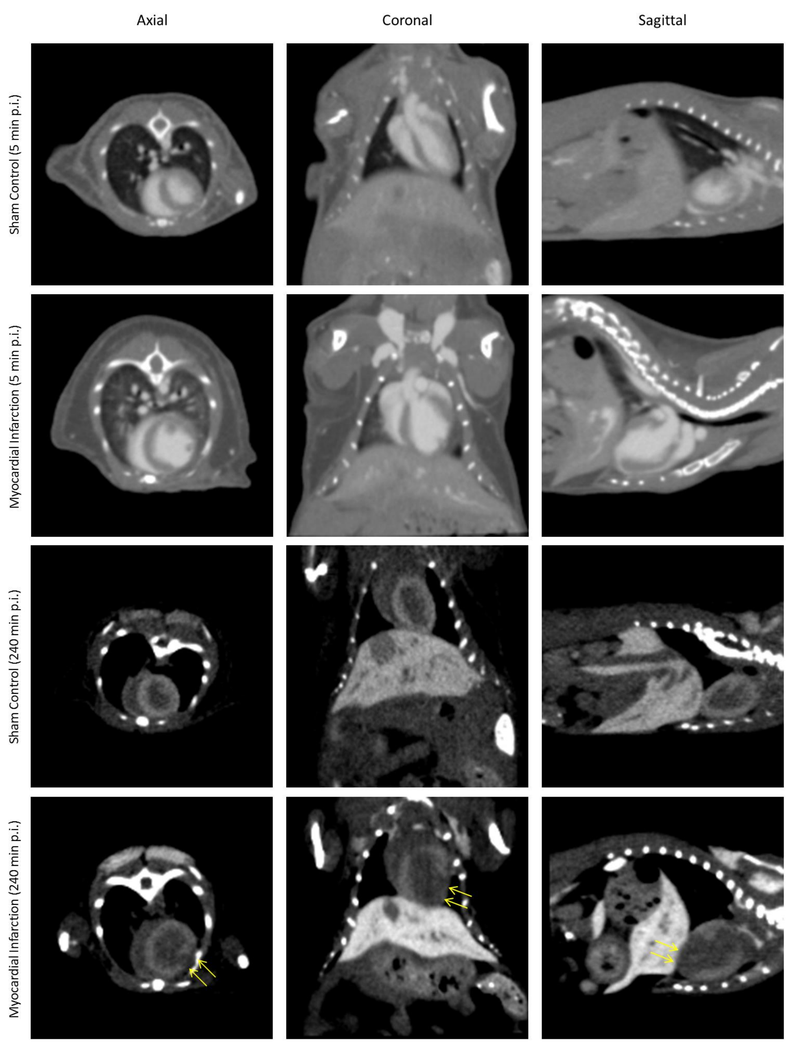

Figure 3: Comparison of the micro-CT reconstructions obtained about 5 min (first and second row) and 240 min (third and forth row) post injection (p.i.) of NPG 4006 in the sham group (first and third row) and in a mouse with myocardial infarction (second and fourth row). Note the enhancement in the healthy myocardium and a lack of enhancement in the infarcted region (yellow arrows). C/W=500 HU/850 HU.

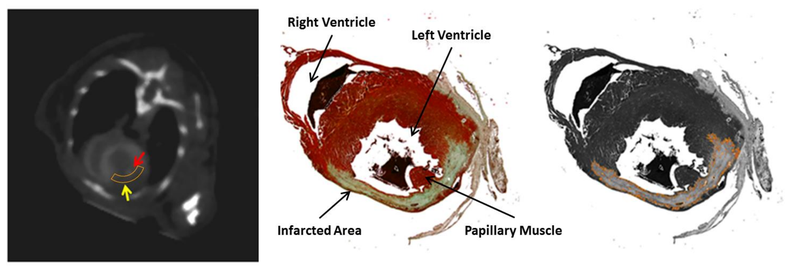

Figure 4: Correlation of the micro-CT reconstruction (left, C/W=500 HU/850 HU) to the histological stains (middle, right). The segmented area of myocardial infarction is indicated in orange. Note the papillary muscle (red arrow) whose viability is indicated in the CT reconstruction by a contrast enhancement and which is verified in the histological stains.

Results

Histology vs. micro-CT

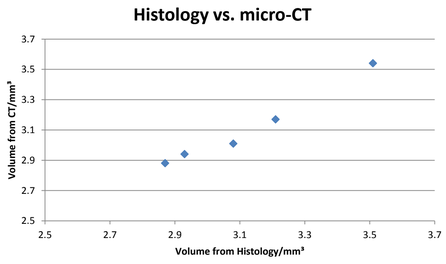

Figure 5: Correlation of the infarcted regions measured from micro-CT and histological slices.

Summary

- NPG 4006 is a novel blood pool agent with an iodine content of 210 mg/mL.
- The blood pool contrast enhancement of an injected volume of 125 µL is about 800 HU for a 25 g mouse.
- The blood pool agent is well tolerated and accumulates in the myocardium and allows for the identification of infarcted regions.
- This renders NPG 4006 the optimal choice for longitudinal studies of cardiac remodelling and other processes.

Results

Blood pool peak enhancement was observed immediately after injection of the contrast agent NPG 4006 with an enhancement of about 800 HU compared to the baseline and a blood half-life of about 120 min (cf. figure 2). The peak enhancement in the myocardium was observed after about 240 min showing an increase in CT values of 400 HU. A qualitative comparison of CT reconstructions and histological staining indicates that the blood pool agent is homogenously distributed in the healthy myocardium (cf. figure 3). Furthermore, a noteworthy enhancement of brown adipose tissue in the neck region was observed. The quantitative comparison of infarct volumes obtained from CT reconstructions and histology are highly correlated (R^2=0.98).

Conclusion

The high contrast enhancement caused by the high iodine content allows for the application of sophisticated image reconstruction method allowing for a reduction in radiation dose and hence allowing for longitudinal studies.

The comparison of infarcted areas obtained from CT scans and histology indicate that the severity of myocardial infarction can be accurately quantified *in vivo* using CT acquisitions after administration of the novel blood pool agent NPG 4006. This allows for the investigation of a variety of processes in longitudinal studies such as cardiac remodeling.

References

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