

Improved detection of pancreatic islets in vivo using double contrast

Vít Herynek^{a,b,*}, Zuzana Berková^{b,c}, Eva Dovolilová^{b,c}, Daniel Jiráček^{a,b}, Jan Kříž^c, Peter Girman^{b,c}, František Saudek^{b,c} and Milan Hájek^{a,b}

The transplantation of pancreatic islets containing β -cells, which produce insulin, is an alternative approach to the treatment of type 1 diabetes mellitus. The non-invasive visualization of transplanted islets can be performed using MRI; however, this requires labeling of the islets with a suitable contrast agent prior to transplantation. The detection of islets labeled by iron oxide-based contrast agents and transplanted into the liver tissue can be significantly improved using the intravenous administration of a suitable gadolinium contrast agent prior to MRI. The applied contrast agent not only improves the contrast-to-noise ratio, but also eliminates artifacts that may lead to an overestimation of the number of hypointense spots and their area; thus it improves the accuracy of automated and semi-automated procedures used for transplanted islet segmentation and quantification. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: pancreatic islets; transplantation; cellular labeling; contrast enhanced MRI; double contrast

1. INTRODUCTION

The transplantation of pancreatic islets is an alternative approach to the treatment of type 1 diabetes mellitus (1). This method has been successfully tested on animal models and recently has been introduced in pilot clinical experiments (2). Most frequently, isolated and purified islets are infused via a transcatheter into the portal vein and are carried by blood flow into the hepatic sinusoids (3). Transplanted islets can be visualized using magnetic resonance imaging. However, this method requires the *in vitro* labeling of islets by a suitable contrast agent in order to distinguish the islets from the surrounding tissue following transplantation. Suitable contrast agents for this purpose are superparamagnetic contrast agents based on iron-oxide nanoparticles with a suitable non-toxic coating. The first reports of imaging cell transplants labeled by superparamagnetic contrast agents appeared in 1992 (4). To date, a number of iron-oxide based contrast agents have been developed, and these compounds are commercially available. Moreover, some of them are approved for clinical use. The commercially available contrast agents Resovist[®] (Schering) and Feridex[®] (Berlex) have also been successfully used for labeling pancreatic islets (5–7). Both contrast agents have a suitable size of the nanoparticles; additionally, a carboxylated dextran coating in the case of Resovist (8) eliminates the necessity of an additional transfection agent, such as poly-L-lysine (9).

Superparamagnetic contrast agents provide 'negative' contrast, i.e. labeled islets are represented by hypointense areas on T_2 -weighted or T_2^* -weighted images. The T_2^* effect is so strong that it may cause signal voids even on images acquired by T_1 -weighted gradient echo sequences. Although the resolution of an MR image is usually not sufficient to distinguish single islets, the strong T_2^* effect has an impact on the broader vicinity of the labeled islet, thus enabling the detection of labeled objects much smaller than an image voxel. However, it is sometimes not easy to determine if signal voids represent transplanted labeled islets

or not because of low contrast between the liver tissue and the labeled islets.

The aim of our study was to enhance the contrast between transplanted islets and the liver tissue by the administration of a suitable gadolinium-based contrast agent prior to measurement. The application of a T_1 -contrast agent might improve the contrast-to-noise ratio in the liver and thus enable the automated evaluation of MR images and islet counting. We used in the experiments reported here the contrast agent Multi-Hance[®] (Bracco Diagnostics, Italy), i.e. Gd-BOPTA. The contrast agent is dedicated for CNS and liver imaging and is also approved for clinical practice. Gd-BOPTA provides a highly selective increase of the liver signal-to-noise ratio in rats due to a higher rate of biliary excretion (10).

2. RESULTS AND DISCUSSION

Isolated pancreatic islets from adult Lewis rats were labeled by the contrast agent Resovist[®] (Schering, Czech Republic) and injected through a portal vein into the liver of both control rats

* Correspondence to: V. Herynek, MR-Unit, Department of Diagnostic and Interventional Radiology, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, Prague 14021, Czech Republic.
E-mail: vit.herynek@medicon.cz

a V. Herynek, D. Jiráček, M. Hájek
MR-Unit, Department of Diagnostic and Interventional Radiology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

b V. Herynek, Z. Berková, E. Dovolilová, D. Jiráček, P. Girman, F. Saudek, M. Hájek
Center for Cell Therapy and Tissue Repair, 2nd Medical Faculty, Charles University, Prague, Czech Republic

c Z. Berková, E. Dovolilová, J. Kříž, P. Girman, F. Saudek
Pancreatic Islet Laboratory, Diabetes Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

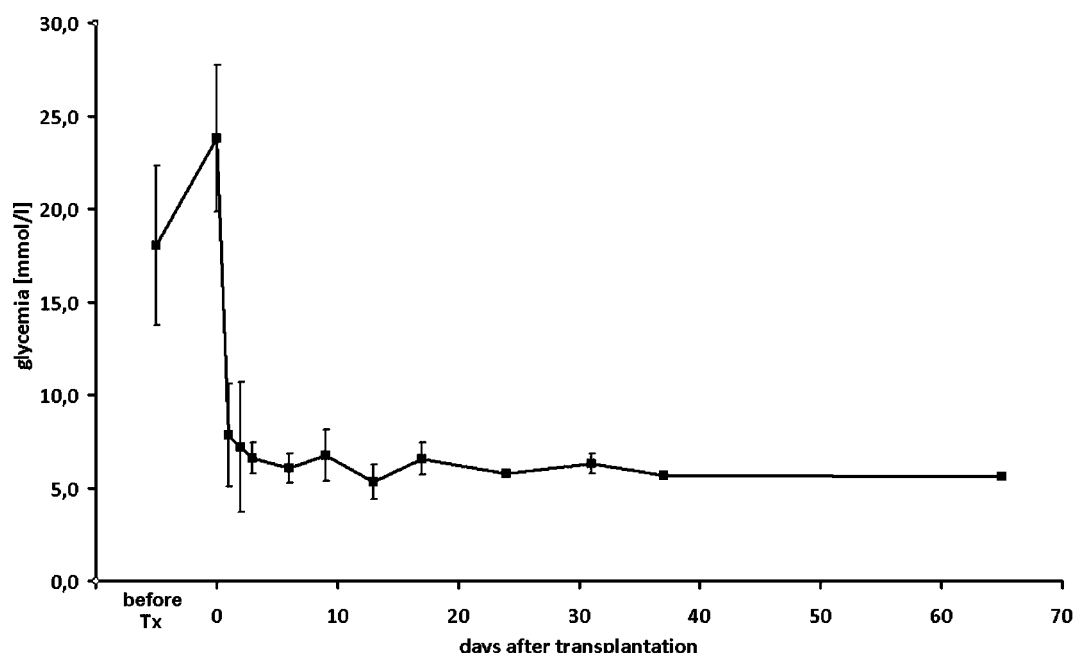


Figure 1. Normalizing of glycemia levels in diabetic rats after islet transplantation during the experiment.

and rats with streptozotocin-induced diabetes. All animals were transplanted successfully, blood glucose levels decreased in animals with induced diabetes after transplantation and normal blood glucose levels were reached 3 days after islet transplantation, which is in accordance with published data (5,11). As the normoglycemia was maintained throughout the whole 3 month experiment (see Fig. 1), we conclude that

the application of MultiHance[®] during MR imaging probably has no significant long-term impact on the function of transplanted pancreatic islets. This finding is also supported by the intravenous glucose tolerance test, which was performed 2, 4, 8 and 12 weeks after transplantation (see Fig. 2).

Pilot measurements showed that the liver was evenly perfused by the gadolinium contrast agent within approximately 30 min.

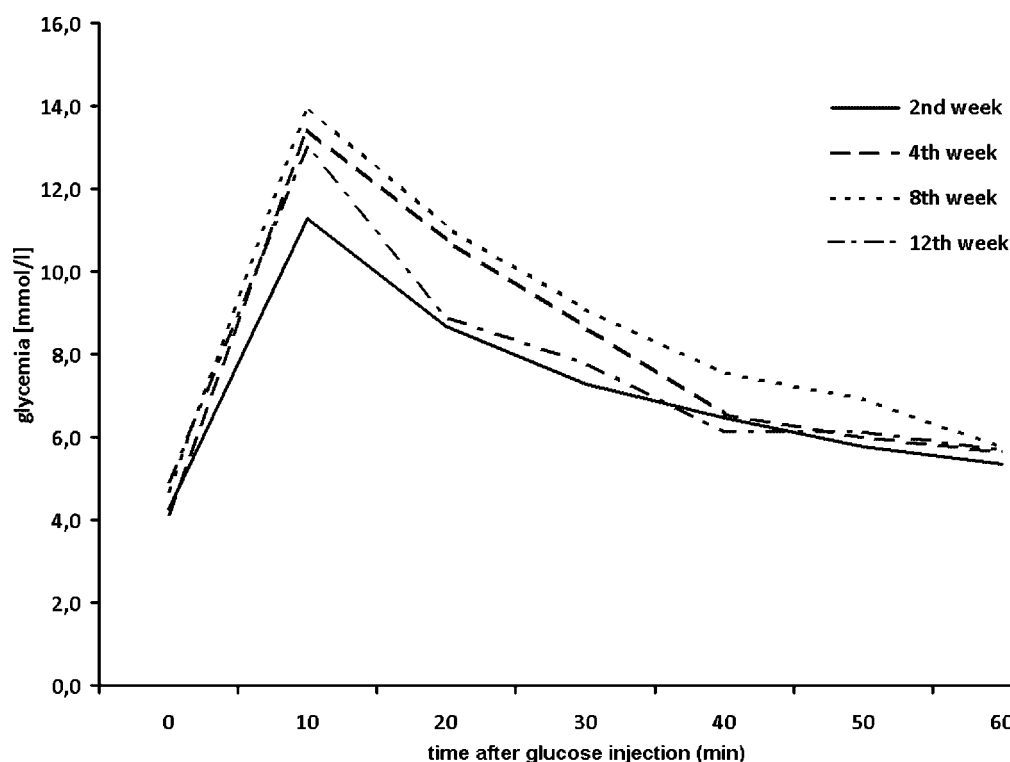


Figure 2. Intravenous glucose tolerance test shows the early response to intravenous glucose load (solid line, second week; dashed line, fourth week; dotted line, eighth week; dotted-and-dashed line, twelfth week).

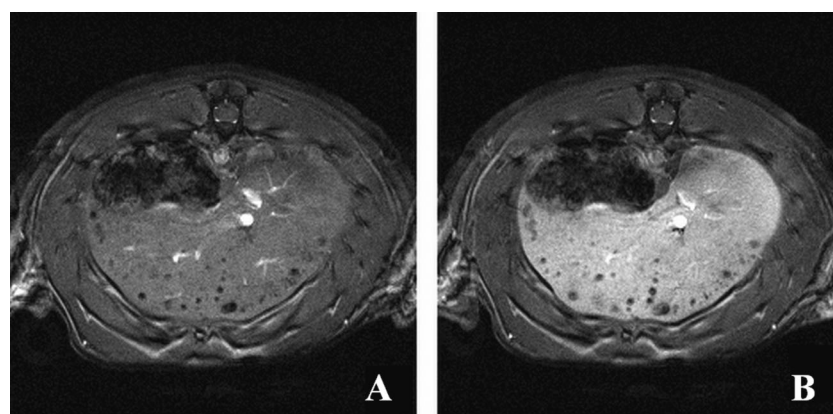


Figure 3. Native (A) and post-contrast (B) MR images of a rat liver in identical slices with transplanted labeled pancreatic islets.

A signal decrease caused by the excretion of the contrast agent was observable approximately 90 min post-injection. Therefore, there was a fairly long time window for image acquisition, and for further evaluation we chose images obtained 30–60 min post-injection. A comparison of native and post-contrast MR images of a rat liver with transplanted pancreatic islets can be seen in Fig. 3.

Superparamagnetic nanoparticles have a strong T_2^* effect and a negligible T_1 effect, therefore they ensure a hypointense signal in the vicinity of the islets even on strongly T_1 -weighted gradient echo images. The strong T_2^* effect of the SPIO nanoparticles is not affected by the administration of a Gd-based contrast agent. However, the application of a Gd-based contrast agent increases the signal in the liver tissue, thus increasing the contrast-to-noise ratio (CNR) between the liver tissue (hyperintense signal) and the labeled islets (hypointense signal). To the eye, the images obtained after the application of the gadolinium-based T_1 contrast agent appear to be considerably better, which corresponds to an improvement in CNR. The average CNR before the application of MultiHance[®] was 24 ± 12 , while the CNR after contrast agent application reached 34 ± 14 .

MR images were processed both semi-automatically (with manual image thresholding) and fully automatically. Fully automated image processing excludes any intervention of the evaluator (11). Both methods evaluated the number of

hypointense spots and their total area in the liver in each animal. The average numbers of hypointense spots and their area detected in the liver by both methods within a time span of 77 days after transplantation are summarized in Tables 1 and 2.

Induced diabetes had no significant effect on the detection of islets. The differences between the numbers and areas of the hypointense spots in normal and diabetic animals either before or after negative contrast agent administration are not statistically significant (see Fig. 4).

Both semi-automated and automated methods detected a lower number of hypointense spots and a smaller area after contrast agent administration than in native images ($p < 0.05$, paired t -test).

The two evaluation methods did not statistically differ in the number of detected hypointense spots, either before or after contrast agent administration. In contrast, the methods did differ in their evaluation of the area of the hypointense spots. The semi-automated method detected a significantly larger total area of hypointense spots than the automated one (paired t -test, $p < 0.05$), both on native images and post-contrast ones.

The substantial decrease in the number of spots during the first week is caused by the fact that a large number of the transplanted islets die within the first week (11). We hypothesize that the dead islets and the free or released iron particles are washed out within this week and that the released iron is

Table 1. Average values (and standard deviations) of the numbers of detected hypointense spots in MR images of the rat liver after Langerhans islet transplantation (sum of all spots in all slices).

Days after transplant	Semi-automated		Automated	
	Before contrast agent application	After contrast agent application	Before contrast agent application	After contrast agent application
1	270 \pm 89	213 \pm 83	407 \pm 228	270 \pm 89
7	227 \pm 100	171 \pm 96	221 \pm 107	227 \pm 100
14	204 \pm 109	162 \pm 101	239 \pm 152	204 \pm 109
21	171 \pm 103	129 \pm 83	175 \pm 85	171 \pm 103
35	105 \pm 23	80 \pm 15	127 \pm 30	105 \pm 23
49	97 \pm 27	71 \pm 19	103 \pm 18	97 \pm 27
63	117 \pm 24	78 \pm 11	96 \pm 10	117 \pm 24
77	118 \pm 14	87 \pm 11	99 \pm 27	118 \pm 14

Table 2. Average values (and standard deviations) of the area (in mm²) of detected hypointense spots in MR images of the rat liver after Langerhans islet transplantation (sum of the areas in all slices)

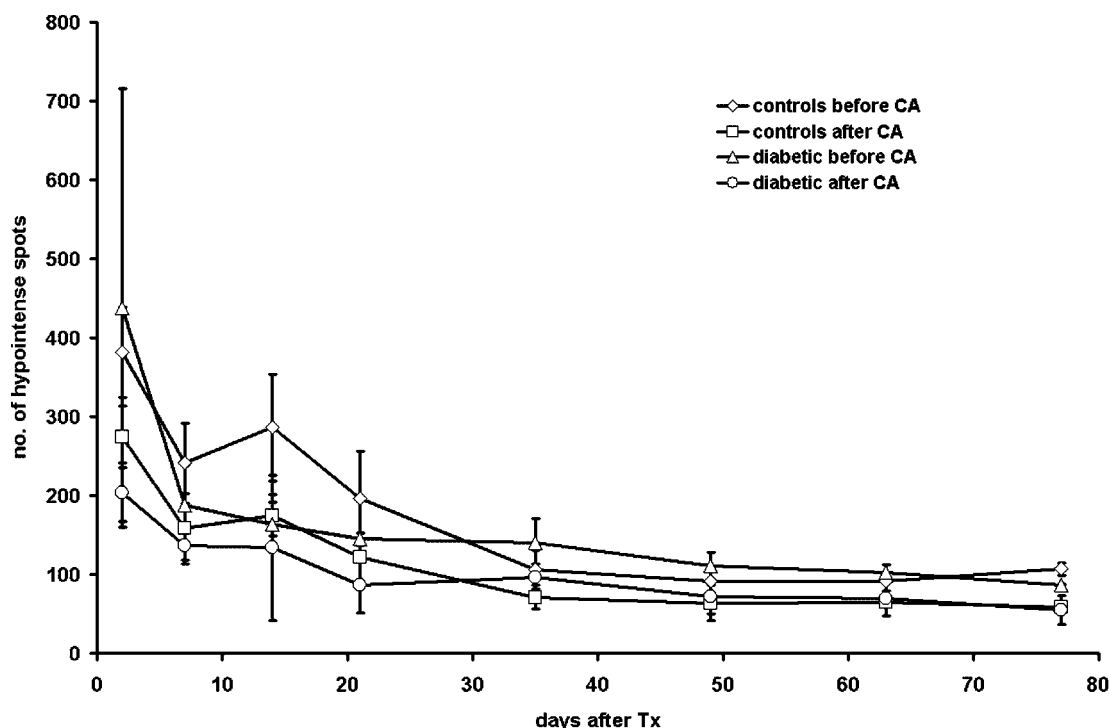
Days after transplant	Semi-automated		Automated	
	Area (mm ²) before contrast agent administration	Area (mm ²) after contrast agent administration	Area (mm ²) before contrast agent administration	Area (mm ²) after contrast agent administration
1	259 ± 117	186 ± 84	97 ± 59	66 ± 40
7	280 ± 119	177 ± 116	58 ± 34	39 ± 27
14	215 ± 121	163 ± 85	57 ± 37	37 ± 26
21	167 ± 120	118 ± 93	42 ± 25	26 ± 19
35	105 ± 58	67 ± 26	29 ± 6	20 ± 6
49	73 ± 49	51 ± 30	26 ± 8	17 ± 8
63	69 ± 30	46 ± 15	20 ± 5	14 ± 3
77	103 ± 33	63 ± 30	22 ± 9	14 ± 4

eliminated normally through the liver. It has already been proven that a free iron-based contrast agent is washed out from the liver tissue within one week (5).

A brighter background and higher CNR alone does not necessarily ensure better results in terms of islet detection. The method of double contrast apparently enables better contrast between the signal of the transplanted islets and the liver tissue background. Nevertheless, the contrast between the hypointense signal caused by the labeled islets and the native liver tissue is usually sufficient. If we compare automated or semi-automated evaluation of images without apparent artifacts, we find no substantial differences in the number of detected particles. However, once some hypointense artifacts

(such as partial volume effect, flowing blood, etc., see Fig. 5) occur in the image, they may be considered as false positive signals, and therefore both image processing methods overestimate both the number and the area of hypointense spots. In this case, positive contrast usually suppresses these artifacts, thus eliminating false positive signals, and the lower detected number of hypointense spots (and smaller area) better corresponds to the real number of hypointense spots caused by the transplanted islets.

The mask size and shape used in the case of fully automatic image processing also eliminates too large and too small objects, which may cause an overestimation of the particle area when a manual threshold is used during semi-automated analysis (11).

**Figure 4.** Time dependence of the number of detected hypointense spots evaluated by the automated procedure in diabetic (△, before; ○, after contrast agent administration) and control rats (◇, before; □, after contrast agent administration). CA, contrast agent; Tx, transplantation.

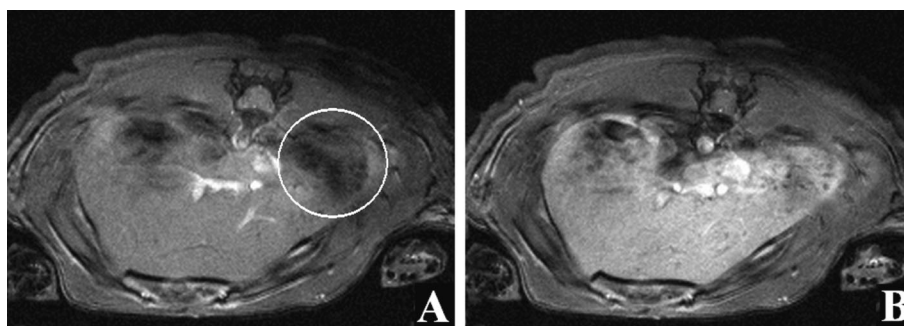


Figure 5. Native (A) and post-contrast (B) MR images of a rat liver with transplanted labeled pancreatic islets. A partial volume effect clearly visible in one liver lobe (in the white circle) makes it impossible to distinguish the pancreatic islets on the native image (A). The artifact is suppressed after contrast agent application (B).

The semi-automated evaluation method also adjusts the threshold in the image globally and cannot eliminate variations in intensity within the image slice caused by uneven transmission or detection of the MR signal. This also results in a greater number of false positive signals when a global threshold is applied. This leads not only to a higher number of spots, but also to the merging of adjacent but separate spots, therefore we did not detect an increased number of spots, but only an increased area of the hypointense regions.

The number of detected hypointense spots on MR images was still low compared with the total number of injected islets. This is caused by several factors: some islets could be destroyed immediately after transplantation, the islets may seed together in clusters and imaged together as a single islet spot, and finally, not all islets possess a sufficient amount of iron and therefore cannot be detected by MR. Quantification could be also affected by the varying size of the islets.

Despite the blooming effect of the superparamagnetic label, which causes the hypointense spots to be much larger than the actual size of the labeled islets, we speculate that the evaluation of the hypointense area may better reflect the dynamics of the intracellularly located iron particles in the islets than the number of hypointense spots. Furthermore, we cannot be sure if an observed hypointense spot represents one or more islets. The total area of the hypointense spots decreased faster than their number, and this could be caused by continuous iron release even from fully functional living islets. Also, we should note that neither of the above-mentioned evaluation procedures can correctly handle the situation in which larger clusters of labeled islets cause hypointense spots on adjacent image slices.

Long-term induced normoglycemia in diabetic animals confirmed that the labeled transplanted islets were functional and viable. Their ability to maintain blood glucose levels in diabetic animals (see Fig. 1) and the early response to increased intravenous glucose load (intravenous glucose tolerance test, see Fig. 2) proved that labeling the islets with Resovist had no adverse effect on their function (5–7) even after repetitive exposure to the gadolinium-based contrast agent Multihance®.

3. CONCLUSIONS

Our results demonstrate that the *in vivo* detection of pancreatic islets labeled by iron oxide nanoparticles can be substantially improved by the application of a suitable T_1 contrast agent prior

to MR imaging, thereby increasing the contrast-to-noise ratio, especially in areas affected by the partial volume effect.

As normal glucose levels were reached within 1 week and were maintained throughout the whole 3-month experiment, we conclude that the application of MultiHance® during MR imaging probably has no long-term impact on the function of transplanted pancreatic islets. The automated procedure for detecting and counting the particles (labeled islets) provides satisfactory and reliable results that are not dependent on the evaluator.

4. EXPERIMENTAL

4.1. Pancreatic islet isolation and labeling

Rat pancreatic islets were isolated according to a standard protocol (12) from adult Lewis rats. The isolated pancreatic islets were cultured in CMRL-1066 medium (37 °C, 5% atm CO₂; Sigma) containing the contrast agent Resovist® (Schering) for 24 h. Resovist® concentration in the medium was 5 µL susp./ml media, which corresponds to a concentration of 140 µg Fe/ml. Subsequently, the islets were removed from the tissue culture medium, centrifuged and washed in HBSS solution (Sigma, St Louis, MO, USA). The size of the implanted islets varied from 150 to 300 µm.

4.2. Diabetes induction

Diabetes was induced in seven animals (Lewis rats) by the intravenous administration of streptozotocin (50 mg/kg; Sigma, USA) and confirmed by blood glucose levels > 18 mmol/l on three consecutive days.

4.3. Islet transplantation

Labeled islet isografts (2000) suspended in 0.5 ml HBSS were injected through a portal vein into the liver of 15 Lewis rats – eight control animals and seven animals with induced diabetes.

4.4. Viability and function tests

Blood glucose levels were monitored before transplantation, on the day of transplantation, and 1, 2, 3, 6, 9, 13, 17, 24, 31, 37 and 65 days after transplantation. Intravenous glucose tolerance tests were performed 2, 4, 8 and 12 weeks after transplantation.

4.5. MR imaging

All MR measurements were performed using a 4.7 T Bruker spectrometer equipped with a standard resonator coil. The rats were anesthetized by passive inhalation of 1.5–2% isoflurane in air. Breathing was monitored during the measurements. The rats were placed on a heated pad and examined in a prone position.

Each MRI session consisted of native image acquisition, then the contrast agent was applied through a tail vein [0.02 ml of gadobenate dimeglumine, Gd-BOPTA, 0.5 M; Multi-Hance[®] (Bracco, Germany) per 100 g body weight]. A standard recommended dosage of the contrast agent was used; the concentration was not optimized separately for this experiment. Post-contrast images were acquired several times within approximately 1 h after contrast agent application. A standard gradient echo sequence ($TE = 3.4$ ms, $TR = 80$ ms, matrix 256×256 , $FOV = 6$ cm, slice thickness = 2 mm) was used for both native and post-contrast imaging. A set of 15 axial slices covering the whole liver was acquired. The whole MRI session took up to 90 min. Each animal was subjected to eight MRI sessions – 1 day after transplantation, then 1, 2, 3, 5, 7, 9 and 11 weeks after transplantation.

4.6. Image processing

ImageJ software (Wayne Rasband, NIH, USA) was used for semi-automated image analysis and islet counting. Semi-automated analysis consisted of hand selecting the liver area on each image, manual thresholding and automated evaluation of the number and area of hypointense spots representing the transplanted labeled pancreatic islets. The evaluator's basic criterion for setting the proper manual threshold was to set the threshold as low as possible without omitting any hypointense spots apparently representing a labeled islet.

Automated image analysis was performed using a homemade software written in Matlab (The Mathworks Inc, USA), which detects hypointense regions representing pancreatic islets independently of the user. First, the software enhanced the contrast between the hypointense regions and the liver tissue. This was based on morphological top-hat and bottom-hat transformations. Then the software set a threshold for islet detection based on a histogram shape. After manual selection of the ROI (circumventing the liver on each image), the area of hypointense spots and their number were determined using ImageJ. For a detailed description of the automated image processing, see Jirak *et al.* (11).

Both image analysis methods were applied to all slices containing liver tissue. The CNR was evaluated in four randomly selected animals before and after the application of Multi-

Hance[®]. All protocols were approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine, and the experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Acknowledgements

The study was supported by grants GACR 203/09/1242, the Czech Science Foundation; MZ0IKEM2005, the Ministry of Health of the Czech Republic; 1M0538, the Ministry of Education, Youth and Sports of the Czech Republic; ENCITE-European Commission Research - 7th Framework Programme No. 201842.

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