Macrophage infiltration into grafts detected non-invasively by MRI: An early marker of allograft chronic rejection in a rat model of kidney transplantation.

N. Beckmann[1], C. Cannet[2], S. Zurbruegg[1], R. Haberthuer[2], N. Stohler[2], C. Pally[2], C. Bruns[2]

[1]Discovery Technologies, Novartis Institutes for BioMedical Research, Basel, Switzerland, [2]Transplantation Research, Novartis Institutes for BioMedical Research, Basel, Switzerland

Introduction:

The non-invasive detection by MRI of macrophage infiltration into kidney grafts in a life-supporting transplantation model, in which Lewis rats received kidneys from Fisher 344 donors (1), is feasible by administering i.v. superparamagnetic particles of iron oxide, SPIO (2). Here we compared the cortical signal of grafts in MR images, acquired 24 h after administering SPIO, to biochemical parameters assessed in the urine and blood at the same time points when MRI measurements were carried out. The aim was to determine which parameter, either the MRI signal intensity or biochemical parameters, could be used to non-invasively detect early signs of chronic allograft rejection in this model. The Banff scores of chronic rejection (3) assessed histologically served as standard for comparisons.

Methods:

Transplantation: Kidneys from male Fisher 344 rats were transplanted into male Lewis rats. Details are provided in (2). Kidneys were preserved for 24h in cold (4ºC) UW prior to transplantation. The grafts were not perfused *ex vivo*. The grafts had life-supporting function, i.e., contralateral nephrectomy was carried out at transplantation time.

MRI: Rats were anaesthetized with forene (1.5-2.0%) in a mixture of O_2/N_2O (1:2), administered via a face mask. Measurements were carried out with a Bruker Biospec 47/40 system. A gradient-echo sequence (TR = 16.8 ms; TE = 8.4 ms; FOV = 6x6 cm^2; matrix = 256x128; slice = 1.5 mm; 20 averages), without respiratory gating, was used throughout the study. SPIO (Endorem, Guerbet, France) was administered i.v. 24 h before image acquisition at three doses: 0.3 ml/kg (n=5 recipients), 1 ml/kg (n=6 recipients) or 3 ml/kg (n=6 recipients).

Biochemical parameters: blood: creatine, urea, platelets, lymphocytes, neutrophils, monocytes; urine: creatine, protein, glomerular filtration rate.

Histology: Carried out for assessing the Banff scores (Verhoeff staining) and the iron amount (Perls' Prussian blue reaction).

Results and Discussion:

A cortical signal attenuation dependent on the SPIO dose was observed in the grafts, as shown in fig. 1 (left) for three recipients. Similarly, a dose-dependent attenuation was also seen in native kidneys from control Lewis rats (data not shown). However, the signal reduction induced by SPIO in native kidneys was invariable, for three administrations of the contrast agent (once every 4 weeks). At each time point, the cortical signal in a graft was reported to the mean cortical signal intensity in native kidneys for the corresponding SPIO dose. The resulting relative cortical signal in the grafts decreased significantly between 8 and 16 weeks after transplantation (fig. 1, right). By contrast, the only biochemical parameter that changed significantly over an extensive period of 32 weeks following transplantation was protein in the urine (starting 16-22 weeks post-transplant). Proteinuria is a characteristic of the present model (1).

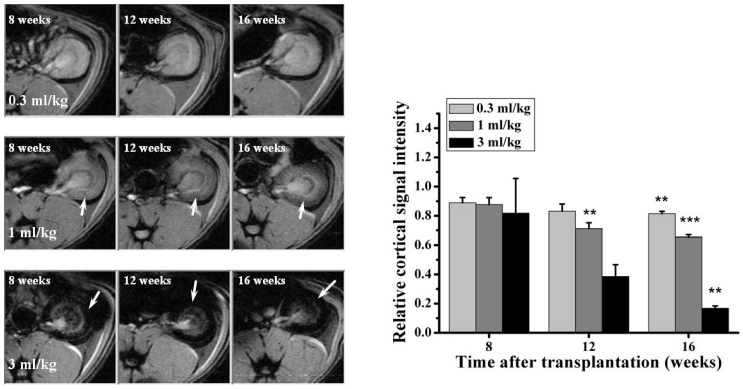

Fig. 1 – *Images from three grafts (left) and relative cortical signal. Anova comparisons carried out with respect to the 8-week values, for each dose.*

At 16 weeks after transplantation, the Banff scores correlated negatively and highly significantly to the MRI cortical signals for the lower doses of SPIO (R=-0.97, p=0.005 and R=-0.95, p=0.003). No correlation was present for the higher SPIO dose since the grafts were saturated by iron as shown histologically. Only occasional correlations, much weaker than those with respect to the MRI signal, were encountered between the Banff scores and a few of the biochemical parameters.

The data reported here indicate that the infiltration of iron-labeled macrophages into grafts detected non-invasively as signal attenuations by MRI provides a readout for detecting early signs of chronic graft rejection, that is more reliable than the biochemical parameters assessed in the blood and the urine. This approach may shorten considerably the duration of the experimental period for the mild life-supporting Fisher 344-to-Lewis kidney transplantation model in particular. Detecting early changes associated with chronic rejection can have an impact in animal and clinical studies by facilitating the investigation of novel therapies for transplantation.

1. Diamond JR, et al. Transplantation 1992;54:710-716.
2. Beckmann N, et al. Magn Res Med 2003;49:459-467.
3. Solez K, et al. Kidney Int 1993;44:411-422.