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BOLD-MRI for the assessment of renal oxygenation in humans: Acute effect of nephrotoxic xenobiotics

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Hypoxia of renal medulla is a key factor implicated in the development of drug-induced renal failure. Drugs are known to influence renal hemodynamics and, subsequently, affect renal tissue oxygenation. Changes in renal oxygenation can be assessed non-invasively in humans using blood oxygenation level-dependent magnetic resonance imaging (BOLD-MRI). This study was designed to test the acute effects of administration of specific drugs in healthy human kidney oxygenation using BOLD-MRI. Acute changes in renal tissue oxygenation induced by the non-steroidal anti-inflammatory drug indomethacin, the iodinated radio-contrast media (RCM) iopromidum, and the calcineurin inhibitors cyclosporine micro-emulsion (CsA-ME) and tracrolimus were studied in 30 healthy volunteers. A modified Multi Echo Data Image Combination sequence was used to acquire 12 T_2*-weighted images. Four coronal slices were selected to cover both kidneys. The mean R_2* ($1/T_2$*) values determined in medulla and cortex showed no significant changes induced by indomethacin and tacrolimus administration. CsA-ME decreased medullary ($P = 0.008$) and cortical ($P = 0.004$) R_2* values 2 h after ingestion. Iopromidum caused a significant increase in medullary R_2* within the first 20 min after injection ($P < 0.001$), whereas no relevant changes were observed in renal cortex. None of the measurements showed left–right kidney differences. Significant differences in renal medullary oxygenation were evidenced between female and male subjects ($P = 0.013$). BOLD-MRI was efficient to show effects of specific drugs in healthy renal tissue. Cyclosporine increased renal medullary oxygenation 2 h after ingestion of a single dose, whereas indomethacin and tacrolimus showed no effect on renal oxygenation. Injection of iodinated RCM decreased renal medullary oxygenation.

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KEYWORDS: BOLD-MRI; non-steroidal anti-inflammatory drug; indomethacin; cyclosporine; tacrolimus; iodinated radio-contrast media

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Adequate renal perfusion and tissue oxygenation is critical for the maintenance of normal renal function. Severe impairment of renal hemodynamics leads to renal hypoxia and failure.[1] Several drugs currently used, like the non-steroidal anti-inflammatory drug (NSAID) indomethacin, the iodinated radio-contrast media (RCM), and the immunosuppressive drugs cyclosporine A (CsA) and tacrolimus, are well-known agents influencing renal hemodynamics and thus affecting renal tissue oxygenation by influencing renal perfusion and/or oxygen consumption.[2] Measurement of renal tissue oxygenation in humans by means of blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) offers a non-invasive tool to assess regional oxygenation of tissue and, subsequently, to study the effect of specific drugs on tissue oxygenation.[3]

BOLD-MRI is sensitive to disturbances of the local magnetic field caused by variations in the oxygen level in hemoglobin molecules, thus using deoxygenated hemoglobin as an endogenous contrast agent. The resulting magnetic susceptibility effect causes a loss of phase coherence, leading to signal attenuation on T_2*-weighted MR images. The ratio of oxyhemoglobin (diamagnetic) to deoxyhemoglobin (paramagnetic) concentrations is proportional to the partial pressure of oxygen (pO_2) of blood, and blood pO_2 is supposed to be in balance with the surrounding tissue. Therefore, BOLD signal estimated by transverse relaxation rate R_2* ($1/T_2$*) can be considered as a sensitive indicator of tissue pO_2.[3,4]

NSAIDs are widely used because of their efficacy and excellent safety profile. However, these drugs have multiple effects on kidney function. NSAID block the synthesis of vasodilatory prostaglandins, which have an intermediatory role in renal hemodynamics. The application of NSAID shifts the balance between vasoconstrictors and vasodilators towards vasoconstrictors and thus reduces renal perfusion reversibly.[1] In patients with pre-existing renal disease, these drugs may worsen renal function.

Calcineurin inhibitors are the mainstay of immunosuppression in renal transplantation. CsA and tacrolimus are currently the most widely used baseline immunosuppressants for prevention of acute rejection following kidney transplantation. However, their use is limited by acute and chronic

toxicity. Acute nephrotoxicity of CsA is reversible and partially caused by a decrease in renal blood flow related to afferent arteriolar vasoconstriction.[5] Tacrolimus nephrotoxic effects seem identical or slightly less than those of CsA. But tacrolimus does not show any vasocontrictory properties and has apparently no hemodynamic effects in the kidney.[5]

Diagnostic and interventional procedures using iodinated RCM are performed with increasing frequency. In parallel, iodinated RCM nephropathy has become a frequent cause of hospital-acquired acute renal failure.[6,7] The overall incidence of contrast-induced nephropathy is estimated to be 0.6–2.3%.[8] In high-risk patients undergoing angiographic procedures or patients with pre-existing renal failure, the incidence is higher: 14.5% after coronary intervention,[9] 16.8% in high-risk patients undergoing cardiac or peripheral angiography,[10] or even 40% in patients with severe pre-existing renal failure.[11] The mechanisms of iodinated RCM nephropathy are not fully understood and a combination of different mechanisms may be involved in the radio-contrast-induced acute renal failure.[6] A shift of the medullary oxygen balance towards elevated hypoxia, induced by an imbalance of vasodilator and vasoconstrictor factors, with a negative effect on renal hemodynamics is believed to play a key role. This reduction of medullary tissue oxygenation is caused by alteration in medullary blood flow or enhanced oxygen requirement. Liss *et al.*[12] have measured medullary oxygenation in rats after injection of RCM.[12] Iopromide (low-osmolal monomer) had the least effect of the contrast media studied (ioxalgate and iotrolan). It is not known if such an effect occurs also in humans.

BOLD-MRI offers the possibility to monitor intra-renal oxygenation non-invasively in humans after drug intake. The aim of this study was to analyze the acute effect of selected drugs (indomethacin, cyclosporine microemulsion (CsA-ME), and tacrolimus) and iodinated RCM (iopromidum) on renal perfusion in healthy subjects by means of BOLD-MRI measurements. Such measurements might ultimatively represent a new approch for a better understanding of the effects of these drugs on kidney oxygenation, and hence open novel possibilities for drug monitoring in kidney transplantation and for prevention of RCM-induced nephropathy.

RESULTS

Baseline R_2^* values ranged from 13.3 to 20.7 s^{-1} (mean $16.8 \pm 2.2\ s^{-1}$) for the medulla and from 9.7 to 13.1 s^{-1} (mean $11.2 \pm 0.8\ s^{-1}$) for the cortex. Mean R_2^* values for the subgroup gender yielded a significance for R_2^* values of the cortex between female and male subjects (Table 1). The mean coefficient of variation (inter-individual coefficient of variation) between volunteers was 13 and 7% in medulla and cortex, respectively. The average intra-individual coefficient of variation for the eight re-investigated subjects was 9% in medulla and 5% in cortex.

Indomethacin administration did not induce significant changes in renal R_2^* for the medulla or for the cortex (Figure 1). Multiple analysis of variance (MANOVA) with repeated measures showed a tendency to lower R_2^* values with time (Table 2). No significant difference was found between right and left kidney (Table 2).

Table 1 | R_2* values

Number	Gender	Age	*R2** medulla	*R2** cortex
30	F+M	30.7 ± 9.7 Range: 18–50	16.8 ± 2.2	11.2 ± 0.8
15	F	30.7 ± 9.3 Range: 20–49	16.1 ± 1.9	10.9 ± 0.6[a]
15	M	30.6 ± 10.3 Range: 18–50	17.4 ± 2.4	11.6 ± 0.9[a]

F: female, M: male; age: average ± s.d.; R_2^*: average ± s.d.
[a]Significant difference between gender (P=0.013).

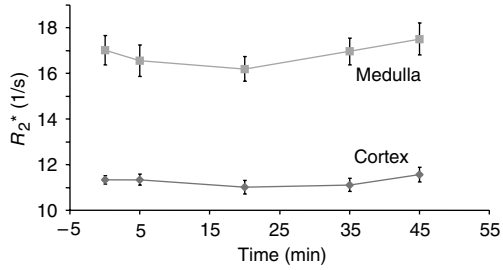

Figure 1 | Time-dependent effect of indomethacin (100 mg p.o., Indocid®) on R_2* value in renal cortex and medulla (mean ± s.e.m., $n = 10$, P = NS).

Table 2 | *P*-values of MANOVA

		Repeated measures		Bonferroni baseline vs *t*			
	Location	Time	P^a	*t*1	*t*2	*t*3	*t*4
Contrast agent	Medulla	<0.001*	0.890	0.432	0.002*	<0.001*	<0.001*
	Cortex	0.45#	0.989	0.008*	0.501*	0.578	0.783
Indomethacin	Medulla	0.096	0.605	0.287	0.148	0.855	0.136
	Cortex	0.066	0.996	0.956	0.173	0.364	0.496
Cyclosporine	Medulla	0.004*	0.771	0.014#	0.934		
	Cortex	0.004*	0.688	0.001*	0.018#		
Tacrolimus	Medulla	0.317	0.300	0.604	0.349		
	Cortex	0.226	0.983	0.315	0.533		

MANOVA, multiple analysis of variance.
[a]P: right vs left; *P<0.01; #P<0.05.

A significant reduction ($P = 0.004$) (MANOVA) in cortical R_2^* values was observed 2 h after CsA-ME administration. This effect was reversed 2 h later (Figure 2 and Table 2). Repeated measures detected significant changes in the medulla, and pairwise comparison between baseline and the third point (t2) after 4 h showed a significant difference (Table 2). No significant differences between right and left kidney were found (Table 2).

No significant correlation was found between R_2^* value and blood CsA concentration [cyclo] (medulla: $R_2^* = -0.0016[\text{cyclo}] + 18.542$, $R^2 = 0.0643$; cortex: $R_2^* = 0.0001[\text{cyclo}] + 11.106$, $R^2 = 0.0028$).

Tacrolimus had no significant effect on R_2^* values for medulla or cortex (Figure 3). Neither right–left differences (Table 2) nor significant correlations between R_2^* index and blood tacrolimus concentrations [tacro] were observed (Figure 3) (medulla: $R_2^* = -0.005[\text{tacro}] + 16.397$, $R^2 = 0.0035$; cortex: $R_2^* = 0.0195[\text{tacro}] + 10.324$, $R^2 = 0.1447$). Regression analysis between the difference of R_2^* values ($\Delta R_2^* = R_2^*$ (2 h after cyclosporine dosing)–R_2^* (at baseline)) and the blood concentrations of cyclosporine or tacrolimus remained negative.

Iodinated RCM iopromidum produced an increase in medullary R_2^* value 20 min after administration (Figure 4). This effect was highly significant in the medulla when analyzed with MANOVA with repeated measures (Figure 5 and Table 2). No relevant changes were observed in cortical R_2^* index, but repeated measures showed a tendency for changes with time in the cortex, mainly because of the first drop (Bonferroni test; Table 2). No significant differences between the right and left kidney were detected (Table 2).

Data obtained after placebo measurements showed no statistically significant difference in the time course for the repeated measures (MANOVA; Figure 6). There was also no significant difference between right and left kidney.

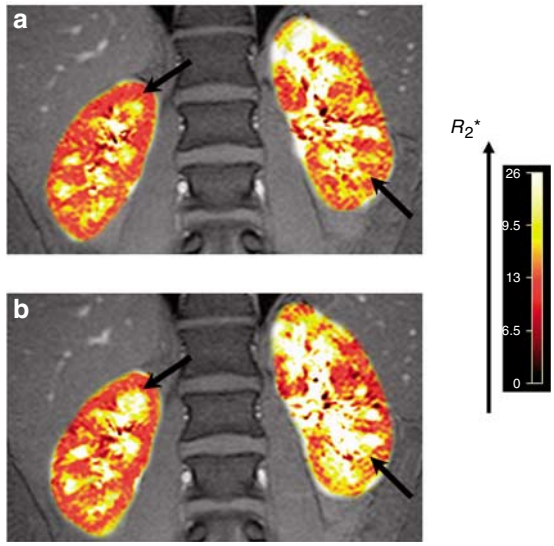

Figure 4 | R_2^* color map (a) before and (b) 20 min after injection of RCM iopromidum in a healthy volunteer. Arrows indicate zones of medullary R_2^* increase after RCM administration.

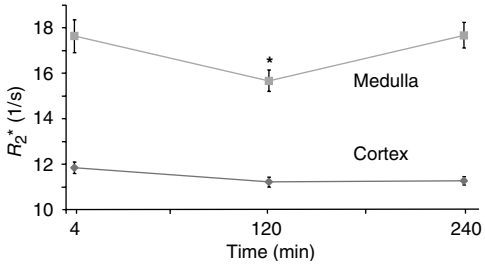

Figure 2 | Time-dependent effect of CsA-ME (6 mg/kg p.o., Sandimmun Neoral®) on R_2^* value in renal cortex and medulla (mean ± s.e.m., $n = 10$, $^*P < 0.002$).

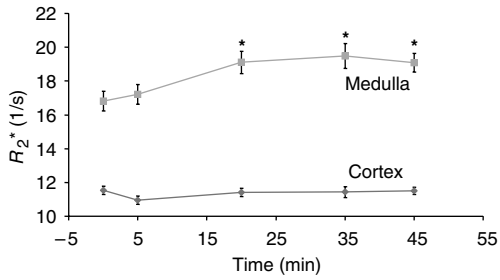

Figure 5 | Time-dependent effect of iodinated RCM iopromidum (2 ml/kg intravenous (bolus) (Ultravist300®) on R_2^* value in renal cortex and medulla (mean ± s.e.m., $n = 10$, $^*P < 0.001$).

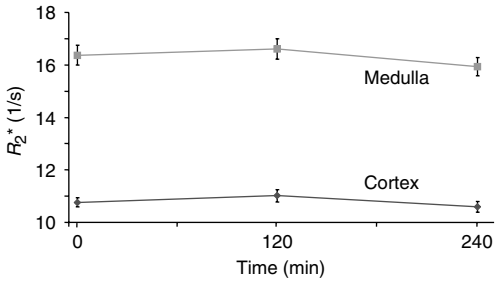

Figure 3 | Time-dependent effect of tacrolimus (0.2 mg/kg p.o., Prograf®) on R_2^* value in renal cortex and medulla (mean ± s.e.m., $n = 10$, P = NS).

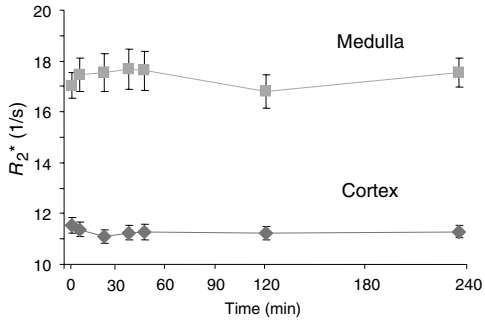

Figure 6 | Time-dependent effect of placebo measurements (i.e. without medication) (mean ± s.e.m., $n = 8$, P = NS).

DISCUSSION

BOLD-MRI is the only technique currently available that allows non-invasive estimation of oxygen content in human kidney and offers the ability to perform measurements dynamically.[3] Beside microscopic field heterogeneity due to deoxy-hemoglobin, R_2^* values are influenced by other factors including macroscopic field homogeneity, vascular volume, and hematocrit. In addition, a nonlinear relationship between $R2^*$ and pO_2 makes a quantitative interpretation of BOLD data in terms of mm Hg (pO_2) difficult.[13] However, as macroscopic field homogeneity and spin–spin relaxation process most probably remain constant during the study presented here, changes in BOLD-MRI signal intensity represented by R_2^* values may reflect the changes in pO_2 of tissues and, therefore, allow to assess the effect of drugs on renal tissue oxygenation.

The mean R_2^* values in medulla ($16.8\,s^{-1}$) and cortex ($11.2\,s^{-1}$) are in the range of those described in the literature for baseline results (i.e. without stimulation) in normal subjects.[3,14–16] Li *et al.*[16] described R_2^* values between 15.2 and $25.3\,s^{-1}$ in baseline medulla and between 10.5 and $16.6\,s^{-1}$ in baseline cortex. In our previous study, the R_2^* values ranged from 15 to $19\,s^{-1}$ in the medulla and 11 to $12.5\,s^{-1}$ in the cortex.[17] Prasad *et al.*[3] obtained mean R_2^* indices between 19 and $20.5\,s^{-1}$ in the medulla and between 12.3 and $13.1\,s^{-1}$ in the cortex. Epstein *et al.*[14,15] found mean R_2^* values of $17.4\,s^{-1}$ for the medulla and $12.9\,s^{-1}$ for the cortex and $17\,s^{-1}$ for the medulla and 12.6 for the cortex in the younger group. The results obtained in the present study are slightly lower than the mean values reported. This difference is partly explained by the study protocol. For logistical reasons, the BOLD-MRI measurements were not performed at the same time of the day in all the volunteers, the subjects were normally hydrated, but not submitted to an acute water load. As expected, R_2^* values are consistently higher in medulla than in cortex, indicating that renal medulla is more hypoxic than cortex in normal subjects.[3] A significant difference in the cortical R_2^* values between female and male subjects was observed (Table 1). This novel finding indicates that the renal medulla of female subjects contains a higher tissue oxygenation content when compared to male subjects. The mechanism and the possible pathophysiological implication are unknown. One might speculate whether estrogens known to modulate renin angiotensin or nitric oxide formation in female subjects might account for the observed gender difference.[18,19]

Hypoxic injury to renal medulla is one of the mechanisms that initiates the phenomenon of acute renal failure.[1] Therefore, it seems important to know whether specific drugs used in clinical practice can affect renal medullary oxygenation in humans and to which extent. Applying BOLD-MRI on eight subjects after administration of placebo medication allowed to establish unambiguously that the measured time course of the R_2^* values after drug administration was an effect of the medication and no random finding. The acute drug exposure revealed xenobiotic-specific results in healthy volunteers. Indomethacin, given as a single oral dose, did not change renal tissue R_2^* (Figure 1). This is a practically relevant observation, indicating that a single dose of indomethacin as prescribed routinely does not influence renal cortical or medullary tissue oxygenation in humans. Nevertheless, some caution must be taken, as patients might react differently and repeated dosing of indomethacin for several days might be more deleterious for kidney oxygenation. In addition, dose and hydration status might be of pivotal relevance, as in rats a decrease in cortical pO_2 and a reduction of 57% in medullary oxygenation was observed after a higher dose of indomethacin (10 mg/kg body weight (bw)) given intravenously in salt-depleted rats.[20] Similar observations were reported by Prasad *et al.*[21] and by Agmon *et al.*,[22] using intravenous injection of indomethacin in rats. In addition, an effect of indomethacin might have been missed in this study because of the relatively short observation time. Nevertheless, the present results indicate that a single dose of indomethacin may have minimal effect on human kidney oxygenation. This observation is in line with other studies in humans. Sturrock *et al.*[23] and Olsen *et al.*[24] found neither a change in renal sodium and water handling nor in glomerular filtration rate, effective renal plasma flow, and renal vascular resistance after 2×100 mg indomethacin administration in healthy male subjects.

Nephrotoxicity, one of the side effects of calcineurin inhibitors, CsA and tacrolimus, contributes to long-term renal graft failure and cardiovascular morbidity in organ recipients.[25] One possible mechanism for this nephrotoxicity might be a decrease of tissue oxygenation by influencing renal hemodynamics. Indeed, Klein *et al.*[5] presented evidence that cyclosporine but not tacrolimus given for 2 weeks decreased renal glomerular filtration rate and effective renal plasma flow in healthy volunteers. The observation of the absence of an effect of tacrolimus in the present investigation is in line with the latter study. However, the reduced renal perfusion, with a decline in the glomerular filtration rate observed by Klein *et al.*,[5] appears to be in contradiction with the increased oxygenation after cyclosporine in our volunteers. The difference between the two studies might be best explained by the much higher total doses of CsA-ME given by Klein *et al.*[5] (single dose of 6 mg/kg bw vs 10 mg/kg bw for 2 weeks).[5] Note that contradiction might only be apparent as the medullary oxygen content depends not only on the perfusion but in addition on active tubular sodium transport, that is, tissue oxygen consumption. If this transport is reduced out of proportion relative to the reduced perfusion, then the medullary oxygen content might increase, despite a decreased perfusion following cyclosporine dosing, a hypothesis that deserves further consideration. In contrast to CsA, tacrolimus showed no effect on medullary oxygenation, suggesting different influence on intrarenal oxygen distribution. Whether this is important in the pathogenesis of calcineurin inhibitor-induced nephrotoxicity remains an open question.

The iodinated RCM iopromidum produces a rapid and sustained increase in medullary R_2^*, indicating a severe

decrease (22 and 28%) in renal medullary oxygenation 20 and 35 min after injection, respectively (Figure 5). To illustrate this effect, R_2^* color maps are shown in Figure 4. Similar observations have been made in animal studies by Liss *et al.*,[12,26] who found in rats injected a high dose of iopromide (2.5 times the dose used in this study), a reduction of pO_2 of 40% in the medulla and a slight, yet not significant decrease in the cortex. Prasad *et al.*[21] reported no change in medullary R_2^* values in animals during the first 15 min following the administration of RCM iothalamate. This lack of a change in medullary R_2^* values directly after intravenous injection of iothalamate might be a consequence of the short observation period of 15 min.[21] In the present study in human volunteers (Figure 4), medullary R_2^* values were increased as early as 20 min after RCM administration and continued to be elevated during the 45 min of observation time. The marked and sustained reduction in medullary pO_2 seen after intravenous injection of iopromide has important clinical consequences. Prevention of RCM-induced medullary hypoxia by drug therapy and/or aggressive volume substitution might reduce the risk of acute renal failure after RCM administration.

Conclusion and outlook

The present study allowed to assess the acute renal effects of several drugs in healthy human volunteers. Cyclosporine (CsA-ME) increased renal medullary oxygenation 2 h after ingestion of a single dose, whereas indomethacin and tacrolimus had no effect on renal oxygenation. Injection of iodinated RCM decreased renal medullary oxygenation in healthy humans. BOLD-MRI is a non-invasive tool to detect early functional changes, owing to changes in tissue content of oxygen, and may therefore be of value to develop new strategies to reduce medullary hypoxia attributable to drugs or RCM. The present investigation was performed in healthy volunteers without apparent risk factors for nephrotoxicity of the xenobiotics given. It is reasonable to assume that the susceptibility of the kidney to these xenobiotics in disease states when the autocrine and paracrine defense mechanisms are not operative in order to preserve medullary integrity and function is completely different from that in healthy volunteers.[1,27,28] As a corollary, the observations made in the present study in healthy subjects are not *a priori* completely predictive for observations in patients. Thus, future studies should focus on the effect of the nephrotoxic agents investigated in the present study in subjects with well-defined pathological entities.

MATERIALS AND METHODS

Study protocol

The study was designed as a prospective, open study. Approval of the institutional Ethical Committee was obtained and all subjects gave informed consent before entering the study.

Fifteen female and 15 male healthy volunteers at an age of 18–50 years (mean age of 30.7 ± 9.7 years) and body mass index $<29\,kg/m^2$ have been included in the study. One volunteer participated in all the four studies and nine in two studies. Physical examination including body height and weight, blood pressure and heart rate, serum creatinine, and urine tests were completed in all patients. For volunteers who received RCM, a normal thyroid stimulating hormone concentration was required before entering the study. Exclusion criteria were impaired renal function (serum creatinine $>100\,\mu M$/l for men and $>90\,\mu M$/l for women), predisposition to atopia (asthma, eczema, urticaria, and allergic conjunctivitis), history of allergy to RCM or iodine allergy, thyroid disease, pregnancy, medical therapy (diuretics, angiotensin-converting enzyme inhibitors, CsA or CsA-ME, NSAID), or infective disease during 2 weeks before the study or contra-indication for MR examination.

The volunteers were allocated to four groups (Table 3): indomethacin, CsA-ME and tacrolimus, and iopromidum, an iodinated RCM. The dosage of medication and RCM corresponds to the amount used in humans for treatment or routine radiological examinations, in particular abdominal CT. The volunteers received either indomethacin 100 mg per os (Merck Sharp & Dohme-Chibret Inc., Whitehouse Station, NJ, USA), CsA-ME 6 mg/kg bw p.o. (Novartis Pharma Ltd, Basel, Switzerland), tacrolimus 0.2 mg/kg bw p.o. (Fujisawa Ireland Limited, Killorglin Co., Kerry, Ireland), or iopromidum 2 ml/kg bw intravenous (Schering, Berlin, Germany) (Table 3).

Steady-state BOLD-MRI measurements were performed on all the volunteers before administration of the drugs. The subsequent procedure depending on the studied drug is outlined in Table 3. Indomethacin was orally applied while the volunteer was lying in the MR scanner. RCM was injected (Medrad Spectris MRI Injector, Medrad Inc., Indianola, PA, USA) at a flow rate of 1.5 ml/s. For both drugs, MR measurements were repeated 5, 20, 35, and 45 min after

Table 3 | Study design and characteristics of volunteers

Drugs	Indomethacin	Radiocontrast iopromidum	Cyclosporine microemulsion	Tacrolimus
Trade name	Indocid®	Ultravist300®	Sandimmun Neoral®	Prograf®
Dosage	100 mg p.o.	2 ml/kg intravenous (bolus)	6 mg/kg p.o.	0.2 mg/kg p.o.
Measurements	0, 5, 20, 35, and 45 min after drug administration	0, 5, 20, 35, and 45 min after drug administration	0, 120, and 240 min after drug administration	0, 120, and 240 min after drug administration
Blood samples	None	0 min: sCreat, TSH	120 min: sCreat, Cyclo; 240 min: sCreat, Cyclo	120 min: sCreat, Tacro; 240 min: sCreat, Tacro
Age (years)	30.7 ± 10.7	30.2 ± 10.3	29.2 ± 11.3	31.9 ± 11.7
Gender (F/M)	4/6	3/7	4/6	7/3
Number	n=10	n=10	n=10	n=10

Cyclo, cyclosporine concentration; F, female; M, male; p.o., per os; sCreat, serum creatinine concentration; Tacro, tacrolimus concentration; TSH, thyroid stimulation hormone.

administration (Table 3). CsA-ME and tacrolimus were orally taken by the volunteers outside the MR scanner, and BOLD measurements were repeated 120 and 240 min after ingesting the drugs. Serum creatinine, CsA, or tacrolimus blood levels were determined immediately after the MR examination.

Eight of the 30 volunteers (randomly selected, three women, five men) underwent a repeated BOLD-MRI study using a placebo (a glass of water or NaCl injection) after 3–7 months.

In this study, the subjects were not asked to abstain from food and water overnight. However, we assume that variations in the overnight water intake would be negligible and that all the subjects were well hydrated at the time of acquisition.

Laboratory methods

Urinary glucose, protein, or hemoglobin was analyzed by stix analysis (Multistix®, Bayer, USA). Serum creatinine was measured by the enzymatic plus-Method on a Hitachi 917 analyzer (Roche, Rotkreuz, Switzerland). Serum thyroid stimulating hormone and CsA levels were assessed by the Abbott-FPIA-Method (Abbott AG, Baar, Switzerland) and tacrolimus was measured by the Abbott-MEIA-Method (Abbott AG, Baar, Switzerland).

MR measurements

All MR measurements were carried out on a syngo-based 1.5 T whole-body MR system (SONATA, Siemens Medical Systems, Erlangen, Germany) using the body coil for transmission and a phased array surface coil for reception. For morphological evaluation of the kidneys, coronal T_2-weighted fat suppressed Half-fourier Acquisition Single-shot Turbo spin-Echo images were acquired (repetition time (TR) 1010 ms, echo time (TE) 81 ms, flip angle 150°, field of view (FOV) $400 \times 400\,mm^2$, voxel size $1.1 \times 0.8 \times 5.0\,mm^3$), followed by T_1-weighted Fast Low Angle SHot (FLASH) gradient-echo sequence images in axial and coronal orientation (TR 65 ms, TE 4.76 ms, flip angle 70°, FOV $400 \times 400\,mm^2$, voxel size $1.1 \times 0.8 \times 5.0\,mm^3$). Four slices with good cortico-medullary differentiation were then selected for functional evaluation with BOLD-MRI.

Twelve T_2*-weighted images were recorded within a single breath-hold of 17 s using a modified Multi Echo Data Image Combination sequence[17] with the following parameters: TR 65 ms, TE 6–52.31 ms (inter-echo spacing 4.21 ms), flip angle 30°, FOV $400 \times 400\,mm^2$, voxel size $1.6 \times 1.6 \times 5\,mm^3$, bandwidth 325 Hz/pixel, matrix 256×256 with an interpolation in both directions to 512×512.

Data were transferred to a standard personal computer. R_2* maps were calculated voxel by voxel, using a home-built IDL program (Interactive Data Language, RSI, Boulder, CO, USA). As the fit of more than 250 000 exponential functions per image was too time consuming, the following function (equation (1)) was minimized instead:[29]

$$\sum_{i=1}^{n} y_i(\ln(y_i) - abx_i)^2 \qquad (1)$$

The weighting of the function by y_i compensates for the logarithmic transformation of the Rician noise that occurs with the linearization of the exponential function. As the longest TE used in this experiment was 52.31 ms with T_2* between 50 and 100 ms, the signal amplitudes were well above noise level and, therefore, Rician noise approached a Gaussian distribution. Regions of interest were selected in the medulla and the cortex, and a mean value of R_2* index was estimated. Because of the heterogeneity of contrast changes within cortex and medulla, the regions of interest were chosen in a homogenous part of the cortex or the medulla.[3]

Statistical significance of the results was analyzed using MANOVA for repeated measures (time) and three variables (volunteer, medulla/cortex, and left/right kidney) with Bonferroni adjustment for multiple comparison (SPSS11.0 for Windows, Chicago, IL, USA). For gender differences, a two-sided unpaired Student's *t*-test was applied (SYSTAT 10.0 for Windows, Systat Software Inc., Richmond, CA, USA). The intra- and inter-individual coefficient of variation represents the s.d. divided by the mean R_2* value for different measurements and/or different subjects.

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