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Evaluation of renographic and metabolic parameters in human kidney transplantation

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Background. **The aim of this work is to demonstrate that the value of the mean transit time (MTT) obtained from the ^{99m}Tc-MAG3 renogram deconvolution is related to the levels of adenine nucleotides determined in cortical biopsies from transplanted kidneys.**

Methods. **The functional state was estimated by means of the MTT and the initial heigt (H0) of the renal retention function obtained from the ^{99m}Tc-MAG3 renogram deconvolution and by the measure of adenine nucleotides obtained from biopsies. We studied 30 kidney graft recipients, 25 normal functioning grafts (NFG) and 5 with acute tubular necrosis (ATN).**

Results. **The MTT is significantly longer for ATN ($p<0.001$). The initial uptake values (H0) are significantly lower for ATN ($p<0.001$). The sum of adenine nucleotides (SAN) is significantly greater for NFG than for ATN ($p<0.001$). The values of the MTT seem to reflect the energy state of the cells in transplanted kidney.**

Conclusion. **The analysis of MTT may be indicative of the functional metabolic recovery and thus it may be predictive of the renal graft function at least in the same extent than the biochemical analysis of a cortical renal biopsy immediately after blood reperfusion of the tissue.**

KEY WORDS: Adenine nucleotides - Kidney transplantation radionuclide imaging - Technetium 99m metiadite diagnostic use - Radioisotope renography methods.

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The functional state of the kidney is reflected by renographic and metabolic parameters. Isotopic renography, which is a non invasive method, allows us to quantify renal function by time parameters related to tracer elimination and by relative function when bi-renal subjects are considered. Renogram deconvolution with the blood curve enables us to complete the standardization of the renographic studies by removing the dependence of the renogram with respect to the tracer input curve and by enabling the calculation of the renal retention function (RRF).[1] The application of the deconvolution for the evaluation of renal transplants [2,3] provides a complementary diagnostic method in renal transplantation. ^{99m}Tc-Mercaptoacetyl-triglycine (MAG3) is widely employed for gamma camera renography and for measurement of renal function. This tracer is mainly excreted in the urine by tubular secretion and only in a minor degree by glomerular filtration. The renal clearance of MAG3 is less than the clearance of iodine-125 labelled orthoiodohippurate (OIH) but about 2-3 times the glomerular filtration rate.[4]

The metabolic changes that take place during the cold storage seem to be determinant in post-transplantation acute tubular necrosis. Changes in adenine nucleotides contents (ATP, ADP and AMP)

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TABELLA I.—*Values for normal funtioning grafts and for acute tubular necrosis.*

Normal functioning grafts (25 kidney units)
Age 44±12 (17-65) years
Serum creatine 258±194 (81-835) mol/l
Effective renal plasma flow 432±106 (236-688) ml/min
Diuresis 2746±1196 (1100-5590) ml
Concentration of ciclosporine in blood 142±56 (49-260) ng/ml
Acute tubular necrosis (5 kidney units)
Age 53.4±7.1 (42-61) years
Serum creatinine 394±379 (335-1035) mol/l
Effective renal plasma flow 184±26 (146-211) ml/min
Diuresis 444±442 (291-1600) ml
Concentration of ciclosporine in blood 225±84 (100-335) ng/ml

have been described in ischemic kidney and play an important role in functional recovery.[5]

The aim of this work is to demonstrate that the value of the mean transit time (MTT) obtained from the ^{99m}Tc-MAG3 renogram deconvolution is related to the levels of adenine nucleotides determined in cortical biopsies from transplanted kidneys.

Materials and methods

Kidney population

We studied 30 kidney graft recipients transplanted in our hospital for 1 year. Twenty-five normal functioning grafts (NFG) and five with acute tubular necrosis (ATN). The kidneys were obtained from corpse donors, perfused with EuroCollins solution and cold stored until transplantation. The clinical and biochemical data are shown in Table I.

Renographic studies

Acquisition.—Renograms were performed into the first week after transplantation in all the cases. The examinations were performed after the intravenous administration of 222-296 MBq of ^{99m}Tc-MAG3, using a gammacamera equipped with a low energy all purpose collimator. The acquisition times were: 1 frame every 2.5 seconds for 2 minutes and 1 frame every 20 seconds for the remaining 22 minutes.

Regions of interest.—The renograms were obtained from regions of interest (ROI) outlining the outer edge of the whole kidney. A ROI outlined over the aorta before renal artery bifurcation was used to standardize the blood disappearance curve. This procedure enabled us to obtain the blood curve regardless of patient height and position and it is always valid for kidney grafts where the position of the kidney is variable. The background ROI was mirrored in the contralateral graft area.

Renographic parameters.—The renal retention function was obtained by deconvolution of the renogram with the aortic blood curve.[2,3] The parameters were obtained from the RRF: the mean transit time (MTT) and the initial height (H0). The MTT, calculated as the ratio between the area and the H0, is related to the functional state of the kidney and reflects the parenchymal function. The H0 is related to the renal perfusion and the tracer extraction.

Adenine nucleotide determinations

A cortical biopsy was obtained from each human kidney during the transplantation process 30 minutes post-reperfusion. Tissue samples were quickly frozen in liquid nitrogen and stored at -80°C. The samples were homogenized in 120 µl (1:10 w/v) of ice-cooled 6% trifluoracetic acid (Merck). The homogenates were centrifuged at 4°C for 10 min (12.000xg) and 20 µl of the neutralized supernatant was injected into an High Performance Liquid Chromatography (HPLC) column. To separate nucleotides (ATP, ADP and AMP), an anionic exchange column (Spherisorb SAX) 5 µm (25x0.4 cm, Tracer) was used with a non-linear gradient, 0 -100%, between a low-strength eluent (0.007M KH_2PO_4+0.014M KCl, pH 4.0) and a high-strength eluent (0.25M KH_2PO_4+0.5M KCl, pH 5.0) both containing 2% of methanol, for 30 minutes at a flow rate of 1 ml/min.

The nucleotides were determined with a UV detector at 254 nm. Analysis and quantification of chromatograms were performed with a Nelson program (Nelson Analytical, Cupertino, CA). The results are expressed in nmol/mg protein±sem. For protein analysis, the precipitates were digested overnight at room temperature with 1 ml of 1M NaOH and determined according to the Bradford method [6] using bovine serum albumina as standard.

Statistical methods

Mean values and standard deviations were calculated for the renographic parameters. Comparisons

between both entities were analyzed using the Mann-Whitney U-test. Evaluations of adenine nucleotide differences were performed with one-way analysis of variance (ANOVA) of the means followed, if significant, by the Scheffé test. Significance was considered to be reached at $p<0.05$.

Results and discussion

The mean values and standard errors of the renographic parameters and the sum of adenine nucleotides are shown in Table II. The MTT is significantly longer for ATN ($p<0.001$). The initial uptake values (H0) are significantly lower for ATN ($p<0.001$). The sum of adenine nucleotides (SAN) is significantly greater for NFG than for ATN ($p<0.001$).

The kidney depends on ATP production for several functions including transport, metabolic processes and Na,K-ATPase, which has a prominent role in this organ. ATP turnover in the kidney has been described to be 18-36 µmol ATP/min·g under normal conditions.[7] During ischemia, mitochondrial oxidative phosphorilation is suppressed by lack of oxygen. ATP levels rapidly falls, followed by a slow metabolism of the total pool of adenine nucleotides (ATP+ADP+AMP). Many attempts have been made to find a reliable test for the estimation of renal injury and its relationship with delayed graft function in kidney transplantation.[8] The metabolic changes that take place during the cold storage and the recovery of metabolic function in the immediate post-transplantation, seem to be determinant in the delayed graft function (acute tubular necrosis). The renal ATP content after reflow, determined in cortical biopsies, has been shown to be a good predictor of the functional recovery of the kidney. The ability of the kidney to synthetize ATP after ischemia seem to be more important than the absolute level to which ATP levels fall. However, a significant decrease in the sum of adenine nucleotides (SAN) has been described in ATN and it has been suggested to be predictive of immediate renal function.[5] This finding is confirmed in the present study.

The renographic findings are in agreement with the functional state of the grafts. Given that the same dose was administered for both NFG and ATN and that H0 is related to the renal tracer extraction capability, the differences in the H0 values (approximately one order of magnitude) are explained by the existing damage of the renal parenchyma.[2 9] The MTT values for NFG do not show significant differences with respect to our results for healthy kidneys [3] and the renal retention functions are consistent with the time behaviour of normality. The MTT values for ATN are longer than those for NFG, which is explained by the low extraction capability and the diminished tracer elimination of the ATN *versus* NFG. These preliminary results show that the values of the mean transit time seem to reflect the energy state of the cells in transplanted kidney.

TABLE II.—*Renographic parameters (MTT, H0), sum of adenine nucleotides (SAN) and significance for NFG and for ATN (mean±standard error and range). MTT are expressed in seconds. SAN are expressed in nmol/mg protein.*

Parameters	Normal functioning grafts (25 kidney units)	Acute tubular necrosis (5 kidney units)	Significance
MTT	195±9.9 (111-277)	420±12.4 (390-454)	<0.001
H0	0.036±0.0024 (0.011-0.068)	0.004±0.0007 (0.002-0.006)	<0.001
SAN	22.2±1.3 (13.6-42)	10.7±1.4 (6.2-13.5)	<0.001

In summary, the MTT values in ATN are likely due to metabolic cell failure already detectable 30 minutes after transplantation. The analysis of MTT may be indicative of the functional metabolic recovery and thus it may be predictive of the renal graft function at least in the same extent than the biochemical analysis of a cortical renal biopsy immediately after blood reperfusion of the tissue.

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