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NEW TRENDS IN CLINICAL BIOCHEMISTRY OF TRANSPLANTATION

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LECTURES

ARTERIAL HYPERTENSION AND RENAL DYSFUNCTION IN LONG-TERM LIVER TRANSPLANT RECIPIENTS

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De novo arterial hypertension and renal dysfunction are two of the most common medical diseases after liver transplantation (LT). Arterial hypertension and renal dysfunction occur in greater than 50-60 % of long term survivors. The natural history of untreated arterial hypertension after LT is not known since it is now treated to avoid cardiovascular complications. As far as renal dysfunction is concerned, the greatest decline in renal function appears to occur in the first 18 months. The decline in renal function is rarely and slowly progressive so that and end-stage renal disease (ESRD) occurs in less than 10 % of long term survivors after LT. The development of ESRD decreases survival. Liver transplant recipients maintained on haemodialysis had a 27 % 6-year survival after the onset of ESRD. The relationship between arterial hypertension and renal dysfunction are complex both on the clinical and on the pathophysiogical level. From a clinical point of view it can be stated the followings: a) there is no difference in the prevalence of renal dysfunction between liver transplant recipients who develop or not arterial hypertension after LT, b) renal dysfunction may persist after the resolution of arterial hypertension after LT and c) renal dysfunction complicates the pharmacological therapy of arterial hypertension after LT. Even though not all arterial hypertension after LT can be ascribed to cyclosporine (CSA) or tacrolimus (TAC), the main pathophysiological mechanisms underlying both arterial hypertension and renal dysfunction are the changes in vascular tone induce by the calcineurin inhibitors. Previous clinical observations suggest also that the changes in the regulation of systemic and renal vascular tone may be to some extent independent and/or divergent. A major mechanism of CSA- and TAC-related arterial hypertension and renal dysfunction is the impairment of the endothelial control of vascular tone. The calcineurin inhibitors reduce the endothelial release of vasodilating systems (nitric oxide and prostacyclin) and increase that of vasoconstrictors (endothelin and thromboxane). The net effect of these changes is an imbalance that favours vasoconstriction. The role for the sympathetic neural pathways leading to arterial vasoconstriction particularly in the renal circulation in calcineurin-induced hypertension and renal dysfunction is still debatable. The intense renal vasoconstriction that renal transplant recipients develop soon after transplantation despite the kidney denervation does not support a primary role. The activation of the renin-angiotensin system occurs late after LT, but its role may be enhanced by a calcineurin inhibitors-related increase of angiotensin II receptors. Other mechanisms has been proposed to explain the calcineurin inhibitors-induced arterial hypertension such as renal sodium retention but their role is thought to be secondary as compared to the disturbances of the vasoactive systems induced by CSA and TAC. Finally most of these mechanisms are not mutually exclusive and may amplify each other.
To prevent the renal dysfunction in long term survivors after LT patients at risk pre-LT should be identified. These include patients with hepatorenal syndrome or perhaps all patients with renal failure at the time of LT. In these patients induction immunosuppresion may delayed the use of the calcineurin-inhibitors until the kidney start to recover. In liver transplant recipients with an increasing creatinine level several therapeutical options are now available. Dose reduction of calcineurin-inhibitors is key, although long-term renal dysfunction seems not to be dose-dependent. Several trials suggest that the use of mycofenolate mofetil (MMF) or rapamycin in place of calcineurin-inhibitors results in an improvement of renal function in liver transplant recipients with renal failure. But, the follow-up in these trials is not long enough to determine whether there was a real positive effect on renal dysfunction without any increase in rejection. So, probably, the renal function can be safely preserved for many years tapering the calcineurin-inhibitors to a minimal dose and adding a third agent such s MMF. Immunosuppression tapering is also a key component of arterial hypertension management after LT. It has been suggested that early prednisone withdrawal and CSA or TAC tapering is safe and significantly decrease the prevalence of arterial hypertension after LT. Calcium channel blockers are the most commonly antihypertensive drugs for use after LT because they inhibit endothelin and cause vasodilation. Nifedipine is usually preferred because verapamil or diltiazem may lead to CSA or TAC toxicity. Other antihypertensive agents, particularly β-blockers, are effective in the treatment

of arterial hypertension in liver transplant recipients. The preliminary results of a randomized controlled trial show that carvedilol, a α1- and β-blocker with anti-oxidative properties, is as effective as nifedipine but safer than the latter in the treatment of arterial hypertension after LT. The efficacy of angiotensin-converting enzyme inhibitors is still debated while their synergism with CSA or TAC to induce hyperkalemia is a fact. Finally, since there is some evidence that CSA causes more frequently arterial hypertension than TAC, hypertensive patients on CSA who are resistant to the previously mentioned therapy may improve by switching to TAC.

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PRACTICAL TDM STRATEGIES FOR MYCOPHENOLIC ACID

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Mycophenolic acid (MPA), the active immunosuppressant metabolite of the prodrug mycophenolate mofetil (MMF), is a potent inhibitor of inosine monophosphate dehydrogenase II (IMPDH II) and has been shown to be effective in reducing the rate of acute rejection in transplant recipients. It is now widely used in kidney and heart transplantation, in particular in combination with cyclosporin and steroids. However, MMF is also being used in association with tacrolimus and sirolimus and is gaining importance as a primary component in long-term immunosuppressive regimens. MMF is rapidly and completely absorbed, undergoing extensive presystemic deesterification to MPA, which is subject to a significant enterohepatic cycling process that contributes about 40% to the AUC of mycophenolic acid. MPA is extensively bound to albumin with a range of protein binding of 97-99% in patients with normal renal and liver function. Factors that can influence this protein binding include hypoalbuminemia, impaired renal function, hyperbilirubinemia, and elevated concentrations of the primary metabolite mycophenolic acid glucuronide (MPAG). Furthermore, MPA is a restrictively cleared drug with a relatively low organ extraction ratio. Consequently drug clearance is dependent on the free fraction of the drug. Several investigations have now shown that when MMF is given in combination with cyclosporine, MPA exposure is lower than when the same dose is given in combination with tacrolimus or sirolimus. Discontinuation of cyclosporine is also associated with an increase in MPA levels despite an unchanged MMF dose. Preliminary evidence suggests that cyclosporine may inhibit biliary secretion of MPAG, thereby reducing the enterohepatic circulation of MPA. High steroid doses have also been shown to reduce MPA exposure, presumably through induction of UDP-glucuronosyltransferase

Although it was recommended that MMF be administered at a fixed dose twice daily, substantial evidence has now accumulated to suggest that therapeutic drug monitoring of MPA may help to guide dosing of this drug to optimise efficacy and reduce side effects.

1) There is pronounced inter-individual variation in the pharmacokinetics of MPA.
2) The degree of MPA exposure is influenced by the concomitant immunosuppressive medication.
3) A correlation has been established between pharmacokinetic parameters of the drug and the incidence of acute rejection and to a lesser extent hematologic toxicity.
4) Validated methods (hplc and EMIT immunoassay) are available for quantification of MPA.

Numerous studies have now demonstrated that low MPA-AUC levels and low pre-dose (C_0) MPA concentrations are associated with an increased risk of acute rejection in renal (pediatric and adult) and heart transplant recipients. In general, the MPA-AUC showed a better discrimination of acute rejection than C_0. Some, but not all studies have also found a relationship between increased MPA levels and hematologic toxicity. For renal transplant patients a target range of 30 - 60 mg*h/L for the MPA AUC has been proposed based on prospective and retrospective concentration-clinical outcome studies in patients receiving concomitant CsA and prednisone therapy. The range of trough concentrations that corresponds to this is 1 - 3.5 mg/L. Results obtained with the EMIT immunoassay are on average around 20-30% higher than those measured with an hplc method. This is due to cross-reactivity with an acylglucuronide metabolite of MPA, that has been shown to be an inhibitor of IMPDH II and to possess antiproliferative properties. The major metabolite of MPA, MPAG does not cross-react in the EMIT assay and is not an inhibitor of IMPDH. The EMIT immunoassay has been shown to have a comparable diagnostic efficacy to hplc for assessing the risk of acute rejection in pediatric renal transplant recipients.

Since determination of a full AUC is impractical in routine TDM, limited sampling strategies have been evaluated that allow a good estimation of the full AUC using algorithms based on 3 sampling time points within the first two or four hours after the MMF dose.

A prospective multicentre trial (FDCC) has been initiated to compare a TDM-based adjustment of MMF doses using a 2 hr limited sampling strategy to a fixed dose regimen.

CLINICAL OUTCOME OF ORTHOTOPIC LIVER TRANSPLANTATION

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Orthotopic liver transplantation (OLT) has become a standard procedure for end-stage cirrhosis. The purpose of this anlysis is to give a brief overview concerning the clinical outcome OLT.
Concerning the current distribution of primary indications for liver transplantation in Europe virus-induced cirrhosis represents the largest porpotion with 25%. Although this indication is undisputed, the high rate of reinfection is often a point of discussion, as the survival rate decreases significantly in the case of clinical hepatitis recidivism.
The next frequent indication is alcoholic cirrhosis with 19%. In the past, many transplant centers have been reluctant to accept alcoholic cirrhosis as an indication for OLT. The main reasons have been a suspected high rate of alcohol relapse, a lack of compliance with required immunosuppressive therapy, a higher rate of multiorgan disease than in other recipients, and, last but not least development of liver disease through fault of their own. In contrast to these suspected arguments, substantial evidence of successful outcomes has emerged and policy has been changed in many centers.
Even in classical indications such as primary biliary cirrhosis and sclerosing cholangitis which represent a porportion of 13%, some aspects are still under discussion. The problem in cholestatic diseases is the timing of transplantation as liver function and synthesis are often found to be almost normal and patients suffer more from secondary complications of cirrhosis.
Liver transplantation for malignancy in cirrhosis (10%) goes back to the beginning of transplantation. Nowadays, the indication is examined critically because experience has shown a 5-year patient survival rate of below 20% in patients suffering from pT4. The main cause of death is recurrence of tumor. Several analyses during the last few years have shown that appropriate patient selection will result in good outcome.
Another traditional indication for liver transplantation is acute hepatic failure (10%). 1-year survival rate of 63% after OLT is low in comparison to elective transplantation, but in fact quiet high, considering the mortality rate of 80% for the spontaneous course.
Patient survival rates demonstrate for cirrhosis at 1-and 5-year about 80% and 70%, respectively. In acute hepatic failure, more patients are lost in the perioperative period and later on, the course is comparable to that in cirrhosis. It does not appear surprising, that patients transplanted for malignancy show decreased long-term survival.
Considering an averange 5-year survival in patients with end-stage liver disease of 20% or less, excellent patient survival can be achieved by liver transplantation.
The causes of death have changed over time with the exception of infection, which still the most common cause of death (42%). With increasing survival rates, an increasing number of long-term socalled non-transplantation-related complications such as cardiovascular or age-related death gain in importance. Unfortunately, the next most frequent causes of death are recurrence of malignancy and de novo tumors (13%). In contrast, primary non-function of the graft (1.2%), hepatic artery thrombosis (1.2%) or rejection (1.1%) are very rare events.
The death rate in the first year post-transplant is 20% and after the second year below 5% annually. Considering an average 5-year survival rate of less than 20% in patients with end-stage liver disease, excellent patient survival may be achieved by means of liver transplantation.

TRYPTOPHAN CATABOLISM IN RENAL ALLOGRAFT RECIPIENTS

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Introduction: Indoleamine (2,3)-dioxygenase (IDO) is the rate limiting enzyme in the degradation of the essential amino acid tryptophan via the kynurenin pathway. IDO inhibits T cell proliferation due to tryptophan depletion and production of pro-apoptotic catabolites, thus leading to T cell anergy. IDO is activated by proinflammatory cytokines such as INF-gamma and is involved in inflammation, host immune defence and maternal tolerance. The role of IDO in solid organ transplantation, however, remains unclear. In this study we we wanted to determine the status of peripheral IDO-mediated tryptophan catabolism in patients after kidney transplantation

Material and Methods: A total of 10 patients (mean age: 43 ±15 years; 1 female, 9 male) who underwent primary cadaveric renal transplantation were followed prospectively during the first four postoperative weeks. Immunosuppression consisted of calcineurin-inhibitor based triple therapy with corticosteroids and MMF. Serum concentrations of kynurenine and tryptophan were analysed by high performance liquid chromatography (HPLC) on reversed phase at serial time points following transplantation. Kynurenine per tryptophan ratios (kyn/trp) were calculated as an indirect estimate of IDO activity. Intragraft expression of IDO was assessed by immunohistochemistry. Serum neopterin was assassed using radioimmunoassay.

Results: Patients with an uncomplicated postoperative course showed a significant decrease of kyn/trp by the end of the first week (day 5: 46 ± 25 ; day 15: 41 ± 19 ; day 19: 47 ± 24) when compared to pretransplant levels (118 ± 52) and remainded thereafter at low levels which were almost similar to normal controls throughout the entire observation periode. However, there was an increase of serum kynurenine and a decrease of tryptophan concentrations as reflected by a significant increase of kyn/trp in patients with acute rejection episodes (81 ± 43). kyn/trp correlated significantly with serum neopterin at any time point suggesting an interferon-gamma induced increase in IDO activity. Intragraft IDO staining was localised predominantly in renal tubular epithelial cells in biopsies classified Banff II, whereas in normal renal biopsy specimens rather few scattered positive areas with nuclear staining were found.

Conclusion: This is the first study to demonstrate that acute rejection is associated with increased serum kyn/trp in patients after kidney transplantation. IDO activity may serve as a new marker of immune activation and via its regulation by INF-gamma offer a potential tool of modulating the alloimmune response. However, the possible effects of this IDO activation and thereby leading to abnormal tryptophan and kynurenine serum levels on graft function will deserve further investigation.

EFFECTS OF RAPAMYCIN IN DIFFERENT TYPES OF ARTERIAL SMOOTH MUSCLE CELLS

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Background: The macrolide antibiotic rapamycin is used as an immunosuppressant in organ-transplanted patients who should not receive calcineurin inhibitors. Recently, much attention has focused on its potential appilication for other therapeutic targets, including accelerated arteriopathy after cardiac transplantation and stent restenosis. Furthermore, there is increasing evidence that rapamycin inhibits tumor growth by curtailing expression of vascular endothelial growth factor (VEGF) in malignant cells.
Aims of this study was to analyse effects of rapamycin in different types of arterial smooth muscle cells in terms of nuclear factor kappa B (NF-κB) signalling and VEGF expression. NF-κB contributes to angioplasty-induced lumen loss, likely via induction of an inflammatory response and a decreased rate of apoptosis. Local application of rapamycin by drug-eluting stents prevents lumen loss after angioplasty by inhibition of neointimal hyperplasia. Antiproliferative effects of rapamycin include blockage of cell-cycle progression at the G_1/S transition, but other mechanisms such as interference with NF-κB signaling may be involved as well. Vascular smooth muscle cells (SMCs) are composed of heterogenous lineages, and the formation of neointima after angioplasty is considered to arise by the proliferation and migration of a subpopulation of SMCs from the synthetic phenotype, referred to as neointimal smooth muscle cells. The effects of rapamycin on neointimal derived, synthetic phenotyped SMCs which are responsible for the occurence of restenosis have not been investigated so far.
Marterial and Results: Rat neointimal SMCs were derived from ballon catheter-injured arteries. As compared to medial SMCs, neointimal SMC elucidated higher basal NF- κB levels. Rapamycin (tested at a concentration 0.1 μg/ml) selectively blocked basal and tumor necrosis factor alpha (TNF-α) stimulated nuclear protein binding to NF-κB concensus oligonucleotides in neointimal but not in medial smooth muscle cells, as assessed by electrophoretic mobility shift assay (EMSA). Rapamycin (tested at concentrations between 0.001 to 100 μg/ml) led to a strong induction of inhibitor kappa B alpha (IκB-α) protein levels in neointimal cells, whereas rapamycin had only a weak stimualtory effect on IκB-α levels in medial smooth muscle cells.
Incubation of 1 μg/ml of rapamycin decreased VEGF mRNA production in cultured human smooth muscle cells within 48 hours: control 138.3 (±9.9; n = 4) μmol/l, after 24h of incubation with rapamycin 118.0 (± 12.6; n = 3; p = 0.11) μmol/l, after 48h of incubation with rapamycin 41.8 (±10.9; n = 4; p < 0.001) μmol/l. Rapamycin at a concentration of 0.1 and 1 significantly decreased VEGF protein secretion within 48 hours of incubation: control 585.9 (± 120.3, n = 8) pg/ml; rapamycin 0.1 μg/ml 460.7 (± 35.0, n = 8; p = 0.011) pg/ml; rapamycin 1 μg/ml 458.5 (± 45.3, n = 8; p = 0.009) pg/ml. As compared to medial SMCs, intimal cells secreted much lower amounts of VEGF protein within 48 hours of incubation (642.8 ± 95.9 pg/ml, n = 8 and 238.6 ± 34.3 pg/ml, n = 8, respectivly). Rapamycin significantly decreased VEGF secretion in both type of cells (318.4 ± 34.5 pg/ml, n = 8; p < 0.001 and 164.9 ± 13.3 pg/ml, n = 8, p < 0.001, respectivly).
Conclusions: The inhibition of NF-κB signaling and VEGF expression in smooth muscle cells by rapamycin supports the concept, that this drug is feasible not only to prevent restenosis formation after angioplasty but may also prevent chronic graft vascular disease or the development of secondary malignomas (due to immunosuppression) by anti-inflammation and anti-angiogenesis.

IN SITU DETECTION OF ROS AND NOS ACTIVITY IN PIG LIVER SUBMITTED TO ISCHEMIA/REPERFUSION: CORRELATION WITH TISSUE METABOLISM ASSESSED BY OPTICAL BIOPSY AND SDH ACTIVITY

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Liver transplantation involves cold ischemic preservation, grafting and reperfusion phases. Ischemia and nutrient depletion affect the metabolism and may cause irreversible damage. Reactive oxygen species (ROS), including nitric oxide (NO), play important roles in these phases (Rauen & De Groot, 2002). We are testing an approach based on the *in situ* demonstration of ROS and NO production and of enzyme activities related to the energy production and on the *in vivo* evaluation of metabolism with an optical biopsy technique to assess non invasively the liver response in the various phases of transplantation. Molecules associated with metabolic processes (*e.g.* NAD(P)H, oxidized flavins, fatty acids) are endogenous fluorophores (Wagnières et al, 1998). Optical biopsy allows to evaluate *in vivo* the autofluorescence spectra of normal and pathological tissues, the contribution of the individual fluorophores to the overall spectrum being assessed by curve fitting (Croce *et al.*, 2003). We report the response of Landrace pig liver preserved for 4h with UW solution at 4°C, grafted (phase comprising a "back-table" period with warm ischemia) and blood perfused for 30 min.

Methodology: Optical biopsy was made *in vivo*, along the transplantation phases. Biopsies were performed and snap frozen at three time points: T0 (pre-transplantation phase, beating heart donor), T1 (end of cold-storage) and T2 (after reperfusion). The following parameters were studied: morphology, glycogen content, ROS production, NOS activity and expression, protein targets of NO (nitrotyrosylated) and SDH activity. Kerver *et al.* DAB-Mn^{2+}-Co^{2+} method (1997) was used to visualize ROS. The production of NO was inferred from the fixation-resistant NADPH diaphorase activity of NO Synthase (NOS), with an improvement of Gossrau's method (Nakos and Gossrau, 1994) obtained by using a tissue stabilizer and a tetrazolium salt optimised for lipid-rich tissues (Freitas *et al.*, 2002). Slides were observed under a light microscope with Nomarkis's differential interference contrast (DIC) (Freitas *et al.*, 2002). SDH activity (index of mitochondrial activity and also of glutaminolysis) was demonstrated with tetrazolium salt method (Van Noorden & Frederiks, 1992). iNOS, eNOS and nitrotyrosine expression were studied with immunofluorescence techniques and multicolour fluorescence detection. Morphology was assessed with H&E and glycogen with PAS reaction. Optical biopsy was performed with excitation at 366 nm with an Optical Multichannel Analyser (OMA) equipped with a fibre-optic probe.

Results: Morphology: With respect to the starting condition (T0), cold preservation (T1), though on the overall preserving tissue morphology, caused portal edema, sinusoid dilatation, hepatocyte shrinkage and presence of leukocytes on the portal connective tissue. Reperfusion (T2) caused partial re-absorption of the edema, decrease of hepatocyte eosinophilia, abundance of leukocytes in sinusoids. Glycogen: Almost nil PAS reaction in T0 (fasted donor) and after cold-preservation (T1); a few PAS^+ hepatocytes in T2. ROS: hepatocytes negative in all time points. In T0 the reaction was positive in a few sinusoidal cells with stellate morphology (fat-storing cells?), especially beneath the Glisson capsule. In T1, the number of ROS^+ sinusoidal cells and leukocytes increased significantly; in T2 it remained high, but with high variability in different tissue sections. iNOS and eNOS expression, activity and targets: Comparison of these reactions indicated that bile duct cells express the eNOS isoform and contain nitrotyrosylated residues and that eNOS expression corresponds to NADPH-diaphorase activity resistant only to pre-fixation, not to fixative in the medium. iNOS was expressed by stellate-like (Ito) cells in T0 and by a few endothelial and Kupffer-like cells, especially in T2; it corresponded to NADPH-diaphorase activity resistant to pre-fixation and fixative in the medium. SDH activity: In T0, SDH was very intense in hepatocytes (periportal-to-centrolobular gradient) and moderately intense in bile duct cells. In T1, SDH activity decreased slightly in periportal and sharply in midzonal and pericentral hepatocytes and in bile ducts to increase slightly after reperfusion (T2), though not

reaching the basal levels. SDH activity was high in leukocytes in the connective tissue and in sinusoids. Autofluorescence: Cold-preservation caused an increase and reperfusion a decrease of the overall autofluorescence intensity, in keeping with first an increase and hence a decrease of free NAD(P)H. The trend of the oxidized flavins and fatty acids signals was in keeping with poor β-oxidation of the latter during the ischemic period with slight resume during reperfusion.
Conclusion: Our data confirm that cold preservation slows down the liver metabolism and preserves its capacity to respond to the ensuing reperfusion. Optical biopsy confirmed its potentiality to detect and follow in real time *in vivo* alterations of organ metabolism. NOS activity in fat-storing cells is compatible with their role of modulators of the sinusoidal tone; eNOS expression and moderate activity in bile ducts might have a similar role. On the contrary, iNOS expression and activity might be an index of injury. (Funds from FAR-UniPV and MIUR-COFIN 1999,2001).

RATG INDUCTION THERAPY: BOLUS VS.STANDARD ADMINISTRATION . MID TERM RESULTS IN CARDIAC TRANSPLANTATION

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Objective: Due to the onset of several possible both immediate and long term complications, optimal induction therapy after heart transplant (HTX) is still under investigation. To evaluate the clinical impact of a bolus R ATG (*Fresenius* ®-S) anti rejection prophylaxis administration vs. standard dosage R ATG (*Fresenius* ®-S) therapy course, a prospective randomised trial was carried out. Here we report and comment seven years follow up clinical outcome.
Materials and methods: July 1997 to March 1999 30 patients (65% males, 35% females, aged 34 to 62 y., mean 43±8y.) underwent to HTX for dilated cardiomyopathy (55%) or other reasons (45%). According to their anti rejection prophylaxis, patients (pt.) population was random subdivided in two groups. Sixteen pts.(54%) receiving R ATG bolus therapy course (a single intra operative pulse followed by three more pulses at a dosage of 8 mg./kg./day) were enrolled in G1, while the remaining 14 pts. (46%) had standard e.v. R ATG (2-5 mg./kg./day for eight days) and they were included in G2. Differences in terms of age, sex distribution and preoperative cause of heart failure were not detected. Pts. were follow up from 63 to 43 mos., m. 49 mos ± 16 mos.).To diagnose acute or persistent acute rejection episodes routine endomyocardial biopsies (EMB) were scheduled at regular time interval. Specimens were classified according ISHLT criteria. Acute rejection episodes exceeding grade 3 were treated with e.v. administration of three pulses of Methilprednisolone. Chronic rejection was assessed though routine annual angiography. Annual CT scan was scheduled in order to find out malignancies. Infective events requiring hospital stay and specific antimicrobic therapy were considered as well. Renal and liver function were assessed through routine lab report and annual abdominal ECHO.
Results: Seven years actuarial survival rate was 100% in G2 vs. 93,8% in G1, P:=0.341. Graft survival comparison showed seven years rate of 93,8% in G1 vs. 100% in G2, P.=0,922.Mean number of rejection episodes /pt. Was 2,50 ± 2,24 in G1 vs. 2,69 ± 2,50 in G2, P.=0,8. Steroid resistant rejection was found in 1 G1 pts., while none of G2 pts. had it. Chronic rejection was found in 1 G1 and in 1 G2 pt., P.=0,922. Infections requiring hospitalisation were 8 in G1 pts. and 8 in G2 pts.,P.=0,696. Malignancies were diagnosed in 2 G1 and in 1 G1pts, P.=0,584. Chronic renal failure affected 11 G1 and 11 G2 pts., P.=0,8. Chronic liver failure was observed in 3 G1 pts. (P.=0,11)
Conclusions: Bolus RATG e.v. administration can be considered safe and effective. Although it does not reach statistical significance it seem to have an inferior rate of complication occurence if compared to standard e.v. administration. A more expanded pts. series is therefore needed.

POSTTRANSPLANT LEVELS OF NEOPTERIN PREDICT LONG-TERM GRAFT SURVIVAL

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Chronic allograft nephropathy (CAN) and death with functioning graft due to cardiovascular disease (CVD) are the key determinants for long-term graft survival. CAN and CVD are seen as manifestations of a single disease entity with a common pathogenesis including inflammation and accelerated atherogenesis. Therefore we investigated the relation between early posttransplant inflammatory burden and long-term graft survival.
In 64 consecutive renal transplant patients the acute phase reactants serum amyloid A (SAA) and serum C-reactive protein (S-CRP) as well as the macrophage product neopterin in urine (U-NEOP) and serum (S-NEOP) were determined daily during the immediate postoperative period (mean p.o.obs. x=29.2±8.7 days, total of Σ =1869 days). SAA and CRP were measured with high-sensitive assays (in mg/l; immune nephelometry, Dade Behring Co., Marburg, Germany), NEOP was measured with ELISA-technique (Brahms, Berlin, Germany) and related to serum and urine creatinine levels, resp. (in umol/mol creatinine). The association between the mean values of these parameters and the survival distribution function of the 64 patients was tested using the log rank test and the Wilcoxon test. In this analysis graft loss was defined as either resumption of dialysis treatment or patient death with functioning graft.
The 1- and 5- year graft survival rates in our patients were 93% and 76%, resp. The markers showed the following mean posttransplant levels: S-CRP x=22.3±21.9 mg/l, SAA x=10.0±9.9 mg/l, U-NEOP x=714±680 umol/mol creatinine and S-NEOP x=89±79 umol/mol creatinine. Both the log rank test and the Wilcoxon test provide evidence that the graft survival is negatively related to the posttranspalnt levels of U-NEOP (p=0.0085 and p=0.0271, resp.) and S-NEOP (p=0.0489 and p=0.0734, resp.). Whereas SAA (p=0.5997 and p=0.7415, resp.) and S-CRP (p=0.3576 and p=0.3814, resp.) did not reach statistical significance.
These findings support the impact of the inflammatory burden on graft survival. In paticular, elevated posttransplant neopterin values, reflecting activated innate and adaptive responses, are predictive for long-term graft outcome.

THE THERAPY WITH MYCOPHENOLATE MOFETIL ALTERS GUANOSINE-5'-TRIPHOSPHATE LEVELS IN RED BLOOD CELLS OF HEART TRANSPLANT RECIPIENTS

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Mycophenolate mofetil (MMF) in combination with cyclosporine A (CsA) and corticosteroids is used for the prevention of rejection in transplant recipients. In-vivo MMF is degraded to mycophenolic acid (MPA), the active metabolite. MPA acts as immunosuppressive drug by inhibiting inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the de-novo-synthesis of guanine nucleotides. IMPDH oxidizes inosine-5'-monophosphate (IMP) to xanthosine-5'-monophosphate (XMP), which is then converted to guanosine-5'-monophosphate (GMP). Monitoring of plasma concentrations of MPA is complicated by the fact that this drug is highly protein bound, and free MPA is presumed to be the pharmacologically active fraction of the drug. In an attempt to find a long-term parameter for MPA monitoring as - for example - glycosylated hemoglobin is used for monitoring diabetes mellitus, we measured the two energy-rich-phosphates guanosine-5'-triphosphate (GTP) and adenosine-5'-triphosphate (ATP) by means of HPLC in the red blood cells (RBCs) of the transplanted patients. HTX-patients treated with the conventional triple therapy including azathioprine (AZA) served as control group. In the MMF-group the mean MPA trough-level was 4.8 µmol/l (range: 0.5-12.9).

MONTHS AFTER HTX	Control Group ATP (pmol/10^6 RBCs)	MMF Group ATP (pmol/10^6 RBCs)	Control Group GTP (pmol/10^6 RBCs)	MMF Group GTP (pmol/10^6 RBCs)
Pre	143±28	139±29	6.4±2.1	7.0±3.1
1-2	151±28	136±281	7.2±2.7	9.4±3.1*
2-6	157±27	133±31*	7.2±4.2	13.0±5.0
6-12	155±30	140±27	6.0±4.4	16.1±6.9
>12	150±29	135±34	6.5±3.3	14.3±6.3*

*=p< 0.001 compared to AZA treated control group

The results, listed in the table, were unexpected, since a continuous increase in GTP levels in the RBCs of MMF treated patients was found. Similar results were seen analyzing whole blood.
In a second step IMP-DH activity was measured in RBCs. Thereby, an increase in IMPDH activity by more than 380% was observed in patients treated with MMF compared to the controls. Against all expectations, our data shows that especially in long-term MPA treated patients, IMP-DH activity increases. Nevertheless, the clinical results show less side-effects and a significant reduction in transplant rejections in comparison to therapies including azathioprine. Thus, the mechanism of action of MPA has to be urgently reassessed.

CELL CYCLE ANALYSIS OF CD133+ AND CD133- CELLS ISOLATED FROM UMBILICAL CORD BLOOD

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Umbilical cord blood cells (stem/progenitor cells) exhibit high proliferative capacities leading to a large expansion of cells in appropriate cell culture conditions.
The aim of this study was to evaluate by flow cytometry the cycling status of CD133+ and CD133- cells depending on various culture conditions, such as sera, stem cell factor (SCF), interleukin 3 (IL-3) and interleukin 6 (IL-6).
An immunomagnetic system was used for cell separation. CD133+ and CD133- cells were seeded in Iscove's modified Dulbecco's medium (IMDM) with different serum concentrations and were stimulated with SCF (100 ng/ml), IL-3 (50 ng/ml) and IL-6 (50 ng/ml).
Our experiments demonstrated that immediately after separation 96.0 ± 0.6% of CD133+ cells and 98.8 ± 0.2% of CD133- cells were in G_0/G_1 phase, while 2.9 ± 0.4% and 0.6 ± 0.05% were in the S-phase, respectively. After one week cultivation in IMDM supplemented with horse serum (25%) 25.0 ± 3.5% of CD133+ and 30.7 ± 1.3% of CD133- cells were detected in the S-phase. A combination of horse and fetal calf serum (12.5% each) yielded 30.0 ± 0.6% and 27.0 ± 5.2% CD133+ and CD133- cells in the S-phase, respectively. Serum concentrations of 12.5% did not change cell cycle characteristics in CD133+ cells while resulting in lower proportions of CD133- cells in S-phase.
In conclusion, our data indicate that the source and concentration of the serum used for cultivation of stem/progenitor cells have an impact on *in vitro* expansion. The cell cycle properties of CD133+ and CD133- seem to be different.

TRANSPLANTATION OF HUMAN ISLETS; IMMUNOSUPPRESSIVE PROTOCOLS AND LABORATORY ASSESSMENTS

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By human islet auto-transplantation after pancreatectomy it has proven that the method of islet isolation and intraportal application works in principle. Isl*et* allo-transplantation with limited success is performed since many years. A new protocol was introduced in 2000 by a group in Edmonton. Standardised enzyme perfusion of the pancreas, the use of albumin instead of xenoproteins, a standardised purification procedure improved the isolation in the laboratory. Selection of patients, the administration of sirolimus and steroid free regimen improved the success of islet transplantation in the clinic.

Islets are derived from organ donors and isolated by enzymatic digestion and mechanical disruption. The most critical step is the digestion process. The maximal number of free islets without disruption and a high viability of cells must be obtained. Since only 2% of pancreatic tissue are hormone producing cell aggregates the acinar tissue must be removed by density gradient centrifugation. This work has to be performed under highly sterile conditions in an GMP facility. Cells are administered into the portal vein by a transcutaneous, transhepatic route. In the immediate posttransplant period islets get stuck in the capillaries where the engraft and find a connection to the vascular system. Longest function of islet is now in the range of more than ten years.

The immunosuppressive protocol for islet transplantation has changed during the years. Induction with antibodies and also the application of steroids has been performed. The most successful and well tolerated steroid sparing protocol has been introduced by the Edmonton group using an induction with a chimeric humanised anti IL-2 antibody, low dose tacrolimus and sirolimus. Blockage of the IL-2 signal by sirolimus has proven to be superior to the immunosuppressive effect of other antimetabolites. Since the main immunosuppressive effect is based on sirolimus an exact monitoring of trough levels has to be performed. Comparable to other immunosuppressive drugs metabolism varies between patients. There is no immunological test available at present. Due to the fact that most of the substance is membrane bound we use a solid phase extraction extraction procedure from whole blood must be performed. After HPLC separation on a reversed phase column detection is performed either by UV detection or with higher sensitivity and specificity by electrospray mass spectrometry. Usually low blood levels of immunosuppressive substances are close to the detection limit and determination of blood levels must be performed under highly controlled conditions.

CLINICAL OUTCOME OF HEART TRANSPLANTATION

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Recent trials using new immunosuppressive regimens revealed that rejection might not be any further a major problem after heart transplantation (HTx).
Results form our studies suggest that the combination therapy with tacrolimus (Tac), MMF, and steroids prevents acute rejection completely after HTx when MMF and tacrolimus doses are adjusted within the target ranges. This strategy also allows complete withdrawal of steroids early after transplantation.
Comparison between the combinations Tac/MMF and cyclosporine (CsA)/MMF indicates that Tac/MMF is more efficacious for prevention of acute rejection episodes, and that CsA treated patients need a higher MMF dosage in order to achieve similar MMF trough levels when compared to patients receiving Tac.
The combination of Tac and Sirolimus (Sir) seems to allow the use of low target trough levels for each compound without increasing the risk for rejection. In addition, viral infections can be almost completely abolished.
Therefore, the transplantation research should shift the focus from rejection incidence and short term survival to improvement of quality of life and long term survival. Those factors, however, depend again primarily on the composition and dosing of the posttransplant rejection therapies used since each of the compounds is associated with a different profile of safety parameters:
Common direct or indirect adverse effects of immunosuppression like nephrotoxicity, diabetes, hypertension are well recognized and documented. Others, such as gastrointestinal toxicity, neurotoxicity, hyperlipidemia, effects on bone metabolism and cosmetic factors are considered less significant since they do not directly affect the survival of the patients. They do, however, have a significant influence on the quality of life and therefore on the compliance of the patients. A third group of factors having a major impact on the long term mortality after HTx - graft vessel disease, number and severity of infectious episodes and incidence of tumors - is directly or indirectly associated with the potency and specificity of the immunosuppressive combination used. Besides suppressing the recipient's immune system, the newer compounds might have an additional direct effect on these diseases resulting in a potential benefit for the patients.
Based on the knowledge of the impact of the different compounds on the long term morbidity, major consideration should be given to the development of a posttransplant treatment protocol, which maintains the immunosuppressive potency necessary to prevent rejection, limits the severity and impact of toxic side effects (rather than treatment of its consequences) and takes advantage of potential beneficial effects.