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FACTOR XII, PLASMA PREKALLIKREIN, α_2-MACROGLOBULIN AND C1-INHIBITOR LEVELS IN RENAL ALLOGRAFT RECIPIENTS DURING IMMUNOSUPPRESSION WITH CYCLOSPORIN A.

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Factor XII clotting activity (FXII), Plasma Prekallikrein amidolytic activity (PK), 2-Macroglobulin (α_2-M) and $C\bar{1}$-Inhibitor ($C\bar{1}$-Inh) antigen have been measured in 17 patients immediately before and sequentially up to four months after kidney transplantation. Based on suspected Cyclosporin A (CyA) induced endothelial damage, activation of the contact system with resulting consumption of the contact activation factors was evaluated. Before transplantation, FXII, PK, α_2-M, $C\bar{1}$-Inh levels were 99±27%, 102±21%, 115±55%, and 129±32%, respectively. In the first two weeks after transplantation FXII decreased to 65±27%, PK to 67±20% and α_2-M to 88±42%; $C\bar{1}$-Inh rose to a maximum of 201±44% (mean ± S.D.)($2p < 0.001$). Mean FXII levels correlated positively with PK, α_2-M and albumin and negatively with CyA level and dose and serum bilirubin. PK and α_2-M correlated positively with each other and with albumin and negatively with creatinine, bilirubin and CyA ($p < 0.01$). The changes of FXII, PK and α_2-M after transplantation suggest an influence of CyA on production or consumption of these factors. The behaviour of the $C\bar{1}$-Inh may be unspecific and related to its action of an acute phase reactant.

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CYCLOSPORIN A (CyA) INDUCED ENDOTHELIAL CELL INJURY.

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The chronic administration of CyA to animals and humans to prevent graft rejection may induce renal arteriolar damage resembling hemolytic uremic syndrome (HUS). This is a syndrome of vascular damage with thrombotic occlusions of the microcirculation. Endothelial damage is considered the first event in the pathogenetic cascade leading to HUS. We have used bovine aortic endothelial cells in culture to address the issue of CyA-induced arteriolar damage. CyA-induced a time (1-24 hours) and dose (1-50 μM) dependent cell damage. CyA-induced injury was characterized by an early cell detachment followed by lysis as documented by the increase in LDH and Cr release. 1 μM CyA did not induce cell detachment and lysis was evident only after prolonged incubations. 10 and 50 μM CyA both induced marked cell detachment and lysis: lysis started 3 hours after incubation of endothelial cells with CyA and was maximal at the end of 24 hour incubation (LDH release, percent specific increase over control values: 10 μM CyA, 47%; 50 μM CyA, 70%; ^{51}Cr release, percent specific increase over control values: 10 μM CyA, 28%; 50 μM CyA, 34%). CyA-induced injury was associated with dose- and time-depedent increase in prostacyclin (PGI2) and thromboxane A2 (TxA2) release by endothelial cells exposed to 10 and 50 μM CyA. CyA-induced generation of PGI2 and TxA2 was inhibited when the incubations were carried-on in the presence of acetyl salicilic acid (500 μM). These studies indicate that CyA exerts a direct toxic effect on endothelial cells and might help to understand the pathogenesis of CyA-induced vascular damage.

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FACTOR VIII:C (F VIII:C) AND VON WILLEBRAND FACTOR ANTIGEN (vWFag) IN RENAL TRANSPLANT RECIPIENTS IMMUNOSUPPRESSED WITH CYCLOSPORIN A (CyA).

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In 17 consecutive cadaveric kidney transplant recipients F VIII:C and vWFag were repeatedly determined before transplantation and during 4 months thereafter. Graft biopsy was performed in 12 patients for deterioration of renal function. F VIII:C was determined by a one stage clotting assay using F VIII:C deficient substrate plasma. vWFag was assayed by electroimmunoassay using specific rabbit anti-human vWFag antibodies. Results of F VIII:C and vWFag are expressed referring to NHP as 100%.

Results:

2/17 patients lost their graft due to irreversible vascular rejection, 2/17 patients had reversible vascular rejection, 2/17 patients developped glomerulonephritis, 6/17 patients showed acute or chronic CyA nephrotoxicity. In 5/17 patients graft biopsy was not necessary. Despite normalisation of renal function (serum creatinine levels <150umol/L) in 9 out of 17 patients F VIII:C (239 ± 66% to 408 ± 74%, mean ± SD) remained elevated in all 17 patients. vWFag (181 ± 29% to 454 ± 84%) was normalised in only 2 out of 17 patients. CyA dosis and CyA blood levels were not correlated with F VIII:C and vWFag. All 4 patients with histological vascular rejection, both patients with later developping glomerulonephritis and 3 out of 6 patients with later developping CyA nephrotoxicity showed F VIII:C/vWFag quotients >1 (1,1 to 1,3) Four out of 5 patients with well functioning graft had F VIII:C/vWFag quotients consistently <1 (0,68 to 0,92).

Conclusion:

1. The elevated F VIII:C and vWFag levels in chronic renal failure are not normalised during 4 months of observation despite normalisation of renal function by transplantation and immunosuppression with CyA.
2. A quotient F VIII:C/vWFag <1 may indicate a good prognosis for kidney allograft function in the absence of CyA nephrotoxicity whereas later developping graft rejection or glomerulonephritis were associated with F VIII:C/vWFag >1.

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THE EFFECTS OF CYCLOSPORIN A (CyA) ON PLATELET FUNCTION IN HUMAN RENAL ALLOGRAFT RECIPIENTS AND NZW RABBITS.

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CyA therapy is associated with microvascular thrombotic complications both in human allograft recipients and experimental animals. We have assessed evidence for increased platelet reactivity, i.e. presence of spontaneous aggregation, responses in platelet rich plasma (PRP) to ADP, total platelet nucleotide content and release to 20μg/ml collagen, and sensitivity to ZK 36,374, a prostacyclin analogue, serially in 21 human renal allograft recipients for 1 year post-transplantation and in 15 O NZW rabbits which received CyA 25mg/kg (n=5), or 'placebo' (the carrier for CyA) (n=5), or N saline (n=5) i.v. for 10 consecutive days. In the humans, spontaneous aggregation was observed on 10 occasions in 5 patients and responses to low doses of ADP, 0.5 and 1.0 μM, were significantly increased compared to normal controls up to 1 year ($p<0.002$) post transplantation. Total platelet nucleotide (ATP + ADP) content was significantly decreased ($p<0.002$) up to 3 months post-transplantation, indicative of in vivo activation, as was nucleotide release. Platelet sensitivity to ZK 36,374 decreased after 2 months ($p<0.01$) and this persisted at 1 year ($p<0.02$) compared to sensitivity at 1 week post transplantation (paired t tests). In the animal model, CyA or 'placebo' administration were unassociated with spontaneous aggregation, responses to ADP 0.5-5.0 μM and collagen 2.0-4.0 μg/ml remained unchanged as did sensitivity to ZK 36,374. In conclusion, CyA-treated renal allograft recipients exhibit spontaneous platelet aggregation, hyperaggregability to low doses of ADP, in vivo activation and decreased sensitivity to ZK 36,374, with abnormalities persisting up to one year post-transplantation. These abnormalities are not reproducible in an animal model.