

## Does cyclosporine inhibit *in vivo* hepatitis C virus replication? – a pilot study

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Hepatitis C virus (HCV)-related recurrent disease is one of the most important comorbidities after liver transplantation (LTx). Clinical analyses of outcome indicate a high rate of post-transplant progressive graft injury leading to fibrosis and cirrhosis, resulting in the graft loss. It is estimated that cirrhosis occurs in 25–33% of HCV-positive recipients within 5 years [1] and 1–5% of patients will develop rapidly progressive fibrosing cholestatic hepatitis [2]. This observation became especially evident since mid 1990s. Although factors influencing poor outcomes are not fully understood, more aggressive immunosuppression and older donor age, have been discussed as possible reasons for HCV-related disease acceleration [3,4,5]. Current aggressive and multidrug immunosuppressive regimens as well as treatment of acute cellular rejection episodes are associated with disease acceleration, with no single immunosuppressive agent considered superior with respect to influence on recurrent HCV disease [6].

An issue of calcineurin inhibitors role in HCV-related disease progression became valid again following publications of a few studies presenting evidence for *in vitro* inhibition of HCV replication by cyclosporine A (CsA) [7,8,9]. There was also data that CsA in combination with interferon improved chronic C hepatitis treatment results [10,11]. However, data on *in vivo* inhibition of HCV replication by CsA remain insufficient. We analysed HCV viral load and liver enzymes activity in patients who were initially on tacrolimus (TAC) based regimens and were subsequently converted to CsA.

Among 84 patients who underwent LTx in years 2001–2005, a total number of 32 subjects was transplanted due to the HCV-related end-stage liver disease (38,1%). Twenty-one patients initially received combination of TAC, mycophenolate mofetil (MMF) and steroids (withdrawn within 6 months), while in four patients CsA, MMF and steroids (withdrawn within 6 months) have been initiated. Pulse steroid therapy for rejection was used in one patient only. All patients were longitudinally followed-up by monthly biochemical evaluation in the first year after transplantation and by bi-monthly evaluation thereafter. Five TAC subjects were converted to CsA due to poor diabetes mellitus (DM) control after a med-

ian time of 17 months (range 3–59 months) post-transplant.

Serum HCV load was measured by the quantitative PCR technique (RT PCR, HCV Test v. 2.0 Cobas AmpliCor Monitor; Roche) at a time point of the first post-transplant aminotransferase increase at least two times the upper limit of normal, and compared to the retrospectively examined pre-transplant viraemia from the –80 °C stored serum samples obtained prior to transplantation in each patient. In five cases, viral load was also measured 1 month after conversion from TAC to CsA. For the purpose of this report control examination of viraemia was performed after a median time of 12 months in all converted patients as well as in the six available TAC patients who were not treated with interferon and ribavirin.

Results were analysed statistically by Mann–Whitney and Wilcoxon's matched pairs test for comparisons of viraemia using licensed Statistica 6.0 PL (Statsoft, Krakow, Poland) program. Normal distribution was checked by Shapiro–Wilk test.

Aminotransferases higher than 2× baseline (>80 U/l), confirmed by histological examination as an HCV-related hepatitis were observed in 21 patients (84%) after a median time of 15 months from LTx (range 3–59 months). Significant increase of the median viral load from pre-transplant value of  $1.27 \times 10^6$  (range  $5.43 \times 10^3$ – $3.65 \times 10^7$  UI/ml) to  $4.45 \times 10^6$  UI/ml (range  $1.11 \times 10^5$ – $6.9 \times 10^7$  UI/ml,  $P < 0.03125$ ) was noted.

In four patients, aminotransferases remained normal. Three of them were HCV-RNA positive and HCV-RNA was repeatedly negative in one case (pretransplant value  $1.11 \times 10^4$  UI/ml, genotype 3a).

In each of five patients with chronic recurrent C hepatitis, converted from TAC to CsA due to instable DM, a significant drop in Alanine aminotransferase (ALT)/aspartate aminotransferase (AST) activity was noted as well as reduction in HCV viraemia measured 1 month after conversion. Median viral load decreased from  $4.37 \times 10^6$  to  $1.69 \times 10^6$  UI/ml (Table 1). This difference, however, was not statistically significant probably due to a high dispersion of results.

**Table 1.** Alanine aminotransferase (ALT)/aspartate aminotransferase (AST) and hepatitis C virus viral load before and after conversion from tacrolimus (TAC) to cyclosporine A (CsA).

Studied parameter	On TAC (median and range)	On CsA (median and range)	Statistical significance
ALT	204 U/l (164–283)	105 U/l (59–129)	$P < 0.0431$
AST	127 U/l (61–283)	78 U/l (56–113)	$P < 0.0431$
Viral load	4.370.000 UI/ml (2.950.000–24.400.000)	1.695.000 UI/ml (162.000–21.800.000)	$P < 0.0797$

**Table 2.** Alanine aminotransferase (ALT) activity and hepatitis C virus viral load in the follow-up period.

Studied parameter	1 month after conversion	12 months after conversion	Statistical significance
ALT on CsA	105 U/l (59–129)	57 U/l (37–108)	$P < 0.01$
Viral load on CsA	1.695.000 UI/ml (162.000–21.800.000)	1.450.000 UI/ml (78.600–10.600.000)	$P = 0.3$
Viral load on TAC	4.370.000 UI/ml (2.950.000–24.400.000)	5.520.000 UI/ml (3.510.000–36.500.000)	$P = 0.22$

CsA, cyclosporine A; TAC, tacrolimus.

After a median time of 12 months since conversion from TAC to CsA, further significant aminotransferase decrease was observed. Now median ALT value in this group is 57 U/l (range 37–108 U/l) and none of these patients implicitly requires antiviral treatment. HCV viral load did not change significantly in the follow-up period neither in the CsA group nor in the TAC patients who were not treated with interferon and ribavirin, still being insignificantly higher in patients on TAC (Table 2).

Recurrence of HCV infection after LTx is imminent. In our study, only one patient with low pre-transplant viral load and genotype 3a remained HCV-RNA negative after LTx. Elevation of liver enzymes and histologically confirmed recurrence of HCV-related hepatitis also concerned majority of patients, although time to hepatitis and severity of reinfection varied widely. Elevated liver enzymes were accompanied by the increase of viraemia in comparison to pre-transplant viral load and onset of DM in 20% of cases. This is consistent with previously published observations [12,13]. Time to the first significant ALT/AST increase did not correlate with the type of calcineurin inhibitor in our study, although some authors reported longer time to hepatitis with CsA than with TAC [14,15]. It must be noted, however, that only four patients in our group received cyclosporine as an induction immunosuppressive therapy after transplantation.

However, we have made an interesting observation concerning decreased ALT/AST activity and viral load soon after conversion from TAC to CsA. Similar results were reported by Lorho *et al.* [16]. Favourable biochemical response in our group could be partly attributed to a better glucose metabolism on CsA which is less diabetogenic than TAC, but direct influence of CsA on HCV replication and subsequently on hepatitis can be also postulated. It is worth emphasizing, that HCV viraemia

remained stable on CsA and a continuous improvement of disease activity has been observed in the follow-up period. Albeit comparisons of viral load did not reach statistical significance, results are encouraging and further examinations are warranted to conclude whether cyclosporine suppresses *in vivo* HCV replication and if so, whether this is related to any clinical implication on HCV-related disease progression.

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