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Successful immunomodulating in AIDS patients with ursodeoxycholic acid—a pilot study

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SUMMARY

Based on the positive therapeutic results with ursodeoxycholic acid (UDCA) in patients with primary biliary cirrhosis, in whom we observed a clinical improvement in conjunction with the normalization of the low pretreatment dipeptidyl peptidase (DPIV, CD26) expression of peripheral blood lymphocytes (PBL), we hypothesized that the very low DPIV expression in AIDS patients could be positively influenced by UDCA. Four young male AIDS patients were therefore treated with 750 mg of UDCA for 4 months. The low CD26 expression (2–8% of the PBL *versus* 18–28% in healthy controls) at the beginning of the study rose to 10–16% after UDCA therapy. Simultaneously we observed a two-to-three-fold elevation of the absolute number of lymphocytes as well as a slight increase of $CD4^+$ cells. These effects were similar in all examined patients. Further investigations should be conducted on this potentially beneficial effect of UDCA.

Keywords ursodeoxycholic acid AIDS dipeptidyl peptidase primary biliary cirrhosis peripheral blood lymphocytes

INTRODUCTION

Infection of cells by HIV is initiated by an interaction of the virus surface envelope glycoprotein gp120 and the virus receptor CD4 on lymphocytes, monocytes and macrophages [1]. However, this interaction alone is not sufficient to produce the infectious process, since the $CD4^+$ cells only bind the virus without definitely leading to their infection. Therefore, it was suggested that other host cell-specific factors would be necessary to interact with the HIV surface protein and activate the fusion domain of the viral gp41. Recent investigations [2–4] have proposed that dipeptidyl peptidase IV (CD26) could be one responsible molecule. CD26, an ectoenzyme in the cell membrane of human T lymphocytes, is an important constituent in the process of lymphocyte activation and proliferation, as well as a functional characteristic of IL-2-producing cells [5,6]. HIV-infected patients were shown to display a decreased expression of CD26 [7–9].

Ursodeoxycholic acid (UDCA), a hydrophilic bile acid, is widely used as an anti-cholestatic drug in primary biliary cirrhosis (PBC) [10]. The immunomodulatory effect of UDCA is proposed as one of the underlying mechanisms for its beneficial effects [11]. Thus, patients with PBC were found to reveal significantly decreased expression of CD26. Therapy with UDCA in these patients leads to normalization of this expression and increased production of IL-2 [10].

Therefore, we hypothesized that UDCA could also play an immunomodulatory role in AIDS patients and increase their reduced CD26 expression. To test this hypothesis we examined the effect of UDCA therapy on several immunological parameters, such as expression of CD26, CD4, CD8, IL-2 and interferon-gamma (IFN-γ) production in HIV-infected patients.

MATERIALS AND METHODS

We conducted a pilot study with UDCA therapy in four young men with AIDS. The patients were recruited from our AIDS clinic and included into the study after giving their written consent. The protocol of the study was approved by the local Ethical Committee of the University Clinic in Dresden. UDCA was applied orally in dosage 10 mg/kg body weight for 4 months. The patients were in a stable clinical phase. Two of them were under regular antiviral therapy with retrovir and videx for 2 years before and during the study. The other patients had not been treated with specific antiviral drugs. The $CD4^+$ lymphocytes were in the range of 162–401 cells/μl at baseline. The expression of dipeptidyl peptidase (DPIV; CD26) of peripheral blood lymphocytes (PBL) was determined before, during and after UDCA therapy. PBL were isolated from blood by density gradient centrifugation [12]. Cells were collected at the interphase and washed twice. Cell numbers

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Table 1. Data of the examined patients before, during and after ursodeoxycholic acid (UDCA) treatment

Variable	Patient	−6 months	−3 months	Baseline*	2 months	3 months	4 months†	7 months	10 months
CD4/μl	1	351	417	351			443	495	312
	2	545	495	401			646	495	658
	3	335	392	324			470	449	522
	4	225	162	162			258	183	158
CD8/μl	1	1035	1392	1035			1748	2546	1514
	2	3016	3509	2642			3078	2546	3455
	3	694	862	938			891	1170	1144
	4	990	658	658			959	987	1040
Ly$\times 10^6$/ml	1			0·5	0·7	4·6	3·6		6·2
	2			1·0	1·6	4·5	4·0		11·3
	3			2·0	1·8	4·6	4·0		6·6
	4			0·8	1·0	2·0	2·3		4·3
CD26 %	1			3	5	10	10		11
	2			3	8	10	13		8
	3			2	2	16	16		16
	4			8	10	12	16		20

*Medication start.
†UDCA discontinuation.

and viability were assessed by acridine orange staining. Viability was $\geq 95\%$. PBL were counted by light microscopy. Cytochemical staining for dipeptidyl peptidase IV-positive PBL ($DPIV^+$) was performed after a modification of the method by Lojda [13]. $DPIV^+$ lymphocytes were detected in a histochemical reaction by incubation with glycyl-proline-4-methoxy-β-naphthylamide. $CD4^+$ and $CD8^+$ T cells were detected by means of flow cytometry.

RESULTS

All patients showed very low expression of DPIV (between 2% and 8%) before UDCA therapy (Table 1). The healthy controls ($n = 20$) exhibited DPIV expression between 18% and 28%. The absolute number of lymphocytes in the examined patients was considerably lower in comparison with the healthy subjects ($1{\cdot}1 \times 10^6$ *versus* $4{\cdot}0 \times 10^6$).

Surprisingly the percentage of $DPIV^+$ ($CD26^+$) PBL increased after UDCA treatment, reaching 10–16% after 4 months. The absolute number of lymphocytes was also improved in all patients after UDCA treatment, with a complete normalization in three of the patients after 4 months therapy. The drug safety and tolerability appeared to be very good, with no side-effects observed. The $CD4^+$ PBL after UDCA treatment were increased in comparison with pretreatment levels in all patients. CD26 expression 6 months after the end of the study remained increased in three patients and fell slightly in one patient, although remaining higher than baseline. The other laboratory parameters showed no significant alterations.

DISCUSSION

In a previous study we demonstrated an immunomodulatory effect of UDCA in 22 patients with PBC [11]. DPIV expression in PBL in every PBC patient was lower than the normal range in healthy controls, but after 4–8 weeks of UDCA treatment reached normal ranges. Simultaneously we observed a significant improvement in liver function. These findings suggest that UDCA exerts a normalizing effect on immunocompetent cells and positively influences their immunoregulatory capacity.

Bile acids were shown to completely inactivate HIV-1 *in vitro* and destroy all the cultured persistently HIV-1-infected T cells, as well as selectively inhibiting the replication of HIV-1 [14,15]. Chan *et al*. showed the positive effect of UDCA therapy on AIDS-associated cholangitis [16]. As UDCA is a well tolerated and safe drug we thought it reasonable to test its therapeutic effect in HIV-infected subjects who have decreased CD26 expression [7,8]. Our results suggest a beneficial effect of UDCA in AIDS, with elevation of DPIV (CD26) expression and of the absolute lymphocyte number. CD26 is a known marker of IL-2-producing lymphocytes. In addition, we observed an increase of the $CD4^+$ PBL. Recently UDCA was found to suppress antigen-specific $CD4^+$ T cell-mediated apoptosis of target cells [17], and apoptosis appears to be the cause of the HIV-associated T cell defect. This could partly explain the immunomodulatory effect of UDCA.

Our study provides preliminary data for a potentially beneficial effect of UDCA in the treatment of AIDS. However, our trial was conducted on a small number of subjects and no virological parameters were examined. Further investigations will be necessary to scrutinize the immunomodulating effect of UDCA in carefully designed clinical trials with larger numbers of patients.

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