Alpha-Lipoic Acid is an Effective Inhibitor of Human Immuno-deficiency Virus (HIV-1) Replication

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Summary. Alpha-lipoic acid, a naturally occurring disulfide-compound that acts as a cellular coenzyme, inhibits replication of HIV-1 in cultured lymphoid T-cells. Alpha-lipoic acid was added 16 hours after infection of the T-cell lines Jurkat, SupT1 and Molt-4 with HTLV IIIB and HIV-1 Wal (a wild type HIV-1 isolate). We observed a dose dependent inhibition of HIV-1-replication in CPE (Cytopathic effect) formation, reverse transcriptase activity and plaque formation on CD4-transformed HeLa-cells. An over 90% reduction of reverse transcriptase activity could be achieved with 70 μg alpha-lipoic acid/ml, a complete reduction of plaque-forming units at concentrations of ≥35 μg alpha-lipoic acid/ml. An augmentation of the antiviral activity was seen by combination of zidovudine and low dose of alpha-lipoic acid (7 μg/ml). Trypan blue staining revealed no toxic effects of alpha-lipoic acids on peripheral blood mononuclear cells and T-cell lines even in concentrations of ≥70 μg/ml. Therefore, we propose the inclusion of alpha-lipoic acid into chemotherapy trials in combination with zidovudine.

Key words: HIV inhibition – Alpha-lipoic acid-therapy

Alpha-lipoic acid, a naturally occurring disulfide-compound that acts as a cellular coenzyme, can be given over longer periods with only few side effects, and it penetrates into most cell types including lymphoid and neuronal cells [4]. Other compounds with mercapto-groups have also been shown to inhibit the replication of HIV in cell culture. However, these drugs are probably not useful for a continued application in HIV-infected individuals because of adverse side effects, or have not been given to HIV-patients in a clinical trial so far [1, 5, 8]. Investigating if alpha-lipoic acid reveals similar effects on HIV, we found that the compound clearly inhibits virus replication in cultured lymphoid T-cells.

Methods

The T-cell lines Jurkat-, SupT1- and Molt-4 were infected with various multiplicities of cell-free virus (MOI = 10; 1; 0.1) for 16 hours. Two HIV strains, HTLV IIIB and HIV-1 Wal were used, the latter isolated from peripheral blood mononuclear cells (PBMC) of a heterosexual female. After 16 hours, the cells were washed twice and distributed in a 24-well cluster plate. Alpha-lipoic acid was added in a single dose at increasing concentrations (7–70 μg/ml) to the culture medium. Control experiments were performed in parallel with the solvent benzyl-alcohol. Cell toxicity was assessed by thymidine incorporation and trypan-blue staining with PHA-stimulated PBMC and T-cell lines. CPE-formation, reverse transcriptase activity [3] and plaque formation on CD4 transformed HeLa cells [2] were determined 4 to 7 days after infection and compared with untreated controls.

Results

An inhibition of cytopathic changes was consistently observed in more than 15 experiments with
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Fig. 1. Dose dependent reduction of reverse transcriptase (RT)-activity and plaque formation in HIV-infected cell cultures by alpha-lipoic acid. Jurkat cells were infected with HIV-1 Wal at a MOI of 1.0. Alpha-lipoic acid was added 16 hours later. Control experiments were done with the drug solvent benzylalcohol in analog concentrations. Production of plaque-forming units and RT-assay were reduced with increasing concentrations (7–70 μg/ml) of alpha-lipoic acid. A decrease of proliferation of the Jurkat cells up to 50% was seen at concentrations of α-lipoic acid > 40 μg/ml

Fig. 2. Reduction of reverse transcriptase (RT)-activity in HIV-1-infected Jurkat cells by AZT and alpha-lipoic acid. Alpha-lipoic acid (7 μg/ml) and AZT (0.1 μM) were given to HIV-1 infected (MOI = 1) Jurkat cells, either alone or in combination. Cultures were kept continuously under AZT starting 8 hours prior to infection. Alpha-lipoic acid was supplemented as a single dose 16 hours post infection. RT-activity was measured 7 days later

both virus strains at all applied concentrations. RT-activity was reduced in a dose dependent manner, reaching levels close to background at 70 μg/ml lipoic acid (Fig. 1). Reduction of plaque forming units was even more significant; no plaques were seen at concentrations of ≥35 μg/ml, with a MOI of 0.1 and 1.0 (Fig. 1). A second line of experiments were done, adding alpha-lipoic acid to permanently infected Molt-4 cells at high doses (70 μg/ml). This showed a significant reduction of plaque-forming units within 3 days, but delayed decrease of RT-activity over 3 weeks. (Data not shown).

Trypan-blue staining of alpha-lipoic acid-treated PBMC and T-cell lines revealed no toxic effects, even in concentrations of ≥70 μg/ml. Proliferation of two T-cell lines (Jurkat and SupT1) was reduced up to 50% within 7 days, if concentrations of ≥40 μg/ml were given. Other cell lines and PHA-stimulated PBMC were less or not affected. A combined treatment of infected Jurkat cells by azidothymidine (zidovudine) and alpha-lipoic acid resulted in a stronger inhibition of HIV-replication than by each drug alone (Fig. 2).

Discussion

HIV-replication is inhibited by alpha-lipoic acid both in acutely and in chronically infected cells. Antiviral effects were observed when alpha-lipoic acid was supplemented 16 hours after infection of cell lines with HIV-1 and in permanently HIV-infected Molt 4-cells. Thus it appears likely, that the compound exerts antiviral activity after reverse transcription and proviral integration. The early reduction of plaque-forming units in comparison to RT-activity may indicate that non-infectious particles accumulate at lower concentrations of the drug. AZT (3'-azido-3'deoxythymidine), a nucleoside analog inhibiting reverse transcription, acted in concert with alpha-lipoic acid. When cells were incubated with AZT, virus expression decreased more significantly, if AZT was combined with a low dose (7 μg/ml) of alpha-lipoic acid. This is a range of concentration that could be reached by oral therapy. In conclusion, alpha-lipoic acid suppresses HIV replication by a mode of action different from AZT. Unlike AZT, alpha-lipoic acid has very few side effects and stimulates certain T-cell functions [6, 7]. We suggest that alpha-lipoic acid should be applied in clinical trials to evaluate its possible effects in preventing progression of HIV disease.

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References