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Preliminary evidence for inhibitory effect of glycyrrhizin on HIV replication in patients with AIDS

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Summary

Glycyrrhizin (GL) at a dose of 400–1600 mg/day (7.2–30.8 mg/kg/day) was administered intravenously for a period of more than a month, on 6 separate occasions, to 3 hemophiliacs with acquired immune deficiency syndrome (AIDS). Human immunodeficiency virus type 1 (HIV-1) p24 antigen was detected at the beginning of 5 of the 6 treatment courses. Viral antigen was not detected at the end of or during 3 of the 5 treatment courses and decreased to a low level following the 2 other courses. These findings suggest that GL might inhibit HIV-1 replication in vivo.

AIDS; HIV-1; Glycyrrhizin; Antigen, p24

Introduction

Glycyrrhizin (GL) ($C_{42}H_{62}O_{16}$, Mw 822.92) has been isolated from the aqueous extract of licorice root *Glycyrrhiza radix*, and consists of one molecule of glycyrrhetinic acid (GA) and two molecules of glucuronic acid. GA has been reported to inhibit the growth and cytopathogenicity of a number of unrelated DNA and RNA viruses in vitro (Pompei et al., 1979). Recently, it has been reported that GL also inhibits the cytopathic effect of human immunodeficiency virus type 1 (HIV-1), viral antigen expression in HIV-1-infected MT-4 cells, as well as giant

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cell formation caused by HIV-1 infection in MOLT-4 cells. This inhibition is achieved at relatively high concentrations of the compound, its 50% inhibitory concentration being about 0.15 mmol/l (123 μg/ml) (Ito et al., 1987, 1988). Acute toxicity of GL for mice is very low, the 50% lethal dose of GL being 405 mg/kg (Ito et al., 1987). To clarify whether GL also has inhibitory activity on HIV-1 replication in vivo, GL was administered intravenously to 3 AIDS patients at 6 occasions.

Materials and Methods

Patients

All patients were hemophiliacs who had been infected with HIV-1 and admitted to either Tokyo Medical College or Kumamoto University Hospital. All patients were HIV-1 antibody positive when examined using Western blot assay (Hattori et al., 1987a). To each patient, GL was administered through continuous intravenous drip infusion, the dose consisting of 0.2% GL dissolved in saline, supplemented with 2% glycine and 0.1% cysteine (kindly provided by Minophagen Pharmaceutical Co., Tokyo). Informed consent was obtained from each patient prior to treatment.

Monitoring the patient's status

Sera obtained upon admission and during therapy were assayed for HIV-1 p24 antigen using an enzyme-linked immunosorbent assay (ELISA) developed by Abbott Laboratories (Chaisson et al., 1986). The data were expressed in terms of cut-off index (COI). A score higher than 1 was interpreted as positive. Positive reactions were confirmed by neutralization assays using soluble p24 antigen. HIV-1 was isolated through inoculation of either plasma or cerebrospinal fluid (CSF) on a human T cell lymphotropic virus type 1 (HTLV-1) infected cell line, designated SKT-1B. The expression of HIV-1 p24 antigen was determined by an indirect immunofluorescence assay using monoclonal antibody (VAK 4) to p24 of HIV-1 (Koito et al., 1987; Hattori et al., 1987b). Subpopulations of lymphocytes were analysed by laser flow cytometry. Fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies [OKT3 (CD3), OKT4 (CD4), OKT8 (CD8) and OKIa1 (HLA-DR) (Ortho Pharmaceutical, Raritan NJ)] were used for analyses using laser flow cytometry (Ortho Diagnostic Systems, Westwood, MA) (Yamamoto et al., 1986).

Serum GL levels were kindly measured by Teijin Bio Science Laboratory (Tokyo, Japan).

Results

Clinical cases

Case 1 was that of a 59-year-old male with recurrent oral candidiasis. T4 count was less than 100/μl and serum p24 antigen was positive upon admission. This patient received 3 courses of GL therapy. He initially received 400 mg/day (7.7 mg/kg/day) for 1 month beginning Jan. 20, 1987. A transient decrease in serum Ag was observed following treatment (Fig. 1A), but CD4-, CD8- HLA-DR-positive cells continued to decrease and GL therapy was discontinued. T4 count was 12/μl and the serum HIV p24 level was high when GL treatment at 1600 mg/day (30.8 mg/kg/day) was instituted in this patient. Serum HIV p24 level decreased to a low level after 1 week, this decrease being sustained through the continued administration of GL at 1600 mg/day. Furthermore, the decrease in p24 was associated with an increase in the number of CD4-, CD8- and HLA-DR-positive cells (Fig. 1B). GL therapy was continued for 50 days. Then the dose of GL was tapered to 400 mg/day to clarify whether the decrease in serum HIV p24 level could be explained by the administration of the high GL dose. This tapering caused an immediate elevation of serum HIV p24 levels, suggesting that the high GL dose was responsible for suppressing HIV-1 replication. One month after discontinuation of the second course of treatment, the patient suffered from interstitial pneumonia of unknown origin, the T4 count being 7/μl and HIV p24 being detected in the serum (3.5 COI) on 29 October 1987 (Fig. 1C). The patient then received the third

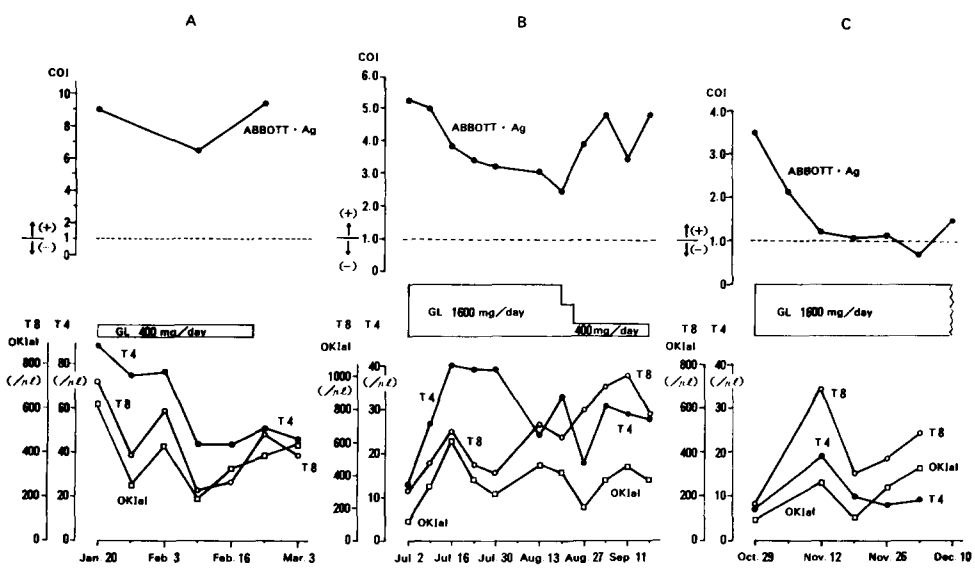

Fig. 1. Clinical course of case 1. Serum HIV p24 antigen and subpopulations of lymphocytes are presented in the upper and lower panels, respectively. Panels A, B and C indicate treatment courses 1, 2 and 3, respectively. OKIal is HLA-DR-positive cells.

course of GL treatment (1600 mg/day) in conjunction with other supportive therapies including antibiotics (sodium cefsoldin, 2 g/day). Serum HIV p24 dropped to a low level (1.2 COI) and a two-fold increase in T4 count was observed after 2 weeks. However, the T4 count dropped to less than 10/μl after 4 weeks, in spite of continuous administration of GL. The pneumonia again resolved. GL therapy (1600 mg/day) was transiently discontinued for one month in February in 1988 upon the patient's request. Thereafter the same dosage of GL was installed until the time of this writing. At the twelfth month of the third course of therapy the serum HIV p24 fell below the level of detection (0.79 COI). During the third course of GL treatment, a gain in body weight of 3–4 kg was noted.

Case 2 was that of a 47-year-old male who was admitted because of *Pneumocystis carinii* pneumonia. The pneumonia was treated through administration of trimethoprim-sulfamethoxazole (960 mg trimethoprim and 4800 mg sulfamethoxazole per day). The serum level of p24 was high (3.6 COI) and T4 count was 34/μl at the time of admission. GL at 400 mg/day (7.3 mg/kg/day) was administered for 2 months beginning 16 February, 1987. HIV p24 decreased to low levels after 1 week (1.2 COI) and became negative after 2 weeks. While these low levels persisted during treatment, no apparent changes in T4 count were observed, so that therapy was discontinued. The patient developed interstitial pneumonia of unknown origin in October, the T4 count being 14/μl. The pneumonia resolved by supportive therapy including antibiotics (sodium piperacillin, 4 g/day) and trimethoprim-sulfamethoxazole (the same dose as described previously). The latter was administered because the possibility of *Pneumocystis carinii* pneumonia could not be ruled out. After resolution of pneumonia, the patient was treated with a high dose of GL (1600 mg/day; 29.1 mg/kg/day) beginning on 28 October. Serum HIV p24 was not detectable upon admission at this time. A slight increase in T4

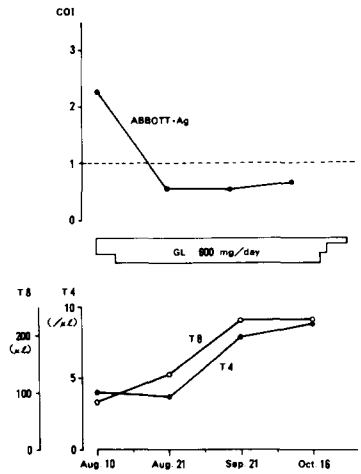

Fig. 2. Clinical course of case 3. Upper and lower panels: as in Fig. 1. The patient died on 23 October 1987.

count (32/μl) was observed after one month. Serum HIV p24 remained negative until 4 months and fluctuated between 1.1–1.3 COI, thereafter. The therapy has been continued for over 11 months to the patient, and the patient is well at the time of this writing. T4 counts decreased to 2/μl and serum HIV p24 has remained negative (0.9 COI) until the eleventh month of therapy.

Case 3 was that of a 35-year-old male who was admitted because of persistent fever, thrush and a low T4 count (4/μl). Dementia and paraplegia were also noted. CT scan revealed atrophy of the frontal brain and dilatation of brain ventricles. GL at 400 mg/day was administered for one week. Since no side effects were observed, the dose was increased to 600 mg/day (12 mg/kg/day). While HIV-antigenemia was evident upon admission, it disappeared by day 10, and remained negative throughout the course of treatment. T4 and T8 counts rose slightly during the therapy period (Fig. 2). The number of HLA-DR-positive cells before therapy was 81/μl, and raised to 275/μl after 55 days of therapy. Paraplegia and dementia, however, progressed and decerebrate rigidity later appeared. After three months, the dose of GL was tapered to 200 mg/day, but the patient died of respiratory arrest a week after the tapering started. During autopsy, plasma and CSF were obtained sterilely and inoculated onto SKT-1B cells. The relative proportion of p24 antigen-positive cells was 12% for SKT-1B cells cultured with CSF but 0% for SKT-1B cells cultured with plasma, when examined after 10 days of cultivation. Furthermore, HIV p24 proved positive in CSF (10.5 COI) and negative in plasma (0.65 COI). Numerous giant cells were observed in subcortical areas with retrovirus-like particles being found in these cells, strongly suggesting the presence of HIV encephalopathy. Interstitial pneumonia was also observed during the autopsy, but the causative agent(s) could not be identified.

Changes of other immunological parameters

Delayed-type skin hypersensitivity to purified protein derivatives proved negative in all three patients when tested upon admission. Furthermore, positive reactions were not observed even after therapy. T8 counts increased (2.1–2.8-fold) in cases 1 and 3 at 45–55 days. The increase was also observed at the seventh month of therapy in case 1. Increases of HLA-DR-positive cells (1.2–4.4-fold) were observed at 45–55 days in all 5 courses when GL was administered at a dose > 600 mg/day.

Side effects and serum GL concentration

Serum GL levels were monitored in the third course of case 1 and in the second course of case 2. The serum GL concentration levels (undetectable before therapy) were 173.3 and 158.4 μg/ml at day 3 and day 9 in case 1, and 271.2 μg/ml at day 6 in case 2. These concentrations are above the 50% inhibitory concentration of GL (123 μg/ml), suggesting that continuous infusion of GL at 1600 mg/day permits to obtain virus-inhibitory drug concentration levels. Significant subjective or objective side effects were not observed during GL treatment.

GL has been reported to have mineralcorticosteroid effects and administration of a large dose of GL has been associated with pseudoaldosteronism (Conn et al., 1986). Serum potassium levels were monitored at least once a week and the levels were often low (3.0–3.5 mEq/l). The administration of spironolactone (100–300 mg/day) and the potassium prevented hypokalemia in all patients. Endocrinological parameters such as serum ACTH, 17KS, 170HCS, cortisol were within normal levels. No other serious side effects, including high blood pressure and liver and kidney dysfunctions, were observed.

Discussion

In this preliminary study, GL was administered at 400–1600 mg/day to three AIDS patients, whose T4 counts were less than 100/μl. Before therapy began, serum HIV p24 was positive in 5 of the 6 treatment courses. Disappearance of serum HIV p24 was observed in 3 courses and a decrease of serum HIV p24 was observed in 2 courses. Thus, GL may have suppressed HIV replication in these patients. This hypothesis is further supported by the fact that the serum HIV p24 level rose again after the dosage of GL was tapered from 1600 mg/day to 400 mg/day during the second treatment of case 1.

No serious side effects were observed even when GL was administered at 1600 mg/day for eleven or twelve months. Serum GL levels were measured in two patients, who had received daily 1600 mg of GL. The serum GL concentrations in these patients were above the 50% inhibitory concentration of GL for HIV replication in vitro. Thus, based on our preliminary findings an effective dosage of GL might be 29.1–30.8 mg/kg/day (1600 mg/day). A dosage of 600 mg/day (12 mg/kg/day) might also be effective, because at this dose GL apparently suppressed HIV-1 replication in case 3. These assumptions are clearly tentative and it is obvious that further extended controlled trials would be necessary to corroborate our findings.

Although the serum HIV p24 levels seem to drop following GL therapy, a sustained increase in T4 counts was not achieved. These observations are similar to those made by Yarchoan et al. (1988) using other anti-HIV agents. An increase in T8 and HLA-DR-positive counts was noted at some points during GL treatment. This could possibly be explained by an immunostimulatory effect of GL, which is known to stimulate interferon production in mice (Abe et al., 1982). This immunostimulatory effect may be beneficial in HIV-1-infected individuals, in that it may increase the activity and/or number of HIV-1-specific cytotoxic T8 cells (Plata et al., 1987).

Our third case does not point to any beneficial effects of GL on the neurological symptoms of AIDS. Dementia and paraplegia continued to progress despite administration of GL. CSF contained a large amount of HIV p24, although p24 levels in the plasma were low. HIV-1 was isolated from CSF but not from plasma. It is questionable that GL is able to penetrate the blood-brain barrier (BBB). In the abeyance of such evidence, GL ought to be combined with anti-HIV agents,

which do penetrate the BBB (Chaisson et al., 1986; Yarchoan et al., 1988), if it were to be used in patients with neurological symptoms of AIDS.

Acknowledgements

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