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SHORT COMMUNICATIONS

Prevention of binding of rgp120 by anti-HIV active tannins

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Abstract—Several tannins with anti-HIV activity have been described previously (Nonaka *et al.*, *J Nat Prod* **53**: 587–595, 1990). We have shown that the tannins chebulinic acid and punicalin were able to block the binding of HIV rgp120 to CD4. These compounds were not toxic to stimulated human peripheral blood lymphocytes at concentrations ten times above their maximal effective concentration.

A wide variety of compounds has been examined in the search for agents with activity against human immunodeficiency virus-1 (HIV*). Recently, a series of tannins with activity against HIV was described [1, 2]. These compounds have been shown to be able to inhibit the growth of HIV in H9 lymphocyte cultures and to inhibit HIV reverse transcriptase (RT) *in vitro*. However, these tannins are charged molecules and may not be able to enter intact cells. Therefore, they may have a mechanism of action other than RT inhibition that is responsible for their anti-HIV activity. The observation that some of these tannins need to be present in the time of infection in order to exert potent anti-HIV activity pointed in this direction [2].

In this report we show that some of the tannins had significant ability to block the binding of recombinant HIV coat protein gp120 (rgp120) to its normal cellular receptor CD4. We compared these results with those obtained with other compounds known to inhibit the binding of HIV to CD4.

Materials and Methods

Reagents. Anti-CD4-FITC (anti-Leu-3a) and anti-TcR α/β were obtained from Becton Dickinson, Mountain View, CA. Recombinant HIV coat protein gp120 and anti-rgp120-FITC (clone 5B6) were gifts of Genentech Inc., San Francisco, CA. The tannins have been described previously [2]. Those tested in this study were: GN-8, 1,3,4-tri-*O*-galloylquinic acid; GN-11, 3,5,-di-*O*-galloylshikimic acid; GN-28, chebulinic acid; GN-29, chebulagic acid; GN-30, punicalin; GN-31, punicalagin; and GN-32, punicacortin C. All tannins were used from a stock solution of 2 mM in dimethyl sulfoxide (DMSO).

Binding assays. Human peripheral blood lymphocytes (PBL) were prepared by density gradient centrifugation and cultured as previously described [3]. Compounds were evaluated as previously described [3]. Briefly, anti-binding activity was measured by determining the ability of compounds to block binding of anti-CD4-FITC or rgp120. PBL were incubated with compounds for 10 min at room temperature, followed by the addition of αCD4-FITC or rgp120, and cells were incubated for 30 min at 4°. Binding of rgp120 was detected using FITC-labeled anti-rgp120. Cell bound fluorescence was measured by flow cytometry.

Viability testing. Human PBL were treated with tannins and stimulated with anti-TcR followed by culture for 4 days. Viability was evaluated by propidium iodide (3 μM) exclusion using flow cytometry.

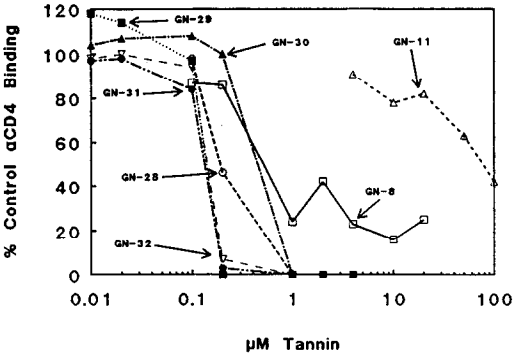

Fig. 1. Concentration–response curves for the ability of tannins to block binding of anti-CD4-FITC to human PBL. Key: (□) GN-8; (△) GN-11; (○) GN-28; (■) GN-29; (▲) GN-30; (●) GN-31; and (▽) GN-32. The order of their effectiveness was: GN-31, GN-29, GN-32 > GN-28 > GN-30 > GN-8 ≫ GN-11. Binding of αCD4-FITC to untreated cells is defined as the 100% response.

Results and Discussion

Binding assays. The tannins were evaluated for their ability to block the binding of anti-Leu-3a-FITC. This antibody binds to and blocks the HIV rgp120 binding site on CD4 [4]. Figure 1 shows that five of the seven tannins tested completely inhibited the binding of αCD4. GN-8 showed partial inhibitory activity, while GN-11 showed minimal activity. We attempted to measure the ability of these tannins to block the binding of rgp120 to PBL. The data (not shown) indicated that GN-8 and GN-11 were unable to block rgp120 binding. For the remaining tannins, GN-28 and GN-30 were definitely inhibitory at a concentration of 2 μM. Both of these compounds have potent anti-HIV activity with an IC_{50} around 1 μM when the compounds are present during the initial virus infection process [2]. The anti-rgp120 binding activities of GN-29, -31, and -32 were not clearly interpretable due to apparent nonspecific binding of assay reagents by the tannin-treated cells. GN-31 and GN-32 were shown previously to inactivate virus [2], which could be due to nonspecific interaction of these two compounds with viral proteins. These results suggest that a major mechanism of action of these tannins could be to block the binding of HIV rgp120 to CD4 on its target cells.

* Abbreviations: HIV, human immunodeficiency virus-1; RT, reverse transcriptase; PBL, peripheral blood lymphocytes; αCD4, monoclonal anti-CD4 antibody; αTcR, monoclonal anti-T-cell receptor antibody; and FITC, fluorescein isothiocyanate.

We have tested this set of tannins for their effects on the viability of stimulated normal human peripheral blood lymphocytes after 4 days of culture. The inactive compound GN-8 showed moderate toxicity (58% viable vs 94% for controls) at the tested concentration of 40 μM. The other inactive compound, GN-11 (20 μM), and all of the active compounds (10 μM) had no effect on the viability of the lymphocytes.

One approach in developing anti-HIV drugs is to look for compounds which could interfere with the interaction between viral rgp120 and cellular CD4. Tannins such as chebulinic acid and punicalin could be excellent lead compounds for further drug development.

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The effect of anion transport inhibitors and extracellular Cl^- concentration on eosinophil respiratory burst activity

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Abstract—Furosemide has been shown recently to protect asthmatic patients against certain bronchoconstrictor challenges. We investigated the effect of furosemide on eosinophil function. Since furosemide may be exerting its inhibitory effect on the eosinophil by inhibiting anion transport, we also assessed the effects of the anion transport inhibitors 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS). Furosemide, NPPB and DIDS inhibited the eosinophil respiratory burst in response to leukotriene B_4 (LTB_4) and, to a smaller extent, inhibited the response to opsonized zymosan (OZ). To assess whether the anion transport inhibitors were achieving their inhibitory effect by inhibiting an influx of Cl^- ions into the eosinophil, the effect of removing extracellular Cl^- on eosinophil function was determined. OZ-induced H_2O_2 production was inhibited by removing extracellular Cl^- whereas the LTB_4 response was not affected by the concentration of extracellular Cl^-.

Recently, it has been shown that the loop diuretic furosemide protects asthmatic patients against various indirect bronchoconstrictor challenges. Inhalation of furosemide was demonstrated to inhibit the airway response to exercise [1], distilled water [2], metabisulphite [3] and adenosine [4], and to inhibit the early and late response to antigen [5]. The mechanisms of action of furosemide on the asthmatic response are yet to be established. One possibility is that furosemide may exert an inhibitory effect on inflammatory cells which may be activated by these challenges. Eosinophils are implicated in a range of allergic and inflammatory disorders, and can cause tissue injury by the generation of active oxygen species (e.g. superoxide anions and H_2O_2) via an oxidative burst, and by the release of toxic proteins [6].

In the present study, we have therefore investigated the effect of furosemide on the respiratory burst of the eosinophil using a continuous assay for H_2O_2. Furosemide may exert an effect on the eosinophil by inhibiting anion transport and we therefore examined the effects of the Cl^- transport inhibitors 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB*) [7] and 4,4′-diisothiocyanatostilbene-

* Abbreviations: DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; LTB_4, leukotriene B_4; NPPB, 5-nitro-2-(3-phenylpropylamino)-benzoic acid; OZ, opsonized zymosan.