Vol. 148, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1987 Pages 206-210

ANTIRETROVIRAL ACTIVITY OF CASTANOSPERMINE AND DEOXYNOJIRIMYCIN, SPECIFIC INHIBITORS OF GLYCOPROTEIN PROCESSING

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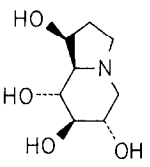
Received August 17, 1987

SUMMARY: The objective of this study was to investigate the antiretroviral activity of specific inhibitors of glycosidases and mannosidases that are involved in N-linked oligosaccharide processing of glycoproteins. Castanospermine and 1-deoxynojirimycin, potent inhibitors of glucosidases I and II, showed significant activity against Moloney murine leukemia virus (IC_{50}: 1.2 μg/ml). Deoxymannojirimycin and swainsonine, inhibitors of mannosidase I and II, respectively, did not show any activity. These observations suggest that removal of the outermost glucose residue from high mannose asparagine-linked oligosaccharide may be essential for the replication of mouse leukemia virus. The relative nontoxic nature of these inhibitors and a novel mechanism of action suggest a potential for compounds of this type as chemopreventive and therapeutic agents in the treatment of acquired immune deficiency syndrome (AIDS).

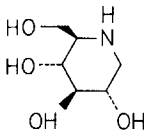
Human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), is an enveloped retrovirus (1,2). This virus is cytopathic for $T4^+$ (CD_4^+) lymphocytes. HIV possesses two envelope proteins that are glycosylated: glycoprotein gp 120 binds to the CD_4 antigen of $T4^+$ T lymphocytes; a transmembrane protein, glycoprotein gp 41, anchors the envelope in the viral membrane. These viral envelope glycoproteins and host CD_4 surface receptors play an important role in virus adsorption, penetration, syncytium formation and spread of the virus to adjacent cells (3,4). Therefore, interference with processing of viral envelope glycoprotein could be an attractive target for chemotherapy against HIV infection. Consistent with this suggestion, Blough et al. (5) have shown that two non-specific glycosylation inhibitors, 2-deoxy-D-glucose and β-hydroxynorvaline at mM

Abbreviations: **AIDS:** Acquired immune deficiency syndrome; **HIV:** Human immunodeficiency virus; **MoLV:** Moloney murine leukemia virus; **AZT:** 3′-Azido-3′-deoxythymidine.

0006-291X/87 $1.50



Castanospermine
(1S,6S,7R,8R,8aR-1,6,7,8-
tetrahydroxyindolizidine)



Deoxynojirimycin
(1,5-dideoxy-1,5-
imino-D-glucitol)

Figure 1. Structural formulae of castanospermine and deoxynojirimycin.

concentrations blocked the expression of enveloped glycoproteins, gp 120 and gp 41, in HIV infected MT-4 and H_9 cells in culture. The availability of specific inhibitors of a number of enzymes involved in the oligosaccharide processing of glycoproteins (6) has made it possible to investigate the role of carbohydrate moieties on the cell surface glycoproteins in cellular interaction, differentiation, immune response and tumor metastases (6,7,8). However, there is a paucity of information on the antiviral activity of these specific inhibitors, except for the work of Pan et al. (9) who has reported a lack of activity against influenza virus by castanospermine. In the present study, we have investigated the antiretroviral activity of a number of these specific enzyme inhibitors, in particular, castanospermine and deoxynojirimycin (Fig. 1), using Moloney murine leukemia virus (MoLV) as a model for the human immunodeficiency virus (HIV).

MATERIALS AND METHODS

Cells:

Mouse SC-1 cells and rat XC cells were maintained in minimum essential medium (MEM) with 10% fetal calf serum.

Virus:

Moloney murine leukemia virus (MoLV) was obtained from C3H10T1/2 (clone 8) cells chronically infected with and constitutively producing MoLV (10). These cells were generously provided by Dr. Max Proffitt (Cleveland Clinic Foundation, Cleveland, Ohio).

Compounds:

Castanospermine (1S, 6S, 7R, 8R, 8aR-1,6,7,8 tetrahydroxyindolizidine) was isolated from seeds of the Australian chestnut tree Castanospermum australe as described earlier (11) and 1-deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol) was synthesized as previously reported (12). Bromoconduritol (6-bromo-3,4,5-trihydroxycyclohex-1-ene), 1-deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol) and swainsonine (1S, 2R, 8R, 8aR-1,2,8-trihydroxyindolizidine) were purchased from Boehringer Mannheim Biochemicals,

Indianapolis, Indiana. 3'-Azido-3'-deoxythymidine and 2',3'-dideoxycytidine were obtained from Sigma Chemical Co., St. Louis, MO.

Murine leukemia virus plaque assay:

The XC plaque assay was performed according to the method of Rowe et al. (13) with modification as previously described (14). Briefly, mouse SC-1 cells (10^5) were seeded into each well of 6-well cluster plates (Costar #3506) in 4 ml MEM with 10% FCS. Following an 18 hr incubation period (37°C), MoLV was then applied at a predetermined titer to give optimal (i.e. countable) numbers of virus plaques, i.e. 58 ± 15/well. Compounds were added 2 hr prior to addition of the virus. Three days later, the culture medium was removed, the SC-1 cell monolayers were exposed to UV irradiation (1800 ergs), and rat XC cells (10^6) were seeded into each well in 4 ml MEM. Following an additional 3 day incubation (37°C), these cells were fixed with ethyl alcohol (95%) and stained with 0.3% crystal violet. Plaques were then counted under low magnification.

RESULTS

The effect of inhibitors of glycoprotein processing on the replication of MoLV is presented in Table 1. The data indicate that castanospermine and 1-deoxynojirimycin have equivalent antiretroviral activity with an IC_{50} of 1.2-2.5 µg/ml. The other inhibitors bromoconduritol, deoxymannojirimycin and swainsonine did not inhibit viral replication even at 100 µg/ml. The standard antiretroviral compounds, dideoxycytidine and azidothymidine, had IC_{50}'s against the virus of 1.0 and 0.001 µg/ml, respectively. The dose response effect of castanospermine and 1-deoxynojirimycin against MoLV replication is depicted in Figure 2.

TABLE 1

EFFECT OF INHIBITORS OF GLYCOPROTEIN PROCESSING ON MURINE LEUKEMIA VIRUS (MoLV) REPLICATION

Compound	IC_{50} for Virus Replication* (µg/ml)
Castanospermine	1.2, 1.2, 2.5
Deoxynojirimycin	1.2, 1.2, 2.5
Bromoconduritol	>100, >100
Deoxymannojirimycin	>100, >100
Swainsonine	>100, >100
2',3'-dideoxycytidine	1.0, 1.0
3'-Azido-3'-deoxythymidine	0.001, 0.001

* The IC_{50} value was determined based on the virus yield in control wells (PFU = 58 ± 15/well). The data presented are individual values from separate experiments.

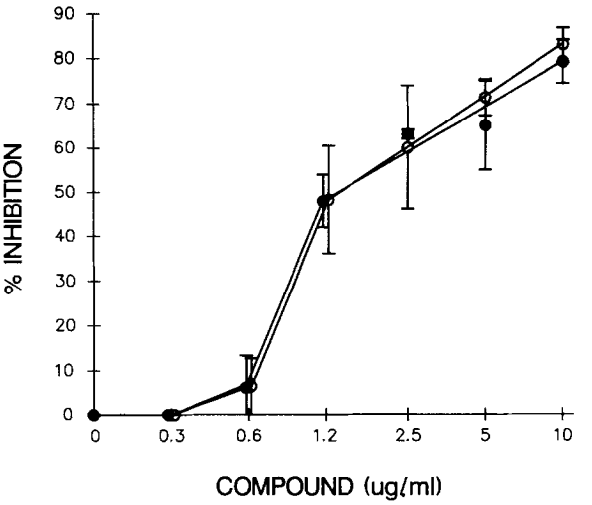


Figure 2. Dose dependent inhibition of Moloney murine leukemia virus (MoLV) infection by castanospermine (●) and 1-deoxynojirimycin (○). Compounds were tested at various dilutions for their ability to inhibit MoLV infection in the XC plaque assay. Compounds were added two hours prior to the addition of virus. Data are compiled from three separate experiments (mean ± S.E.; n=3) and expressed as percent of virus control (58 ± 15 p.f.u./well).

DISCUSSION

Castanospermine is a potent inhibitor of glucosidase I. Bromoconduritol inhibits only glucosidase II, whereas 1-deoxynojirimycin is a potent inhibitor of both glucosidase I and II in the pathway of oligosaccharide processing of glycoproteins (6,8). The data presented in Table 1 and Fig. 2 clearly indicate that castanospermine and 1-deoxynojirimycin showed significant antiretroviral activity while bromoconduritol was not active against the replication of MoLV. Deoxymannojirimycin and Swainsonine, inhibitors of mannosidase I and II respectively, did not show activity. These observations suggest that removal of the outermost glucose residue from high mannose asparagine-linked oligosaccharides is critical for the viability of the mouse leukemia virus, in sharp contrast to the influenza virus which is not affected by glucosidase I inhibitors (9). Accumulation of immature glycoproteins due to the inhibition of glucosidases I by castanospermine and deoxynojirimycin might have a role in inhibiting the replication of MoLV by inhibiting fusion, virus attachment to the receptor of the host cell, virus penetration and/or syncytia formation. In the course of this work, Walker et al. (15,16) reported data consistent with the observations described here that castano-

spermine prevented the syncytium formation of HIV infected cells and inhibited the replication of HIV in cell culture.

Reverse transcriptase has been considered as the most attractive target in the design of antiretroviral agents. However, AZT, a potent inhibitor of reverse transcriptase, has shown significant untoward side effects. The relative non-toxic nature of glucosidase inhibitors (castanospermine LD_{50}: for mice >500 mg/kg) and their novel mechanism of action suggest a potential for compounds of this type as chemopreventive and therapeutic agents in the treatment of AIDS.

ACKNOWLEDGMENTS

The authors thank Mr. C. Hull for his technical help and Mrs. R. Hoeper for her secretarial assistance.

REFERENCES

1. Gallo, R., Sarin, P.S., Gelmann, E.P., Robert-Guroff, M., Richardson, E., Kalyanaraman, V.S., Mann, D., Sidhu, G.D., Stahl, R.E., Zolla-Pazner, S., Leibowitch, J., and Popovic, M. (1983) Science 220, 865-867.
2. Barré-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vézinet-Brun, F., Rouzioux, C., Rozenbaum, W., and Montagnier, L. (1983) Science 220, 868-871.
3. Montagnier, L., Clavel, F., Krust, B., Chamaret, S., Rey, F., Barré-Sinoussi, F., and Chermann, J.C. (1985) Virology 144, 283-289.
4. McDougal, J.S., Kennedy, M.S., Sligh, J.M., Cort, S.P., Mawle, A., and Nicholson, J.K.A. (1986) Science 231, 382-385.
5. Blough, H.A., Pauwels, R., DeClercq, E., Cogniaux, J., Sprecher-Goldberger, S., and Thiry, L. (1986) Biochem. Biophys. Res. Commun. 141, 33-38.
6. Elbein, A.D. (1984) CRC Crit. Rev. Biochem. 16, 21-49.
7. Olden, K., Bernard, B.A., Humphries, M. J., Yeo, T.K., Yeo, K.T., White, S.L., Newton, S.A., Bauer, H.C., and Parent, J.B., (1985) Trends Biochem. Sci. 10, 78-82.
8. Humphries, M.J., Matsumoto, K., White, S.L., and Olden, K. (1986) Cancer Res. 46, 5215-5222.
9. Pan, Y.T., Hori, H., Saul, R., Sanford, B.A., Molyneux, R.J., and Elbein, A.D. (1983) Biochemistry 22, 3975-3984.
10. Proffitt, M.R., Hirsch, M.S., Ellis, D.A., Gheridian, B., and Black, P.H. (1976) J. Immunol. 117, 11-15.
11. Liu, P., Rhinehart, B.L. (1986). Eur. Patent Appl. EP 202,661.
12. Inouye, S., Tsuruoka, T., Ito, T., and Niida, T. (1968) Tetrahedron 23, 2125-2144.
13. Rowe, W.P., Pugh, W.E., and Hartley, J.W. (1970) Virology 42, 1136-1139.
14. Bowlin, T.L. and Proffitt, M.R. (1983) J. Interf. Res. 3, 19-31.
15. Walker, B.D., Kowalski, M., Goh, W.C., Rohrschneider, L., Haseltine, W.A., and Sodroski, J. (1987) Proc. Int. Conf. on AIDS. 3, 54.
16. Walker, B.D., Kowalski, M., Goh, W.C., Rosen, C., Rohrschneider, L., Haseltine, W.A. and Sodroski, J. (1987). Proc. Natl. Acad. Sci. (in press).