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SHORT COMMUNICATION

Inhibition of the production of HIV-1 from chronically infected H9 cells by metal compounds and their complexes with L-cysteine or N-acetyl-L-cysteine

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Summary

A number of metal compounds and their complexes with cysteine and N-acetyl-cysteine (NAC) were tested for their ability to inhibit HIV replication *in vitro*, specifically in chronically infected H9 cells (which produce virus continuously). Out of seven metal compounds tested, only bismuth nitrate and bismuth sodium tartrate inhibited virus production in chronically infected H9 cells. The complexes made with metals and cysteine or NAC had slightly improved selective indices.

Key-words: antiviral activity; cysteine; HIV; metals; NAC.

Compounds currently used for AIDS therapy, such as AZT, ddI and other nucleoside analogues, are inhibitory to HIV infection of T cells *in vitro* at low concentrations. They are, however, ineffective as anti-HIV agents when the virus is already integrated into the T-cell DNA, so that the cells are chronically or latently infected.

Recently it has been demonstrated that NAC may produce beneficial effects in AIDS patients by increasing the intracellular glutathione concentration and by inhibiting tumour necrosis factor-α (TNF-α)-stimulated replication of HIV in cell lines and PBMCs (Roederer *et al.*, 1991). This may protect latently infected cells from TNF-α, which is thought to have a role in the progression of AIDS, partly through its stimulation of the production of destructive reactive oxidative intermediates (Folks *et al.*, 1989; Poli *et al.*, 1990). Moreover, complexes with cysteine and NAC have been reported previously to have much reduced cytotoxicity compared to the metals alone (Hussain *et al.*, 1992).

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Several HIV-related proteins contain cysteine-rich regions which can bind to metals (Frankel *et al*, 1988; Leonard *et al.*, 1990). A typical metal-binding protein is the tat protein, which is essential for viral replication *in vitro*. It contains a cysteine-rich region which is conserved in several HIV isolates and is very similar to metallothiones. The protein forms tight metal-linked dimers with Zn^{2+} or Cd^{2+}. Since dimerization could be essential for the tat protein to function, we have investigated the possibility of inhibition of HIV expression by different metal compounds and also by metal complexes with cysteine and NAC.

The compounds tested were antimony (III) chloride, bismuth (III) nitrate, cadmium acetate dihydrate, mercurous (I) chloride and silver nitrate (Fluka-Buchs, Switzerland); NAC, L-cysteine, sodium arsenite, and copper (II) sulphate (Merck ABS-Geneva, Switzerland); and bismuth sodium tartrate (ICN Biomedicals Inc., Aurora, USA). Metal derivatives of cysteine and NAC were prepared by adding a 100-mM solution of the metal compound in bidistilled water to an equal volume of the amino acids dissolved in bidistilled water at concentrations of 100 mM (for silver), 200 mM (for cadmium, copper and mercury) and 500 mM (for bismuth nitrate). Bismuth nitrate becomes soluble by cysteine addition which may confirm the formation of a complex. A typical example of the simple reaction is shown in Fig. 1.

The inhibition of virus yield from chronically infected H9 cells was measured in 96-well plates by mixing 5-fold dilutions of compounds with 4×10^4 cells per well in 200 µl of RPMI 1640 medium supplemented with 10% foetal calf serum. After 5 days of incubation at 37 °C the progeny virus was titrated on C8166 cells using doubling dilutions of freshly collected supernatants. The end-point was determined by examining syncytia and by the XTT-formazan method (Weislow *et al.*, 1989), and was compared with the untreated control. p24 antigen was measured by ELISA using combinations of anti-p24 monoclonal antibodies and pooled human anti-HIV-1 sera. The cytotoxicity to chronically infected and uninfected H9 cells was measured by the XTT-formazan method.

None of the compounds tested inhibited infection of C8166 cells (human T-lymphoblastoid cell line) significantly (data not shown). Anti-HIV activity in C8166 cells was measured as described in detail previously (Mahmood *et al.*, 1993).

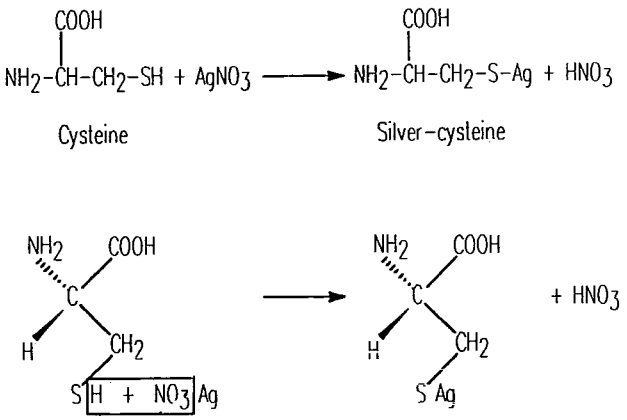

Fig. 1. Formation of metal thiol compounds. The complex is generated by mixing equal volumes of equimolar solutions of silver nitrate and cysteine.

There was, however, a definite reduction in the virus yield and antigen (p24) produced from chronically infected H9 cells (clone derived from the HUT78 cell line, human cutaneous T-cell lymphoma, infected with HIV-1_{IIIB}) treated with bismuth nitrate or more soluble bismuth compounds, such as bismuth sodium tartrate, at concentrations that were not toxic to growing cells (both uninfected and chronically infected) (Table 1). Bismuth compounds were non-toxic at millimolar concentrations to freshly isolated human lymphocytes or to renal Na,K-ATPase (data not shown), in contrast to silver which was toxic to both biological systems in the absence of cysteine. Antimony, cadmium and copper also produced inhibition, whereas silver and arsenic were inactive. Arsenic was extremely toxic, even at low concentrations (Table 1).

Cytotoxicity was generally reduced when metals were complexed with cysteine or NAC (Table 1). Although there was no apparent increase in the antiviral activity, the addition of cysteine or NAC to these compounds may confer a double advantage by both maintaining anti-HIV activity in chronically infected H9 cells and promoting anti-TNF-α activity. NAC inhibits the TNF-α-stimulated HIV expression *in vitro*. Neither cysteine nor NAC showed any effect in the chronically infected H9 cells used here which did not require TNF-α stimulation to produce virus. A complex of bismuth nitrate and cysteine was not toxic at 2 mM, although this concentration led to a reduction in virus production of nearly 100%.

The mode of action of metals in HIV suppression is unclear, but the reduction of antigen (p24) suggests that the decreased virus infectivity may be caused by inhibition at the level of virus production, rather than by the production of uninfectious virions. It is unlikely that proteases or glycosidases are inhibited by these compounds as they show negligible anti-HIV activity in C8166 cells. Further studies are required to investigate the possible interaction of these metals with the tat protein to inhibit its function and virus production. It is unclear why there was marginal activity against HIV-1_{IIIB} infection of C8166 cells.

Although metal compounds, especially bismuth, have been used in humans for the eradication of *Helicobacter*

Table 1. Inhibition of HIV-1_{IIIB} expression in chronically infected H9 cells by metals and their complexes with cysteine or NAC

Compound	EC_{50}	TC_{50}	SI
Bismuth nitrate	300	2000	7.5
Bismuth sodium tartrate	200	1000	5
Antimony chloride	22	110	5
Sodium arsenite	3.8	3.8	1
Copper sulphate	20	40	2
Cadmium acetate	1.6	5	3.1
Silver nitrate	NM	20	NA
Mercurous chloride	NM	20	NA
Cysteine	NM	>2000	NA
NAC	NM	>2000	NA
Silver cysteine complex	NM	>1000	NA
Silver NAC complex	NM	>1000	NA
Mercury cysteine complex	NM	>300	NA
Bismuth nitrate cysteine complex	400	>2000	>5
Bismuth nitrate NAC complex	400	>2000	>5
Copper cysteine complex	100	100	1
Cadmium cysteine complex	10	25	2.5

EC_{50} is the concentration that reduces the p24 antigen or virus yield by 50% in cell cultures.
TC_{50} is the concentration of drug that reduces cell growth by 50% (XTT assay).
SI (selective index) is $[TC_{50}/EC_{50}]$.
NM means not measurable (EC_{50} was not reached at non-toxic concentrations), and hence SI is not applicable (NA) for these compounds.
All concentrations are in μM except for bismuth sodium tartrate which is in μg ml^{-1}.
The results shown are the means of three independent determinations with a range of values ≤33% in all cases.

pylori (Glupczynski and Burette, 1992), the toxicity to humans remains a problem, especially where the therapeutic index of the compounds is low. However, these are interesting anti-HIV agents which should be studied further to develop compounds with higher anti-HIV activity and selectivity.

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