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Short Communication

Effect of *N*-methylisatin-β-4′:4′-diethylthiosemicarbazone on intracellular Moloney leukemia virus constituents

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Summary

N-Methylisatin-β-4′:4′-diethylthiosemicarbazone (M-IBDET) inhibits intracellular production of viral constituents in a mouse cell line, 3T3/MLV, chronically infected wih Moloney leukemia virus. Electron microscopic observations confirmed that inhibition of virus production by the drug was not associated with any structural changes in the cell morphology or any damage to the plasma membrane, the site of viral assembly and 'budding'. Treatment of the cells with 17 μM M-IBDET for 6 h inhibited extracellular virus production by 80% but did not affect the level of viral RNA in the cytoplasm or in the plasma membrane. Intracellular reverse transcriptase activity and levels of viral structural proteins were significantly inhibited. Thus, although the drug did not affect viral RNA, it reduced viral protein synthesis.

murine leukemia virus; protein synthesis; RNA synthesis; thiosemicarbazone derivative

The antiviral activity of thiosemicarbazone derivates has been reviewed by Bauer [2]. *N*-Methylisatin-β-thiosemicarbazone (M-IBT), also known as methisazone, is a potent and specific inhibitor of pox virus and one of the few synthetic antiviral drugs known

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to be clinically effective [2]. Isatin-β-thiosemicarbazone (IBT) inhibits adenovirus multiplication, and some of its derivatives inhibit the multiplication of certain enteroviruses [12]. Only its antipox activity has been investigated in detail [9]. M-IBDET was found by us to be the only compound out of nine thiosemicarbazone derivatives, including M-IBT and IBT, which specifically inhibited the production of Moloney leukemia virus (MLV) [5], a member of the retrovirus family [3,7]. Suppression of MLV release from the 3T3/MLV cells into the culture medium was confirmed by various parameters of viral assay [14] and was dependent on drug concentration and length of treatment. In the present study, we have examined the effect of the drug on intracellular reverse transcriptase activity, viral RNA levels and viral structural protein synthesis.

Rates of inhibition of virus production in 3T3/MLV cells [15,18] by various concentrations of M-IBDET were examined. Reverse transcriptase activity served as a specific assay for intracellular virus production [15]. Fig. 1 shows that 50% inhibition levels were obtained with 34 μM of M-IBDET after 3 h of treatment, with 17 μM after 6 h and with 8.5 μM M-IBDET after 9 h. Under identical experimental conditions, the DNA, RNA and protein syntheses of the 3T3/MLV cells were also assayed by following the incorporation of radioactive precursors into 10% TCA-precipitated material. Treatment of the cells with 17 μM of M-IBDET for 6 h showed a 26%

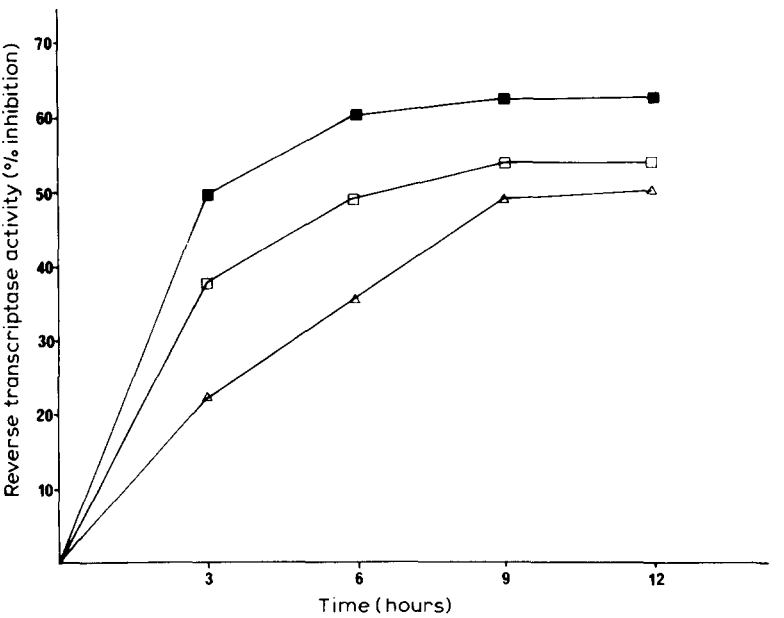

Fig. 1. Inhibition of intracellular reverse transcriptase activity by different doses of M-IBDET. Confluent 3T3/MLV cultures were incubated in medium with M-IBDET at 34 μM (■———■), 17 μM (□———□) or 8.5 μM (△———△). At indicated times, cytoplasmic preparations were obtained from drug-treated and untreated cells, and reverse transcriptase activity was determined in samples containing 10 μg protein. Results are expressed as percent inhibition in enzyme activity.

inhibition in [^{3}H]thymidine incorporation and a 5% inhibition in uridine incorporation, while cellular protein synthesis was not affected at all [14]. Parallel electron microscope (EM) observations were carried out on drug-treated and untreated cells to ensure that virus inhibition was not merely a result of morphological damage to the cells or their plasma membrane. Fig. 2 is an electron micrograph of 3T3/MLV cells treated with M-IBDET. Typical C-type particles can be seen budding from the host cell membrane (left inset) indicating that the plasma membrane remained intact after drug treatment. The morphology of the virus particles released from the treated cells was in no way different from normal C-type particles (right inset).

Next, the effect of M-IBDET on the level of intracellular virus RNA was examined. RNA was extracted using methods described previously [8] from cytoplasmic preparations made from 2×10^8 3T3/MLV drug treated and untreated cells. The amount of virus specific RNA in each preparation was determined employing the hybridization technique [8] using a viral specific [^{3}H]cDNA probe complementary to the MLV 70 S RNA. It can be seen from Fig. 3 that in control cells virus-specific RNA represents

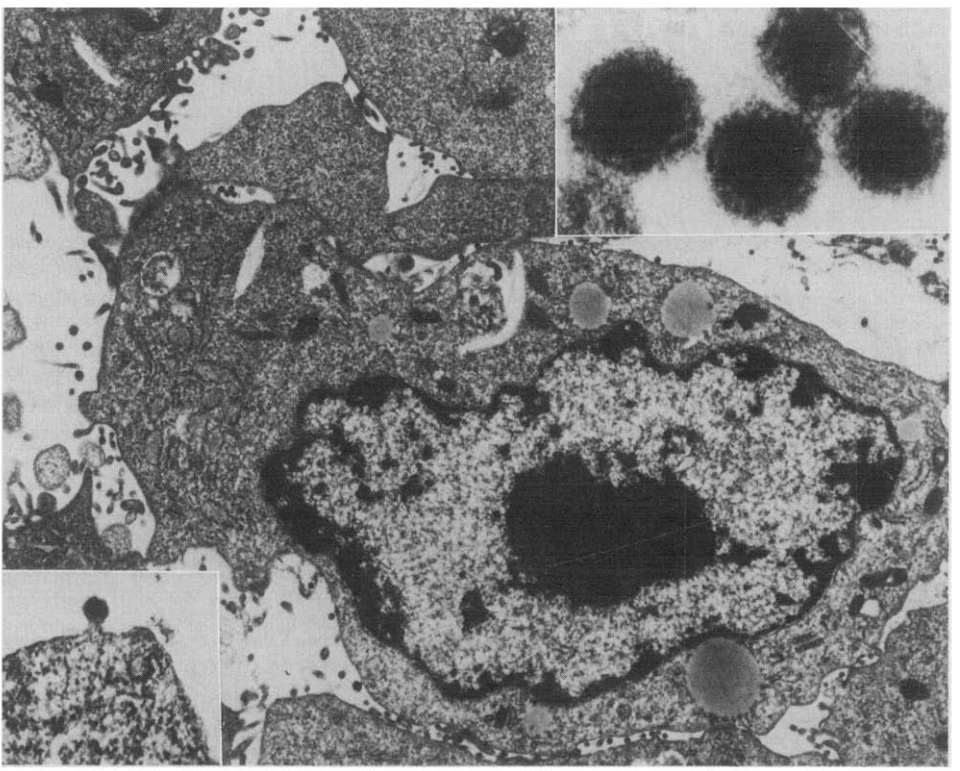

Fig. 2. Transmission electron micrograph of 3T3/MLV cells treated with 17 μM M-IBDET for 6 h (original magnification ×1500). Right inset: Typical C-type particles (original magnification ×9000). Left inset: A C-type particle budding from the plasma membranes of the drug-treated cells (original magnification ×4500).

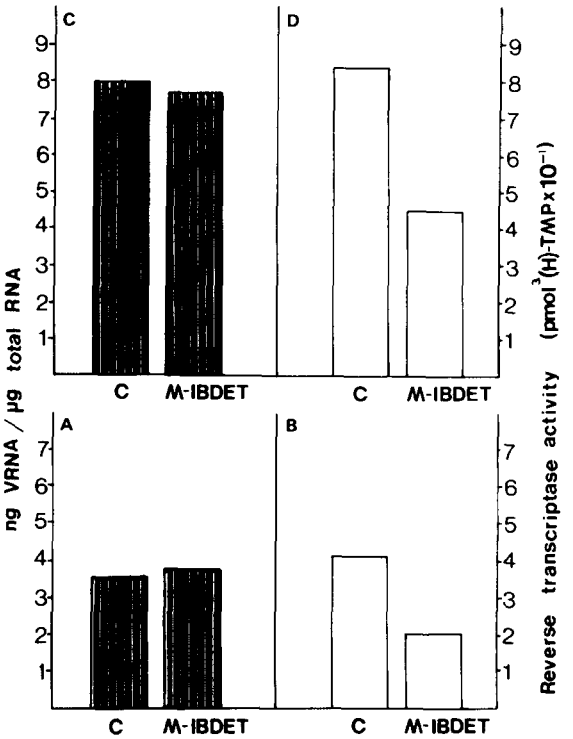

Fig. 3. Levels of viral RNA and reverse transcriptase activities in subcellular fractions of 3T3/MLV control and M-IBDET-treated cells. 3T3/MLV control cells (C), and M-IBDET-treated cells (M-IBDET), were harvested and fractionated into cytoplasmic and plasma membrane fractions. RNA was extracted and analyzed for virus-specific RNA (▮▮▮▮), by hybridization with [^{3}H]cDNA. Reverse transcriptase activity (▭) was assayed in the same subcellular fractions in samples containing 10 µg protein, using a standard exogenous reaction [17]. Results are expressed as picomoles of [^{3}H]TMP incorporated per sample in a reaction containing 1 µCi of [^{3}H]TTP (usual yield 220 cpm/pmol). (A) Viral RNA in the cytoplasmic fractions; (B) reverse transcriptase activity in the cytoplasmic fractions; (C) viral RNA in the plasma membrane fractions; (D) reverse transcriptase activity in the plasma membrane fractions.

0.36% (3.6 ng viral RNA/µg total RNA) of the total cellular RNA. This level was entirely unaffected by treatment with M-IBDET. Nevertheless, in the same cellular preparation cytoplasmic reverse transcriptase activity was reduced by 50% (Fig. 3B). As the drug did not affect the total amount of viral RNA in the whole cytoplasmic fraction, we examined its effect on the cellular plasma membrane associated viral RNA. This cell fraction, although relatively poor in cellular RNA, is rich in viral RNA that is being assembled into budding virus particles [4]. Therefore whole membranes were isolated from 2×10^9 treated or untreated cells by methods previously described [13,15]. The preparations were characterized by the activity of 5'-nucleotidase, a specific plasma membrane marker enzyme [11,15]. RNA was extracted and assayed employing the method described previously [8]. Results shown in Fig. 3C show that the

plasma membrane fraction from the drug-treated cells contained the same level of viral RNA as control cells (8 ng viral RNA/μg total RNA). In contrast, reverse transcriptase activity was reduced by 53% (Fig. 3D). Since the intracellular activity of viral reverse transcriptase was inhibited by M-IBDET we further examined the effect of the drug on other viral structural proteins. 3T3/MLV cells were pulse-labelled with [^{3}H]leucine in the presence of 17 μM M-IBDET for 6 h. Labelled cytoplasmic preparations from drug-treated and untreated cells were prepared [1] and reacted with rabbit anti-MLV serum, as described before [16]. The immunoprecipitates were analysed on SDS-polyacrylamide gel electrophoresis using the buffer system described by Laemmli [10]. Results are summarized in Table 1. It can be seen that the total amount of radioactivity in the viral glycoprotein gp-70, as well as in the viral structural proteins p-30, p-15-12-10 was reduced in drug-treated cells. A mean value of 63.4% inhibition in the total viral structural proteins was found. It should be emphasized that total cellular protein synthesis was not affected under these experimental conditions.

In conclusion, intracellular inhibition of MLV replication in 3T3/MLV cells by M-IBDET is associated with significant inhibition of the virus-specific reverse transcriptase activity in the cytoplasm. This inhibition testifies to the specificity of the effect. Levels of viral structural proteins in the cytoplasm were also significantly reduced as determined by the radioimmunoprecipitation assays. Interestingly, glycoprotein gp-70 as well as p-30 and p-15-12-10, were all reduced to the same extent, although they are known to be formed from two separate protein precursors [6]. Levels of virus-specific RNA were 2.2-fold higher in the plasma membrane fraction than in the whole cytoplasmic fraction, a result which is in accord with the fact that the plasma membrane serves as an assembly site for viral genomic RNA. M-IBDET failed to affect total cytoplasmic or plasma membrane levels of virus-specific RNA. The EM pictures showing budding of C-type particles from the intact PM of treated cells served as a particularly sensitive indicator for the lack of toxic effects of the drug on the cells. The observation that M-IBDET does not affect the amount of virus-specific RNA, but specifically inhibits MLV protein production, calls for further experimentation to reveal the exact mode of action.

TABLE 1

Effect of M-IBDET on intracellular viral structural proteins

Viral structural proteins	Amount of radioactivity/peak (cpm × 10^{-3})		Inhibition (%)
	Control	Treated	
gp-70	3.5	1.2	65.7
p-30	2.8	0.95	66.1
p-15-12-10	1.2	0.5	58.4

3T3/MLV cells were labeled for 6 h with 10 μCi/ml [^{3}H]leucine in the presence of 17 μM M-IBDET. Cytoplasm was prepared from control and treated cells, and viral proteins were immunoprecipitated. The immunoprecipitates were analysed by SDS-PAGE. The amounts of radioactivity of peaks corresponding to viral structural proteins were calculated.

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References

1 Arcement, L.J., Karshin, W.L., Naso, R.B., Jamjoom, G. and Arlinghaus, R.B. (1976) Biosynthesis of Raucher leukemia viral proteins: presence of p-30 and envelope p-15 sequences in precursor polypeptides. Virology 69, 763–774.

2 Bauer, D.J. (Ed.) (1972) Thiosemicarbazone in chemotherapy of virus disease, Vol. I. Pergamon Press, Oxford.

3 Bishop, J.M. (1978) Retroviruses. Annu. Rev. Biochem. 47, 35–88.

4 Bolognesi, D.P. Montelaro, R.C., Frank, H. and Schäfer, W. (1978) Assembly of type C oncornaviruses: A model. Science 199, 183–186.

5 Edelstein, F., Avramoff, M., Shtacher, G. and Teitz, Y. (1980) New thiosemicarbazones of mono and bicyclic α-ketolactams. Eur. J. Med. Chem. Ther. 15, 566.

6 Eisenman, R.N. and Vogt, V.M. (1978) The biosynthesis of oncovirus proteins. Biochim. Biophys. Acta. 473, 187–239.

7 Fan, H. (1977) Expression of RNA tumor viruses at translation and transcription levels. Curr. Topics Microbiol. Immunol. 79, 1–41.

8 Fan, H. and Baltimore, D. (1973) RNA metabolism of murine leukemia virus. Detection of virus specific RNA sequences in infected and uninfected cells and identification of virus-specific messenger RNA. J. Mol. Biol. 80, 97–177.

9 Katz, E. and Felix, H. (1977) Synthesis of vaccinia viral antigens in Hela cells in the presence of isatin-β-thiosemicarbazones. Antimicrob. Agents Chemother. 11, 202–208.

10 Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227, 680–685.

11 Oseroff, A.R., Robbins, W.P. and Burger, M.M. (1973) The cell surface membrane: Biochemical aspects and biophysical probes. Annu. Rev. Biochem. 42, 647–682.

12 Pearson, G.D. and Zimmerman, E.F. (1969) Inhibition of poliovirus replication by *N*-methylisatin-β-4′:4′-dibutylthiosemicarbazone. Virology 38, 641–650.

13 Quigley, J.P., Rifkin, D.B. and Reich, E. (1973) Phospholipid composition of Rous sarcoma virus, host cell membranes and other enveloped RNA viruses. Virology 46, 106–116.

14 Sherman, L., Edelstein, F., Shtacher, G., Avramoff, M. and Teitz, Y. (1980) Inhibition of Moloney leukemia virus production by *N*-methylisatin-β-4′:4′-diethylthiosemicarbazone. J. Gen. Virol. 46, 195–203.

15 Sherman, L., Eylan, E. and Teitz, Y. (1980) Localization of reverse transcriptase in interferon-treated mouse cells chronically infected with Moloney leukemia virus. Arch. Virol. 66, 143–155.

16 Sherman, L. and Teitz, Y. (1981) Glycoprotein enrichment in Moloney leukemia virus structural proteins released from interferon-treated cells. Exp. Cell Biol. 49, 185–194.

17 Teitz, Y. (1971) RNA dependent DNA polymerase in C-type particles from normal rat thymus cultures. Nat. New Biol. 232, 250–252.

18 Teitz, Y., Lennette, E.H., Oshiro, L.S. and Cremer, N. (1971) Release of C-type particles from normal rat thymus cultures and those infected with MLV. J. Nat. Cancer Inst. 46, 11–23.