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SITE OF ACTION OF N,N′-BIS(METHYLISATIN-β--THIOSEMICARBAZONE)-2-METHYLPIPERAZINE IN THE VACCINIA VIRUS REPLICATION CYCLE

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Summary. — N,N′-Bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine inhibits late function or synthesis of a late component in the replication cycle of vaccinia virus. The kinetics of formation of the component sensitive to the inhibition with N,N′-bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine precedes that of appearance of infectious virus by 30 min. The finding is in accord with the site of action of unsubstituted isatin--β-thiosemicarbazone.

Key words: vaccinia virus; N,N′-bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine; mechanism of action

Introduction

Newly synthesized Mannich N-bases were obtained by aminoalkylation of isatin-β-thiosemicarbazone by Lucka-Sobstal and Zejc (1973) in the Department of Pharmaceutical Chemistry, Medical Academy in Cracow. The compound N,N′-bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine (bis--IBTMP) which was most effective in inhibition of vaccinia virus replication in vitro (Zgórniak-Nowosielska *et al.*, 1973, Borysiewicz and Lucka-Sobstel, 1978) and showed high antiviral activity also in in vivo studies (Zgórniak-Nowosielska *et al.*, 1976, 1980) was selected for the current investigation.

The aim of this report was to determine the site of action of this derivative in the vaccinia virus rep ication cycle.

Materials and Methods

Virus and cells. The WR strain vaccinia virus was obtained from Dr. N. P. Salzman, National Institute of Allergy and Infectious Diseases, Bethesda, U.S.A. For the estimation of dose-response curve and for virus inhibitory experiments, suspension cultures of HeLa cells in basal Eagle's medium supplemented with 5% calf serum were used. The suspension of HeLa cells (5×10^6 cells per ml) was infected with 10 plaque-forming units (PFU) of vaccinia virus per cell. Adsorption

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proceeded at 37 °C for 1 hr. The suspension was then washed three times to remove unadsorbed virus. The infected cells were resuspended at a density 5×10^5 cells per ml. Unless otherwise stated, the cultures were incubated for 24 hr. All virus samples were subjected to 4 cycles of freezing and thawing prior to virus titration. The virus titres were determined by plaque assay in monolayers of chick embryo cells in Petri dishes and expressed in PFU.

Compound. The stock solution of bis-IBTMP, m. w. 564 (10 mM) was prepared in dimethylformamide; the further dilutions were made in basal Eagle's medium.

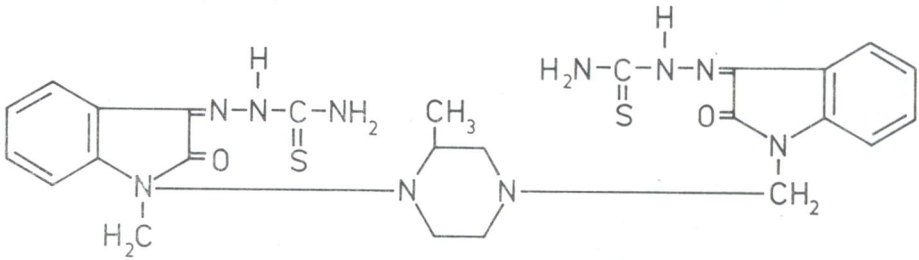

Fig. 1.

The structure of N,N′-bis-(methylisatin-β-thiosemicarbazone)-2-methylpiperazine

Results and Discussion

The toxic effect of bis-IBTMP for HeLa cells was determined in monolayer cultures in test tubes. No significant alteration in growth or morphology of HeLa cells were seen after 3 days exposure to bis-IBTMP up to concentration 30 μM confirming thereby the previous data (Zgórniak-Nowosielska *et al.*, 1973). At higher concentration (100 μM) cell rounding appeared, however this symptome was present also in control cultures containing the same concentration of dimethylformamide in the absence of the tested compound.

The maximum nontoxic concentration (30 μM) exerted higher than 2 log units inhibition of virus replication under conditions of one-step growth experiments. Dose-response curve is shown in Fig. 2.

For the experiments on action of bis-IBTMP in vaccinia virus replication cycle the cells were pelleted 1 hr after infection to remove unadsorbed virus, resuspended in medium and replicate cultures were established. The rate of formation of infectious virus was determined by sampling one culture at various times. New infectious virus was detected 6 to 8 hr after infection and the rise in titre was logarithmic for the next several hours. To other cultures bis-IBTMP (30 μM) was added at various times after infection; all cultures were sampled at 24 hr and titrated. Thus any virus formed after addition of the inhibitor had to contain the bis-IBTMP-sensitive component of the virus synthesized prior to the addition of bis-IBTMP. The experiments revealed (Fig. 3) that synthesis of a very late component of the virion or a very late function necessary for the formation of infectious virus was inhibited by bis-IBTMP. This event took place about 30 min before appearance of infectious virus.

This result is in accord with the data of Woodson and Joklik (1965) who studied the mode of action of not substituted isatin-β-thiosemicarbazone

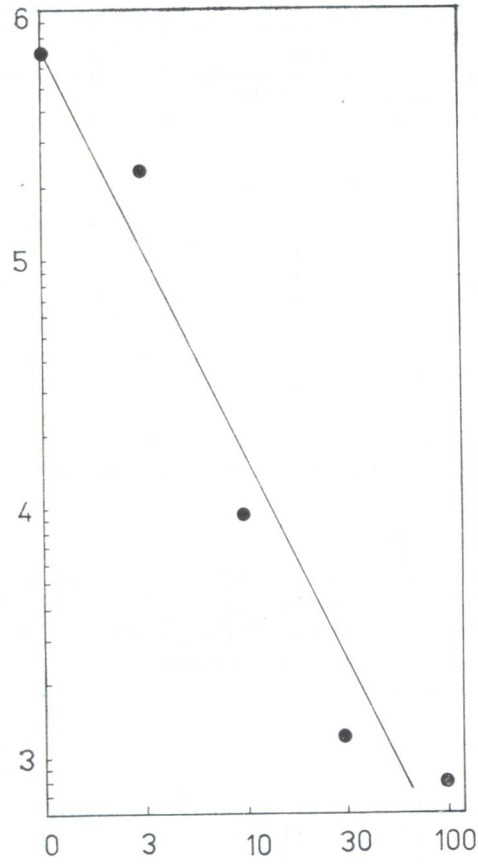

Fig. 2.
Relationship between bis-IBTMP concentration and single-cycle yield of vaccinia virus in HeLa cell cultures
Abscissa: bis-BITMP (μmol/l)
Ordinate: log PFU/ml

(IBT). IBT did not affect vaccinia virus DNA replication or viral mRNA synthesis, or functioning of early viral mRNA. However, in the presence of IBT the sedimentation coefficient of late viral mRNA, i.e. viral mRNA synthesized after first 3 hr of infection cycle, decreased from the normal value of 16S to about 8S within about 5 min of synthesis. As a result the number of polyribosomes formed by viral mRNA was greatly reduced and viral proteins programed to be translated from then on were not formed (Woodson and Joklik, 1965). These results were confirmed by Katz et al. (1978b) and Cooper *et al.* (1979). Katz *et al.* (1978a) also found that immature virus particles formed in the presence of IBT lacked two of the main core polypeptides: 4a and 4b. This block in core polypeptide integration must be a reflection of the inhibition of either messenger RNA synthesis, the translation process or interference with processing of the polypeptide precursor.

Bauer and Sadler (1960*a*) established that sulphur in the side chain of IBT was necessary for antipox activity and suggested that metal chelation may

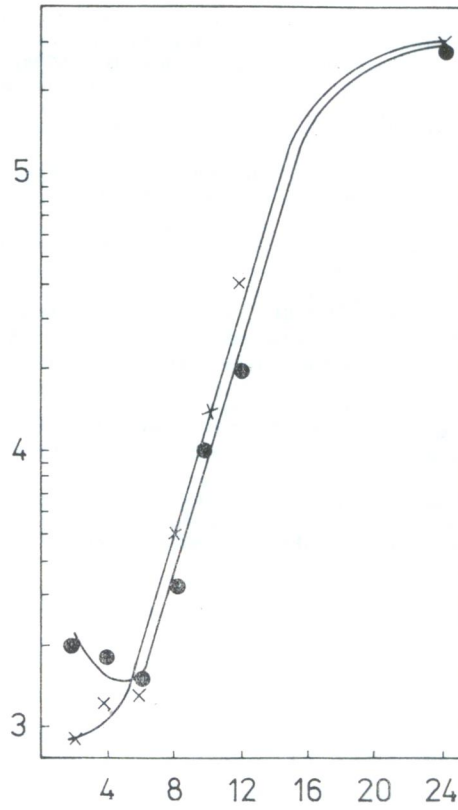

Fig. 3.
The curves of the formation of vaccinia virus component sensitive to inhibition by bis-IBTMP
Abscissa: hr after infection; ordinate: virus titre in log PFU/ml
● Virus titres in untreated control cultures
× Virus titres obtained at 24 hr after addition of bis-IBTMP (30 μM) at the times indicated by the position of the point.

be of importance for antiviral activity since vaccinia virus contains copper (Hoagland *et al.*, 1941). It is attractive to hypothesize (Pfau, 1982) that IBTs would interfere with the late mRNA stability or function, because it is only late in the infection when copper is brought to the "factory" or "virosome" areas of the cytoplasm to be incorporated into maturing virions.

Thus the present results show that the site of action of bis-IBTMP in the vaccinia virus replication cycle might be similar to the mode of action of unsubstituted parent IBT compound.

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