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# Inhibition of herpes simplex virus types 1 and 2 replication in vitro by mercurithio analogs of deoxyuridine

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## Summary

The in vitro antiviral activity of several 5-mercurithio analogs of 2′-deoxyuridine (dUrd) on the replication of herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) were examined. Of those compounds tested, the thioglycerol analog of 5-mercuri-2′-deoxyuridine (HgdUrd) was most effective in inhibiting the replication of HSV-1 in KB cells with a 50% inhibitory dose ($ID_{50}$) of 0.001 μg/ml while the glutathione analog of HgdUrd was the most effective in inhibiting the replication of HSV-2 with a $ID_{50}$ of 0.075 μg/ml. Conversely in HeLa $TK^-$ cells, the mercaptoguanosine analog of HgdUrd was the most effective compound in inhibiting virus replication with $ID_{50}$s of 0.098 and 0.001 μg/ml for HSV-1 and HSV-2 respectively. These results suggest that these mercurithio analogs of dUrd are as effective as acyclovir in preventing the replication of these herpesviruses.

Herpes simplex virus; dUTPase; Mercurithio-deoxyuridine analogs

## Introduction

Numerous nucleoside analogs have been developed for the treatment of infections caused by herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) (Cheng et al., 1976; De Clercq et al., 1980, 1986; Machida, 1986). Most of these compounds selectively inhibit HSV replication due to their preferential phosphorylation by the

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HSV encoded thymidine kinase (TK). Once converted to the triphosphate these nucleoside analogs act as inhibitors and/or alternative substrates of the HSV-encoded DNA polymerase thus preventing viral DNA synthesis.

In addition to TK and DNA polymerase, HSV encodes for other enzymes such as ribonucleotide reductase (Cohen, 1972), uracil-DNA glycosylase (Caradonna and Cheng, 1981), alkaline deoxyribonuclease (Hoffmann and Cheng, 1978) and deoxyuridine triphosphate nucleotidohydrolase (dUTPase; Caradonna and Cheng, 1981) that are involved in HSV DNA biosynthesis and replication. However, except for ribonucleotide reductase, there have been very few studies to determine whether these other HSV-encoded enzymes could be potential targets for antiviral compounds. Recently, it was reported that the HSV-encoded dUTPase could be a potential target site for the development of antiviral agents (Williams, 1988) and that certain 5-mercurithio analogs of 2′-deoxyuridine triphosphate inhibited the activity of the purified HSV-encoded dUTPases but not the cellular enzyme (Williams, 1986). Thus, the purpose of the present study was to determine whether 5-mercurithio analogs of 2′-deoxyuridine (dUrd) could inhibit the replication of HSV-1 and HSV-2 in vitro.

## Materials and Methods

HSV-1 (strain KOS) and HSV-2 (strain HG-52) were used in these studies. The procedures used for the growth of viral stocks and their maintenance have been described previously (Williams, 1984). KB and HeLa $TK^-$ (Bu25) cells were grown as monolayers at 37°C in a 5% $CO_2$ atmosphere in DMEM and RPMI 1640 respectively. Media were supplemented with 5% (v/v) heat-inactivated bovine serum, 1% (v/v) nonessential amino acids and gentamicin (50 μg/ml). HeLa $TK^-$ cells were maintained in medium supplemented with 5-bromodeoxyuridine (Budr, 25 μg/ml). Before use, the HeLa $TK^-$ were washed with PBS and allowed to grow in medium lacking Budr for at least 48 h.

The 5′-mercuric analog of dUrd (HgdUrd) was synthesized and purified according to the procedures described by Dale et al. (1973, 1975). HgdUrd was then used for the synthesis of the various 5-mercurithio-dUrd analogs (Dale et al., 1973, 1975; Williams 1985). Briefly, HgdUrd was mixed with at least a three-fold excess of the mercaptan and allowed to react at 37°C for 15 min. Conversion (>98%) of HgdUrd to the respective mercurithio analog was determined as described previously (Dale et al., 1975). The structures of these compounds are shown in Fig. 1.

The effect of the various compounds on the replication of HSV-1 and HSV-2 was determined using yield reduction plaque assays. Cells were infected with either HSV-1 or HSV-2 at an MOI (multiplicity of infection) of 5 PFU/cell. After an absorption period of 1 h at 37°C, the medium was removed. Cells were washed with PBS and fresh medium containing the test compound was added. The cells were then incubated an additional 23 h at 37°C. The cells were collected, washed twice with PBS and stored at −70°C until use in the plaque assays. Plaque assays were performed as described previously (Cheng et al., 1976) using Vero cells.

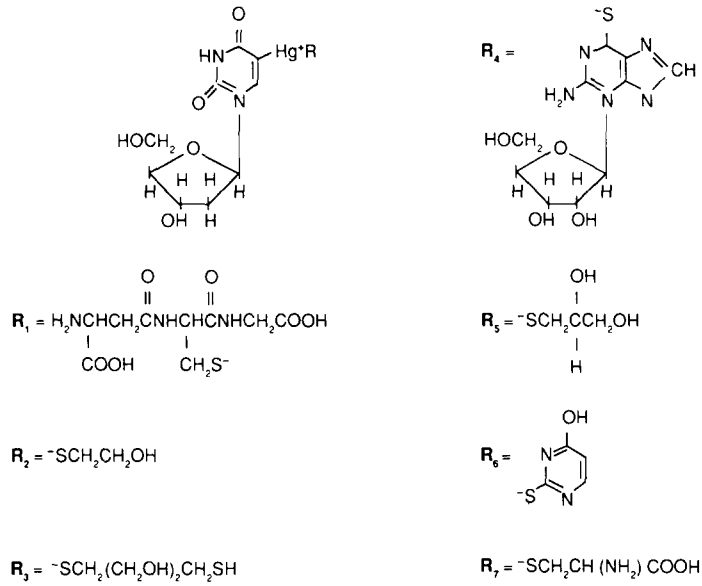


Fig. 1. Chemical structures of the 5-mercurithio–2′-deoxyuridine analogs: $R_1$, glutathione; $R_2$, mercaptoethanol; $R_3$, dithiothreitol; $R_4$, mercaptoguanosine; $R_5$, thioglycerol; $R_6$, thiouracil; $R_7$, cysteine.

The cytotoxicity of the test compounds was determined by assessing the effect of the compound on cell growth and viability. Cells were counted at 6, 18, 24, and 48 h post-treatment and viability was determined by trypan blue exclusion.

## Results and Discussion

All of the mercurithio analogs of dUrd exhibited antiviral activity (Tables 1 and 2). However, the degree of inhibition was not only dependent upon the specific compound but also upon the cell line and type of virus. For example, in KB cells the thioglycerol analog of HgdUrd was the most effective compound tested in inhibiting the replication of HSV-1, while the glutathione analog of HgdUrd was the most effective in inhibiting the replication of HSV-2. Conversely, in HeLa $TK^-$ cells, the mercaptoguanosine analog of HgdUrd was the most effective compound in inhibiting the replication of HSV-1 and HSV-2. These results suggest that the host cell may play a role in regulating the chemotherapeutic efficiency of the mercurithio analogs. Similar results concerning the role of the host cell in determining the effectiveness of other anti-herpes agents have been reported previously (De Clercq et al., 1980; De Clercq 1982).

Further support for this hypothesis was demonstrated by the cytotoxicity studies on uninfected HeLa $TK^-$ cells and KB cells. We had assumed that the mercurithio analogs of dUrd would need to be phosphorylated by TK and other cellular enzymes to the triphosphate derivative in order to be effective, and since these

TABLE 1

Effect of 5-mercurithio analogs of deoxyuridine on the replication of HSV-1 and HSV-2 in KB cells

| Compound | HSV-1 | | HSV-2 | |
|---|---|---|---|---|
| | $ID_{50}$[a] | Selectivity factor[b] | $ID_{50}$ | Selectivity factor |
| HgdUrd | 0.590 ± 0.140 | 7 | 0.112 ± 0.040 | 8 |
| Glutathione-HgdUrd | 1.252 ± 0.310 | 111 | 0.075 ± 0.003 | 1860 |
| Mercaptoethanol-HgdUrd | 0.003 ± 0.002 | 5,910 | 0.394 ± 0.121 | 48 |
| Dithiothreitol-HgdUrd | 1.204 ± 0.126 | 41 | 0.144 ± 0.070 | 340 |
| Mercaptoguanosine-HgdUrd | 0.148 ± 0.018 | 40 | 0.461 ± 0.04 | 170 |
| Thioglycerol-HgdUrd | 0.001 ± 0.001 | 54,000 | 0.050 ± 0.005 | 1100 |
| Thiouracil-HgdUrd | 0.040 ± 0.006 | >2,875 | 4.608 ± 0.179 | >25 |
| Cysteine-HgdUrd | 0.899 ± 0.131 | >127 | 0.176 ± 0.040 | >640 |

[a]Concentration of analog in μg/ml required to decrease virus yield by 50% when compared to untreated controls. Values represent the average ± the standard deviation for at least three different assays. At least three different concentrations of each analog were examined.
[b]Determined by dividing the amount (μg/ml) of analog required to kill 50% of the cells ($LD_{50}$) by the $ID_{50}$ of the analog for the virus.

TABLE 2

Effect of 5-mercurithio analogs of deoxyuridine on the replication of HSV-1 and HSV-2 in HeLa $TK^-$ cells

| Compound | HSV-1 | | HSV-2 | |
|---|---|---|---|---|
| | $ID_{50}$[a] | Selectivity factor[b] | $ID_{50}$ | Selectivity factor |
| HgdUrd | 0.448 ± 0.139 | 15 | 0.148 ± 0.030 | 45 |
| Glutathione-HgdUrd | 1.833 ± 0.550 | 31 | 0.377 ± 0.098 | 150 |
| Mercaptoethanol-HgdUrd | 0.074 ± 0.030 | 260 | 0.053 ± 0.020 | 370 |
| Dithiothreitol-HgdUrd | 0.403 ± 0.066 | 121 | 0.602 ± 0.187 | 81 |
| Mercaptoguanosine-HgdUrd | 0.098 ± 0.018 | 325 | 0.001 ± 0.001 | 53300 |
| Thioglycerol-HgdUrd | NT[c] | | NT | |
| Thiouracil-HgdUrd | NT | | NT | |
| Cysteine-HgdUrd | NT | | NT | |

[a]Concentration of analog in μg/ml required to decrease virus yield by 50% when compared to untreated controls. Values represent the average ± the standard deviation for at least three different assays. At least three different concentrations of each analog were examined.
[b]Determined by dividing the amount (μg/ml) of the analog required to kill 50% of the cells ($LD_{50}$) by the $ID_{50}$ of the analog for the virus.
[c]Not tested.

HeLa cells lack a functional cytoplasmic TK, we assumed that this cell line would be more resistant than KB cells to the mercurithio analogs of dUrd. However, some of the mercurithio analogs of dUrd were more cytotoxic to the HeLa $TK^-$ cells. While this could be due to differences in the amounts of sulfhydryl-reducing agents (glutathione) in the cells, we do not believe that this is the case as it has been previously reported that the mercurithio analogs of various nucleotides are relatively resistant to demercuration (Dale et al., 1973, 1975). However, it may reflect differ-

TABLE 3

Cytotoxic effect of 5-mercurithio analogs of deoxyuridine on KB and HeLa TK$^-$ cells

| Compound | Cell line $LD_{50}$[a] | |
|---|---|---|
| | KB | HeLa TK$^-$ |
| HgdUrd | 4.20 ± 1.30 | 5.70 ± 0.86 |
| Glutathione-HgdUrd | 139.00 ± 5.80 | 56.60 ± 4.30 |
| Mercaptoethanol-HgdUrd | 18.90 ± 1.80 | 19.40 ± 0.88 |
| Dithiothreitol-HgdUrd | 48.80 ± 2.40 | 48.80 ± 1.70 |
| Mercaptoguanosine-HgdUrd | 79.30 ± 3.20 | 32.00 ± 1.23 |
| Thioglycerol-HgdUrd | 53.80 ± 0.85 | 15.00 ± 1.60 |
| Thiouracil-HgdUrd | >115 | >115 |
| Cysteine-HgdUrd | >114 | >114 |

[a]The amount (μg/ml) of analog required to kill 50% of the cell population as measured by trypan blue staining. Values represent the average of at least three determinations ± the standard deviation. At least three different concentrations of the inhibitors were examined.

ences in the incorporation and subsequent metabolism of the compound and/or alternative target sites in the host cell.

The function of the HSV-encoded dUTPase in virus replication is unknown. While mutants of HSV-1 have been constructed which are defective in expressing the HSV-encoded dUTPase in infected cells (Fisher and Preston, 1986; Lirette and Caradonna, 1990), these mutants are double mutants in that they also fail to 'shut-off' cellular dUTPase activity in the infected cells (Williams, 1988; Lirette and Caradonna, 1990). Thus it is not possible to determine at this time whether this enzyme is 'essential' for normal virus replication and/or pathogenesis.

We have previously reported that the HSV-encoded dUTPase has a role in regulating the chemotherapeutic efficiency of specific agents and have suggested that this enzyme could be used as a target site for the development of specific antiviral agents (Williams, 1988). The mercurithio analogs used in this study were chosen because their triphosphate derivatives inhibit the activities of the purified HSV-1- and HSV-2-encoded dUTPase (Williams, 1986). The results of this study demonstrate that these mercurithio analogs of dUrd inhibit the replication of both HSV-1 and HSV-2 in cultured cells. Some of these analogs, the mercaptoguanosine, thioglycerol, thiouracil and mercaptoethanol derivatives, inhibited the replication of HSV-1 and HSV-2 at concentrations which are comparable to those reported for acyclovir (De Clercq et al., 1980, 1986; Machida et al., 1986). While the data do not demonstrate that these analogs inhibit the replication of HSV-1 and HSV-2 by inhibiting the HSV-encoded dUTPase, it is possible that these agents do inhibit the activity of this enzyme and that this results in the inhibition of some function that is required for virus replication. Alternatively, the inhibition of the dUTPase may allow for the accumulation of the triphosphate derivative in the cell, resulting in the inhibition of the virus-encoded DNA polymerase or in the incorporation of the analog into viral DNA by the DNA polymerase. However, additional studies are necessary to determine the mechanisms by which these mercurithio analogs of dUrd inhibit the replication of HSV. Furthermore, while some mercuric com-

pounds are used for the topical treatment of bacterial infections (Hyndiuk et al., 1990) mercury compounds are not used to treat systemic infections due to the associated risk of cytotoxicity. Thus, while these mercurithio analogs of dUrd may act as base-mechanism inhibitors (Dale et al., 1975; Williams et al., 1986), further studies in animals are necessary to determine not only the chemotherapeutic effectiveness of these compounds for treating infections caused by these viruses but also their potential toxic effects.

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