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HERPES AND HUMAN RIBONUCLEOTIDE REDUCTASES

INHIBITION BY 2-ACETYLPYRIDINE 5-[(2-CHLOROANILINO)-THIOCARBONYL]-THIOCARBONOHYDRAZONE (348U87)

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Abstract—The mode of inactivation of herpes simplex virus type 1 and human ribonucleotide reductases by 2-acetylpyridine 5-[(2-chloroanilino)-thiocarbonyl]-thiocarbonohydrazone (348U87) was determined and compared to that described previously [Porter *et al. Biochem Pharmacol* **39**: 639–646, 1990] for 2-acetylpyridine 5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone (A1110U). 348U87 inactivated herpes ribonucleotide reductase faster than did A1110U. Moreover, iron-complexed 348U87 was a considerably more potent inactivator than iron-complexed A1110U. It appeared to efficiently form an initial complex with the viral enzyme prior to rapid enzyme inactivation. The combination of 348U87 and iron-complexed 348U87 inactivated with a rate constant that was slightly greater than the sum of their individual rate constants of inactivation. The corresponding combination of A1110U species inactivated with a rate constant that was much greater than the sum of the individual rate constants of inactivation. Herpes ribonucleotide reductase that had been inactivated by either species of 348U87 was reactivated by diluting the enzyme and inactivators into assay medium containing excess iron. 348U87 was also an effective inactivator of herpes simplex virus type 2 and varicella zoster virus ribonucleotide reductases. The iron-complexed forms of 348U87 and A1110U exhibited very different modes of inactivation of human ribonucleotide reductase. Iron-complexed 348U87 was a tight-binding inactivator, whereas iron-complexed A1110U was only a weak, non-inactivating, inhibitor. Furthermore, the inactivation by iron-complexed 348U87 was not stimulated by either 348U87 or A1110U, whereas the weak inhibition by iron-complexed A1110U was converted to rapid inactivation by A1110U. Excess iron prevented the inactivation by iron-complexed 348U87. Uncomplexed 348U87 was similar to uncomplexed A1110U in that it was not an inhibitor of the human enzyme.

In 1985, 2-acetylpyridine 4-(2-morpholinoethyl)-thiosemicarbazone (A723U), an inactivator of herpes viruses ribonucleotide reductase, was shown to potentiate the antiviral activity of acyclovir (ACV†) *in vitro* [1, 2]. Subsequently, 2-acetylpyridine 5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone (A1110U) (Fig. 1), a more potent inactivator of the viral ribonucleotide reductases, was found to potentiate ACV *in vivo* [3, 4] as well as *in vitro* [5]. The mode of inactivation by A1110U and the basis for its preferential inhibition of the viral versus human ribonucleotide reductase were studied in detail [6]. In brief, either A1110U or $(A1110U)_2Fe$, the iron-complex of A1110U, inactivates herpes simplex virus type 1 (HSV-1) ribonucleotide reductase. Moreover, the combination of A1110U and $(A1110U)_2Fe$ produces a very rapid (much greater than the sum of the individual constants) rate constant of inactivation. Conversely, A1110U is not an inhibitor of human ribonucleotide reductase, and $(A1110U)_2Fe$ is only a weak (non-inactivating) inhibitor. The human enzyme is inactivated only when A1110U and $(A1110U)_2Fe$ are present simultaneously. Furthermore, the second-order rate constant for inactivation of viral ribonucleotide reductase by $(A1110U)_2Fe$ in the presence of A1110U is significantly larger than the rate constant for inactivation of the human enzyme ($25 \times 10^6\ M^{-1}\ hr^{-1}$ at 30° vs $3.8 \times 10^6\ M^{-1}\ hr^{-1}$ at 37°).

Recently, 2-acetylpyridine 5-[(2-chloroanilino)-thiocarbonyl]-thiocarbonohydrazone (348U87) (Fig. 1) was shown to potentiate the antiviral activity of ACV as effectively as A1110U. However, 348U87 has a safer toxicological profile.‡ The Burroughs Wellcome Co. is currently developing the combination of 348U87 and ACV for topical therapy of cutaneous herpetic disease in humans. The present report describes the mode of inhibition of herpes and human ribonucleotide reductases by 348U87 and its iron complex. Although 348U87 and A1110U are structurally similar, and form similar complexes with iron, they inhibit these enzymes by significantly different modes.

MATERIALS AND METHODS

Reagents.

A1110U was synthesized as described elsewhere

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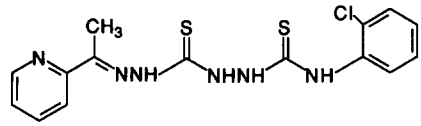
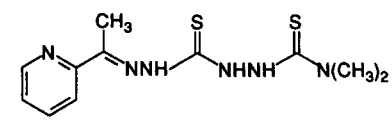
† Abbreviations: ACV, acyclovir (9-[(2-hydroxyethoxy)-methyl]guanine); ACV-TP, acyclovir triphosphate; HSV, herpes simplex virus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VZV, varicella zoster virus; DTT, dithiothreitol; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; A723U, 2-acetylpyridine 4-(2-morpholinoethyl)thiosemicarbazone; A1110U, 2-acetylpyridine 5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone; and 348U87, 2-acetylpyridine 5[-(2-chloroanilino)-thiocarbonyl]-thiocarbonohydrazone.

‡ Spector T, Lobe DC, Ellis MN and Szczech G, data to be published.



348U87



A1110U

Fig. 1. Structures of 348U87 and A1110U.

[2]. 348U87 was synthesized in these laboratories by T. Blumenkopf by a method to be published. All reagents not described below were obtained as described previously [7].

Ribonucleotide reductase purifications and assays. HSV-1 [7], herpes simplex virus type 2 (HSV-2) [8], varicella zoster virus (VZV) [2] and human [9, 10] ribonucleotide reductases were partially purified as described in the indicated references. The enzymes were assayed by monitoring the reduction of purified [^{14}C]CDP [7]. Viral ribonucleotide reductases were assayed in glass vials containing 200 mM Hepes Na^+ at pH 7.7, 10 mM dithiothreitol, and 2 μM (500 Ci/mol) CDP (3–4 × K_m [2, 7, 8]) as described previously [7]. Human ribonucleotide reductase was assayed in glass vials containing 100 mM Hepes at pH 7.4 [11], 5 mM dithiothreitol, 12 μM CDP (5 × K_m [11]) with a specific activity of 75 Ci/mol, 5 mM ATP (rendered iron free [6]), and 6 mM $MgCl_2$. The Hepes and dithiothreitol solutions were stored with Chelex 100 to minimize contamination by iron [6]. Multiple reactions were assayed simultaneously as described elsewhere [12]. The reactions were terminated with hydroxyurea and EDTA, which quenched the reaction and decreased the blank rates to an insignificant level [7, 9]. The product was dephosphorylated and isolated by chromatography on a Dowex-borate column according to the modified [7] method of Steeper and Steuart [13]. The reaction temperature was 37°.

Preparation of iron complexes of inactivators. Either A1110U or 348U87 was mixed with $FeSO_4$ in a molar ratio of 2:1.1 to yield $(A1110U)_2Fe$ or $(348U87)_2Fe$ and a 10% molar excess of iron. This ensured that all the inactivator was complexed with iron. The excess iron was sequestered subsequently with 2 molar equivalents of EDTA (0.1 mol EDTA per mol 348U87). A sample prepared to mimic the 10% excess $FeSO_4$ and EDTA had no effect on the enzymatic reactions of either HSV-1 or human ribonucleotide reductase. Solutions of A1110U and 348U87 were prepared daily and were quantitated by their UV absorbance; ε_{310} pH 7 = 23.1 mM^{-1}, cm^{-1} and ε_{311} in methanol = 25.7 mM^{-1}, cm^{-1}, respectively.

Sequestering iron with EDTA or 1,10-phenanthroline. To demonstrate that either EDTA or 1,10-phenanthroline complexed iron was not readily accessible to 348U87, the following experiments were performed. Stock solutions of $FeSO_4$ with the iron chelators were diluted (final concentration: 10 μM $FeSO_4$ and either 25 μM EDTA or 2 mM 1,10-phenanthroline) into a cuvette containing 10 μM 348U87 and 0.05 M Hepes Na^+, pH 7.7. The visible absorbance spectra were recorded versus a reference cuvette containing 10 μM 348U87 and 0.05 M Hepes Na^+, pH 7.7. The spectrum of 10 μM $FeSO_4$ and either 25 μM EDTA or 2 mM 1,10-phenanthroline was subtracted from these recorded spectra to yield a flat baseline. Since no spectral changes occurred over 50 min in the region where 348U87-iron absorbs (340–700 nm, ε_{412} = 8.7 mM^{-1}, cm^{-1}), EDTA and 1,10-phenanthroline successfully sequestered iron from 348U87. Trace amounts of the 348U87–iron complex would have been readily detected by this method.

Miscellaneous. First-order rate constants for enzyme inactivation, k_{inact}, and initial velocities, v_o, of decelerating time courses of product formation were calculated by iterative non-linear least squares analysis [14] of the following equation:

$$P(t) = P_f[1 - e^{-kt}]$$

where $P(t)$ is the product formed at time t, P_f is the amount of product formed at infinite time, k is k_{inact}, and $k \cdot P_f$ is v_o. The k_{inact} values for inactivators are reported as the net increase over the k_{inact} (inherent instability) of the uninhibited controls.

Plots of k_{inact} versus the concentration of inactivator were fitted to a hyperbola according to the computer program of Cleland [15] to calculate the maximum k_{inact} and the K_d (concentration producing 1/2 maximum k_{inact}).

RESULTS

Formation of 348U87–iron complex. As previously observed with A1110U, a complex between 348U87 and iron was detected by $FeSO_4$-induced perturbations in the absorbance spectrum of 348U87. A stoichiometry of 2 mol 348U87 to 1 mol iron was determined by titrating these spectral changes at 412 nm, ε_{412} = 8.7 mM^{-1}, cm^{-1} (data not shown). Furthermore, the iron remained bound to 348U87 even in the presence of a 250-molar excess of EDTA. Freshly prepared (see Materials and Methods) iron complexes of 348U87 and A1110U were used in the following studies.

Inactivation of HSV-1 ribonucleotide reductase. 348U87, $(348U87)_2Fe$, and the combination of these species were studied as inhibitors of herpes ribonucleotide reductase. Submicromolar concentrations of either $(348U87)_2Fe$ or 348U87 appeared to inactivate the enzyme (Fig. 2). The concentration of $(348U87)_2Fe$ was varied to produce

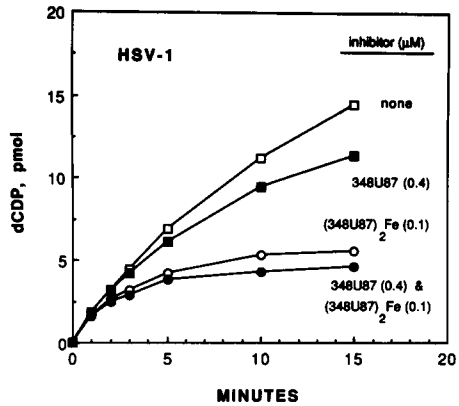


Fig. 2. Inactivation of HSV-1 ribonucleotide reductase. HSV-1 ribonucleotide reductase was assayed in the presence of 348U87, $(348U87)_2Fe$, and the combination of these species as indicated.

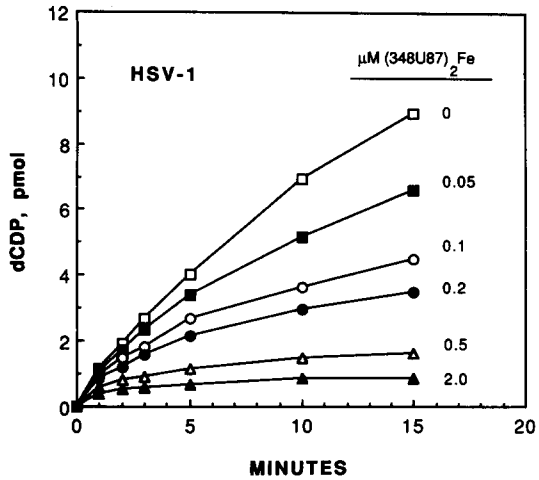


Fig. 3. Inactivation of HSV-1 ribonucleotide reductase by $(348U87)_2Fe$. HSV-1 ribonucleotide reductase was assayed in the presence of the indicated concentrations of $(348U87)_2Fe$.

the data shown in Fig. 3. A plot of the first-order rate constant for inactivation (k_{inact}) versus the concentration of $(348U87)_2Fe$ was fitted to a hyperbola to reveal a maximum k_{inact} of $27 \pm 2\ hr^{-1}$ and a K_d (concentration producing 1/2 maximum k_{inact}) of $0.57 \pm 0.1\ \mu M$. The analogous constants determined for 348U87 were $20 \pm 2\ hr^{-1}$ and $1.1 \pm 0.3\ \mu M$, respectively.

It was possible that the apparent inactivation of HSV-1 ribonucleotide reductase by 348U87 was caused by $(348U87)_2Fe$, which could have been generated from 348U87 and minute amounts of adventitious iron. Concentrations of iron as low as $0.05\ \mu M$ could generate sufficient $(348U87)_2Fe$ to effectively inactivate the enzyme. Therefore, the buffer, enzyme, and substrate were pretreated with $1\ \mu M$ EDTA to chelate and sequester residual iron (see Materials and Methods). Nevertheless, this iron-depleted medium did not prevent $2\ \mu M$ 348U87 from readily inactivating the enzyme; the k_{inact} was $15.1\ hr^{-1}$ in standard assay medium, and $14.1\ hr^{-1}$ in EDTA-treated assay medium. Thus, we conclude that the standard assay conditions with Chelex 100-treated buffer depleted residual iron, and that 348U87 was an inactivator of herpes ribonucleotide reductase.

The combination of 348U87 and $(348U87)_2Fe$ produced a k_{inact} of $18\ hr^{-1}$, which was slightly greater than the sum of the k_{inact} of the individual species ($15\ hr^{-1}$). This slightly synergistic relationship, which was reproduced in three similar experiments, contrasts to the strongly synergistic pattern previously observed with the combination of A1110U and $(A1110U)_2Fe$ [6]. Therefore, we retested the latter compounds under the present conditions and, as before, observed the considerably stronger synergy. The k_{inact} for $1\ \mu M$ A1110U and $0.5\ \mu M$ $(A1110U)_2Fe$ together was $17\ hr^{-1}$ versus $11\ hr^{-1}$ for the sum of the individual k_{inact} values of $2\ \mu M$ A1110U ($8\ hr^{-1}$) and $1\ \mu M$ $(A1110U)_2Fe$ ($3\ hr^{-1}$). We note that to measure reliable k_{inact} values for the individual species, the concentrations of A1110U and $(A1110U)_2Fe$ were twice the concentrations used when both species were present.

Since herpes ribonucleotide reductase was highly sensitive to inactivation by $(348U87)_2Fe$, the reaction buffer was pretreated with Chelex 100 to minimize contamination by iron (see Materials and Methods). This precaution was not necessary with $(A1110U)_2Fe$ as it is a considerably weaker inactivator [6]. However, the enzyme was less stable and less active in the more stringently iron-depleted medium. When this reaction medium was supplemented with very low concentrations of $FeSO_4$, the enzymatic activity and stability were restored. For example, concentrations of $FeSO_4$ as low as $0.2\ \mu M$ increased the initial velocity and reduced the rate constant for the inherent loss of enzyme activity. A maximal 1.5- to 2-fold stimulation of the initial velocity was achieved with 2–5 μM $FeSO_4$.

Reactivation of HSV-1 ribonucleotide reductase. Enzyme inactivated by 348U87, $(348U87)_2Fe$, or the combination of these species was reactivated by dilution into fresh medium supplemented with $FeSO_4$. HSV-1 ribonucleotide reductase was incubated with the inactivators and then diluted 22-fold by adding assay medium containing either no available iron (EDTA treated) or $5\ \mu M$ $FeSO_4$. The activity of the enzyme in iron-depleted medium reflected the degree of inactivation that occurred during the preincubation. The results (Table 1) show that removing the inactivators by dilution and adding excess iron completely regenerated active enzyme.

We noticed that the enzyme appeared less sensitive to inactivation during these preincubations than it was during catalytic cycling (see Fig. 2). Therefore, an additional preincubation experiment was performed with cycling enzyme. When the substrate, CDP, was included in the preincubation mixture, the degree of inactivation was enhanced from 78 to 97%.

Inactivation of HSV-2 and VZV ribonucleotide reductases. Abbreviated studies were performed with HSV-2 and VZV ribonucleotide reductases. Since

Table 1. Reactivation of HSV-1 ribonucleotide reductase

	Relative activity after 22-fold dilution into:	
Preincubated 10 min with*:	1 μM EDTA	5 μM $FeSO_4$
No addition	100	100
348U87	33	98
$(348U87)_2Fe$	49	96
348U87 and $(348U87)_2Fe$	22	91

* Reaction mixtures (10 μL) were incubated at 37° in the standard assay medium less CDP. The concentrations of inhibitors were: 348U87, 1 μM; and $(348U87)_2Fe$, 0.5 μM. After 10 min, 210 μL of complete medium, equilibrated to 37°, was added to dilute the inhibitors and initiate the reaction. Samples were taken at four time points and analyzed for product formation. The velocity of the control reaction was 34 pmol/hr. No activity was lost during preincubation of the control.

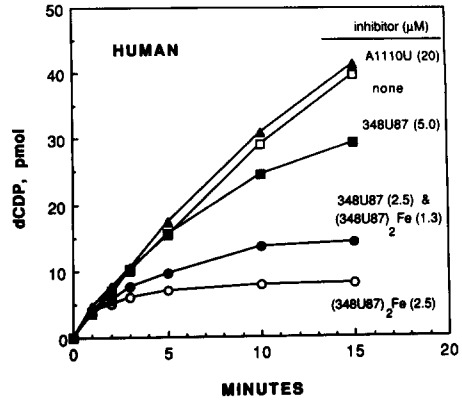


Fig. 5. Inhibition of human ribonucleotide reductase. Human ribonucleotide reductase was assayed in the presence of 348U87, $(348U87)_2Fe$, the combination of these species, and A1110U as indicated.

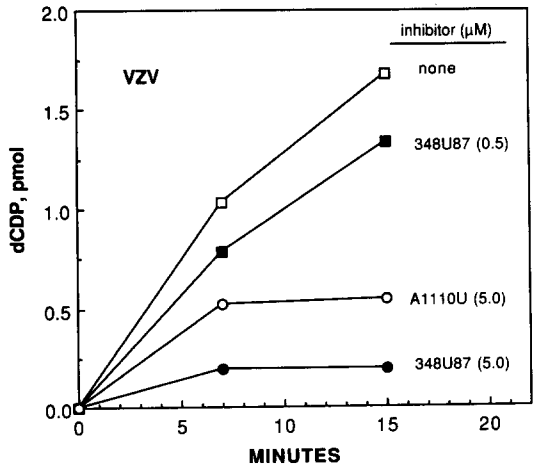


Fig. 4. Inactivation of VZV ribonucleotide reductase. VZV ribonucleotide reductase was assayed in the presence of the indicated concentrations of 348U87 and A1110U.

iron depletion would cause the inherent low stability of HSV-2 ribonucleotide reductase [8] and the low activity of VZV ribonucleotide reductase [2] to deteriorate to unacceptable levels, these enzymes were assayed in buffer that was not pretreated with Chelex 100. 348U87 inactivated HSV-2 ribonucleotide reductase at concentrations very similar to those required to inactivate the HSV-1 enzyme. A maximum k_{inact} of 24 ± 3 hr^{-1} and a K_d of 0.6 ± 0.3 μM were calculated from data not shown. In addition, 348U87 also appeared to inactivate VZV ribonucleotide reductase (Fig. 4).

Inactivation of human ribonucleotide reductase. 348U87, $(348U87)_2Fe$, the combination of these species, and A1110U were also tested as inhibitors of human ribonucleotide reductase. The results shown in Fig. 5 clearly demonstrate that $(348U87)_2Fe$ was an effective inactivator and, as previously observed [6], A1110U was not an inhibitor. A titration experiment showed that the observed k_{inact} was hyperbolically dependent on the concentration of $(348U87)_2Fe$. A maximum k_{inact} of 34 ± 4 hr^{-1} and a K_d of 0.70 ± 0.2 μM were obtained (data not shown). In contrast to A1110U, 348U87 appeared to be a weak inactivator. However, 0.25 μM adventitious iron could generate enough $(348U87)_2Fe$ to produce this rate of inactivation. Therefore, studies with 348U87 were repeated in the presence of 2 mM 1,10-phenanthroline (Sigma), which would chelate and sequester residual iron* (see Materials and Methods). Phenanthroline, which alone had no effect on the enzymic activity, totally eliminated the appearance of inactivation and inhibition of human ribonucleotide reductase by 348U87 (data not shown). Thus, in an iron-depleted assay medium, 348U87 was neither an inhibitor nor an inactivator of human ribonucleotide reductase.

The data of Fig. 5 indicate that 348U87 did not enhance the rate of inactivation by $(348U87)_2Fe$. This finding contrasts with the earlier finding that A1110U promoted $(A1110U)_2Fe$ from a weak inhibitor to an inactivator of human ribonucleotide reductase [6]. The experiment was repeated with $(348U87)_2Fe$ in combination with 348U87 and three other iron chelators. The rate of inactivation of the enzyme by 1 μM $(348U87)_2Fe$ (k_{inact} = 16 hr^{-1}) was not increased by including 2 μM 348U87 (k_{inact} = 15 hr^{-1}), 2 μM A1110U (k_{inact} = 12 hr^{-1}), 2 μM 1,10-phenanthroline (k_{inact} = 15 hr^{-1}), or 1 μM desferal (Ciba) (k_{inact} = 14 hr^{-1}) in the reaction mixtures with 1 μM $(348U87)_2Fe$.

Prevention of the inactivation of human ribonucleotide reductase. Human ribonucleotide reductase lost about 60% of its activity during preincubations for 10 min at 37°. Therefore, reactivation experiments similar to those performed with the HSV-1 enzyme could not be performed. However, very low concentrations of $FeSO_4$

* The magnesium in the assay medium of human ribonucleotide reductase prevented the use of EDTA to chelate iron.

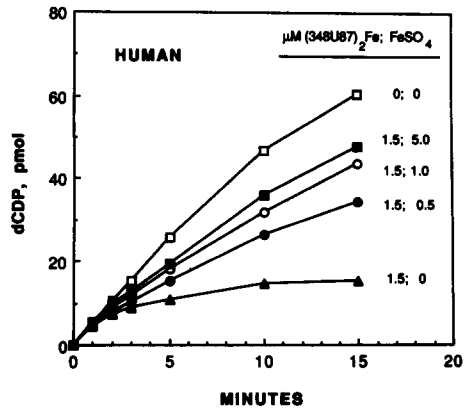


Fig. 6. Prevention of the inactivation of human ribonucleotide reductase by $(348U87)_2Fe$. Human ribonucleotide reductase was assayed in the presence of 1.5 μM $(348U87)_2Fe$ and the indicated concentrations of $FeSO_4$.

Table 2. Summary of the effects of 348U87 and A1110U

	Ribonucleotide reductase	
	HSV-1	Human
A1110U	Inactivation*	No effect
348U87	Inactivation*	No effect
$(A1110U)_2Fe$	Inactivation	Inhibition
$(348U87)_2Fe$	Inactivation*	Inactivation*
A1110U plus $(A1110U)_2Fe$	Highly synergistic inactivation	Inactivation
348U87 plus $(348U87)_2Fe$	Slightly synergistic inactivation	Inactivation by $(348U87)_2Fe$

* K_d of initial complex was ≤ 1 μM.

completely prevented $(348U87)_2Fe$ from inactivating human ribonucleotide reductase. The rates of inactivation by $(348U87)_2Fe$ were measured in the presence of various concentrations of $FeSO_4$ (Fig. 6). In the absence of iron, 1.5 μM $(348U87)_2Fe$ had a k_{inact} of 14 hr^{-1} and produced 30% inhibition of the initial velocity. When 0.5 μM $FeSO_4$ was included in the reaction mixture, the k_{inact} was reduced to 1 hr^{-1}, but the initial inhibition remained unaffected. $FeSO_4$ at concentrations ranging from 1 to 10 μM prevented $(348U87)_2Fe$ from inactivating the enzyme without affecting its inhibitory potency. Thus, low concentrations of iron convert $(348U87)_2Fe$ from an inactivating inhibitor to a non-inactivating inhibitor. Accordingly, $(A1110U)_2Fe$ was retested under the present conditions to ensure that the previously noted [6] lack of inactivation was not due to the presence of trace amounts of adventitious iron. As before, 10 μM $(A1110U)_2Fe$ produced inhibition (33%) of human ribonucleotide reductase, but no inactivation. The effect of $(A1110U)_2Fe$ was totally unaltered by 5 μM $FeSO_4$ (data not shown).

DISCUSSION

Compound 348U87 evolved from an extensive study to find an inactivator of herpes ribonucleotide reductase that potentiates the antiviral activity of ACV without toxicity to host organisms. As 348U87 is similar to A1110U with respect to structure and antiviral properties, we expected these compounds to display similar modes of inactivation of the viral and human enzymes. However, we detected significant differences between their modes of inactivation of herpes ribonucleotide reductase, and even greater differences in their modes of inactivation of human ribonucleotide reductase. For comparison, the effects of A1110U and 348U87 and their iron complexes on HSV-1 and human ribonucleotide reductases are summarized in Table 2.

As an iron chelator, 348U87 was similar to A1110U and the other iron chelators [6] that independently inactivated herpes ribonucleotide reductase. Since iron restores the activity of 348U87-inactivated enzyme, the chelators probably inactivated this enzyme by directly removing its catalytically essential iron. Interestingly, $(348U87)_2Fe$ and $(A1110U)_2Fe$ also inactivated the viral enzyme. Since these compounds are already complexed with iron, they cannot directly remove enzyme-bound iron. Instead, they more likely bind the enzyme in a manner that labilizes the enzyme-bound iron to dissociation. Since plots of k_{inact} versus the concentration of $(348U87)_2Fe$ or $(A1110U)_2Fe$ [6] were hyperbolic, an initial complex between the inactivators and the enzymes was formed prior to inactivation [16]. One can postulate that the tightness of the initial complex determines the degree to which the essential iron is labilized. Thus, the higher affinity of $(348U87)_2Fe$ in these initial complexes with viral and human enzymes may account for the different modes of inactivation by 348U87 and A1110U. The K_d of $(348U87)_2Fe$ with herpes ribonucleotide reductase is 1/10 the K_d of $(A1110U)_2Fe$. Consequently, the tight-binding of $(348U87)_2Fe$ to the viral enzyme may have labilized extensively the enzyme-bound iron. Since 348U87 and $(348U87)_2Fe$ were only slightly better than additive inactivators, it seems that 348U87 may have (1) modestly facilitated the removal of highly labilized essential iron from $(348U87)_2Fe$-bound enzyme, or (2) removed iron from other enzyme molecules not bound by $(348U87)_2Fe$. In contrast, the weaker binding of $(A1110U)_2Fe$ less extensively labilized the essential iron and thereby provided a greater opportunity for A1110U to facilitate iron removal. Thus, A1110U and $(A1110U)_2Fe$ may act in concert for synergistic inactivation.

Similarly, $(348U87)_2Fe$ was a tight-binding inactivator, and $(A1110U)_2Fe$ was only a weak inhibitor of human ribonucleotide reductase. As postulated above, the tight binding of $(348U87)_2Fe$ to human enzyme probably inactivated it by promoting the release of enzyme-bound iron. On the other hand, $(A1110U)_2Fe$, which weakly binds the enzyme, seems incapable of independently forcing the release of catalytically essential iron. However, it probably labilized the iron for removal by A1110U and other iron chelators. Thus, $(A1110U)_2Fe$ inactivates

human ribonucleotide reductase only in the presence of an iron chelator. Neither 348U87 nor A1110U [6] is an independent inhibitor of the human enzyme.

The present study and the earlier investigation [6] indicate that inactivation involves the removal of a catalytically essential iron from both enzymes. Apparently, the iron bound to the herpes enzymes is more accessible to removal since 348U87, A1110U, and other iron chelators independently inactivated these enzymes. Furthermore, herpes ribonucleotide reductase appeares more susceptible to inactivation by 348U87 and $(348U87)_2Fe$ when it is catalytically cycling. Catalytically active enzyme is also more sensitive than resting enzyme to inactivation by A723U [1] and by A1110U and $(A1110U)_2Fe$ [6].

In conclusion, both 348U87 and $(348U87)_2Fe$ were potent independent and additive inactivators of herpes ribonucleotide reductase. However, only $(348U87)_2Fe$ inactivated human ribonucleotide reductase.

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