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HP 0.35, a cephalosporin degradation product is a specific inhibitor of lentiviral RNAses H

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ABSTRACT

Penicillins, cephalosporins and other betalactam antibiotics are widely used antibacterial drugs. Recently it was found that some of them also have effects on proliferating eukaryotic cells (Neftel,K.A. and Hübscher,U. (1987) *Antimicrob. Agents Chemother.* 31, 1657–1661), and one such effect was shown to be the inhibition of DNA polymerase α (Huynh Do,U., Neftel,K.A., Spadari,S. and Hübscher,U. (1987) *Nucl. Acids Res.* 15, 10495–10506). The data suggested that degradation products of betalactam antibiotics were responsible for the inhibitory effect on DNA polymerase α. There is some confirmation at the structural level, since we found that penicillin binding proteins, the natural target of the cephalosporins, share amino-acid homologies to DNA polymerases and also to reverse transcriptase from HIV1 (Hafkemeyer,P., Neftel,K.A. and Hübscher,U. *Meth. Find. Exp. Clin. Pharmacol.* 12, 43–46, 1990).We have purified and determined the structure of one product from the cephalosporin Ceftazidim and found one molecule (HP 0.35) that did not interfere with eukaryotic cell proliferation but rather had a specific inhibitory effect on the RNase H activity of human immunodeficiency virus 1 (HIV1) and feline immunodeficiency virus (FIV) reverse transcriptases, while the DNA polymerising activity of these enzymes was not affected. RNases H from HeLa cells, calf thymus and *Escherichia coli* on the other hand were much less affected by HP 0.35. The inhibitory concentration of 50% (IC_{50}) was more than 10 times lower compared to those of all cellular RNases H. We therefore tested the effect of HP 0.35 on in vitro lentivirus infection as exemplified by FIV-infection of CD4$^+$-cat lymphocytes in cell culture. Under conditions where cell proliferation was absolutely unaffected, HP 0.35 was able to inhibit FIV-infection in CD4$^+$-cat lymphocytes. Moreover, preincubation of these lymphocytes with HP 0.35 rendered the cells completely unsusceptible to FIV-infection. These data suggest that a degradation product of a clinically used betalactam antibiotic might represent an effective inhibitor class for lentiviral RNase H.

INTRODUCTION

Penicillins and cephalosporins (Betalactam antibiotics, BLA) are the most widely used antibacterial drugs. They were for a long time believed to be strong immunogens but practically non-toxic to eukaryotic cells, nevertheless they induce a variety of adverse reactions, which clearly depend on both, daily dose and duration of treatment (1–3). It was observed that patients treated with BLA over a long period of time had the more severe toxic effects due to BLA compared to an application over a short period of time (1–3). Later it was found that BLA specifically affected the proliferation of cultured eukaryotic cells in the S-phase (4). Moreover the effect of these BLA on proliferative cells was enhanced if they were degraded in aqueous solutions before testing (5). These initial observations were confirmed at the biochemical level, when experiments showed that BLA and especially some of their degradation products inhibited the cellular DNA polymerase α (6). There was an identical hierarchy of toxicity of various BLA at two levels, namely the antiproliferative effect on cells and the inhibitory activity on DNA polymerase α (7).

The specific targets of BLA in the bacterial cell wall are the penicillin binding proteins (8). Some of these penicillin binding proteins were recently found to have amino-acid sequence homology to DNA polymerase α and also to HIV1-reverse transcriptase (9). The striking results were that (i) those penicillin binding proteins exclusively binding to penicillin binding protein 2 did not inhibit DNA polymerase α, while penicillin binding protein 2, as an exception, did not exhibit homology and that (ii) the homologous amino-acid sequence of DNA-polymerase

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α corresponded to the dNTP-binding domain while the inhibitory BLA-derivatives apparently are competitive with dTTP (9). In addition, there was an amino-acid sequence homology (9) to the RNA-binding domain of the human immunodeficiency I (HIV1)-reverse transcriptase (11). Due to this homology and to the fact that BLA can be degraded to many different compounds upon aqueous hydrolysis (12), we decided to isolate and probe the products that might have an effect also on the HIV1-reverse transcriptase.

In this paper we present a degradation product (HP 0.35) that has the capacity to specifically inhibit the RNases H activities of HIV1 and FIV reverse transcriptases. The corresponding cellular enzymes were mainly unaffected. These observations were furthermore corroborated by the ability of HP 0.35 to prevent lentivirus infection in vitro as exemplified by the FIV infection, which is a recognized AIDS model system (13). Our data suggested that a BLA lentivirus infection in vitro as exemplified by the FIV infection, which is a recognized AIDS model system (13). Our data suggested that a BLA degradation product might be a new prototypical class of potential anti-HIV compound.

MATERIALS AND METHODS

Materials

Ribo-and deoxyribonucleoside triphosphates and poly- and oligonucleotides were from Amersham (radioactively labelled with [^{3}H]) or from Pharmacia (unlabelled). Pharmacia was the supplier for DEAE-cellulose, Sephacryl S 300 HR, heparin-sepharose CL-6B, poly(rA)/oligo(dT), *E. coli* RNA polymerase and *E. coli* RNase H. Phosphocellulose was purchased from Whatman. (2-[N-Cyclohexylamino]ethane sulfonic acid, was from Sigma Chemical Corporation St. Louis, USA.) All other chemicals and reagents were analytic grade and purchased from local suppliers.

Isolation and structure determination of the Ceftazidim degradation product HP 0.35

HP 0.35 was obtained by degradation of Ceftazidim (Glaxo) in an aqueous solution for 96 hours (100 mM, pH 7.5) at 37°C and purification by flash chromatography (14) on silica-gel and high performance liquid chromatography. Details of its purification and determination of its structure by ^{1}H-NMR and ^{14}C-Spectroscopy will be published elsewhere.

Preparation of nucleic acids

(i) [^{3}H]poly(A)/poly(dT): The reaction mixture contained in a final volume of 1.0 ml: 20 mM 4-(2-hydroxyethyl)-piperazin-1-ethenesulfonic acid (HEPES) (pH 7.5), 5 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin (nuclease free), 0.5 mM [^{3}H]ATP (350 cpm/pmol), 0.36 mg/ml poly(dT) and 100 U *E. coli* RNA polymerase. The reaction mixture was incubated for 30 min at 37°C. The product was phenolized, precipitated with ethanol and the [^{3}H]poly(A)/poly(dT) dissolved in 10 mM Tris/1 mM EDTA (pH 7.5) at a concentration of 60 μg/ml. (ii) [^{3}H]RNA/M13 DNA: The reaction mixture contained in a final volume of 1.0 ml: 20 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 0.1 mM dithiotreitol, 0.1 mg/ml bovine serum albumin (nuclease free), 0.4 mM [^{3}H]ATP (500 cpm/pmol), 1 mM each of CTP, UTP, GTP, 0.18 mM single-stranded M13 mp8 DNA and 70 U of *E. coli* RNA-polymerase. The reaction was incubated for 90 min at 37° resulting in about 50% transcription. The product was phenolized, precipitated with ethanol and the [^{3}H]RNA-DNA hybrid dissolved in 10 mM Tris/1 mM EDTA (pH 7.5) at 60 μg/ml. (iii) To measure 5'-3' exonuclease activity on single-stranded DNA a M13 sequencing primer (24-mer; Promega) was labelled at its 5'-end by polynucleotide kinase according to ref 15. To determine 5'-3' nuclease activity on double-stranded DNA a labelled 24-mer polylinker primer (Promega) was annealed to single-stranded M13 mp8 DNA.

Enzymatic assays

Reverse transcriptase assay. A final volume of 25 μl contained: 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 140 mM KCl, 0.05% (v/v) Triton X-100, 0.5 mM EGTA, 10 μM [^{3}H]dTTP (500 cpm/pmol) and 1.5 μg poly(A)/oligo(dT) (base ratio 5:1) and enzyme fractions to be tested. Incubation was for 30 min at 37°C. Trichloroacetic acid insoluble radioactivity was determined as described by Hübscher and Kornberg (16). One unit is defined as 1 nmol dTTP incorporated in 30 min at 37°C.

RNase H assay for cellular and E. coli enzymes. A final volume of 25 μl contained: 50 mM Tris-HCl (pH 7.5), 5 mM 2-mercaptoethanol, 10 mM ammonium sulfate, 10 mM $MgCl_2$, 4 mg/ml bovine serum albumin (nuclease free), 10 ng of [^{3}H]poly(A)/poly(dT) or 120 ng [^{3}H]RNA-M13 DNA (9500 cpm/pmol) and enzyme fractions to be tested. Incubation was for 15 min at 37°C. Trichloroacetic acid insoluble radioactivity was determined as described by Hübscher and Kornberg (16). One unit is defined as 1 nmol [^{3}H](AMP) hydrolysed in 15 min at 37°C.

RNase H assay for lentiviral enzymes. A final volume of 25 μl contained: 50 mM Tris-HCl (pH 7.5), 5 mM $MnCl_2$, 4 mM dithiothreitol, 4 mg/ml bovine serum albumin (nuclease free), 10 ng [^{3}H]poly(A)/poly(dT) (300 cpm/pmol) or 120 ng [^{3}H]RNA-M13 DNA (9500 cpm/pmol) and enzyme to be tested. Incubation was for 15 min at 37°C. Determination of the activity and units are as for the cellular enzymes.

Assay for Herpes simplex virus 1 (HSV) DNA polymerase and its associated RNase H. The assay for DNA polymerase (17) contained in a final volume of 25 μl: 50 mM Tris-HCl (pH 8.5), 250 mM KCl, 10 mM $MgCl_2$, 4 mM dithiothreitol, 1.25 mg/ml bovine serum albumin (nuclease free), dATP, dCTP, and dGTP each at 48 μM, [^{3}H]dTTP (500 cpm/pmol) at 18 μM and 3 μg DNase 1-treated calf thymus DNA and enzyme to be tested. Incubation was for 15 min at 37°C. The activity was determined as mentioned for the reverse transcriptase. One unit is defined as incorporation of 1 nmol dNTP in 15 min at 37°C. The assay for determination of the RNase H activity was the same as for the cellular RNases H with the exception that (2-[N-Cyclohexylamino]ethane sulfonic acid (pH 9.0) was used as the appropriate buffer. This high pH is necessary to detect the RNase H from HSV1 DNA polymerase (17).

Other enzymatic assays.

The 3'-5' exonuclease and DNA polymerases α and δ assays were as described in (18). DNA helicase was determined as outlined in ref. (19). The 5'-3' exonuclease was carried out in a final volume of 25 μl containing: 20 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM ATP, 4% (w/v) sucrose, 8 mM dithiothreitol, 80 μg/ml bovine serum albumin (nuclease free), 0.25 ng substrate

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and enzymes to be tested. Common RNase activity was determined by measuring trichloroacetic acid soluble activity on single-stranded [^{3}H]poly(A) and common DNase by incubating double-stranded supercoiled pBR 322 DNA with the fractions to be tested by subsequent separation and analysis of the DNA on agarose gels.

Purification of RNases H and reverse transcriptases.

Reverse transcriptases from HIV1 and FIV pellets. All buffers contained 1 mM dithiothreitol and 1 mM phenylmethanesulfonylfluoride (PMSF). Buffer A contained 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 10% (v/v) glycerol. Buffer B contained 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA and 10% (v/v) glycerol. Preparation of the FIV-lysate was as follows: 8 ml FIV containing lymphocytes from infected cell culture (20) were collected and centrifuged at 500 g for 15 min. The supernatant was further centrifuged at 35,000 g for 30 min. The virus pellets were collected and resuspended in 1.2 ml of a buffer containing 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA and 0.04% (v/v) polyaethylenglycolhexadecylaether. 14 HIV pellets (obtained from Dr. Jendis; Swiss National Center for Retroviruses) were lysed with 280 μl of a buffer composed of 0.925% (v/v) Triton X-100, 1.5 M KCl and 560 μl of 25 mM Tris-HCl (pH 7.5), 50 mM KCl, 5 mM dithiothreitol, 0.25 mM EDTA, and 50% (v/v) glycerol. Both lysates (HIV and FIV) were loaded on a 1 ml DEAE-cellulose column that was previously equilibrated with buffer A containing 50 mM KCl. The column was washed with 10 ml buffer A containing 50 mM KCl. Reverse transcriptase and RNase H activities were eluted with a 50–500 mM KCl-gradient in buffer A. The pooled activity was diluted with buffer A (no KCl) to the appropriate conductivity and loaded onto a 1 ml phosphocellulose column that had previously been washed with buffer B containing 50 mM KCl. The column was washed with 10 ml of the same buffer, eluted with a 100–600 mM KCl gradient in buffer B. Active fractions were pooled and used for inhibitory studies.

Reverse transcriptase from a bacterial expression vector. HIV1-reverse transcriptase from the expression vector pJS 3.7 (21) was induced, processed and purified to homogeneity as a p66/p51 heterodimer as outlined in ref. 22.

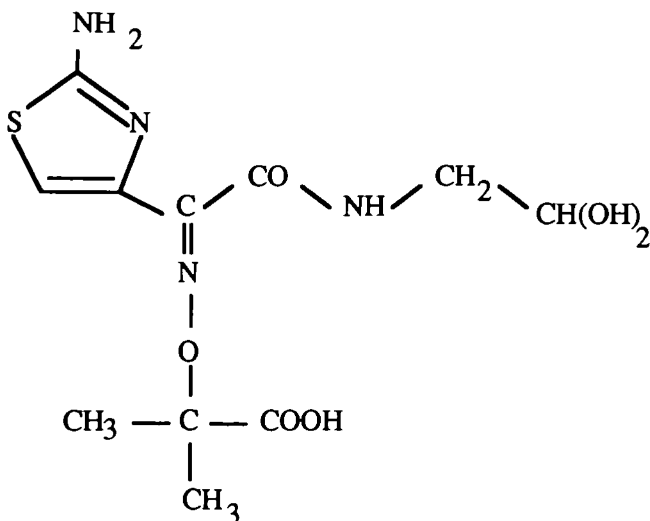

Figure 1. Structure of HP 0.35

Purification of other RNases H. Purification of three forms of RNase H from HeLa cells. All buffers used in this procedure also contained 0.5 mM dithiothreitol, 0.2 mM phenylmethanesufonylfluoride, 4 mM sodium metabisulfite. The separation and purification of three forms of RNase H from HeLa cytoplasmatic extracts is outlined in Fig. 3A. The cytoplasmatic fractions were centrifuged at 25,000 g, prepared and freed from nuclei and mitochondria according to ref. 23 and absorbed at 5 mM potassium phosphate (pH 7.2) on a DEAE-cellulose column at 6 mg of protein per ml of DEAE-cellulose. The column was washed with 20 mM potassium phosphate and eluted with 10 column volumes of a linear gradient from 0.02 to 0.4 M potassium phosphate. The RNase H fractions eluting at 20 (form A), 100 (form B and 175 mM (form C) potassium phosphate were adsorbed at 20 mM potassium phosphate on three separated phosphocellulose columns at 15–20 mg of proteins per ml of phosphocellulose. The columns were eluted with 10 column volumes of a linear gradient from 0.02 to 0.4 M potassium phosphate (pH 7.2) containing 10% (v/v) glycerol. A single peak of activity was eluted from column C (at 300 mM potassium phosphate) and A (at 400 mM potassium phosphate) and two

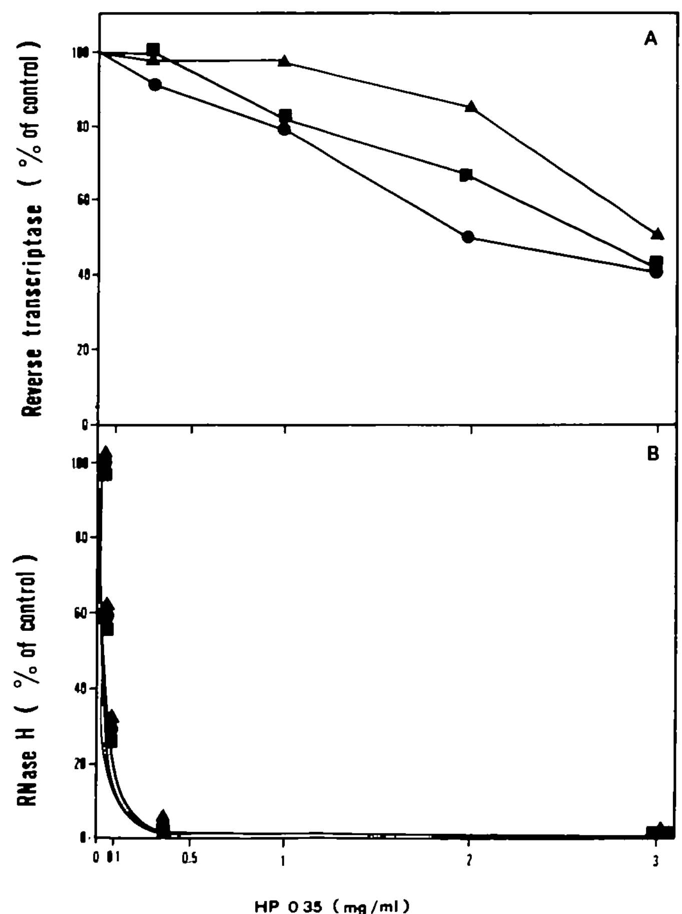

Figure 2. HP 0.35 inhibits RNases H but not reverse transcriptase activities from HIV1 and FIV. A: Reverse transcriptase. B: RNase H. The enzymatic assays were carried out by using 0.2 units reverse transcriptase and 0.06 units RNase H as described in Materials and Methods. HP 0.35 was dissolved in 20 mM Tris-HCl (pH 7.5) and added to the reaction mixture before the enzymes. ● Reverse transcriptase/RNase H from HIV1; ■ Reverse transcriptase/RNase H from FIV; ▲ Reverse transcriptase/RNase H from the expression vector pJS 3.7.

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peaks from column B (at 150 and 300 mM potassium phosphate, respectively). These two peaks were combined. All these forms were then adsorbed at 20 mM potassium phosphate, 20% (v/v) glycerol on heparin-sepharose columns at approximately 10 mg of proteins per ml of heparin-sepharose. The columns were then eluted with 10 column volumes of a linear gradient between 0.02 and 0.4 M potassium phosphate, 20% (v/v) glycerol. The three RNase H activities eluted essentially as single peaks from each column at 160 (form B), 220 (form C) and 250 (form A) mM potassium phosphate, 20% (v/v) glycerol (Figure 3B).

Purification of RNase H from calf thymus. RNase H from calf thymus was isolated according to Hagemeier and Grosse (24).

Purification of HSV1 DNA polymerase. The HSV-1 DNA polymerase was purified from nuclear extract of HSV-1 infected HeLa cells essentially as described by O'Donnell *et al.* (25) up to the DNA cellulose step.

FIV infection of cat lymphocytes

Uninfected lymphocytes were isolated from blood samples of specific pathogen free cats (Merieux). Lymphocytes were extracted by the Ficoll-Hypaque method. Medium for the lymphocyte primary culture consisted of 50 ml RPMI (10x), 13.45 ml sodium bicarbonate (7.5%), 50 ml fetal calf serum, 350 ml Aqua bidest, 0.25 μM 2-mercaptoethanol, 0.01 μg/ml polybrene, 100 mg/l pyruvat, 26.5 mg/l oxalacetate, 16 mg/l insulin, 0.4 mM NaOH, supplemented with 0.25 ml glutamine, 0.0625 ml phytohaemagglutinine, 0.64 ml Ultroser HY and 20 μg/ml interleukin-2. All cultures were maintained at 37°C with humidified 5% CO_2. FIV stocks were prepared from medium containing extracellular virus release from cat lymphocytes that

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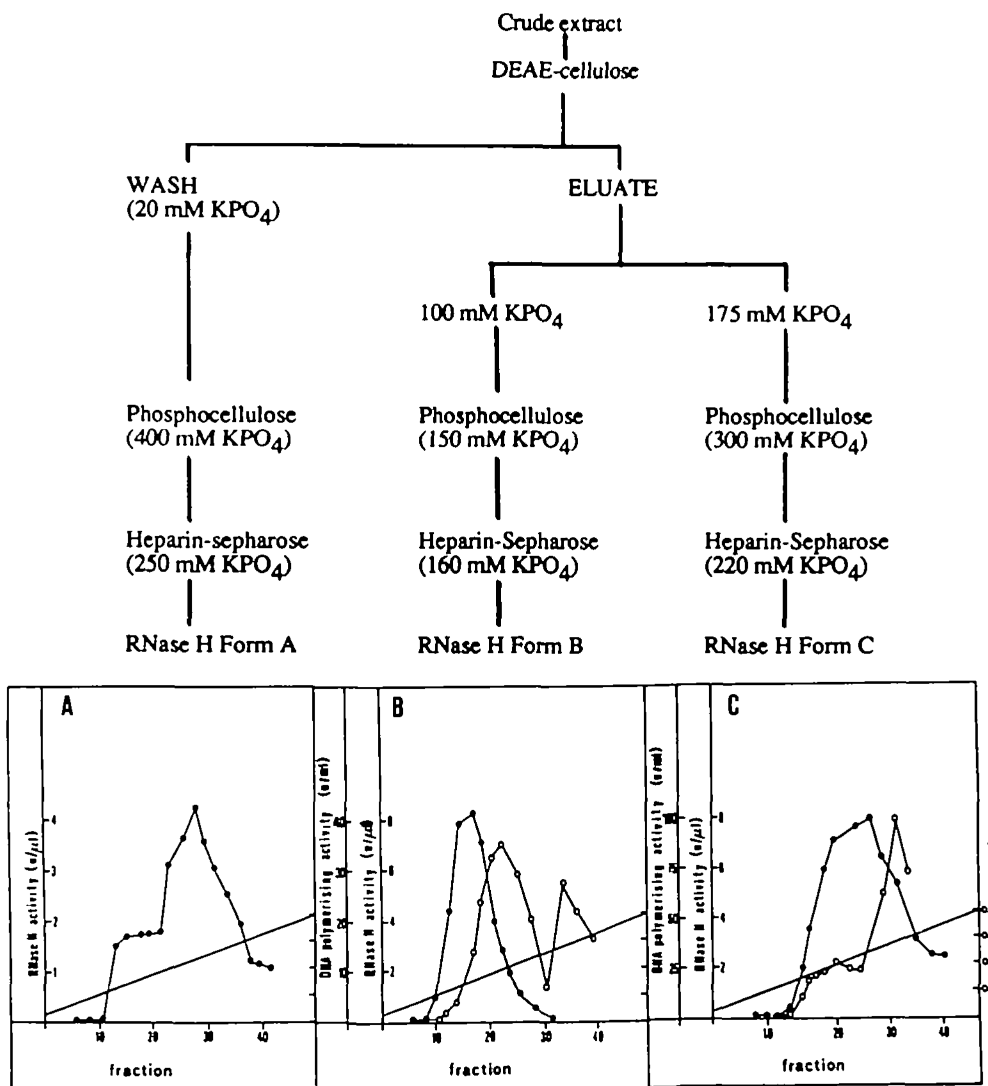

Figure 3. Isolation of three different RNases H from HeLa cells. A: Purification scheme for three RNases H from HeLa cells. B. Heparin-sepharose chromatography of RNase H Form A, Form B and Form C, respectively. The enzymes were extracted, purified and enzymatic activities (RNase H, DNA polymerase α and δ) determined as described in Materials and Methods. ●: RNase H; ○: DNA polymerase

had been infected with FIV for more than 21 days (26). Medium was removed from cultures and cells removed by centrifugation at 500 g for 15 min. Dimethylsulfoxide was added to the supernatant to a final concentration of 10% (v/v). The FIV stocks were thawed and added at a ratio 1:50 to the cat lymphocyte primary cell culture. To monitor reverse transcriptase activity in cell supernatants 5 ml aliquots of FIV infected lymphocyte cell cultures were first centrifuged at 500 g for 15 min to remove the cells and then at 35,000 g for 30 min to pellet the virus. The viral pellet was dissolved in 100 μl of 10 mM Tris-HCl (pH 7.5) containing 100 mM NaCl and 1 mM EDTA. To each viral pellet 2 μl 2% (v/v) polyethylenglycolhexadecylether were added and the reverse transcriptase activity was determined as described above.

Other methods

Protein determination was performed according to Bradford (27).

RESULTS

Structure of HP 0.35

Cephalosporins were previously found to inhibit cellular and viral DNA polymerases (6) and this effect was enhanced if these compounds were previously degraded in aqueous solutions (6). HP 0.35 is the predominant product of the particular cephalosporin Ceftazidim obtained in a degradation pathway which seems to be quite common to Cephalosporins (12). HP 0.35 was purified to homogeneity after degradation of Ceftazidim during 96 hours in aqueous solution (37°C, 100 mM) by flash column and by high performance liquid chromatography to yield a product of more than 90% purity. Figure 1 demonstrates the structure of HP 0.35 (as determined by H^1 and ^{13}C-NMR Spectroscopy). In aqueous solution the aldehyde function is completely in the hydrated form.

HP 0.35 inhibits the RNase H activity of HIV1 and FIV reverse transcriptases

The degraded unpurified Ceftazidim had a pronounced effect on cell proliferation (4) and on cellular DNA polymerases (6). However, the purified HP 0.35 molecule did not affect cell proliferation up to 10 mM and only inhibited DNA polymerase α and δ by 50% at 10 mM (Hafkemeyer,P. and Hübscher,U., unpublished data), suggesting that other compounds than HP 0.35 were responsible for our earlier observations (6). Figure 2 shows that HP 0.35 inhibited RNases H activity inherent in HIV1 reverse transcriptase (IC_{50} <50 μg/ml), but not its DNA polymerising activity (IC_{50} >2 mg/ml). The inhibition pattern between enzymes isolated from HIV1 and FIV were virtually identical. Furthermore, a reverse transcriptase isolated from the expression vector pJS 3.7 (21) behaved identically to the enzyme isolated from the two lentiviruses. In sum, the data suggested that the RNase H but not the reverse transcriptase activity was affected by HP 0.35.

HP 0.35 does not inhibit RNases H from mammalian cells or bacteria

RNases H from calf thymus, HeLa cells and *Escherichia coli* were used as control enzymes. Table 1 lists an overview of the RNases H used in this study. Three RNases H were isolated from HeLa cells (Figure 3). All forms (A; B, and C) specifically degraded RNA from a RNA/DNA hybrid. They were all devoid of common RNase, endonuclease, 3′-5′ exonuclease and 5′-3′ exonuclease (data not shown) and contained in two cases (B and C form) trace amounts of DNA polymerase α. (compare the scales of RNases H and DNA polymerases in Figure 3B). None of these three human RNases H were inhibited to a great extent by HP 0.35, the K_i being at least more than an order of magnitude higher than for the HIV1 enzyme (Figure 4). An identical result was achieved with calf thymus RNase H as well as for the *Escherichia coli* enzyme that was tested because the HIV1 reverse transcriptase was isolated from a bacterial expression system. Two RNase H assays were compared, namely the artificial substrate [^{3}H]poly(A)/poly(dT) (Figure 4A) and the M13 substrate [^{3}H]RNA/M13 DNA (Figure 4B). The inhibitory effect on both substrates was identical. This suggested that HP 0.35 interacts with RNase H also on a natural four base RNA. Finally, we isolated the DNA polymerase from HSV1, a DNA polymerase possessing an RNase H in its polypeptide. This RNase H activity can only be measured at an alkaline pH (17). HP 0.35 did not affect HSV1 RNase H (IC_{50} 0.4 mg/ml). These data indicated that HP 0.35 did not interfere with the corresponding enzymes from human, animal and bacterial cells.

HP 0.35 can prevent the FIV-infection in cat CD4$^+$ lymphocytes

FIV is a widely recognized animal model test system for potential anti-AIDS drugs (see e.g. ref 13 for a rationale). Since the inhibitory behaviour of HP 0.35 on HIV1 and FIV reverse transcriptase were identical we decided to establish the FIV in vitro cell culture system (28). Reverse transcriptase indicating active infection can be detected 9–15 days after inoculation with FIV (Figure 5). HP 0.35 dose-dependently reduced FIV virus production in cat CD4$^+$ lymphocytes as measured by the amount of reverse transcriptase measured for a given amount of cells. At day 15 after infection the reverse transcriptase was only slightly reduced as compared to the control but at day 19, when virus production is on its exponential phase there was a pronounced reduction of virus production. A 48% reduction resulted at concentrations of 10 μM and 100 μM and 77% at 1 mM HP 0.35, respectively. All HP 0.35 concentrations tested

TABLE 1. RNases H used in this study*

Enzyme activity	HIV-RT (vector)	HIV-RT (virus)	FIV-RT (virus)	E.coli	HSV1	calf thymus	HeLa (A)	HeLa (B)	HeLa (C)
RNase H (U/mg)	2220	759	732	7140	282	13466	2250	5000	7.0
Reverse transcriptase (U/mg)	6150	3600	3600	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

*The enzymes used were isolated and the enzymatic activities determined as outlined in Materials and Methods

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had no effect on cell proliferation as measured by the [^{3}H]thymidine uptake of the cells and on viability assessed by the trypan blue exclusion (data not shown). In an additional experiment HP 0.35 was added at a concentration of 100 μM 24 hours prior to infection with FIV (Figure 5B). No virus production was seen with treated cells, while the control infection was normal as expected. Three consecutive repetitions of the experiments mentioned in Figure 5A and B gave identical results.

DISCUSSION

In this paper we have presented a novel compound as a specific inhibitor of HIV1 and FIV-RNase H activities. HP 0.35 is a major aqueous degradation product of the cephalosporin Ceftazidim. Subsequent purification on silica-gel and high performance liquid chromatography yielded homogenous HP 0.35 in reasonable amounts. The inhibitory capacity of HP 0.35 on RNase H from HIV1, FIV and genetically engineered HIV1 reverse transcriptase was compared to its effect on RNase H from HeLa cells, calf thymus and *Escherichia coli* and it was shown that HP 0.35 was able to inhibit the retroviral RNase H activities, whereas substantially no effect was evident on the corresponding retroviral reverse transcriptase polymerisation activities (Figure 2). The inhibitory effect was restricted to HIV1 and FIV RNase H, since the RNases H from cellular origin such as HeLa cells (3 different forms), calf thymus, and *Escherichia coli* had an IC_{50} of at least one order of magnitude higher. The enzymatic data were confirmed in an in vitro cell culture system. FIV infection on cat CD4$^+$ lymphocytes was inhibited by HP 0.35 under conditions where cell proliferation was unaffected. Interestingly, addition of the drug prior to infection with FIV rendered the cells completely unsusceptible to infection with the virus.

HP 0.35 belongs to a completely new class of drugs having a potential to act as an anti-HIV compound. It is derived from a cephalosporin (Ceftazidim) i.e. from an antibiotic containing the betalactam-ring. To our knowledge no betalactam compounds

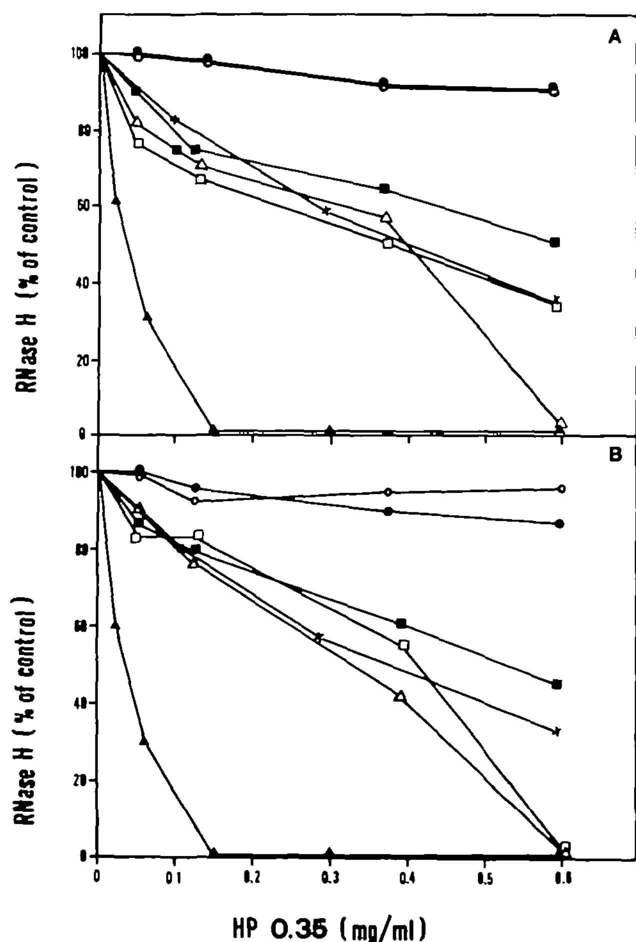

Figure 4. RNases H from HeLa cells, calf thymus and *Escherichia coli* were not affected by HP 0.35. RNase H activities were determined with [^{3}H]poly(A)/poly(dT) (A) or [^{3}H]RNA/M13 DNA (B) by using 1.1 units of RNase H as described in Materials and Methods. Addition of HP 0.35 was as described in Legend to Figure 3. ▲: RNase H from the expression vector pJS 3.7; △ calf thymus RNase H; ■: HeLa RNase H, Form A; ●: HeLa RNase H, Form C; □ HeLa RNase H, Form B; ○: *Escherichia coli* RNase H; HSV1 RNase H.

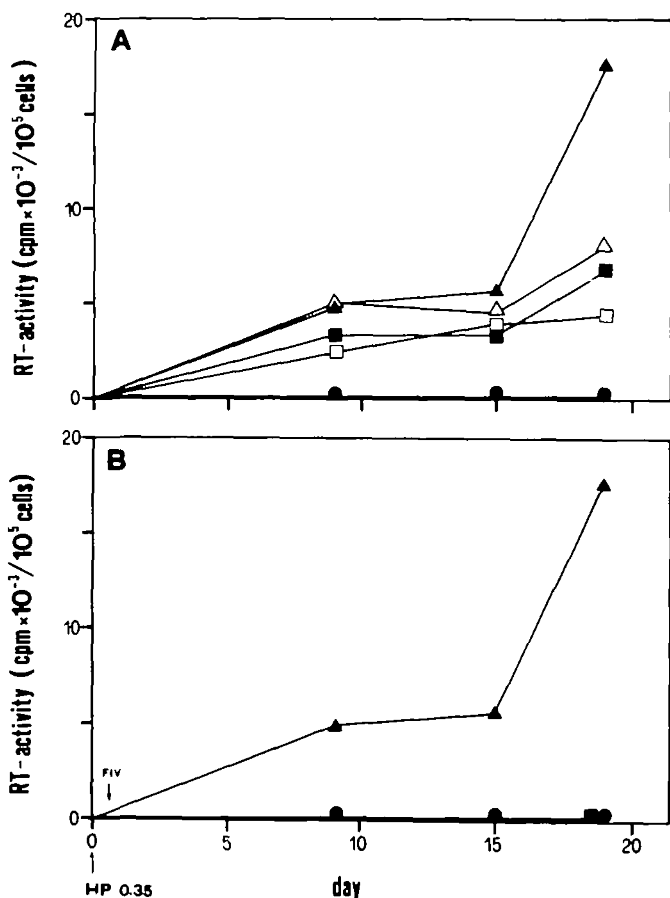

Figure 5. HP 0.35 can prevent the FIV infection of cat CD4$^+$-lymphocytes. Cat CD4$^+$-lymphocytes were grown, treated with interleukin-2 and infected by FIV as described in Materials and Methods. The reverse transcriptase activity was monitored in the cell supernatant at the indicated points. A: Various concentrations of HP 0.35 were added after infection with Materials and Methods. The reverse transcriptase activity was monitored in the cell supernatant at the indicated points. A: Various concentrations of HP 0.35 were added after infection with FIV and supplemented when the media were changed to keep a constant concentration. ▲: no HP 0.35; △: 10 μM HP 0.35; ■: 100 μM HP 0.35; □: 1.0 mM HP 0.35; ●: no virus. B. HP 0.35 was added 24 hours before the FIV was added to the cat CD4$^+$-lymphocytes, ▲: control FIV infection, no HP 0.35; ■: control, no FIV infection; ●: FIV-infection, 100 μM HP 0.35

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or derivatives thereof have been tested for this potency so far.

Most experience so far in anti-retroviral chemotherapy is with nucleoside analogues, in particular with 3′-Azido-2′3′-dideoxythymidine (AZT) (29). AZT as well as dideoxythymidine, dideoxyguanosine and some other nucleoside analogues have great potential to inhibit HIV1 replication in vitro. They also interfere to some extent with the DNA polymerases α, β, γ, δ and ϵ, (Hafkemeyer,P. and Hübscher,U.; unpublished data, see ref. 30 for a revised nomenclature of eukaryotic DNA polymerases). These effects are most likely to be responsible for the toxicity of such compounds on bone-marrow precursors of blood cells, neuronal and other cells (29). Other toxicity problems of e.g. dideoxyinosine and dideoxyadenosine are linked to their cleavage in acid reactions to form a purine base and dideoxyribose probably causing renal damage (31, 32). In addition, development of AZT resistant HIV1 isolates from patients who had been treated with AZT for over six months has already been reported (33, 34).

Since the coding region of RNase H, the carboxyterminal end of the p66 polypeptide of reverse transcriptase, belongs to the most highly conserved regions of HIV (35) the HIV1 RNase H inhibitors look to be very promising drugs in the treatment of AIDS. HP 0.35 exhibits no cytotoxicity in the FIV-cell culture experiments despite its presence for up to three weeks in the culture medium. This has the advantage in that HIV1-replication is abolished and HIV1 is killed even after leaving the proviral state in sometimes very late stages, if it has been quiescent over a longer time period. In addition we used a cell culture system that is recognized for testing potential anti-HIV compounds (36, 37). The mammalian lentiviruses share similarities in genome organization, biological properties and the kind of persistent infection in the host. FIV shares important features with HIV1 being a tropism for T-lymphocytes with association of feline AIDS (36).

In conclusion, we have described a degradation product (HP 0.35) of a clinically widely used betalactam antibiotic as a specific inhibitor of lentiviral RNases H and the compound prevented FIV infection in cell culture. The well documented chemistry of the betalactam antibiotics might help to find more potent RNase H inhibitors that could eventually be tested in an animal model such as FIV infection in cats (37) or HIV infection in SCIDhu mice (38).

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