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Inhibition of HSV-1 and vaccinia virus replication by cephalosporin derivatives

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Summary

Derivatives of betalactam antibiotics of the cephalosporin type at 0.02–1 mM concentrations interfered with in vitro replication of two DNA-containing viruses, herpes simplex I and vaccinia, but showed no effects on two RNA-viruses, lymphocytic choriomeningitis virus and vesicular stomatitis virus, or on cell viability. The exact structure of the active compounds remains unknown, but opening of the betalactam ring appears to be a prerequisite for their formation. Whereas cephalosporin derivatives were most active, no active products were obtained from penicillins and monobactams. The potential of these unexpected antiviral effects of widely used betalactam antibiotics remains subject of further study.

Cephalosporin derivative; DNA virus

Introduction

The antibacterial activity of betalactam antibiotics results from specific and covalent binding to so called penicillin-binding proteins on the plasma membrane of susceptible bacteria; these are enzymes involved in the final steps of peptidoglycan synthesis and hence betalactam antibodies disturb bacterial cell wall formation (Spratt, 1983). Other micro-organisms as well as eukaryotic cells do not bear such targets and are believed to be insusceptible to betalactam antibiotics. Accordingly,

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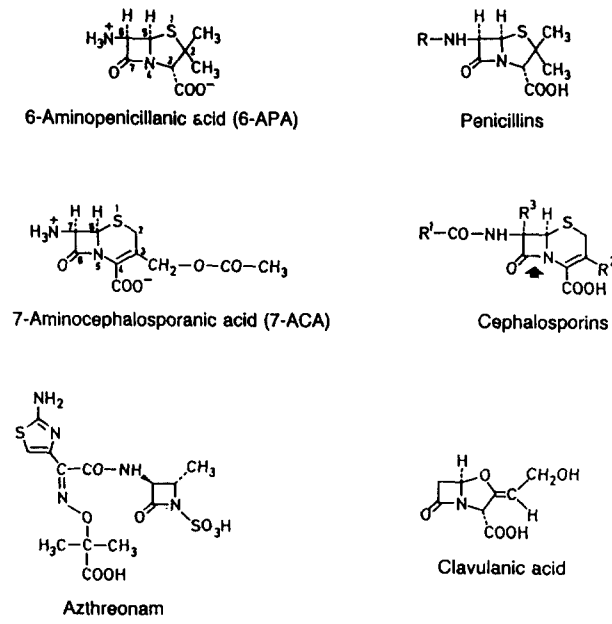

Fig. 1. Structures of various key betalactam antibiotics.

betalactam antibiotics (Fig. 1) have been widely used in clinical and veterinary medicine without causing apparent cell toxicity or obvious antiviral effects.

Recently we have observed that betalactam antibiotics (Fig. 1), via some of their degradation products, may inhibit proliferation of cultured eukaryotic cells in a dose dependent fashion (Cottagnoud and Neftel, 1986; Hügin et al., 1986; Neftel et al., 1983, 1985). Various compounds containing the betalactam ring were found to affect cell viability and biosynthetic activity of cultures differentially when assessed by measuring uptake of radiolabelled thymidine, uridine and amino acids, suggesting that betalactams interfere with DNA replication (Neftel and Hübscher, 1987). Degradation products of betalactam antibiotics (particularly cephalosporins) were shown in a cell free DNA replication system to inhibit calf thymus DNA polymerase α (Do Huynh et al., 1987). We now report that degradation products of cephalosporins but not of other betalactams (monobactams, penicillins and clavulanic acid; see Fig. 1) block in vitro replication of the DNA-containing viruses herpes simplex I (HSV-1) and vaccinia, but do not affect replication of RNA viruses such as lymphocytic choriomeningitis virus (LCMV) and vesicular stomatitis virus (VSV).

Materials and Methods

Betalactams and betalactamase

The following compounds were purchased or received as a gift from the manufacturers: aztreonam (Squibb), ceftriaxone (Roche), ceftazidime (Glaxo), clavulanic acid (Beecham), moxalactam (Shionogi), penicillin G (Hoechst), 6-aminopenicillanic acid, 7-aminocephalosporanic acid and penicilloic acid (Fluka).

7-Desacetyl-aminocephalosporanic acid and 7-desacetoxyaminocephalosporanic acid were a kind gift from Prof. Dr. Roland Reiner, Pharmaceutical Research Department F, Hoffmann–La Roche, Basel, Switzerland.

The broad-spectrum betalactamase mixture prepared from *B. cereus* was from Genzyme Biochemicals, Batch Nr. 3103, Maidstone, Kent, U.K.).

Cell lines

Vero-cells (an African green monkey fibroblast cell line) and MRC-5 cells (30–34 passages) were obtained from Dr. F. Wunderli, Institute of Immunology and Virology, University of Zürich.

Vaccinia assay

1.5×10^5 Vero-cells were plated in a 24-well plate (Costar 3024) for 24 h in 2 ml minimum essential medium (MEM) with 5% FCS. The supernatant was then aspirated off and about 100 plaque forming units (PFU) of vaccinia virus Lancy isolate (Impfinstitut Bern, Switzerland) were added to the monolayer in 200 µl MEM.

After 1 h of incubation at 37°C the virus was aspirated off and 2 ml medium with or without betalactams were added and again incubated at 37°C in 5% CO_2 in a humid atmosphere; 48 h later the supernatant was aspirated and the cell monolayers were stained with crystal violet for 10 min.

Alternatively, the supernatant and cell monolayer were frozen and thawed, put into a glass vial and sonicated for 30 s, then titrated on Vero cells to determine the number of PFU per culture unit.

HSV-1 assay

Approximately 5×10^1 PFU of herpes simplex virus I (HSV-1) (provided by Drs H. Kirchner and S. Opitz, German Cancer Institute, Heidelberg) were added to Vero cell or MRC-5 monolayers in 200 µl MEM for 1 h at 37°C. The supernatant was then aspirated and samples were overlaid with 1 ml of a mixture of 2 × MEM and 1.5% methylcellulose and incubated for 2 days). Plaques were enumerated as described above.

Proliferation assay

$1–2 \times 10^4$ Vero-cells were grown in 96-microwell plates. The supernatant was aspirated off and 200 µl of fresh complete medium with or without betalactams was added, before incubating the plates at 37°C for 36 or 48 h. After various specific timepoints the monolayers were gently washed with PBS and fresh medium

without betalactams was added. After the indicated times 1 μCi of [^{3}H]thymidine (83 Ci/mmol, *methyl*-[^{3}H]thymidine, batch 39, code TRK 686, Amersham International), or 0.5 μCi [^{14}C]leucine, (code CFB.183, batch 41, specific activity 342 mCi/mmol, Amersham International) was added to each well for 6–12 h. The samples were then harvested on cellulose filters and the radioactivity was measured in a Beta-Counter. Viability of cells was assessed by trypan blue exclusion.

Vesicular stomatitis virus (VSV) and lymphocytic choriomeningitis virus (LCMV)

VSV-Indiana (Mudd-Summers isolate) was originally obtained from Dr. D. Kolakovsky, Department of Microbiology, University of Geneva, and was grown and quantitated exactly as described previously (Charan and Zinkernagel, 1986). About 70 PFU of VSV-Indiana were added to 1.5×10^5 Vero-cells in 24-well plates. VSV

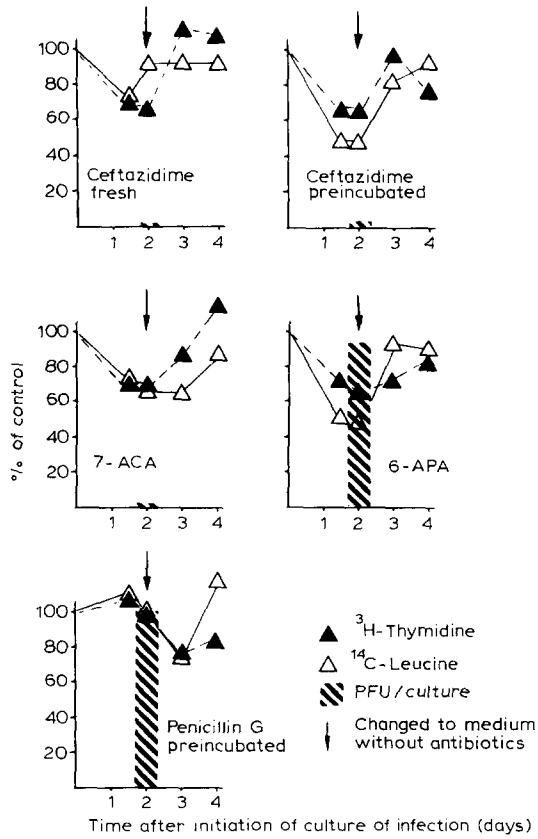

Fig. 2. Comparison of the effects of betalactams on replication and metabolic activity of Vero cells and their effects on vaccinia virus replication. Compounds were added at 1 mM after virus had absorbed for 1 h. Label was added during the last 12 h of culture. Virus plaques were counted after 48 h. Medium with antibiotics was aspirated off at 48 h and replaced by the same medium not containing antibiotics to evaluate reversibility of the observed effects on [^{3}H]thymidine and [^{14}C]leucine incorporation.

TABLE 1

Effect of freshly dissolved, spontaneously degraded or betalactamase treated betalactams on HSV-1 or vaccinia virus plaque formation in Vero cells

Antibiotics	Pretreatment of antibiotics	% Plaque formation at antibiotic concentration (mM)[a]								
		Expt 1: HSV-1				*Expt 2*: Vaccinia virus				
		1	0.5	0.2	0.1	1	0.5	0.2	0.1	0.02
None		100[b]				100				
Ceftazidime	none	<1	<1	30±5	60±3	<1	6±3	4±4	26±4	70±5
Ceftazidime	72 h 37°C[c]	<1	<1	5±3	35±9	<1	<1	5±3	18±3	72±6
Ceftazidime	β-lactamase[d]	<1	<1	<1	33±4	<1	<1	<1	14±7	76±8
7-Aminocephalosporanic acid	none	<1	<1	ND[e]	ND	<1	<1	ND	ND	ND
	β-lactamase	<1	2±2	8±4	25±8	<1	<1	<1	12±10	73±7
6-Aminopenicillanic acid	none	88±12	95±10	ND[e]	ND	93±10	95±11	ND	ND	ND
	β-lactamase	95±11	93±10	ND	ND	97±10	99±12	100±14	96±2	97±4
Penicillin G	none	100±5	96±7	ND	ND	90±9	96±5	ND	ND	ND
Penicillin G	β-lactamase	97±6	104±2	ND	ND	92±7	98±5	ND	ND	ND

[a] 100 PFU of vaccinia virus (Lancy isolate) or about 60 PFU of HSV-1 (Kirchner et al., 1976; Zawatzky et al., 1982) were absorbed to Vero-cell monolayers for 1 h and then overlaid for 48 h with medium or with a mixture of 2× medium and 1.5% methylcellulose respectively, containing the indicated concentrations of antibiotics.

[b] Expressed as % of plaques found in wells containing medium without antibiotics.

[c] Preincubation of 10 mM antibiotics at 37°C for 72 h in minimum essential medium (MEM) at pH 7.2; remaining original substance was less than 50%.

[d] 50 ml of a 2 mM solution of betalactams in MEM with 5% FCS were incubated for 24 h at 37°C with 1 ml of a betalactamase broad spectrum mixture solution containing 100 U/ml betalactamase from *B. cereus* at pH 7.2. After treatment, the concentration of the intact original antibiotic was estimated by HPLC to be less than 1%.

[e] ND, not determined.

replication was assessed by titrating 10-fold dilutions of culture supernatants on Vero cells in 24-well plates for 48 h using a methylcellulose overlay. LCMV (WE isolate) was originally obtained from Prof. F. Lehmann-Grube, Heinrich-Pette-Institut, Hamburg. About 10^2 PFU of LCMV-WE were added to 1.5×10^5 Vero cells in 24-well plates and cultured for 48 h. LCMV culture supernatants were quantitated as follows: aliquots of 10-fold dilution steps of culture supernatants were injected into the footpads of 2 mice. Swelling of the footpads on days 7–9 after injection signals presence of LCMV (Hotchin, 1971). LCMV was also titrated in a routine 5 day plaque assay using Vero cells and Seakem ME agarose (FMC Corporation, Rockland, ME 0484, USA) (Pfau et al., 1983).

Results

Effects of betalactam antibiotics on DNA virus replication

Antiviral activities of betalactam antibiotics (Fig. 1) were evaluated by monitoring plaque formation in vitro by two DNA and two RNA viruses. Freshly dissolved cephalosporins, but not penicillin G, at 0.1–1 mM inhibited plaque formation and virus replication by vaccinia virus in Vero cells in a dose dependent fashion during a 48 h assay (Fig. 2 and Tables 1–3). There was no significant effect of cephalosporins on the viability of Vero cells or of MRC-5 cells (data not shown) at 1 mM or less (Tables 3, 4). Effects on Vero cells and MRC-5 cells (data not shown) were assessed by measuring [^{3}H]thymidine and [^{14}C]leucine incorporation (Fig. 2, Table 3). Both parameters decreased by 30–40% of controls when Vero cells were cultured with 1 mM freshly dissolved or preincubated ceftazidime or 1 mM 7-aminocephalosporanic acid (7-ACA) or 6-aminopenicillanic acid (6-APA) and was unaffected by 1 mM penicillin G. No effects were seen with lower (< 0.05 mM) concentrations of any of the compounds tested. Metabolic activities of Vero

TABLE 2

Inhibition of replication of vaccinia virus by ceftazidime

	Concentration of antibiotics (mM)		
	none	0.5	0.1
	Number of PFU of vaccinia virus generated per unit culture		
Ceftazidime: fresh	$2 \pm 1 \times 10^6$ [a]	$< 10^1$	$3 \pm 1 \times 10^4$
Preincubated for 72 h at 37°C		$< 10^1$	$2 \pm 2 \times 10^2$
Penicillin G		$2 \pm 1 \times 10^6$	$3 \pm 1 \times 10^6$

[a] About 3×10^3 PFU of vaccinia virus were absorbed on about 2×10^5 Vero cells per well in 24-well Costar plates for 1 h; after aspiration of unabsorbed virus, medium with or without antibiotics was added for 48 h. Cell cultures and medium were then frozen, thawed, and sonicated and the PFU content per unit culture was titrated on Vero cells. Incubation of ceftazidime (see Table 1) caused degradation of >50% of the original substance.

TABLE 3

Comparison of effects of freshly dissolved ceftazidime or penicillin G on proliferation and viability of Vero cells and on cytopathic effect of vaccinia virus

	% of control				Actual values for 100%
	1 mM	0.3 mM	0.1 mM	Control	
Ceftazidime					
[^{3}H]Thymidine incorporation[a]	62±6	86±10	98±10	100	8090±180 cpm
% Viable cells	99±5	108±10	109±7	100	2.1±1.8×10^5 cells
PFU of vaccinia	< 1%	19±4	50±6	100	85±2 PFU
Penicillin G					
[^{3}H]Thymidine incorporation	90±7	93±6	90±10	100	6260±500 cpm
% Viable cells	100±5	97±4	105±9	100	2.3±1.3×10^5 cells
PFU of vaccinia	86±5	95±7	93±8	100	76±6 PFU

[a] The indicated parameters were measured 36–48 h after 2×10^4 Vero cells had been seeded into microwell cultures ([^{3}H]thymidine incorporation) or after 1.5×10^5 Vero cells had been put into 24-well Costar plates (viability and vaccinia plaque formation). All determinations represent means of 3 values ± SEM.

cells were evaluated under identical culture conditions by monitoring [^{14}C]leucine incorporation (Fig. 2). Up to 30–40% inhibition was observed when betalactams were used at 1 mM, whereas at ≤ 0.5 mM the inhibitory effects were insignificant (data not shown). In contrast to these small effects on cell function, ceftazidine and 7-ACA had a pronounced effect on vaccinia virus replication in parallel cultures (Table 1, Fig. 2). Virus plaque formation was reduced by a factor of 100 with 1 mM ceftazidime (Table 1, Fig. 2) or by a factor of 3–5 wth 0.1 mM ceftazidine. The comparison of effects of 7-ACA vs. 6-APA on the 3 parameters measured is particularly revealing (Fig. 2). Although both cell proliferation and metabolic activity were inhibited to a comparable extent (30–40%) by both compounds, plaque formation was reduced to undetectable levels by 7-ACA but not at all by 6-APA. This result suggests that replication of vaccinia virus is considerably more susceptible to 7-ACA than are cellular replication and metabolic activity.

The experiments in Fig. 2 also show that impairment of 30–40% of cell proliferation or metabolic activities of cells was readily reversible when antibiotics were eliminated after 48 h of culture. Both [^{3}H]thymidine or [^{14}C]leucine incorporations were monitored 1 and 2 days thereafter and rapidly rose to control values. Although not studied in detail, similar effects were seen when replication of HSV-1 (Table 1) was examined in Vero cells or MRC-5 cells (data not shown). The effects of ceftazidime on vaccinia virus replication were much more dramatic if virus yields per culture were monitored (Table 2). Total numbers of PFU generated in standard cultures of Vero cells inoculated with about 2×10^3 PFU of vaccinia virus during 48 h were as follows (Table 2): control cultures generated around 2×10^6 PFU, whereas cultures containing ceftazidime 0.5 mM or 0.1 mM generated less than 10 PFU or between 2×10^2 to 3×10^4 PFU, respectively, dependent upon whether ceftazidime was degraded or fresh. Penicillin G had no significant

TABLE 4

Summary of antiviral activity of various betalactam antibiotics

	Approximate concentration for 50% inhibition		
	Plaque formation		Cell viability[b] [^{3}H]thymidine or [^{14}C]leucine incorporation (mM)
	vaccinia virus, herpes simplex I virus[a] (mM)	LCMV[a] VSV (mM)	
Cephalosporin-derived			
Ceftazidime β-lactamase treated	0.05	>1	>1
fresh	0.2	>1	>1
Moxalactam	0.5	>1	>1
Ceftriaxone	1.0	>1	>1
7-Aminocephalosporinic acid (ACA)			
β-lactamase treated	0.05	>1	>1
fresh	0.2	>1	>1
7-Desacetyl-ACA	>1.0	>1	>1
7-Desacetoxy-ACA	>1.0	>1	>1
Penicillin-derived			
Penicillin G	>1.0	>1	>1
6-Aminopenicillanic acid	>1.0	>1	>1
Penicilloic acid	>1.0	>1	>1
Monobactam-derived			
Azthreonam	>1.0	>1	>1
Clavulanic acid (β-lactamase inhibitor)	>1.0	>1	>1

[a] About 10^2 PFU of virus were absorbed on Vero cell monolayers (1.5×10^5 per 16 mm well), unabsorbed virus was aspirated off and medium with varying concentrations of antibiotics was overlaid. Plaque formation was evaluated 48 h later.

[b] Cell viability was determined by trypan blue exclusion.

[c] [^{3}H]thymidine or [^{14}C]leucine incorporation was assessed by labeling Vero cells seeded into microwells (2×10^4/well) with medium containing varying concentrations of antibiotics; label was added at 36 h of culture for 12 h.

effect on any of these parameters if used under identical conditions (Tables 1–3).

The minimal effective concentration of cephalosporins or derivatives thereof for antiviral activity (Tables 1, 4) was between 0.05–0.3 mM, dependent upon the particular antibiotic tested and its degree of degradation; these concentrations were at least 3–20 times lower than necessary for impairment of cell proliferation tested in various culture systems (Cottagnoud and Neftel, 1986; Hügin et al., 1986; Neftel and Hübscher, 1987). The inhibitory effect on vaccinia virus replication was identical when Vero cells were irradiated with 2000 rads or 5000 rads (data not shown) before the assay, further suggesting that inhibition of virus replication was not simply caused by impairment of cell proliferation.

Whereas freshly dissolved or degraded cephalosporins at 0.05–1 mM inhibited replication of DNA viruses such as vaccinia, ectromelia (mouse pox: data not

shown) and HSV-1, they had no detectable effect on replication of RNA viruses such as LCMV or VSV (Table 4). This finding also indicates that the antiviral effects were not simply due to impairment of cellular functions required for both DNA and RNA virus replication.

Whereas the effects of higher concentrations of betalactams on cell proliferation were readily reversible for up to 24–48 h (Fig. 2) (Neftel et al., 1983), the effects of cephalosporins on vaccinia virus replication were irreversible when the substances were left in the test well for at least 12 h (Fig. 3); if, however, antibiotics were washed out 4 h (not shown) or 6 h after initiation of infection, the antiviral effects were not significant. Identical results were obtained with HSV-1 on MRC-5 cells.

Structure–activity relationships

The relationship between the structure of various betalactams (Reiner, 1982) and their dose-dependent effects on vaccinia virus and HSV-1 replication was evaluated (Fig. 2, Table 4). Betalactams with cephalosporin structure, namely ceftazidime > 7-aminocephalosporanic acid (7-ACA) > moxalactam > ceftriaxone (in decreasing order) all inhibited vaccinia virus or HSV-1 plaque formation. On the other hand, penicillin G and its hydrolysis product penicilloic acid, as well as 6-aminopenicillanic acid (6-APA), the betalactamase inhibitor clavulanic acid and monobactams (monocyclic betalactams) were inactive at 1 mM, the highest concentration tested. Ceftazidime or 7-aminocephalosporanic acid freshly prepared

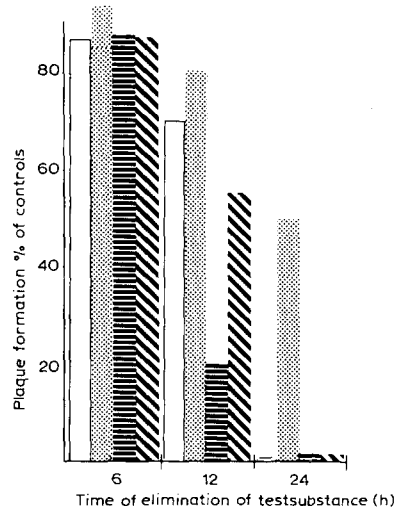

Fig. 3. Evaluation of the time intervals necessary to inhibit plaque formation of vaccinia virus on Vero cells effectively. Vero cells were plated and exposed to 3 × 10^2 PFU of vaccinia virus. The medium containing antibiotics (ceftazidime: fresh 1 mM (▭), 0.5 mM (▒), preincubated 72 h at 37°C 1 mM (▤), 0.5 mM (▨) was replaced by medium without antibiotics at the times indicated. Plaque formation was determined at 48 h, SEM were < 8%. Results are expressed as % of the number of plaques formed in cultures without antibiotics.

from the dry substance were less effective than after preincubation in aqueous solution for 24–72 hrs at 37°C, pH 7.2, or when treated with betalactamase just before use. Efficiency of betalactamase pretreatment was proven by absence of the native compound in HPLC. Since the active degradation products are not yet known, the inhibitory concentrations are given as equivalents of the respective original compounds.

Discussion

Our experiments established marked effects of some derivatives of cephalosporins but not of other betalactam antibiotics on HSV-1 and vaccinia virus replication in Vero or MRC-5 cell cultures. Replication of LCMV and or VSV virus was not affected under identical conditions. Several questions are raised by these findings: firstly, can the effect on the two DNA viruses be distinguished from direct effects on the cell either with respect to the dose of the antibiotics or the kinetics of inhibition; secondly, what may be the pharmacological mode of action and thirdly, what structure of the examined antibiotics may be the active one?

The inhibitory effects of cephalosporins on HSV-1 and vaccinia virus replication do not seem simply to reflect a direct toxic effect on host cells; whereas replication of two DNA viruses was reduced drastically, the replication of two RNA viruses seemed unaffected. Also, although the inhibitory effects on DNA virus replication were effective and irreversible within 12–48 h, effects on cell viability and cell proliferation were minimal and reversible during the same period. These arguments cannot formally invalidate the notion that both DNA virus replication and cell replication are affected via identical pharmacological pathways, but at least there is a difference in susceptibility as reflected by the different dose response curves and the differences in the kinetics of inhibition.

Whether the inhibitory action of some derivatives of betalactam antibiotics on the replicative eucaryotic DNA polymerase α (Do Huynh et al., 1987) may explain all the findings is the subject of continued studies. Meanwhile, examination of purified DNA-polymerase from both HSV-1 and vaccinia virus formally demonstrated that they are similarly but not more sensitive to inhibition by betalactams than are replicative polymerases from various eucaryotic sources (Do Huynh et al., 1987). Therefore, direct effects of betalactam antibiotics or their degradation products observed in a cell free in vitro system cannot readily explain the effects of betalactams on vaccinia virus or HSV-1 replication in living cells.

Our attempts at analyzing the structure function relationship of the various betalactams have led us to the following conclusion: cephalosporin-type compounds were clearly the most active; some as yet unidentified degradation products apparently are much more active than unaltered compounds. In fact, the unmodified compounds may not be active at all and active derivatives may be continuously formed under the culture conditions (Neftel and Hübscher, 1987). Opening of the betalactam ring appears to be crucial, since its hydrolysis by betalactamase promptly produced derivatives that were at least as active as the most potent product formed

during spontaneous degradation for several days in solution. Monobactams, containing no second ring fused to the betalactam ring, were not active under any condition. Hence, the betalactam ring alone or fused to a 5-membered thiazolidine ring (penicillins) does not give rise to active products. The side chain on C_7 of cephalosporins appears not to be important, because 7-ACA, which lacks it, is as active as cephalosporins with various side chains. However, stepwise shortening of the methyl-acetoxy side chain at C_3 of 7-ACA (see Fig. 1) leading to 7-desacetyl-ACA or 7-desacetoxy-ACA, abolished activity. Besides true loss of affinity or activity, the latter finding may simply reflect differential penetration behavior of the 7-ACA derivatives. The hierarchy of antiviral efficiency of the various betalactams tested seems to largely parallel that observed in eucaryotic cell cultures and in cell-free DNA replication assays, where cephalosporins as a group were more potent than penicillins, and monobactams were inactive (Neftel and Hübscher, 1987). It must be pointed out, however, that in the latter assays considerably higher concentrations had to be used as compared to those needed for antiviral activity (Hügin et al., 1986; Neftel et al., 1983, 1985; Hübscher et al., 1986; Neftel and Hübscher, 1987) and the difference in efficiency between cephalosporins and other betalactams is much more pronounced when considering antiviral activity rather than anti-proliferative effects on cultured cells. Therefore, one may speculate that the effects on replication of HSV-1 and vaccinia virus are caused by compounds with an opened betalactam ring that are still fused to a 6-membered second ring.

Overall, these observations reveal unexpected pharmacological actions of betalactam-antibiotic derivatives that merit further examination.

Acknowledgements

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References

Charan, S. and Zinkernagel, R.M. (1986) Antibody mediated suppression of secondary IgM response in nude mice against vesicular stomatitis virus. J. Immunol. 136, 3057–3061.

Cottagnoud, P. and Neftel, K.A. (1986) Betalactams act on DNA synthesis in K-562 cells. Cell Biol. Toxicol. 2, 523–529.

Do Huynh, U., Neftel, K.A., Spadari, S. and Hübscher, U. (1987) Betalactam antibiotics interfere with eucaryotic DNA-replication by inhibiting DNA-polymerase α. Nucleic Acids Res. 15, 10495–10506.

Hotchin, J. (1971) Persistent and slow virus infections. Monogr. Virol. 3, 1–211.

Hübscher, U., Do Huynh, U., Hässig, M. and Neftel, K.A. (1986) Effects of betalactams on DNA replication. Cell Biol. Toxicol. 2, 541–548.

Hügin, A.W., Cerny, A., Zinkernagel, R.M. and Neftel, K.A. (1986) Suppressive effects of betalactam antibiotics on in vitro generations of cytotoxic T cells. Int. J. Immunopharm. 8, 723–729.

Kirchner, H., Hirt, H., Kleinicke, C. and Munk, K. (1976) Replication of herpes simplex virus in mouse spleen cell cultures stimulated by lipopolysaccharide. J. Immunol. 117, 1753–1756.

Neftel, K.A., Müller, M.R., Wälti, M., Erni, J., Gugler, M. and Arrenbrecht, S. (1983) Penicillin G degradation products inhibit in vitro granulopoiesis. Br. J. Haematol. 54, 255–260.

Neftel, K.A., Hauser, S.P. and Müller, M.R. (1985) Inhibition of granulopoiesis in vivo and in vitro by betalactam antibiotics. J. Infect. Dis. 52, 90–98.

Neftel, K.A. and Hübscher, U. (1987) Effects of betalactam-antibiotics on proliferating eucaryotic cells. Antimicrob. Agents 31, 1657–1661.

Pfau, C.J., Gresser, I. and Hunt, K.D. (1983) Lethal role of interferon in lymphocytic choriomeningitis virus-induced encephalitis. J. Gen. Virol. 64, 1827–1830.

Reiner, R. (1982) Antibiotics. Georg Thieme Verlag, Stuttgart.

Spratt, B.G. (1983) Penicillinbinding proteins and the future of betalactam antibiotics. J. Gen. Microbiol. 129, 1247–1260.

Zawatzky, R., Engler, H. and Kirchner, H. (1982) Experimental infection of inbred mice with herpes simplex virus. III. Comparison between newborn and adult C57BL/6 mice. J. Gen. Virol. 60, 25–29.