NEPLANOCIN A INHIBITION OF *S*-ADENOSYLHOMOCYSTEINE HYDROLASE IN *ALCALIGENES FAECALIS* HAS NO EFFECT ON GROWTH OF THE MICROORGANISM

ERIC W. FISHER, CHARLES J. DECEDUE*,†, BRADLEY T. KELLER and RONALD T. BORCHARDT

Departments of Biochemistry and Pharmaceutical Chemistry and †Biochemical Research Service Laboratory, University of Kansas, Lawrence, Kansas 66045, U.S.A.

(Received for publication February 10, 1987)

Neplanocin A, a cyclopentenyl analog of adenosine, is a naturally occurring antibiotic possessing potent inhibitory activity toward the enzyme *S*-adenosylhomocysteine (AdoHcy) hydrolase. In the present study, we examined whether there was a correlation between the inhibition of prokaryotic AdoHcy hydrolase and the reported antibacterial activity of neplanocin A, *e.g. Alcaligenes faecalis* (YAGINUMA *et al.*, J. Antibiotics 34: 359~366, 1981). Of 16 bacterial species screened, only 2 organisms (both of which contained AdoHcy hydrolase) were sensitive to 10 nM neplanocin A when grown on agar plates. None of the 16 strains showed any growth sensitivity in broth culture to concentrations of the antibiotic as high as 4 mM. However, treatment of *A. faecalis* in broth culture with 14 μM neplanocin A resulted in complete inhibition of cellular AdoHcy hydrolase and subsequent elevation of intracellular AdoHcy. No alternative method for degrading or removing the excess AdoHcy from these cells was detected. *Bacillus subtilis*, which exhibited no AdoHcy hydrolase activity showed no alteration of AdoHcy metabolism when treated with the same concentration of the antibiotic. These results indicate that inhibition of AdoHcy hydrolase is not related to the antibacterial activity of neplanocin A and suggest that using this enzyme as a target for the design of antimicrobial agents is not likely to prove a productive approach.

Neplanocin A [(−)-9-(*trans*-2′, *trans*-3′-dihydroxy-4′-(hydroxymethyl)cyclopent-4′-enyl)adenine] is a cyclopentenyl analog of adenosine which was isolated from the culture filtrate of the microorganism *Ampullariella regularis*[1,2]. This novel antibiotic was initially observed to be cytotoxic against cultured L-5178Y lymphoma cells[1] and exhibited significant antitumor activity in mice bearing L1210 leukemia[1,3]. Neplanocin A has also been shown to have antiviral activity[4~7], antimalarial activity[8] and antibacterial activity against organisms grown on nutrient agar plates, including the organism under study here, *Alcaligenes faecalis*[1].

Based on the structural similarity to adenosine, our laboratory has investigated the possibility that some of the pharmacological effects of neplanocin A in eukaryotic cells involve an alteration in cellular transmethylations mediated through an effect on *S*-adenosylhomocysteine (AdoHcy) hydrolase (EC 3.3.1.1). This enzyme, by catalyzing the reversible hydrolysis of AdoHcy to adenosine and homocysteine, plays a pivotal role in eukaryotic cells in regulating cellular levels of AdoHcy, a potent product inhibitor of *S*-adenosylmethionine (AdoMet)-dependent methylation reactions[9,10]. We have observed that neplanocin A is a potent inhibitor of bovine liver AdoHcy hydrolase (Ki=8.39 nM)[4] and that the mechanism of inactivation involves reduction of enzyme bound NAD^+ to NADH[11]. Inhibition of AdoHcy hydrolase by neplanocin A, which has also been demonstrated in a number of cultured cell lines[4,8,12,13], produces an intracellular accumulation of AdoHcy, an elevation of the

AdoHcy/AdoMet ratio and inhibition of AdoMet-dependent methylation reactions. This alteration in cellular metabolism of AdoHcy and AdoMet-dependent methylations has been correlated with the antiviral effects of neplanocin A[4,5,7].

In addition to the effects on AdoHcy hydrolase, our laboratory also reported that neplanocin A could be utilized as a substrate for adenosine kinase, subsequently leading to the formation of *S*-neplanocylmethionine (NpcMet)[14]. Although this analog of AdoMet was not observed to be an efficient substrate for transmethylation[15], it does appear to be responsible for the cytotoxic action of neplanocin A in some cultured cell lines[16,17]. Whether or not this is due to an alteration in the synthesis of the energy-bearing adenosine nucleotides (*i.e.*, ATP) remains to be established.

Recently, SHIMIZU *et al.*[18] reported the occurrence of AdoHcy hydrolase in some prokaryotic organisms including *A. faecalis*. Other prokaryotic organisms degrade AdoHcy to *S*-ribosylhomocysteine and adenine using AdoHcy nucleosidase (EC 3.2.2.9)[19~23], with *S*-ribosylhomocysteine subsequently being degraded by *S*-ribosylhomocysteine hydrolase (EC 3.3.1.3)[23,24]. Our laboratory has since shown that neplanocin A is also a potent inhibitor (Ki=6 nM) of AdoHcy hydrolase isolated from *A. faecalis* and that the mechanism of inactivation again involves reduction of the enzyme bound NAD^+ to NADH[25].

Based on these findings, it was of interest to determine whether there existed a relationship similar to that seen for antiviral activity[7], between the reported antibacterial activity of neplanocin A[1] and the ability of this compound to inhibit AdoHcy hydrolase[25]. In this paper we have investigated the antibacterial effects of neplanocin A against a series of microorganisms possessing or lacking AdoHcy hydrolase. In addition, we have examined the effect of the compound on AdoHcy hydrolase activity and on the intracellular levels of AdoHcy and AdoMet in *A. faecalis*, which contains AdoHcy hydrolase, and in *Bacillus subtilis*, which lacks this enzyme.

Materials and Methods

Growth of Microorganisms

A. faecalis was grown in the enriched medium of SHIMIZU *et al.*[18]. All other microorganisms were cultivated in Tryptic soy broth. Plates and slants were prepared by addition of 1.5% (w/v) bacteriological grade agar to the appropriate medium. Bacterial growth was measured turbidometrically at 715 nm.

Materials

Bacteriological media were purchased from Difco. HPLC grade solvents were purchased from Fisher Scientific. Other chemicals were of reagent grade and commercially available. Neplanocin A was kindly donated by the Toyo Jozo Co., Ltd., Japan.

Cell Extract Preparation

Cells were harvested from 100 ml cultures by centrifugation at 15,000×g for 15 minutes, washed by resuspending the pellets in 10 ml of 0.02 M potassium phosphate, pH 7.5, containing 0.5 mM dithiothreitol, recentrifuged at 8,000×g for 15 minutes, resuspended in 10 ml of wash buffer, and sonicated 3 minutes. These suspensions were then centrifuged at 15,000×g for 10 minutes to remove cell debris and the supernatant cell extracts were immediately assayed.

Enzyme Assays

AdoHcy hydrolase activity was assayed in the hydrolytic direction by the method of BORCHARDT *et al.*[4] using [2,8-^{3}H]AdoHcy prepared as described below. AdoHcy nucleosidase activity was measured using a modification of the procedure of SHIMIZU *et al.*[18]. Reaction mixtures contained 415 μl

of 0.2 M triethanolamine HCl buffer, neutralized to pH 6.5 with NaOH and HCl, 50 μl of 2.5 mM AdoHcy and 25 μl of enzyme extract. Mixtures were incubated at 37°C for 10 minutes in a shaking water bath, and reactions terminated by the addition of 37 μl of 30% $HClO_4$. Excess acid was neutralized by the addition of 1.0 ml triethanolamine buffer. Analysis was conducted by HPLC using a Spectra-Physics Spherisorb 10-ODS, 25 cm reverse-phase column, and a solvent of 50 mM sodium acetate, pH 3.5, at a flow rate of 1.4 ml/minute. Protein was assayed by the BCA (bicinchonic acid) method of Pierce Chemical Company, U.S.A.

Antibacterial Activity

Each strain was cultivated on agar plates on which were placed 7-mm paper discs impregnated with neplanocin A at concentrations ranging from 10^{-2} to 10^{-8} M. These plates were examined after incubation overnight at 27°C. The criterion for determining whether an organism was sensitive to neplanocin A was whether any clearing of the area around the disc was observed. Those strains which produced zones of clearing around the discs were incubated overnight in 1.0 ml broth in a shaking incubator at 30°C, the broth containing neplanocin A at concentrations ranging from 10^{-2} to 10^{-13} M.

Intracellular AdoHcy and AdoMet Metabolism Studies

A. faecalis and *B. subtilis* were cultivated in 10 ml broth cultures, with and without 14 μM neplanocin A. At designated times after inoculation, two aliquots each of 0.5, 5 or 10 ml were removed from each culture, centrifuged 2 minutes at 12,000$\times g$ in a Micro-Centaur centrifuge and the pellets washed in 1.0 ml of 0.02 M potassium phosphate, pH 7.5, 0.5 mM dithiothreitol. This wash was discarded, the cells were lysed with 100 μl of 0.2 M $HClO_4$, and were allowed to sit on ice 20 minutes to precipitate all protein. The cell debris was removed by centrifugation for 10 minutes at high speed and the supernatants were then analyzed immediately or stored at −70°C. Analysis was conducted by reverse-phase HPLC using the method of BORCHARDT *et al.*[4].

Preparation of Radiolabeled and Unlabeled AdoHcy for Enzymatic Assays

AdoHcy was prepared enzymatically using a modified procedure of PALMER and ABELES[26] employing the reverse (synthetic direction) reaction of AdoHcy hydrolase. The reaction mixture contained 1.0 g of adenosine (or [2,8-^{3}H]adenosine purchased from New England Nuclear, 10 mCi/mmol), 1.0 g of *dl*-homocysteine, 20 mg of dithiothreitol, 20 ml of 0.5 M sodium phosphate, pH 7.5, 5 ml of purified bovine liver AdoHcy hydrolase, 0.35 ml of 0.05 M *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA, Burroughs Wellcome Co.) to inhibit adenosine deaminase in the AdoHcy hydrolase preparation, and 30 ml of water. The mixture was incubated at 37°C for 16 to 24 hours in a shaking water bath. The reaction was terminated by plunging the flask repeatedly for short periods into boiling water. The solution was centrifuged to remove the precipitate, and the supernatant was collected and frozen at −20°C. Dowex 50 (formate form) was used for isolation of the AdoHcy. The pH of the supernatant was lowered to between 2.0 and 3.0 with formic acid and the solution was loaded onto the 2.5×10.0 cm Dowex column. The column was washed with 2.0 liters of 0.1 M HCOOH, then 1.0 liter of 0.1 M ammonium formate, pH 4.5. AdoHcy was eluted with two column volumes of 0.4 M NH_4OH. The NH_4OH fraction was lyophilized and the AdoHcy was recrystallized from water. The crystals of AdoHcy were collected by centrifugation, the mother liquor lyophilized, and a second crop of crystals prepared from water as before.

Analysis of AdoHcy in Culture Medium

A 100-ml culture of *A. faecalis* grown to stationary phase in 14 μM neplanocin A was centrifuged to remove the cells and concentrated $HClO_4$ was added to the supernatant at a final concentration of 0.4 M to precipitate the protein. The solution was placed on ice for 20 minutes to complete the precipitation and then neutralized to pH 7.0 with 10 M KOH. The precipitated protein was removed by centrifugation at 15,000$\times g$, 15 minutes, and the solution was acidified to pH 2.0 with concentrated HCl. AdoHcy was separated from the remainder of the medium on SP-Sephadex C-25 cation exchange resin by the method of GLAZER and PEALE[27]. The 150 mM HCl fraction was lyophilized and resuspended in 1.0 ml of 1.0 M potassium phosphate, pH 7.6, and divided into halves. One half was analyzed directly while the other was treated with a small amount of bovine liver AdoHcy hydrolase

prior to being analyzed. The analysis was performed by HPLC using the same method as that in the AdoHcy nucleosidase assay and changing the elution solvent to 50 mM sodium acetate, pH 4.5, in 5% methanol.

Results

Antibacterial Effects of Neplanocin A

Table 1 shows the relationship between the sensitivity to neplanocin A and the presence of AdoHcy hydrolase activity in several bacterial strains. Of the sixteen strains tested in this study, only two were found which had a clear zone in the bacterial lawn around the neplanocin A impregnated disc on the agar plates. The two sensitive strains were shown to contain AdoHcy hydrolase, but with considerably different specific activities. The remaining strains, all of which were insensitive to neplanocin A under identical conditions, consisted of a variety of organisms containing amounts of AdoHcy hydrolase activity ranging from 0 to 12.5 nmol/minute/mg. Repeated attempts to determine the minimum inhibitory concentration of neplanocin A for the susceptible strains were not definitive. When various concentrations of neplanocin A were included in broth cultures, no difference in growth between the treated and untreated cultures could be detected by turbidometric techniques, nor was the density of the treated culture visibly distinguishable from that of the control. Neither of the neplanocin A-sensitive strains (*i.e.*, on agar) exhibited growth inhibition in broth culture containing the antibiotic at concentrations as high as 4.0 mM. In contrast, the two sensitive strains were inhibited on agar plates at concentrations of the compound (on the filter disc) as low as 10 nM.

Intracellular AdoHcy and AdoMet Metabolism Studies

Analysis of intracellular AdoHcy in *A. faecalis* by reverse-phase HPLC demonstrates that growth in broth culture in the presence of 14 μM neplanocin A causes AdoHcy levels in these cells to rise 2-fold above that of the untreated controls (Fig. 1). In contrast, in the hydrolase-negative organism, *B. subtilis*, AdoHcy levels in the control cultures are barely detectable and no increase was observed by growth in the presence of neplanocin A (Fig. 1).

Similar analysis of AdoMet levels in *A. faecalis* and *B. subtilis* demonstrated that for both organisms the AdoMet levels increase during exponential growth, and that this increase is unaffected by the presence of neplanocin A in the growth medium (data not shown). Consequently, despite the fact that the AdoHcy level rises in *A. faecalis* grown in the presence of

Fig. 1. Comparison of the intracellular AdoHcy levels in *Alcaligenes faecalis* and *Bacillus subtilis* cells.

Cells were grown in broth culture in the presence or absence of 14 μM neplanocin A and, at the indicated times, samples were withdrawn, extracted and analyzed by reverse phase HPLC for their content of AdoHcy.

△ Cell density measured at 715 nm, neplanocin A-treated and untreated cultures, ● *A. faecalis* treated with 14 μM neplanocin A, ○ *A. faecalis* untreated control, ■ *B. subtilis* treated with 14 μM neplanocin A, □ *B. subtilis* untreated control.

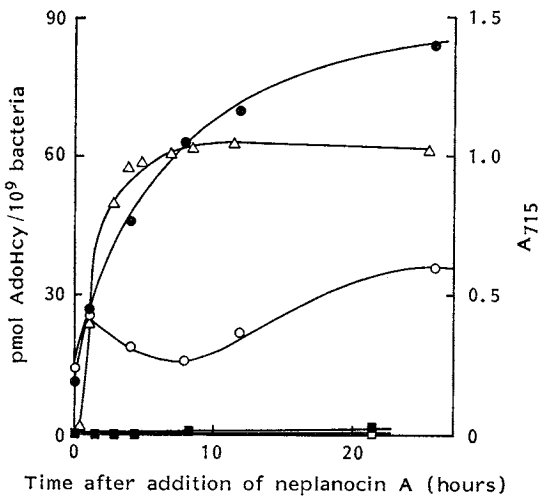


Table 1. Antibacterial effects of neplanocin A.

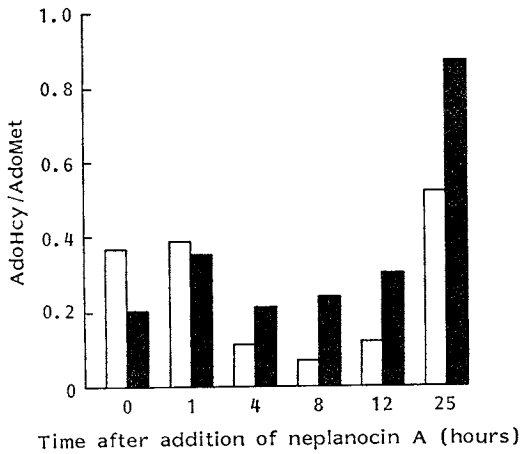
Cells from each species were either harvested, extracted and assayed for AdoHcy hydrolase as described in the Materials and Methods or incubated on plates in the presence of neplanocin A to determine their sensitivity to this inhibitor. The criteria for determining sensitivity to neplanocin A is described in the Materials and Methods.

Species	Sensitivity to neplanocin A	AdoHcy hydrolase activity (nmol/minute/mg)
Acinetobacter calcoaceticus strain (r)	−	1.479
Escherichia coli wild	−	0.056
E. coli K-12	−	0.151
E. coli HA12 GA7	−	0.708
E. coli CSH 61	−	0.218
E. coli MRE 600	−	0.078
E. coli W	−	0.257
Proteus vulgaris	−	12.52
Staphylococcus epidermidis	−	0.201
Salmonella enteritidis	+	0.524
Pseudomonas aeruginosa	−	5.521
Bacillus cereus	−	0.517
B. subtilis	−	0.000
Serratia marcescens	−	0.109
Enterobacter aerogenes	−	8.782
Alcaligenes faecalis	+	4.533

Fig. 2. Intracellular ratio of AdoHcy/AdoMet in *Alcaligenes faecalis*.

Cells were grown in broth culture in the presence or absence of 14 μM neplanocin A and samples were removed at the indicated times and analyzed by reverse-phase HPLC as described in the Materials and Methods.

Solid bars, neplanocin A-treated; open bars, untreated controls.

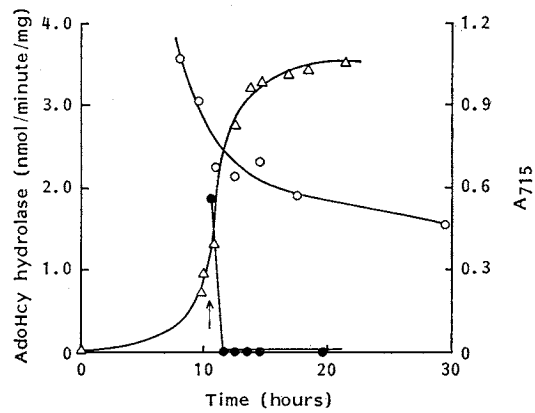


neplanocin A as shown in Fig. 1, the ratio of AdoHcy/AdoMet remains fairly constant except for very late in the growth curve. During this later stationary phase, the ratio begins to decline

Fig. 3. AdoHcy hydrolase activity in *Alcaligenes faecalis*.

A. faecalis was grown in broth culture to early log phase. At this time (arrow), 14 μM neplanocin A was added to one culture and, at the indicated times thereafter, aliquots were removed from each culture and assayed for AdoHcy hydrolase activity by a modification of the method of BORCHARDT *et al.*[4]. Potassium phosphate was used instead of sodium phosphate, the reactions were incubated for 10~15 minutes instead of 60 minutes and Safety Solve scintillation fluid (Research Products International) was used in place of 3a70 counting fluid.

△ Cell density measured at 715 nm, neplanocin A-trea and untreated cultures, ● neplanocin A-treated cells, ○ untreated control cells.



as the intracellular level of AdoMet decreases. In the absence of neplanocin A this ratio is somewhat more variable but follows the same relative pattern (Fig. 2). By comparison, the ratio in *B. subtilis* remains very low throughout the entire growth cycle (data not shown). It is also important to note that in these analyses we observed little, if any, appearance of the NpcMet analog of AdoMet, such as has been observed in several eukaryotic cell lines[12,14,18]. This is a particularly relevant finding considering the apparent toxicity associated with one or more of the phosphorylated metabolites leading to the formation of NpcMet[16,17,28].

AdoHcy Hydrolase in *A. faecalis*

Treatment of *A. faecalis* with neplanocin A in broth culture results in the complete inhibition of AdoHcy hydrolase (Fig. 3). Despite total inhibition of AdoHcy hydrolase, the growth of the organism was indistinguishable from that of the untreated control (Figs. 1 and 3). Interestingly, in the absence of neplanocin A the AdoHcy hydrolase activity at the beginning of the log phase is fairly high (approximately 3.5 nmol/minute/mg) then declines rapidly as the cells progress well into log phase and finally, reaches a plateau at approximately 50% of the initial level within 12 hours. In contrast, the treated cells show a rapid loss of hydrolase activity and the enzyme levels remain fully suppressed for the duration of the experiment (Fig. 3). The loss of AdoHcy hydrolase activity, as shown in Fig. 3, occurs within the 1 hour after the addition of neplanocin A and presumably occurs much faster (*i.e.*, within minutes) based on the sensitivity of the *A. faecalis* enzyme[25] and other similar studies in eukaryotic cell lines[4,12].

AdoHcy Nucleosidase in *A. faecalis* and *B. subtilis*

Assays for AdoHcy nucleosidase in *A. faecalis* indicated no activity in the cell extracts (data not shown) confirming the findings of SHIMIZU *et al.*[18]. In contrast, *B. subtilis* cell extracts completely hydrolyzed AdoHcy to adenine under identical assay conditions (data not shown).

Analysis for the Presence of Extracellular AdoHcy

The findings that AdoHcy hydrolase was completely inhibited by the addition of neplanocin A in actively growing *A. faecalis* and that such cells did not exhibit AdoHcy nucleosidase activity could not explain the mechanism by which the level of AdoHcy is controlled in neplanocin A-treated *A. faecalis* cells. In order to determine if the organism was excreting AdoHcy, the culture medium was analyzed for AdoHcy as described in the Materials and Methods. The analysis yielded no AdoHcy above the lower limit of detection of 100 nmol per liter of medium. Although a small peak appeared with a retention time similar to AdoHcy, treatment of samples of this peak with purified bovine liver AdoHcy hydrolase resulted in no reduction in peak size, indicating that the compound or compounds giving rise to this peak did not include authentic AdoHcy.

Discussion

Complementing the initial observations that neplanocin A was an effective antitumor and antimicrobial agent, our laboratory demonstrated that this antibiotic also exhibited potent antivital activity[4]. It has since been well established that the antiviral activity of neplanocin A is directly related to the inhibitory activity of the compound against AdoHcy hydrolase[7], thereby inhibiting AdoMet-dependent methylation of a virus-specific molecule critical to viral replication (*i.e.*, the 5′-cap structure of viral mRNAs)[29]. Furthermore, there now exists firm evidence that the cytotoxic activity of this antibiotic (as observed with several eukaryotic cell lines[16,17]) is related to its ability to be phosphoryl-

ated by adenosine kinases[17,28], which can eventually lead to the synthesis of NpcMet. It is not firmly established, however, whether this cytotoxicity is directly due to the formation of NpcMet or instead, due to a general effect on the biosynthesis of adenosine nucleotides and the energy state of the cell.

Based on the observation that disks of neplanocin A placed on a lawn of *A. faecalis* have a zone of clearing (ref 1 and Table 1) and the known inhibitory effect of the antibiotic on *A. faecalis* AdoHcy hydrolase[25], we proposed that similar to its antiviral activity, this antimicrobial activity might be a result of the inhibition of the AdoHcy in this organism. In contrast, the results of the present investigation clearly show that in broth culture, *A. faecalis* can grow in the presence of neplanocin A, even though under such conditions there is total inhibition of the cellullar AdoHcy hydrolase. It is also demonstrated that inhibition of the hydrolase by neplanocin A causes a buildup of AdoHcy in *A. faecalis*. This buildup, however, is accompanied by a normal temporal increase in AdoMet levels with the result being that the ratio of AdoMet/AdoHcy remains relatively low throughout most of the growth cycle. Except for the very late stationary phase, the ratio is below 0.4, whereas for mouse L929 cells exposed to neplanocin A the ratio increases to 1.5[4]. This 10-fold increase in the AdoHcy/AdoMet ratio in L929 cells is responsible for an inhibition of AdoMet-dependent methylation and consequently, the antiviral activity of neplanocin A[4~7].

The elevated level of AdoHcy in *A. faecalis* caused by neplanocin A's inhibition of AdoHcy hydrolase apparently is not sufficient to produce lethal effects on the microorganism. Growth curves for the untreated cells were identical to those of the cells treated with neplanocin A, thereby demonstrating that the inhibitor does not affect the rate nor the extent of the organisms's growth. It is interesting that only on agar plates can one observe growth inhibition of bacteria by neplanocin A. Considering the fact that 400,000 times as much neplanocin A could not inhibit growth in broth when compared to agar (4.0 mM for the highest concentration tested in broth vs. 10 nM for the lowest concentration which showed a zone of clearing on a disk), it is unlikely that the concentration at the surface of the disk can explain the inhibition on agar. The only difference in the growth medium was the presence of agar (*i.e.*, the same medium was used with or without agar for plates and broth cultures, respectively). Experiments which altered the flow of air to broth cultures failed to induce neplanocin A sensitivity suggesting that the greater exposure of solid cultures to air is not a factor in the growth inhibition seen on agar plates (data not shown). Thus, it is not immediately obvious why neplanocin A is toxic to these organisms on agar plates but not in broth cultures.

It is clear from our studies that the corresponding analog of AdoMet, NpcMet, is not formed in *A. faecalis* in broth culture. Whether or not neplanocin A undergoes phosphorylation in these cells remains to be determined before any conclusion can be drawn about this metabolic route and the antibacterial effects of the compound. It seems unlikely, however, that these metabolic processes would be dramatically altered in going from broth cultures to agar plate cultures and thus, be responsible for the selective action of neplanocin A under the latter conditions.

A screening procedure for the presence of AdoHcy nucleosidase in *A. faecalis* treated with neplanocin A was carried out to determine whether some of the cellular AdoHcy is metabolized by an alternative pathway. The results of the study verified the findings of SHIMIZU *et al.*[18], indicating the absence of any nucleosidase activity and eliminating this possibility. Analyses for AdoHcy in the culture medium of *A. faecalis* grown in neplanocin A were conducted to determine whether there is an excretory mechanism for the disposal of AdoHcy into the medium, as has been reported for some eukaryotic cells[30,31]. Our results indicate that there is no significant excretion of excess AdoHcy by this bacterium when grown in the presence of neplanocin A. Thus, the study of an alternative disposal route for AdoHcy substantiates the assumption that all AdoHcy produced in *A. faecalis* was detected in the analyses of the intracellular levels of the compound. It appears, therefore, that these cells are not able to accumulate enough AdoHcy in response to neplanocin A so as to elevate their intracellular AdoHcy/AdoMet ratio to a level which has cytotoxic effects.

From the results of our present studies it is clear that the growth of *A. faecalis*, which is inhibited by neplanocin A on agar plates, is not affected by enormous concentrations of the antibiotic when the organism is cultured in broth medium. This is true despite the result that, under these conditions, AdoHcy hydrolase is totally inhibited with the resultant accumulation of AdoHcy as would be ex-

pected. Furthermore, no additional mechanism for the destruction or removal of AdoHcy from *A. faecalis* other than by the principal metabolizing enzyme, AdoHcy hydrolase, could be detected as an alternative possibility for regulating intracellular AdoHcy. Either it is not the accumulation of AdoHcy following inhibition of the hydrolase by neplanocin A that leads to the growth effects on *A. faecalis*, or cultures grown on agar plates have a unique and yet unrecognized mechanism for accumulating greater amounts of AdoHcy than cells grown in broth culture. These results suggest that inhibition of AdoHcy hydrolase in bacteria is not likely to be a productive approach in the design of antimicrobial agents.

Acknowledgments

This work was supported in part by a grant from the Inez-Jay Bequest (CJD) and United States Public Health Service Grant GM 29332 (RTB).

References

1) YAGINUMA, S.; N. MUTO, M. TSUJINO, Y. SUDATE, M. HAYASHI & M. OTANI: Studies on neplanocin A, new antitumor antibiotic. I. Producing organism, isolation and characterization. J. Antibiotics 34: 359~366, 1981
2) HAYASHI, M.; S. YAGINUMA, H. YOSHIOKA & K. NAKATSU: Studies on neplanocin A, new antitumor antibiotic. II. Structure determination. J. Antibiotics 34: 675~680, 1981
3) TSUJINO, M.; S. YAGINUMA, T. FUJII, K. HAYANO, T. MATSUDA, T. WATANABE & J. ABE: Neplanocins, new antitumor agents: Biological activities. *In* Current Chemotherapy and Infectious Disease. Proc. of the 11th ICC & the 19th ICAAC. Vol. II. *Eds.*, J. D. NELSON & C. GRASSI, pp. 1559~1561, The American Soc. Microbiology, Washington, DC, 1980
4) BORCHARDT, R. T.; B. T. KELLER & U. PATEL-THOMBRE: Neplanocin A: A potent inhibitor of *S*-adenosylhomocysteine hydrolase and of vaccinia virus multiplication in mouse L929 cells. J. Biol. Chem. 259: 4353~4358, 1984
5) KELLER, B. T. & R. T. BORCHARDT: Metabolism and mechanism of action of neplanocin A—A potent inhibitor of *S*-adenosylhomocysteine hydrolase. *In* Biological Methylation and Drug Design. *Ed.*, R. T. BORCHARDT *et al.*, pp. 385~396, Humana Press, Inc., Clifton, 1986
6) DECLERCQ, E.: Antiviral and antimetabolic activities of neplanocins. Antimicrob. Agents Chemother. 28: 84~89, 1985
7) DECLERCQ, E. & M. COOLS: Antiviral potency of adenosine analogues: Correlation with inhibition of *S*-adenosylhomocysteine hydrolase. Biochem. Biophys. Res. Commun. 129: 306~311, 1985
8) WHAUN, J. M.; G. A. MIURA, N. D. BROWN, R. K. GORDON & P. K. CHIANG: Antimalarial activity of neplanocin A with perturbations in the metabolism of purines, polyamines and *S*-adenosylmethionine. J. Pharmacol. Exp. Ther. 236: 277~283, 1986
9) BORCHARDT, R. T.: *S*-Adenosyl-L-methionine-dependent macromolecule methyltransferases: Potential targets for the design of chemotherapeutic agents. J. Med. Chem. 23: 347~357, 1980
10) CANTONI, G.; H. H. RICHARDS & P. K. CHIANG: Inhibitors of *S*-adenosylhomocysteine hydrolase and their role in the regulation of biological methylation. *In* Transmethylation. *Ed.*, E. USDIN *et al.*, pp. 155~164, Elsevier/North-Holland, New York, 1979
11) MATUSZEWSKA, B. & R. T. BORCHARDT: The role of nicotinamide adenine dinucleotide in the inhibition of bovine liver *S*-adenosylhomocysteine hydrolase by neplanocin A. J. Biol. Chem. 262: 265~268, 1987
12) RAMAKRISHNAN, V. & R. T. BORCHARDT: Adenosine dialdehyde and neplanocin A: Potent inhibitors of *S*-adenosylhomocysteine hydrolase in neuroblastoma N-2a cells. Neurochem. Int. 2: 1987, in press
13) AARBAKKE, J.; R. K. GORDON, A. S. CROSS, G. A. MIURA & P. K. CHIANG: Correlation between DNA hypomethylation and differentiation of HL-60 promyelocytic cells induced by neplanocin A and 3-deaza nucleosides. Fed. Proc. 44: 464 (Abstract No. 313), 1985
14) KELLER, B. T. & R. T. BORCHARDT: Metabolic conversion of neplanocin A to *S*-neplanocylmethionine by mouse L929 cells. Biochem. Biophys. Res. Commun. 120: 131~137, 1984
15) KELLER, B. T.; R. S. CLARK, A. E. PEGG & R. T. BORCHARDT: Purification and characterization of some metabolic effects of *S*-neplanocylmethionine. Mol. Pharmacol. 28: 364~370, 1985
16) GLAZER, R. I. & M. C. KNODE: Neplanocin A. A cyclopentyl analog of adenosine with specificity for inhibiting RNA methylation. J. Biol. Chem. 259: 12964~12969, 1984

17) INABA, M.; K. NAGASHIMA, S. TSUKAGOSHI & Y. SAKURAI: Biochemical mode of cytotoxic action of neplanocin A in L1210 leukemic cells. Cancer Res. 46: 1063~1067, 1986
18) SHIMIZU, S.; S. SHIOZAKI, T. OHSHIRO & H. YAMADA: Occurrence of *S*-adenosylhomocysteine hydrolase in prokaryotic cells. Characterization of the enzyme from *Alcaligenes faecalis* and role of the enzyme in the activated methyl cycle. Eur. J. Biochem. 141: 385~392, 1984
19) DUERRE, J. A.: A hydrolytic nucleosidase acting on *S*-adenosylhomocysteine and on 5'-methylthioadenosine. J. Biol. Chem. 237: 3737~3741, 1962
20) DUERRE, J. A.; C. H. MILLER & G. G. REAMS: Metabolism of *S*-adenosyl-L-homocysteine *in vivo* by the rat. J. Biol. Chem. 244: 107~111, 1969
21) JAKUBOWSKI, H. & A. GURANOWSKI: *S*-Adenosylhomocysteinase from yellow lupin seeds: Stoichiometry and reactions of the enzyme·adenosine complex. Biochemistry 20: 6877~6881, 1981
22) UELAND, P. M.: Pharmacological and biochemical aspects of *S*-adenosylhomocysteine and *S*-adenosylhomocysteine hydrolase. Pharmacol. Rev. 34: 223~253, 1982
23) WALKER, R. D. & J. A. DUERRE: *S*-Adenosylhomocysteine metabolism in various species. Can. J. Biochem. 53: 312~319, 1975
24) MILLER, C. H. & J. A. DUERRE: *S*-Ribosylhomocysteine cleavage enzyme from *Escherichia coli*. J. Biol. Chem. 243: 92~97, 1968
25) MATUSZEWSKA, B. & R. T. BORCHARDT: Inhibition of *S*-adenosylhomocysteine hydrolase from *Alcaligenes faecalis* by neplanocin A. Arch. Biochem. Biophys., 1987, in press
26) PALMER, J. L. & R. H. ABELES: The mechanism of action of *S*-adenosylhomocysteinase. J. Biol. Chem. 254: 1217~1219, 1979
27) GLAZER, R. I. & A. L. PEALE: Measurement of *S*-adenosyl-L-methionine levels by SP Sephadex chromatography. Anal. Biochem. 91: 516~520, 1978
28) HASOBE, M.; J. G. MCKEE, D. R. BORCHERDING & R. T. BORCHARDT: 9-(Trans-2', trans-3'-dihydroxycyclopent-4'-enyl)-adenine and 3-deazaadenine: Analogs of neplanocin A which retain potent antiviral activity but exhibit reduced cytotoxicity. Antimicrob. Agents Chemother., to submitted
29) KELLER, B. T. & R. T. BORCHARDT: Adenosine dialdehyde: A potent inhibitor of vaccinia virus multiplication in Mouse L929 cells. Mol. Pharmacol. 31: 485~492, 1987
30) BARTEL, R. M. & R. T. BORCHARDT: Effects of adenosine dialdehyde on *S*-adenosylhomocysteine hydrolase and *S*-adenosylmethionine-dependent transmethylations in mouse L929 cells. Mol. Pharmacol. 25: 418~422, 1984
31) SVARDAL, A. M.; R. DJURHUUS, H. REFSUM & P. M. UELAND: Disposition of homocysteine in rat hepatocytes and in nontransformed and malignant mouse embryo fibroblasts following exposure to inhibitors of *S*-adenosylhomocysteine catabolism. Cancer Res. 46: 5095~5100, 1986