REVERSAL OF INHIBITING ACTION OF SHOWDOMYCIN ON THE PROLIFERATION OF *ESCHERICHIA COLI* BY NUCLEOSIDES AND THIOL COMPOUNDS

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The growth inhibiting activity of the antibiotic showdomycin, a nucleoside derivative of maleimide, on *E. coli* UMEZAWA was specifically reversed by ribo- and deoxyribo-nucleosides and thiol compounds such as L-cysteine, glutathione and β-mercaptoethanol. No effect on the reversal of the antibacterial activity of showdomycin was observed for purine, pyrimidine bases, nucleoside phosphates, ribose, deoxyribose and phosphate derivatives of ribose. Of the 19 amino acids tested, L-cysteine was the only one which is able to reverse the activity of showdomycin. All other amino acids were ineffective.

The antibiotic showdomycin was first isolated from *Streptomyces showdoensis* in our laboratory[1], and was shown to inhibit the growth of both gram-positive and gram-negative bacteria, especially streptococci. This antibiotic is a potent inhibitor of the EHRLICH mouse ascites tumor *in vivo*[1], and HeLa cells in tissue culture[2]. Recently, the structure, 3-β-D-ribofuranosylmaleimide, has been proposed for showdomycin by DARNALL, TOWNSEND and ROBINS[3], and independently by KANO *et al.*[4] of our laboratory.

In the previous study[1], it was observed that showdomycin shows much higher activity in the synthetic medium than that in the organic medium. A preliminary search into the cause of this fact showed that the addition of yeast extract into the culture medium results in apparent decrease of the activity of showdomycin and that some components of the yeast extract, such as adenosine and uridine, will reverse the activity of showdomycin.

The purpose of this study is to determine the potential interference of the antibacterial property of showdomycin by nucleic acid related compounds, amino acids, and thiol compounds.

Materials and Methods

As test organism, *Escherichia coli* UMEZAWA was used. This was grown in a glucose mineral medium (1 % glucose, 0.5 % NaCl, 0.1 % $(NH_4)_2HPO_4$, 0.1 % KH_2PO_4 and 0.04 % $MgSO_4 \cdot 7H_2O$) supplemented with 1 % NZ-amine type A, 0.5 % Bacto yeast extract and 0.5 % Difco casamino acids (the pH is adjusted to 7.0) under aerobic conditions at 37°C for 3~4 hours. The 3~4 hour-old cells were then resuspended in the following assay medium to give an initial optical density of 0.114~0.120 at 660 mμ (E 660), to which had been concomitantly added a definite concentration of showdomycin and the test compounds. A modified MACILVAINE buffer medium (pH 6.8) containing glucose (1 %) and casamino acids

(0.2 %) was selected for the culture medium of the *E. coli* for the assay of reversal effect of the antibacterial activity of showdomycin. By the supplements of glucose and casamino acids, fairly rapid growth of *E. coli* was retained and this small amount of casamino acids did not show any significant reversal effect of the activity of showdomycin. In the routine assay, bacterial growth was measured turbidimetrically after 3 hours incubation at 37°C with a COLMAN spectrophotometer at a wave length of 660 mμ.

Showdomycin and other test compounds were sterilized by filtration through Millipore filter.

Nucleic acid related compounds, amino acids, and thiol compounds were obtained from commercial sources.

Results

In the test medium, the growth of *E. coli* was inhibited at very low concentration of showdomycin (2×10^{-8} moles/ml) and a gradual decrease in optical density readings was observed during the incubation of *E. coli* with this antibiotic, owing to lysis of the cells (Fig. 1).

Fig. 1. Effect of showdomycin (2×10^{-8} moles/ml) on 3-hour old cells of *E. coli* at 37°C for different times.

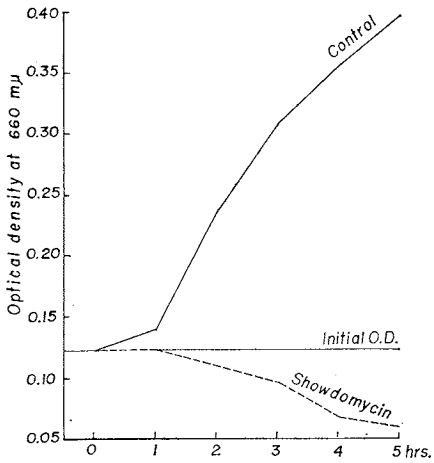
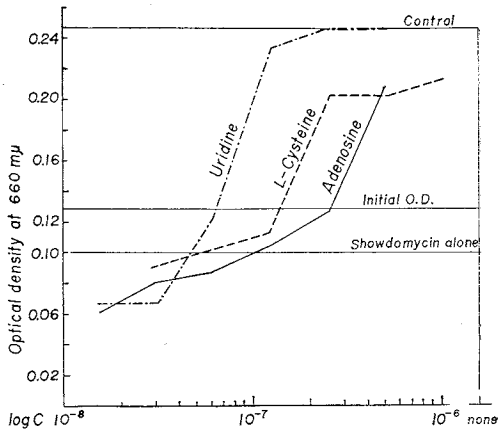


Fig. 2. Concentration of adenosine, uridine and L-cysteine required for reversal of showdomycin inhibition of *E. coli* growth.

(In all cases, showdomycin concentration was 2×10^{-8} moles/ml.)



As shown in Fig. 2, the inhibitory action of showdomycin (2×10^{-8} moles/ml) on the growth of *E. coli* was reversed with the increase of the molar concentrations of ribonucleosides and L-cysteine, and the maximum reversal effect was observed at a concentration in neighborhood of 10^{-6} moles/ml of these compounds.

The effect of test compounds on the antibacterial activity of showdomycin (2×10^{-8} moles/ml) was then assayed at the concentration of 10^{-6} or 2×10^{-6} moles/ml.

The following 18 amino acids were inactive when tested at the concentration of 2×10^{-6} moles/ml: L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. Cysteine was the only amino acid which reversed the activity of showdomycin. These results led us to the consideration that the thiol group of cysteine may have a specific

role in reversing showdomycin activity. This was confirmed by the finding that all the thiol compounds tested are as effective as cysteine (Fig. 3).

As shown in Fig. 3, all the ribonucleosides and desoxyribonucleosides have a strong reversing effect on showdomycin activity. No significant difference in effectiveness was observed among the tested nucleosides having variations in the sugar moeity or base component. Although guanosine induced exceptionally stimulative action of the growth of *E. coli*, the inhibitory action of showdomycin (2×10^{-8} moles/ml) was almost

Fig. 3. Reversal of showdomycin inhibition of *E. coli* growth by nucleosides and thiol compounds.

In all cases, showdomycin concentration was 2×10^{-8} moles/ml. Test compounds (2×10^{-6} moles/ml), dAd=deoxyadenosine, dGu=deoxyguanosine, dCy=deoxycytidine, dUr=deoxyuridine, dTh=deoxythymidine, Ad=adenosine, Gu=guanosine, In=inosine, Cy=cytidine, Ur=uridine, Th=thymidine, Cys=L-cysteine, GL=glutathione, BM=β-mercaptoethanol.

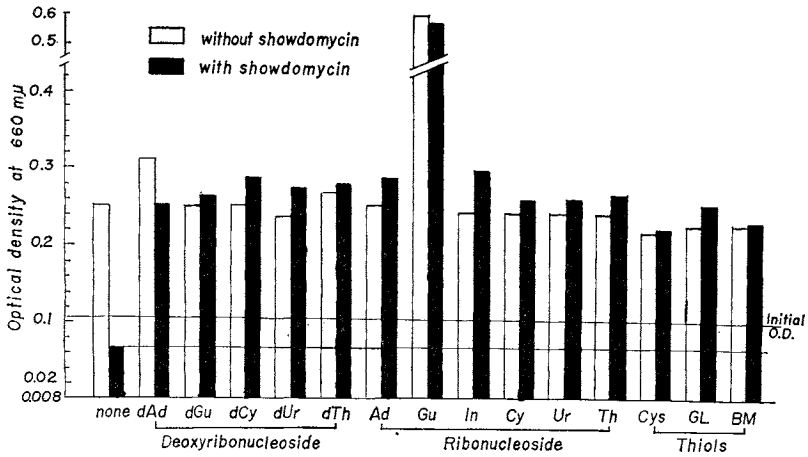
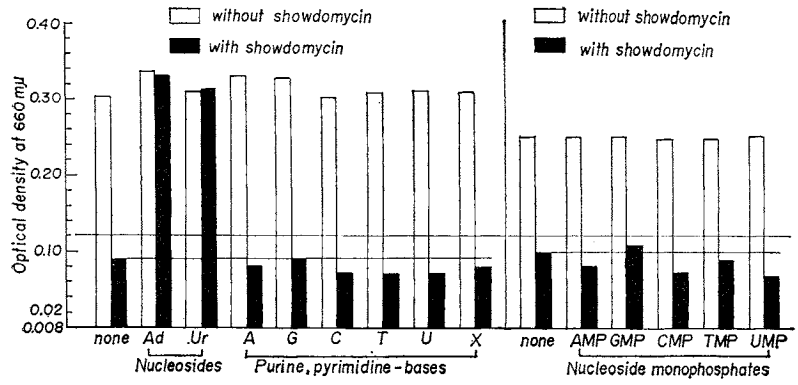


Fig. 4. Effect of purine- and pyrimidine-bases and nucleoside monophosphates on showdomycin inhibition of *E. coli* growth compared with that of nucleosides (adenosine and uridine).

In all cases, showdomycin concentration was 2×10^{-8} moles/ml. Test compounds (2×10^{-6} moles/ml). Ad=adenosine, Ud=uridine, A=adenine, G=guanine, C=cytosine, T=thymine, U=uracil, X=xanthine, AMP=adenosine-5′-monophosphate, GMP=guanosine monophosphate isomer (2′ and 3′), CMP=cytidine-5′-monophosphate, TMP=thymidine-5′-monophosphate, UMP=uridine-monophosphate isomer (2′ and 3′).



completely reversed by supplementation with this ribonucleoside (2×10^{-6} moles/ml) as with other nucleosides. As illustrated in Fig. 4, purine- and pyrimidine-bases and ribonucleoside monophosphates had no effect on the activity of showdomycin. Other nucleotides, such as deoxyribonucleoside monophosphates, ribonucleoside diphosphates, ribonucleoside triphosphate were all inactive in reversing the inhibition of the growth of *E. coli* by showdomycin (Table 1). Moreover, the following compounds were tested in similar conditions and also no reversing effect was observed on the activity of showdomycin: D-ribose, deoxyribose, phosphoribosyl pyrophosphate, ribose-5'-phosphate and orotic acid.

Table 1. Effect of deoxyribonucleotides and ribonucleotides on showdomycin inhibition of *E. coli* growth

Compound	O. D. at 660 mμ (initial O. D.=0.114)	
	with showdomycin	without showdomycin
Deoxyadenosine-5'-monophosphate	0.087	0.276
Deoxyguanosine-5'-monophosphate	0.091	0.312
Deoxycytidine-5'-monophosphate	0.088	0.273
Deoxythymidine-5'-monophosphate	0.089	0.276
Deoxycytidine-5'-triphosphate	0.087	0.272
Adenosine-5'-diphosphate	0.092	0.288
Guanosine-5'-diphosphate	0.091	0.272
Uridine-5'-diphosphate	0.073	0.287
Adenosine-5'-triphosphate	0.087	0.273
Guanosine-5'-triphosphate	0.089	0.268
Cytidine-5'-triphosphate	0.088	0.263
Uridine-5'-triphosphate	0.084	0.277
Control (none)	0.088	0.273

Note: In all cases, showdomycin concentration was 2×10^{-8} moles/ml. The monophosphates and the diphosphates (2×10^{-6} moles/ml). The triphosphates (1×10^{-6} moles/ml).

Discussion

It is apparent from the present studies that the inhibitory action of showdomycin on the proliferation of *E. coli* UMEZAWA was specifically reversed by nucleosides and thiol compounds. The most interesting finding is that all the common nucleosides are able to reverse the activity of showdomycin regardless of variations in their sugar or base component, while purine- and pyrimidine-bases, ribose, deoxyribose, nucleoside phosphates and sugar phosphates are not. Since in the case of phosphate derivatives, especially that of nucleoside phosphates, the permeability of these compounds might be an important consideration, final proof of ineffectiveness of these compounds must await the assay in a cell-free system. It is quite interesting, however, that all the nucleosides are able to reverse the activity of showdomycin, whereas, all the components of these nucleosides (bases and sugars) were ineffective even when base and sugar were concomitantly added to the assay system (data not shown in this paper). This may suggest that the specific shape of the nucleoside defined by the linkage of the base and the sugar is needed for their ability to reverse the action of showdomycin. It is also noteworthy that the thiol compounds, which have a completely different structure from the nucleosides, were equally effective in reversing the showdomycin activity. The recent studies on the structure of

showdomycin demonstrated that this antibiotic is a nucleoside derivative of maleimide[3,4]. DARNELL *et al.*[3] have suggested that this antibiotic may act as an alkylating agent, and the stoichiometric reaction of this antibiotic with thiol compounds was recently observed in our laboratory[5].

We are not in a position as yet to make any conclusions on the mechanism of the reversing effect of thiol compounds and nucleosides on showdomycin activity. However, further investigation of these compounds on showdomycin activity should reveal important information concerning the mechanism of action of this antibiotic.

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