DRUG RESISTANCE OF STAPHYLOCOCCI. XI

INDUCTION OF CHLORAMPHENICOL RESISTANCE BY ITS DERIVATIVES AND ANALOGUES

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Antibacterial activity of 16 chloramphenicol derivatives and their induction ability for chloramphenicol resistance were examined. Three chloramphenicol derivatives; CM 16 (DL-α-dichloroacetamido-β-bromo-*p*-nitropropiophenone), CM 17 (DL-α-dichloroacetamido-α-methylene-*p*-nitropropiophenone) and CM 18 (DL-α-acetamido-α-methylene-*p*-nitro-propiophenone) were found to be highly active against S-1477 but their inducible activity for chloramphenicol resistance was rather low. CM 26 (chloramphenicol monophosphate) and CM 29 (D-*threo*-2,2-diethyl-5-dichloroacetamido-6-(*p*-nitrophenyl)-1,3-dioxane) has no antibacterial activity and CM 15 (DL-*threo*-2-dichloromethyl-4-*p*-nitrophenyl-hydroxymethyl-Δ^2-oxazoline) has very low activity against S-1477, but they have low inducible activity for chloramphenicol resistance in spite of no activity of other derivatives.

Many semisynthetic penicillins derived from 6-aminopenicillanic acid have been prepared and some are already used as chemotherapeutic agents, such as ampicillin (aminobenzyl penicillin) which is active against gram-negative bacilli and methicillin (dimethoxyphenyl penicillin) which is a penicillinase resistant penicillin derivative[1]. As for chloramphenicol, numerous studies on the effect of modifications in its structure on biological activity were reported. A large number of chloramphenicol derivatives and analogues has been prepared, but a useful derivative has not been reported thus far in contrast to the successful results with penicillins[2,3,4]. Continuing from a previous paper[5], this report deals with the antibacterial activity of chloramphenicol derivatives and their ability to induce chloramphenicol resistance in *Staphylococcus aureus*.

Materials and Methods

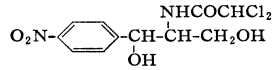
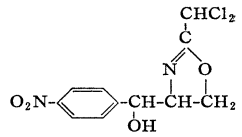
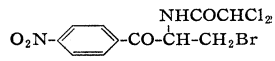
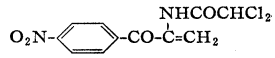
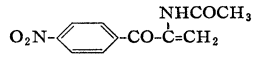
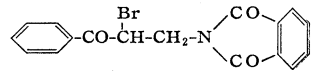
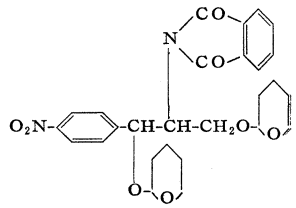
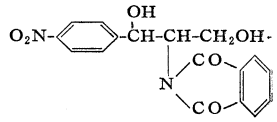
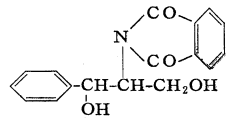
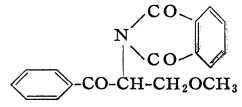
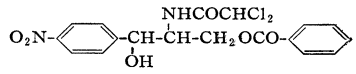
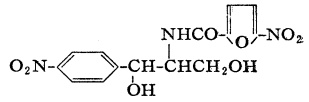
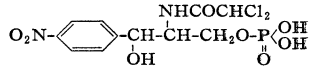
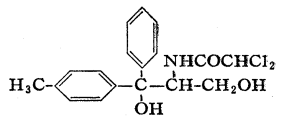
Bacterial Strain: A coagulase-positive strain of *Staphylococcus aureus* S-1477, which was isolated from a patient in 1963, was used exclusively. Drug resistance of the strain was 100 mcg/ml tetracycline, 100 units/ml penicillin and 25 mcg/ml chloramphenicol[6].

Drugs: Chloramphenicol and its derivatives and analogues listed in Table 1 were kindly supplied by the Sankyo Co., Tokyo.

Media: Penassay broth (Difco) was used routinely for the propagation of bacteria.

Determination of Antibacterial Activity: An overnight broth culture of S-1477 was diluted ten-fold with fresh broth and shaken at 37°C for 2 hours. One tenth ml of the

Table 1. Chloramphenicol and its derivatives and analogues

	Abbreviation	Chemical structure
Chloramphenicol	C M	
DL-*threo*-2-Dichloromethyl-4-*p*-nitrophenyl-hydroxymethyl-Δ^2-oxazoline	C M 15	
DL-α-Dichloroacetamido-β-bromo-*p*-nitropropiophenone	C M 16	
DL-α-Dichloroacetamido-α-methylene-*p*-nitropropiophenone	C M 17	
DL-α-Acetamido-α-methylene-*p*-nitropropiophenone	C M 18	
α-Bromo-β-phthalimido-propiophenone	C M 19	
D-*threo*-1-(*p*-Nitrophenyl)-2-phthalimido-1,3-propanediol-ditetrahydropyramyl ester	C M 20	
DL-*threo*-1-(*p*-Nitrophenyl)-2-phthalimido-1,3-propanediol	C M 21	
DL-*threo*-1-Phenyl-2-phathalimido-1,3-propanediol	C M 22	
α-Phthalimido-β-methoxy-propiophenone	C M 23	
Chloramphenicol benzoate	C M 24	
D-*threo*-1-(*p*-Nitrophenyl)-2-(5′-nitrofuroylamido)-1,3-propanediol	C M 25	
Chloramphenicol monophosphate	C M 26	
threo-2-Dichloroacetamido-1-phenyl-1-(*p*-tolyl)-propane-1,3-diol	C M 27	

(to be continued)

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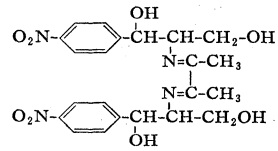
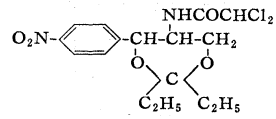
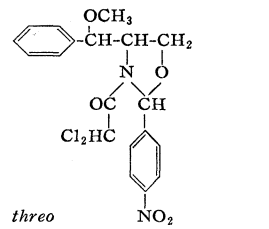
	Abbreviation	Chemical structure
D-Base diacetyl shiff base	CM 28	
D-*threo*-2,2-Diethyl-5-dichloro-acetamido-6-(*p*-nitrophenyl-1,3-dioxane	CM 29	
4-*α*-Methoxybenzyl-oxazolidine	CM 30	

culture at exponential growth was inoculated in 10 ml of broth containing various concentrations of chloramphenicol derivatives and analogues in cylindrical culture cell (pass length 30 mm), and incubated for 6 hours at 37°C. Bacterial growth was determined turbidimetrically with a self-recording Jouan Biophotometer (Etablissement Jouan, 113, Boulevard Saint-Germain, Paris, France).

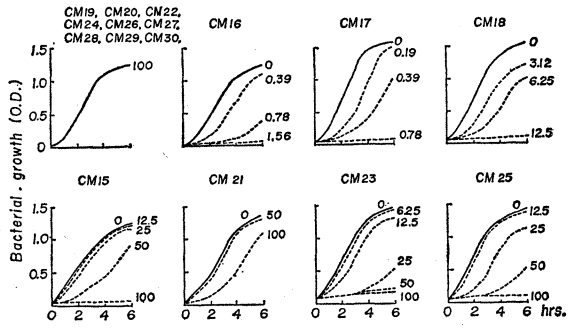
Induction of Chloramphenicol Resistance by its Derivatives and Analogues: An overnight broth culture of S-1477 was diluted ten-fold with fresh broth and shaken at 37°C for 2 hours. Three tenths ml of the culture was then inoculated into 10 ml of broth containing various concentrations of chloramphenicol derivatives and analogues. After incubation for 2 hours at 37°C with shaking, 0.1 ml of the culture was diluted with fresh broth containing 50 mcg/ml of chloramphenicol and incubated for 6 hours at 37°C. Bacterial growth was determined as mentioned above. Normal culture of this strain without induction could not grow in the medium containing 50 mcg/ml of chloramphenicol.

Results

Antibacterial Activity of Chloramphenicol Derivatives and Analogues

Growth curve of *Staphylococcus aureus* S-1477 in broth containing various concentrations of chloramphenicol derivatives and analogues was shown in Fig. 1. Bacterial growth was not inhibited by 100 mcg/ml of CM 19, CM 20, CM 22, CM 24, CM 26, CM 27, CM 28, CM 29 and CM 30. CM 15, CM 21 and CM 25

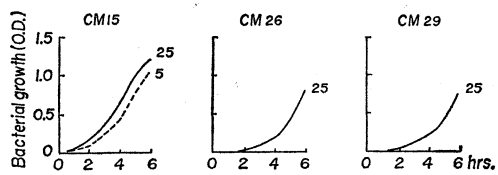
Fig. 1. Growth of an inducible strain in broth containing various concentrations of chloramphenicol derivatives and analogues.



An overnight culture of S-1477 was diluted with fresh broth and shaken at 37°C for 2 hours; 0.1 ml of the culture in early phase of exponential growth was inoculated in 10 ml of broth containing various concentrations of chloramphenicol derivatives and analogues, and shaken at 37°C in Jouan Biophotometer. Bacterial growth was determined according to the method described in Materials and Methods. Concentrations of each drug are shown in the figure.

showed less antibacterial activity than chloramphenicol, and CM 23 had almost the same activity as chloramphenicol. It was found that CM 16, CM 17 and CM 18 have higher antibacterial activity than that of chloramphenicol, their minimum inhibitory concentrations (MIC) being 1.56, 0.78 and 12.5 mcg/ml, respectively. Consequently, antibacterial activity of CM 17 was found to be 64-fold higher than that of chloramphenicol under the conditions used.

Fig. 2. Growth of induced populations in broth containing 50 mcg/ml of chloramphenicol.



An overnight culture of S-1477 was diluted with fresh broth and shaken at 37℃ for 2 hours; 0.3 ml of the culture in early phase of exponential growth was inoculated in 10 ml of broth containing various concentrations of chloramphenicol derivatives and analogues and shaken at 37℃. Concentrations of each drug for induction are shown in the figure. After 2-hour incubation, 0.1 ml of the induced culture was inoculated in 10 ml of broth containing 50 mcg/ml of chloramphenicol and shaken at 37℃ in Jouan Biophotometer.

Ability of Induction for Chloramphenicol Resistance

Induction ability of chloramphenicol derivatives and analogues for chloramphenicol resistance was then examined. Pretreatment of S-1477 with 25 (or 5.0) mcg of 15 ml chloramphenicol solution and with 25 mcg of CM 26 and CM 29 could induce chloramphenicol resistance, and induced populations could grow in the broth containing 50 mcg/ml of chloramphenicol. But induction did not occur by treatment with 25 mcg/ml of CM 19, CM 20, CM 21, CM 22, CM 23, CM 24, CM 25, CM 27, CM 28 and CM 30. Similarly, pretreatment with subinhibitory concentrations of CM 16, CM 17 and CM 18 could not induce resistance to chloramphenicol under the conditions used (Fig. 2).

Discussion

It was found that CM 16 (DL-α-dichloroacetamido-β-bromo-p-nitropropiophenone), CM 17 (DL-α-dichloroacetamido-α-methylene-p-nitropropiophenone) and CM 18 (DL-α-acetamido-α-methylene-p-nitropropiophenone) have much higher antibacterial activity than that of chloramphenicol against S-1477, *i.e.*, a strain carrying inducible resistance to chloramphenicol.

From the data described above, it may likely be concluded that: (1) the nitrophenyl moiety is essential for its antibacterial activity, and CM 18 having acetamido side chain is less active than that of CM 17, carrying dichloroacetamido side chain; (2) eminent antibacterial action of chloramphenicol derivatives and analogues is dependent on carbonyl substitution of carbon atom 1 of propanediol chain of authentic chloramphenicol (CM 16, CM 17, CM 18); (3) superiority of action is given by the substitution of Br in place of OH at carbon atom 3 of propanediol chain (CM 16) and by double bond C=C between carbon atoms 2 and 3 (CM 17, CM 18).

It has been reported that the chloramphenicol resistance of naturally isolated staphylococci is due to a chloramphenicol-acetyltransferase, which converts hydroxy radical on the adjacent carbon atom(s) of propanediol chain of chloramphenicol to 3-acetoxy (3-acetoxy-chloramphenicol) and/or to 1,3-diacetoxy (1,3-diacetoxy-chloramphenicol)[7,8,9].

Therefore, high antibacterial activity of chloramphenicol-derivatives, CM 16, CM 17 and CM 18, against S-1477 carrying inducible resistance to chloramphenicol is accounted for by the substituted chemical structures, which are hardly affected by chloramphenicol acetyltransferase. The fact that induction ability for the chloramphenicol-resistance of CM 16, CM 17 and CM 18 is much lower than that of chloramphenicol, will be another reason for their antibacterial acitivity against S-1477.

The relationship between induction ability for chloramphenicol-resistance and chemical structure of the chloramphenicol derivatives and analogues is now under investigation and will be described elsewhere.

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