ANALOGY BETWEEN *IN VIVO* AND *IN VITRO* BIOLOGICAL EFFECT OF CHLORAMPHENICOL AND ITS ACETYLATED DERIVATIVES

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Received 19 August 1970
Revised version received 17 September 1970

1. Introduction

It has been shown that wild type strains of *E. coli* resistant to chloramphenicol (Cm) are able to acetylate this drug. The synthesis of the acetylase is controlled by an extrachromosomal element (= plasmid), which can be transferred, from the resistant *E. coli,* to sensitive cells. In some cases, the plasmid is not transferable although the resistance is due to the same mechanism. With chloramphenicol resistant mutants, selected *in vitro,* we have not been able to demonstrate such a reaction [1]. The acetylation occurs mainly in positions 1 and 3 (1,3-diacetoxy-chloramphenicol) or in position 3 (3-monoacetoxy-chloramphenicol) [1, 2]. Fig. 1. represents a molecule of the drug with the positions 1, 2 and 3 indicated.

To investigate further the mechanism of resistance to chloramphenicol, the effect of the acetylated compounds of the drug was tested on protein synthesis in *E. coli, in vivo* and *in vitro* with polyuridylic acid or viral RNA as messenger. It was found that chloramphenicol and the acetylated compounds showed the same pattern of inhibition *in vitro,* as they did *in vivo*: the inhibition decreased with mono-acetylation and nearly disappeared with di-acetylation.

2. Materials and methods

The 3-acetoxy-Cm and 1, 3-diacetoxy-Cm were a kind gift from Dr.Suzuki (Japanese National Institute of Health). We also made some preparations of these compounds as follows: R265, a Cm-resistant wild type strain of *E. coli* (a clinical isolate) was grown in a 5 liter nutrient broth (Difco) in presence of chloramphenicol (200 μg/ml) to late log phase; Cm and its acetylated derivatives were extracted by ether; the organic phase was evaporated *in vacuo*, redissolved in 5 ml ethanol, purified on charcoal, filtered and finally evaporated to a 1 ml volume. The acetylated compounds were separated by thin layer chromatography [1]. The corresponding spots were eluted with ethanol and these solutions evaporated again to 1 ml volume.

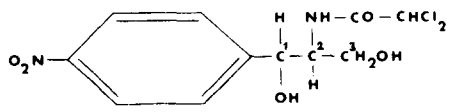

Fig. 1. The chloramphenicol molecule with positions 1 and 3.

3. Experiments and results

We first determined the Minimum Inhibitory Concentration (MIC) of chloramphenicol (ARCO Ltd., Switzerland) and of its acetylated compounds in nutrient broth. The values are reported in table 1. The growth of Cm-sensitive strains of *E. coli* was inhibited by 6 to 12 μg/ml of chloramphenicol, whereas 100 μg/ml of the mono-acetylated compound were necessary to obtain the same order of inhibition. At a higher concentration (200 μg/ml), the diacetylated derivative was inactive.

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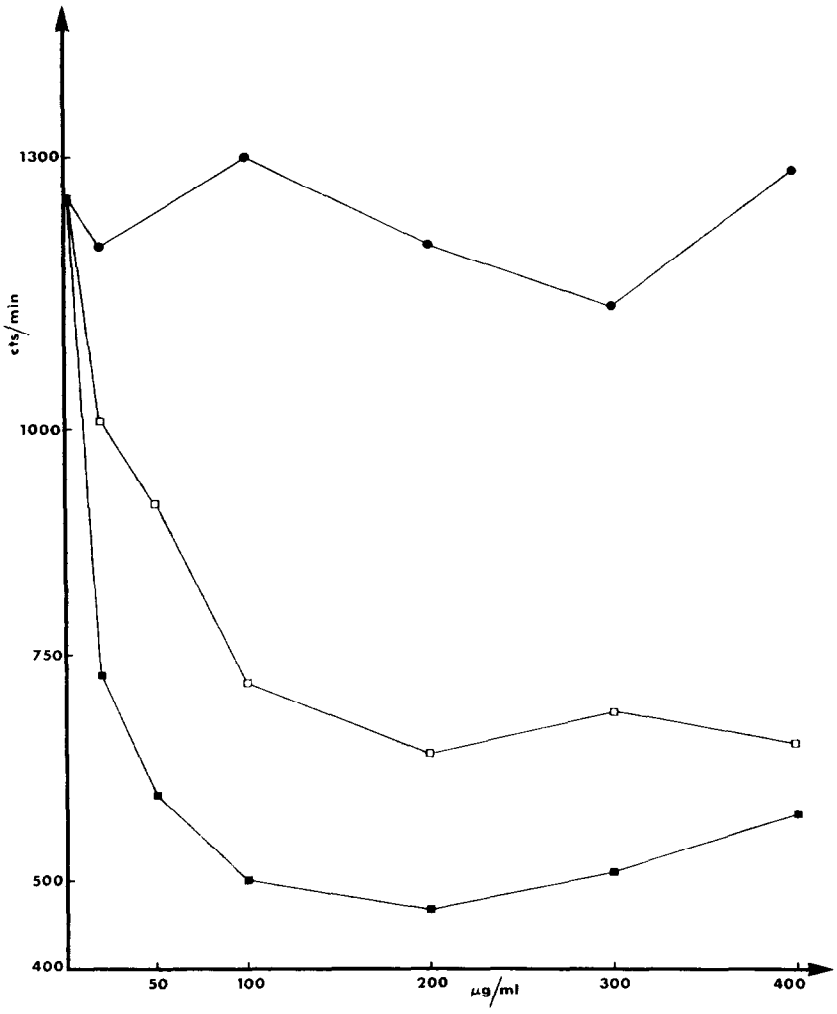

Fig. 2. Incorporation of labeled amino acids (cts/min) in the presence of ■—■ Cm, □—□ 3-Cm and ●—● 1,3-Cm (synthetic compounds) as a function of concentrations of Cm and Cm derivatives. The 200 µl reaction mixture contained: 100 µl "mix" (1 ml tris-HCl, pH 7.4; 0.5 ml tRNA, 50 mg/ml; 0.4 ml ATP 0.05 M; 0.3 ml GTP 0.02 M; 1 ml phosphoenolpyruvate 0.07 M; 1.4 ml $MgCl_2$ 0.1 M; 0.5 ml NH_4Cl 4 M; 4.9 ml H_2O); 10 µl pyruvate-kinase (1 mg/ml); 40 µl S100 (crude extract-ribosome dialysed against tris 0.01 M, KCl 0.1 M and concentrated on G25 coarse); 100 µg 70 S ribosomes in 20 µl buffer $MgCl_2$ 0.01 M; 10 µl ethanol 50% with or without Cm or its derivates: 10 µl phage R17 RNA; 10 µl amino acid mixture (mixture 1/1 v/v of ^{14}C ULmixture and of a solution of the 20 cold amino acids, 2 µmoles/ml); the mixture was adjusted to pH 7.0. Ribosomes and S100 are extracted from *E. coli* MRE 600. The system was incubated 20 min at 32°; 2 ml of a solution of TCA 5%, casamino acids 3% are added and the tRNA is made soluble by heating at 90°for 15 min. The samples are then filtered on Millipore GFC and the tubes washed twice with TCA 5%, casamino acids 3%. The filters are dried and the radioactivity counted on Nuclear Chicago.

The effect of Cm and its derivatives was then tested on an *in vitro* protein synthesizing system. With poly-U as messenger, no significant differences were observed between samples with or without Cm and the acetylated compounds; similar results were obtained with Cm by Kucan and Lipmann [3]. We then used a native RNA messenger in our system. The incorporation of labelled amino-acids in the presence of

Table 1
The minimum inhibitory concentrations of Cm and its acetylated compounds (μg/ml).

	E. coli K12	*E. coli* wild type R376
Cm	3–6	6–12
3-Cm	50–100	50–100
1,3-Cm	> 200	> 200

The sensitive strains used are: *E. coli* K12 F^+ met^- azi^S str^S $T1^S$ $T6^S$ and *E. coli* R376, wild type (clinical isolate).

chloramphenicol and its derivatives are reported in fig. 2. The values obtained show that the relative inhibitory activities of the three compounds tested are the same *in vitro* and *in vivo*: again *in vitro* the 3-acetoxy compound is less active than Cm, whereas the 1,3-diacetoxy is inactive. We observed no difference between the behaviour of the compounds we prepared biologically and those synthesized by Dr.Suzuki. An unexpected finding was the regain of amino-acid incorporation with increasing concentrations of Cm. Identical results for the inhibition of amino acid incorporation were obtained if the ribosomes used in the cell-free system were obtained from resistant strains.

4. Discussion

The difference of activity between Cm and its acetylated derivatives and the decrease of effect of Cm with increasing concentrations cannot easily be explained at this stage. However, from these two observations, one could imagine a model in which two specific sites on the Cm molecule (carried on C_1 and C_3, fig. 1) correspond to two other sites in the protein synthesizing system. According to Weisblum and Davies [4], these sites could be localized on the 50 S ribosomal subunit. In our model, both sites of the same Cm molecule would be attached to the other corresponding sites in order to be completely active. If only one of the sites is free, the activity of the antibiotic is decreased. This would explain the activities found *in vivo* and *in vitro* with the mono-acetylated compound. In excess of Cm, the two sites of the protein synthesizing system would be occupied by two different Cm molecules and thus the antibiotic activity would be lessened. This would explain the lessening activity of Cm with increasing concentrations.

To obtain further information on the reliability of our model, two types of experiments will have to be carried out. The binding of acetylated derivatives to the ribosomes should be determined in comparison with that of chloramphenicol and a quantitative study of the binding of chloramphenicol to the ribosomes should be done in relation to the ratio chloramphenicol/ribosomes.

Acknowledgements

We thank Prof. Spahr for a gift of phage R17 RNA, and Prof. Tissières for help.

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