The inactivation of chloramphenicol and thiamphenicol by anaerobic Bacteroides fragilis

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Antibiotics are an important means of managing infections disease, and the suitable choice of which is crucial. In serious infections, the combination of penicillin and an aminoglycoside is often considered but not in the cases of Bacteroides or Chlamydia. While some anaerobes are sensitive to chloramphenicol, clindamycin and high does of penicillin, the chlamydia and bacteroides are more sensitive to thiamphenicol.\(^{(1,2,3)}\)

Thiamphenicol is a broad-spectrum antibiotic and an analog of chloramphenicol with the substitution of the p-nitro-group by a methyl sulfonyl moiety.\(^{(4,5,6)}\) Its chemical formula is D\((=\)-threo-2\((p\)-methyl sulfonylphenyl\))-dichloraceta-mino-1, 3-propanediol. Its mechanism of antibacterial action is probably similar to chloramphenicol's which lies in the inhibition of protein synthesis within the bacterial cells.\(^{(7)}\)

The present paper reports our Comparative Bioassay Study on the inactivation of thiamphenicol and chloramphenicol by Bacteroides fragilis from November 1981–May 1982.

Materials

\textbf{a – Bacterial indices}

\textbf{a.1} The chosen strain of thiamphenicol–choloramphenicol resistant–Bacteroides fragilis was a gift from Dr. Keneth Goodner, Department of Microbiology, Jefferson Medical College.

Two thiamphenicol–choloramphenicol sensitive strains were isolated from our patients.

\textbf{a.2} The specific strain of Aeromonas hydrophila was supplied by the Aerobic unit, Department of Medical Microbiology, Chulalongkorn Hospital Medical School.

\textbf{b – The synthetic drugs}

The pure thiamphenicol (T P) used was lot. No. 80836/846, with the potency of 1015 mcg./mg. and the expired date of October, 1986. The pure chloramphenicol (C P) used was lot No. 53124 with the potency of 996 mcg./mg., and the expired date of August 1986.

\textbf{c – Equipments}

Equipments at the Anaerobic unit, Department of Medical Microbiology* included an anaerobic incubator, chamber CO\(_2\) cabinet, jar with the Oxoid gas generator kit with other accessories capable of various biochemical tests according to qualified procedures.\(^{(8,9,10)}\)

The specific Bacteroides fragilis strains were therefore easily identified and survived longer at \(-42^\circ\)C.\(^{(10,11)}\)

\textbf{Methods}\(^{(12,13,13a)}\)

1. The pure cultures of three Bacteroides fragilis strains were anaerobically incubated and grown in fresh thioglycollate broths overnight.

2. With the Mc Farland’s turbidity 1.0 standard, the overnight broths were diluted to \(2 \times 10^8\) CFU*/ml by fresh sterile trypticase soy broth.

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* CFU/ml. = Colony forming unit/ml.
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3. The diluted broths were then incubated at 37°C for 4 hours.

4. In the mean time (within 4 hours), 2 x 5 ml. of 100 mcg./ml. of each of the chloramphenicol (CP) and thiamphenicol** (TP) were prepared by dilution with sterile, distilled water (deminerized water).

5. Fresh sterile trypticase soy blood agars with the “well” in the blood media were prepared as described by Nara-thorn et al. (18,18a)

6. The standard inhibition zones (diameters) (SIZ) of chloramphenicol and thiamphenicol against aerobic Aeromonas hydrophila in the wells were performed and measured (control).

Next, each antibiotic was inactivated with the Bact. fragilis broths (No. 3) and anaerobically incubated for 3 or 6 hours before their residual activities were determined by bioassay with the A. hydrophila aerobically. After a re-incubation, the inhibition zones were measured and compared with the standard zones.

Results

1. The given strain of resistant-CP–TP–Bact. fragilis and 2–local sensitive–CP and TP–Bact. fragilis strains were used.

2. The SIZ for CP is 26 mm., while the SIZ for TP is 24 mm. in diameters (12,13,18a).

Table 1 Showing the residual activities (after inactivation) of chloramphenicol (CP) and Thiamphenicol (TP) by bioassay with A. hydrophila.

<table>
<thead>
<tr>
<th>Regimens</th>
<th>CP</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- The two sensitive strains of Bact. fragilis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1 After 3 hours incubation</td>
<td>22 (84.6%)*</td>
<td>23.6 (98.3%)*</td>
</tr>
<tr>
<td>1.2 After 6 hours incubation</td>
<td>18 (69.2%)*</td>
<td>23 (95.8%)*</td>
</tr>
<tr>
<td>2- The resistant strain of Bact. fragilis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 After 3 hours incubation</td>
<td>16 (61.5%)</td>
<td>20 (83.3%)</td>
</tr>
<tr>
<td>2.2 After 6 hours incubation</td>
<td>0 (0%)</td>
<td>18 (75%)</td>
</tr>
</tbody>
</table>

* mean residual activity of the antibiotics.

** Methyl alcohol is the only primary diluent of pure chemical CP and TP drugs.
Figure 1  Mean residual activities of thiamphenicol (T P) and chloramphenicol (C P) incubated with the sensitive strains of Bact. fragilis
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![Graph showing residual activity (%) over incubation time (hr.)](image)

**Figure 2** Residual activity of thiamphenicol (T P) and chloramphenicol (C P) incubated with the resistance strain of *Bact. fragilis*.

*Note: T P = Thiamphenicol, C P = Chloramphenicol*
Figure 3  Showing the inhibition zone of A. hydrophila by an antibiotic in the “well”.

Discussion

Chloramphenicol, a substance originally isolated from a culture of Streptomyces venezuelae, is now manufactured synthetically. Chloramphenicol is potent inhibitor of protein synthesis in microorganisms. It blocks the attachment of amino-acids to the nascent peptide chain on the 50S unit of ribosomes by interfering with the action of peptidyl transferase. (7)

The mechanism of thiamphenicol, the analog of chloramphenicol with substitution of the p-nitrogroup by a methylsulfonyl moiety, is probably the same, or more advantageous.

Although chloramphenicol can be inactivated by chloramphenicol acetyltransferase (14, 15), chloramphenicol hydrolase (16) and nitroreductase (17, 18) in specific pathogens, chloramphenicol resistance is mostly due to the drug's destruction by the acetyltransferase under a plasmid control. (7)

From Table 1 and Figure 1 of the sensitive—Bact. fragilis strains, one can interpret that C P is inactivated more than T P.

Our findings also support the work of Britz et al. in 1978 that the major active product of Bact. fragilis in chloramphenicol in activation is the acetyl transferase. (18)

In Table 1 and Figure 2, thiamphenicol retained more activity 83.3%,
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75% at 3 and 6 hrs. respectively towards the anaerobic resistant B. fragilis strain than chloramphenicol.

In these events, the study shows that the modes of inactivation of thiamphenicol are different to chloramphenicol.

In this study of anaerobic Bact. fragilis, the inactivation of chloramphenicol is by acetyl transferase\(^{(15)}\) while thiamphenicol by other means.

The fact is that thiamphenicol has no p-nitrogroup.* The bacterial nitro reductase is probably non-significant to thiamphenicol as it is to chloramphenicol.\(^{(17,18)}\) Therefore, the chloramphenicol resistant strain of Bact. fragilis may be sensitive to thiamphenicol.

Not only do many anaerobic bacteria possess the Beta-lactamase to destroy Betalactam antibiotics,\(^{(2)}\) but these drugs are too expensive** for our patients and the general practitioners. Neither metronidazole nor thiamphenicol is approved by F D A,*** but they are widely used by many clinicians.

Although chloramphenicol infrequently causes gastrointestinal upsets, prolonged administrations of more than 3 gm. daily to adults regularly result in abnormalities of the early forms of red blood cells, the elevation of serum iron and anemia. These changes are reversible upon discontinuance of the drug. However, very rare individuals exhibit apparent idiosyncrasy to chloramphenicol and develop severe or fatal depression of bone marrow function. The mechanism of this aplastic anemia is not understood but is distinct from the dose-related reversible effect described above.\(^{(7)}\)

In premature and newborn infants, chloramphenicol can induce collapse (Gray's syndrome) because its normal mechanism of detoxification (glucuronide conjugation in the liver) is not yet developed. However, the mentioned side effects are not seen clinically in thiamphenicol.\(^{(5,8,7)}\)

Acknowledgements

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* substitution of nitrogroup by a methyl sulfonyl group.

** Natimycin of Schering-Essex Phamaceutical Co. and Ceftriaxone, a new cephalosporin of Roche Phamaceutical Co.

*** Food and Drug Administration, U.S.A.
References


