The Effects of Isoniazid on the Growth Cycle of *Mycobacterium avium* NCTC 8559

SURIYATI MOHAMAD AND PAZILAH IBRAHIM

School of Pharmaceutical Sciences
Universiti Sains Malaysia
Penang, MALAYSIA
INTRODUCTION

*Mycobacterium avium*

- Belongs to group *M. avium* complex (MAC)
- Intracellular pathogenic, slow-growing acid-fast bacilli
- Pre-HIV era, localized pulmonary infections and cervical lymphadenitis
- Advent of AIDS, common opportunistic infections causing disseminated disease
- Clinical management of patients a formidable problem
- Resistant to most antibiotics including isoniazid

**Isoniazid (INH)**

- Mainstay antimycobacterial agent
- Knowledge of resistance in *M. avium* still lacking
- Previous work (Pazilah, 1996):
  - *M. avium* had growth cycle of 3 different stages
  - Susceptibility to INH varied throughout the cycle
OBJECTIVES

As an extension to work by Pazilah (1996):

- To elucidate INH action at cellular level on growth cycle of *M. avium* NCTC 8559
- To recognize susceptible stages
- To further understand isoniazid resistance
METHODS

Effects of INH

- MIC of drug determined using 1% proportion method
- *M. avium* NCTC 8559 cells in Middlebrook media
- Harvested at different stages (initial, filamentous & fragmentation)
- Exposed to MIC level of INH
- Incubated at 37 °C in CO$_2$ / 1,2,3,4,5,6 and 7 days
  - Cellular morphological changes
  - Acid-fastness properties
  - Colony counts for viability
RESULTS AND DISCUSSION

I. Minimum inhibitory concentration (MIC) of INH

- *M. avium* NCTC 8559 = 200 µg / ml
- *M. tuberculosis* H37Rv = 0.050 µg / ml
II. GROWTH EFFECTS OF INH

Effects of INH assessed by comparing results to that of untreated control presentations

1. Cellular morphology and acid-fastness properties (Fig. 1 – 3)

- Cells viewed using a bright-field microscope equipped with digital camera
- Analysis of images using Pixera Visual Communication Suite
Fig. 1. Effects of INH on the cellular morphology and acid-fastness properties of M. avium NCTC 8559 cells.

Exposure to INH at initial stage affected normal progression in growth cycle and acid-fastness properties of M. avium NCTC 8559 cells.

- Untreated culture
  - Initial stage
  - Filamentous stage
  - Fragmentation stage
  - Most cells stained bright red and retained their acid-fastness

- INH-treated culture
  - Initial stage
  - Fragmentation stage
  - Main loss in acid-fastness due to inhibition of mycolic acids syntheses
  - Elongated cells appeared less extensive
  - Presence of ‘ghost’ cells from day 1 in increasing abundance
  - No filamentous forms

Sequence of events in growth cycle of M. avium NCTC 8559 exhibited 3 different morphological changes due to INH treatment at the initial stage.
Fig. 2. Effects of INH on the cellular morphology and acid-fastness properties of *M. avium* NCTC 8559: addition at filamentous stage of the growth cycle.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>CONTROL</th>
<th>INH</th>
</tr>
</thead>
</table>

**Untreated culture**

- Starting cells mixture of small and elongated rods became longer and filamentous until day 3
- Fragmented on day 4 and coccobacilli by day 7

**INH-treated culture**

- Cells remained a mixture of small and elongated rods in first 3 days followed by fragmentation into coccobacilli from day 4 onwards
- Elongated cells not as extensive
- Filamentous forms not observed
- Most cells retained acid-fastness properties

Exposure to INH at filamentous stage affected normal progression in growth cycle but not acid-fastness properties of *M. avium* NCTC 8559 cells
**Fig. 3. Effects of INH on the cellular morphology and acid-fastness properties of *M. avium* NCTC 8559: addition at the fragmentation stage**

<table>
<thead>
<tr>
<th>DAYS</th>
<th>CONTROL</th>
<th>INH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td><img src="CONTROL_0.png" alt="Image" /></td>
<td><img src="INH_0.png" alt="Image" /></td>
</tr>
<tr>
<td>1</td>
<td><img src="CONTROL_1.png" alt="Image" /></td>
<td><img src="INH_1.png" alt="Image" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="CONTROL_2.png" alt="Image" /></td>
<td><img src="INH_2.png" alt="Image" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="CONTROL_3.png" alt="Image" /></td>
<td><img src="INH_3.png" alt="Image" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="CONTROL_4.png" alt="Image" /></td>
<td><img src="INH_4.png" alt="Image" /></td>
</tr>
<tr>
<td>5</td>
<td><img src="CONTROL_5.png" alt="Image" /></td>
<td><img src="INH_5.png" alt="Image" /></td>
</tr>
<tr>
<td>6</td>
<td><img src="CONTROL_6.png" alt="Image" /></td>
<td><img src="INH_6.png" alt="Image" /></td>
</tr>
<tr>
<td>7</td>
<td><img src="CONTROL_7.png" alt="Image" /></td>
<td><img src="INH_7.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Untreated culture**

- Starting cells mixture of coccobacilli and small rods became elongated on day 1 and filamented on day 2
- Fragmented into smaller rods from day 4 and eventually in the forms of tiny coccobacilli from day 6

**INH-treated culture**

- Elongation not as extensive
- No filamentous forms through out experiment
- Presence large number of ‘ghost’ cells from day 2 onwards

Exposure to INH at fragmentation stage affected normal progression in growth cycle and acid-fastness properties of *M. avium* NCTC 8559 in same ways as initial stage.
2. Colony counts (Fig. 4 – 6)

Growth curves were followed based on percentage of colony counts on day 0
**Fig. 4. Effects on colony counts: Addition at initial stage**

**Untreated culture**
- Above starting counts on day 0 through out study period
  - Indicating active cellular proliferations

**INH-treated culture**
- Lowered colony counts through out study period

*M. avium* NCTC 8559 cells at initial stage susceptible to inhibitory effects of INH and prevented from undergoing further divisions
**Untreated culture**
- Above starting count throughout experiment
- Normal growth kinetics with starting cells in filamentous forms

**INH-treated culture**
- Counts also above starting count throughout experiment
  - Cells in both cultures went through active proliferation throughout study period

**INH had no bactericidal effect on** *M. avium* NCTC 8559 cells at filamentous stage
**Fig. 6. Effects on colony counts: Addition at fragmentation stage**

**Untreated culture**
- Normal growth curve with starting cells in fragmented forms

**INH-treated culture**
- Lag period 1st 24 hours little change in colony counts
  - Starting cells on day 0 not all in fragmented units
  - Some cells still in filamentous form continued to undergo division balancing number of colonies
- Sharp decline from day 1 onwards

Even though effects did not occur immediately, *M. avium* NCTC 8559 cells at fragmentation stage susceptible to inhibitory effects of INH and prevented from undergoing further divisions.
CONCLUSION

Exposures to INH

- Affected normal progression in growth cycle of *M. avium* NCTC 8559 most obviously at initial and fragmentation stages

- A correlation existed between acid-fastness properties and colony counts