Effects of weak acids, UV and proton motive force inhibitors on pyrazinamide activity against *Mycobacterium tuberculosis* in vitro

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**Background:** Pyrazinamide is a paradoxical frontline tuberculosis drug characterized by high sterilizing *in vivo* activity but poor *in vitro* activity. Pyrazinamide is thought to act by the entrapment of pyrazinoic acid in the bacterial cell, leading to acidification and membrane damage. Consequently, the effects of weak acids and molecules affecting membranes added to pyrazinamide were studied.

**Objectives:** To examine the effects of weak acids, UV, oxidative stress and additional energy inhibitors on pyrazinamide activity *in vitro* against *Mycobacterium tuberculosis* as well as the effect of pyrazinamide on *Escherichia coli* ampicillin persisters.

**Methods:** Drug exposure experiments followed by cfu counts were performed to determine the effects of the above various factors on pyrazinamide activity *in vitro* against *M. tuberculosis*.

**Results:** Some weak acids such as benzoic acid, sorbic acid and propyl hydroxybenzoic acid could enhance the activity of pyrazinamide *in vitro* against old tubercle bacilli but not young bacilli whereas other weak acids such as salicylic acid and lactic acid did not appear to enhance pyrazinamide activity. While energy inhibitors carbonylcyanide *m*-chlorophenylhydrazone and dinitrophenol enhanced pyrazinamide activity for old tubercle bacilli but not young bacilli, valinomycin and KCN increased pyrazinamide activity for both young and old bacilli. Oxidative stresses due to *H*2*O*2 and menadione did not have a significant effect on pyrazinamide activity. UV, which presumably damages the membrane, enhanced the activity of pyrazinamide. Pyrazinamide, which otherwise has no activity against actively growing *E. coli* bacteria, could kill non-growing starved *E. coli* and also ampicillin-tolerant persisters.

**Conclusions:** Some weak acids, UV and various energy inhibitors were found to enhance the activity of pyrazinamide *in vitro* against *M. tuberculosis*. Pyrazinamide shows preferential activity against both *M. tuberculosis* and *E. coli* persisters over the growing forms.

Keywords: TB, mycobacteria, *E. coli*, persisters

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**Introduction**

Pyrazinamide is an important frontline tuberculosis (TB) drug and plays a key role in shortening TB therapy from 9–12 months to the current 6 months.1,2 The ability of pyrazinamide to shorten the TB therapy is related to its activity against a population of non-growing, persister tubercle bacilli residing in an acid pH environment that are not killed by other TB drugs.1–3 Despite its high *in vivo* sterilizing activity, paradoxically, pyrazinamide is not active *in vitro* under normal culture conditions near neutral pH,4 but is only active under acidic conditions (e.g. pH 5.5),5 with an MIC of 50–100 mg/L at pH 5.5–6.0.2

The activity of pyrazinamide is inversely related to pH with higher activity at more acidic pH. This relationship can be expressed by the Henderson-Hasselbalch equation.6 We have shown that besides acid pH, various factors such as the age of the culture, inoculum size, serum albumin,6 iron,7 and hypoxic conditions8 could affect the activity of pyrazinamide *in vitro*. We have proposed a model for the mode of action of pyrazinamide where pyrazinamide as a prodrug is converted into the active form pyrazinoic acid (POA) inside the bacilli, which then gets to the cell surface by diffusion and a weak efflux mechanism and then forms protonated HPOA at acid pH and gets back into the bacilli.9 The entry of HPOA disrupts the membrane potential, which could

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eventually cause energy depletion and inhibition of membrane transport function. This model of pyrazinamide action can best explain the various peculiar properties of pyrazinamide, such as acid pH requirement, preferential killing of non-growing old tubercle bacilli and higher activity against tubercle bacilli under anaerobic conditions. Indeed, this model has also led us to the finding that energy inhibitors such as N,N′-dicyclohexyl carbodiimide (DCCD) (H-ATPase inhibitor), azide (cytochrome c oxidase inhibitor) and rotenone (NADH dehydrogenase inhibitor) could all increase the activity of pyrazinamide, but not other control drugs isoniazid and rifampin, indicating the specificity of the enhancement of activity by the energy inhibitor for pyrazinamide.

_Mycobacterium tuberculosis_ is uniquely susceptible to pyrazinamide and the unique pyrazinamide susceptibility is related to a deficient POA efflux mechanism and a poor ability to maintain the membrane proton motive force. In contrast, non-tuberculous mycobacteria such as _Mycobacterium smegmatis_ and other bacteria such as _Escherichia coli_ are intrinsically resistant to pyrazinamide (MIC > 2000 mg/L) at least partly due to a more active efflux mechanism in these bacteria and perhaps a better ability to maintain membrane energy and pH homeostasis. In the present study we examined the effects of weak acids, oxidative stress conditions, DNA-damaging agents and various other energy inhibitors on pyrazinamide activity in vitro. We identified additional factors that could potentially enhance pyrazinamide activity. Furthermore, we also explored the possibility that pyrazinamide, which is ineffective in killing growing metabolically active tubercle bacilli, might be more active against slow metabolizing _E. coli_ persisters not killed by ampicillin.

Materials and methods

**Drugs and chemicals**

Pyrazinamide, DCCD, sodium azide, carbonylcyanide _m_-chlorophenylhydrazone (CCCP), dinitrophenol (DNP), valinomycin, KCN, norfloxacin, sodium nitroprusside and _H_2O_2_ were obtained from Sigma-Aldrich Co. Pyrazinamide and sodium azide were dissolved in deionized water at stock concentrations of 10,000 mg/L and 10 mM and filter-sterilized. DCCD was dissolved in 95% ethanol at a stock concentration of 200 mM.

**Mycobacterial growth**

_M. tuberculosis_ H37Ra was grown in 7H9 liquid medium (Difco) supplemented with 0.05% Tween 80 and 10% bovine serum albumin dextrose-catalase (ADC) enrichment (Difco) at 37°C for ~7–10 days (mid- to late-exponential phase) with occasional agitation as described previously. Old cultures of _M. tuberculosis_ were prepared by incubating the cultures at 37°C for 7–12 weeks without shaking.

**Effect of weak acids, oxidative stresses, UV, DNA-damaging agents and proton motive force (PMF) inhibitors on pyrazinamide activity**

* M. tuberculosis H37Ra was grown in 7H9 + ADC for 1–2 weeks (young cells) or 2 months (old cells) at which time 1.0 mL aliquots were placed in microtube tubes and then cells were harvested and washed in 1 mL of PBS buffer and resuspended in 1.0 mL of 7H9 + ADC or citrate buffer at pH 5.5. Various agents, such as weak acids benzoic acid (2 mM), salicylic acid (1 mM), propyl hydroxybenzoic acid (1 mM), sorbic acid (1 mM) and lactic acid (1 mM), DNA-damaging agents norfloxacin (1 mg/L), sodium nitroprusside (1 mM) and _H_2O_2_ (4 mM), or PMF inhibitors DCCD (1 mM), DNP (1 mM), sodium azide (1 mM), CCCP (0.1 mM), valinomycin (1 μM) and KCN (10 mM), with or without pyrazinamide (100 mg/L) were added to young or old cultures. Following drug exposure for 5–7 days, the viability of the tubercle bacilli after washing was determined by plating on 7H11 plates. The cfu count was determined from triplicate samples of different drug exposed cells and the average cfu is presented.

UV exposure was performed by exposing tubercle bacilli in a Petri dish to UV for 15, 30 or 60 s with or without pyrazinamide in citrate buffer (pH 5.5). A control with no drug was included along with a pyrazinamide (100 mg/L) alone control. The treated bacterial suspensions were allowed to incubate for 5 days when the bacteria were harvested, washed and resuspended in PBS buffer prior to determining the cfu/mL for each sample by plating on 7H11 + ADC plates.

**Activity of pyrazinamide against persister bacteria and starved bacteria of _E. coli_**

To determine the effect of pyrazinamide on persister bacteria, _E. coli_ strain DH5α was grown overnight at 37°C with shaking. The next day, the stationary phase culture was diluted 1:100 into fresh Luria–Bertani medium and the culture was incubated with shaking for about 2–3 h to obtain log phase culture. The log phase cultures were subjected to cfu determination before treatment with ampicillin (50 mg/L) for 1 h at 37°C. The ampicillin-treated cultures were harvested and washed in PBS. The cells were resuspended in citrate buffer at pH 5.0 followed by pyrazinamide addition at a final concentration of 2000 mg/L or with no pyrazinamide addition as a control. The tubes were incubated for 5 days at 37°C when 100 μL aliquots were removed, washed and resuspended in PBS and diluted and plated on LB agar plates for cfu counts. To determine the effect of pyrazinamide on starved log phase culture, the bacterial cells were resuspended in citrate buffer pH 5.0 and treated with 2000 mg/L pyrazinamide or with no pyrazinamide for 5 days before cfu was determined.

**Statistical treatment**

Pairwise comparison of the cfu data for statistical significance was performed using the Student’s _t_-test.

**Results**

**Effect of weak acids on pyrazinamide activity**

To determine the effect of weak acids on pyrazinamide activity in vitro, we exposed _M. tuberculosis_ cells from a 7-week-old culture resuspended in 7H9 medium at acid pH 5.5 to pyrazinamide alone, or weak acids benzoic acid (2 mM), salicylic acid (1 mM), propyl hydroxybenzoic acid (1 mM), sorbic acid (1 mM) and lactic acid (1 mM) alone, and pyrazinamide plus weak acids, and drug free control. Following drug exposure for 7 days, the viability of the tubercle bacilli was determined by plating on 7H11 plates. The average cfu is presented in Tables 1 and 2 (data not shown for lactic acid). Pyrazinamide alone and weak acids benzoic acid and propyl hydroxybenzoic acid alone reduced the cfu by ~10-fold compared with the control;
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Table 1. Effect of weak acids on pyrazinamide (PZA) activity against old (7-week-old) cultures

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PZA</th>
<th>BA</th>
<th>BA + PZA</th>
<th>SA</th>
<th>SA + PZA</th>
<th>PH</th>
<th>PH + PZA</th>
</tr>
</thead>
<tbody>
<tr>
<td>cfu/mL</td>
<td>3.00 × 10^6</td>
<td>1.67 × 10^5</td>
<td>3.00 × 10^5</td>
<td>4.33 × 10^4</td>
<td>1.33 × 10^3</td>
<td>1.57 × 10^3</td>
<td>1.33 × 10^3</td>
<td>3.3 × 10^4</td>
</tr>
</tbody>
</table>

BA, benzoic acid; SA, salicylic acid; PH, propyl hydroxybenzoic acid.

Table 2. Effect of weak acids on pyrazinamide (PZA) activity for young (1-week-old) and old (2-month-old) cultures

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PZA</th>
<th>BA</th>
<th>BA + PZA</th>
<th>Sorbic acid</th>
<th>PZA + sorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young cells</td>
<td>8.00 × 10^4</td>
<td>5.30 × 10^3</td>
<td>1.50 × 10^4</td>
<td>2.00 × 10^4</td>
<td>5.00 × 10^4</td>
<td>2.00 × 10^4</td>
</tr>
<tr>
<td>Old cells</td>
<td>2.00 × 10^5</td>
<td>4.00 × 10^4</td>
<td>7.00 × 10^4</td>
<td>1.00 × 10^4</td>
<td>4.00 × 10^4</td>
<td>1.00 × 10^4</td>
</tr>
</tbody>
</table>

Results are given in terms of cfu/mL.

BA, benzoic acid.

Table 3. Effect of UV on pyrazinamide (PZA) activity for young cultures (1–2 weeks old)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>PZA</th>
<th>15 s UV</th>
<th>15 s UV + PZA</th>
<th>30 s UV</th>
<th>30 s UV + PZA</th>
<th>60 s UV</th>
<th>60 s UV + PZA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.50 × 10^8</td>
<td>2.30 × 10^8</td>
<td>3.30 × 10^8</td>
<td>2.70 × 10^8</td>
<td>3.00 × 10^8</td>
<td>1.00 × 10^8</td>
<td>2.00 × 10^8</td>
<td>1.00 × 10^8</td>
</tr>
<tr>
<td>5</td>
<td>9.00 × 10^7</td>
<td>3.00 × 10^7</td>
<td>1.30 × 10^7</td>
<td>1.30 × 10^7</td>
<td>1.00 × 10^7</td>
<td>2.70 × 10^7</td>
<td>4.70 × 10^7</td>
<td>3.70 × 10^7</td>
</tr>
</tbody>
</table>

Results are given in terms of cfu/mL.

however, the combination of weak acids benzoic acid and propyl hydroxybenzoic acid with pyrazinamide reduced the cfu by an additional 10-fold over that of pyrazinamide alone or weak acids alone for the 7-week-old cultures (Table 1). The reduction in cfu in bacilli treated with weak acids plus pyrazinamide was statistically significant when compared with pyrazinamide or weak acids alone (P < 0.01; determined by the Student’s t-test). This suggests that weak acids benzoic acid and propyl hydroxybenzoic acid could enhance the activity of pyrazinamide. However, the weak acid salicylic acid (Table 1) alone had a pronounced antimycobacterial activity but salicylic acid or lactic acid did not appear to have a significant enhancement effect on pyrazinamide activity (P > 0.05).

Since the age of the culture influences the activity of pyrazinamide, we also evaluated the effect of age of culture on the enhancement of pyrazinamide activity by weak acids. Benzoic acid enhanced the activity of pyrazinamide for the 7-week-old culture (Table 1) in one experiment and a 2-month-old culture in a second experiment (Table 2) but had no such enhancement effect for the 1–2-week-old young culture (Table 2). Similarly, sorbic acid (1 mM) had a more pronounced enhancement effect on pyrazinamide activity for the old culture than for the young culture (Table 2).

Effect of DNA-damaging factors and oxidative stress conditions on pyrazinamide activity

Agents that produce oxidative stress such as menadione (1 mM) and H_2O_2 (4 mM) did not have a significant effect on synergizing pyrazinamide activity for both young and old M. tuberculosis cultures (P > 0.05) (data not shown). However, sodium nitroprusside (1 mM), a reactive nitrogen intermediate (RNI) generator, enhanced the pyrazinamide activity for old cultures as shown by a 10-fold decrease in cfu compared with pyrazinamide or nitroprusside alone (P < 0.05), but its enhancement effect on pyrazinamide activity was not obvious for young cultures (P > 0.05). Norfloxacin, a quinolone drug that can damage DNA, did not have any significant effect on enhancing pyrazinamide activity (P > 0.05).

We also evaluated the effect of UV on pyrazinamide activity by exposing young M. tuberculosis cultures (1–2 weeks old) to UV for 15, 30 and 60 s alone, pyrazinamide alone and UV in combination with pyrazinamide and assessed the treated bacilli for cfu counts at day 0 and day 5. Pyrazinamide alone had little effect on the young culture as shown previously.6,8 Interestingly, when the M. tuberculosis culture was exposed to UV for 60 s there was a 10-fold reduction in cfu for the UV plus pyrazinamide combination compared with the UV exposure alone at day 5 (P < 0.05) but not at day 0 (P > 0.05) (Table 3). The UV enhancement effect of pyrazinamide activity was not obvious at 15 s of exposure and somewhat less so at 30 s of exposure and most obvious at 60 s of exposure.

Effect of various energy inhibitors on pyrazinamide activity

We have shown previously that energy inhibitors such as DCCD, an ATPase inhibitor, rotenone, a Complex 1 inhibitor, and sodium azide, a cytochrome c oxidase inhibitor, could enhance pyrazinamide activity in vitro against M. tuberculosis.8,9 In the present study we further evaluated the effect of additional energy inhibitors including CCCP, DNP, KCN and valinomycin (1 μM) on possible enhancement of pyrazinamide activity. As shown in Table 4, as a positive control, azide increased the activity of pyrazinamide as expected; however, CCCP, which is a weak organic acid that acts as a proton carrier and uncoupler, reduced the cfu by at least a 100-fold at 60 s of exposure.
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Table 4. Effect of CCCP on pyrazinamide (PZA) activity for young (1-week-old) and old (2-month-old) cultures

<table>
<thead>
<tr>
<th>5 day exposure</th>
<th>Control</th>
<th>PZA</th>
<th>Azide</th>
<th>PZA + azide</th>
<th>CCCP</th>
<th>PZA + CCCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young cells</td>
<td>2.20 \times 10^3</td>
<td>4.00 \times 10^7</td>
<td>9.00 \times 10^6</td>
<td>4.70 \times 10^5</td>
<td>2.10 \times 10^7</td>
<td>2.50 \times 10^8</td>
</tr>
<tr>
<td>Old cells</td>
<td>1.00 \times 10^6</td>
<td>1.00 \times 10^6</td>
<td>1.00 \times 10^4</td>
<td>0</td>
<td>1.00 \times 10^5</td>
<td>1.00 \times 10^3</td>
</tr>
</tbody>
</table>

Results are given in terms of cfu/mL.

Table 5. Effect of valinomycin on pyrazinamide (PZA) activity for young (1-week-old) and old (2-month-old) cultures

<table>
<thead>
<tr>
<th>5 day exposure</th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.00 \times 10^6</td>
<td>1.40 \times 10^6</td>
</tr>
<tr>
<td>PZA</td>
<td>1.30 \times 10^6</td>
<td>2.60 \times 10^5</td>
</tr>
<tr>
<td>Valinomycin</td>
<td>2.90 \times 10^6</td>
<td>2.00 \times 10^6</td>
</tr>
<tr>
<td>PZA + valinomycin</td>
<td>5.30 \times 10^5</td>
<td>2.70 \times 10^5</td>
</tr>
</tbody>
</table>

Results are given in terms of cfu/mL.

Table 6. Effect of KCN on pyrazinamide (PZA) activity for young (1-week-old) cultures

<table>
<thead>
<tr>
<th>5 day exposure</th>
<th>H37Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00 \times 10^5</td>
</tr>
<tr>
<td>PZA</td>
<td>1.00 \times 10^5</td>
</tr>
<tr>
<td>KCN</td>
<td>8.30 \times 10^2</td>
</tr>
<tr>
<td>PZA + KCN</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are given in terms of cfu/mL.

uncoupler DNP also increased the activity of pyrazinamide for the old cultures as demonstrated by a 10-fold reduction in cfu with DNP plus pyrazinamide compared with pyrazinamide alone or DNP alone. However, the enhancement effect of DNP on pyrazinamide activity was not obvious for young *M. tuberculosis* cultures (data not shown). Valinomycin, an agent that disrupts membrane potential, increased the activity of pyrazinamide against *M. tuberculosis* (*P* < 0.05) for both young and old bacilli (Table 5). The enhancement effect of valinomycin for pyrazinamide activity was more obvious for the old culture than for the young culture as shown in Table 5. KCN, a compound that inhibits the cytochrome *c* oxidase in the respiratory chain, completely eliminated the cfu in combination with pyrazinamide (Table 6).

**Effect of pyrazinamide on ampicillin-tolerant *E. coli* persisters and starved bacteria**

*E. coli* is known to be completely resistant to pyrazinamide with an MIC of pyrazinamide of more than 2000 mg/L at pH 5.0.13 Pyrazinamide is known to be more active against non-growing tubercle bacilli while growing young bacilli are less susceptible to the drug.5 Since pyrazinamide is a drug that kills *M. tuberculosis* persisters,6,8 we wondered whether pyrazinamide has any activity on *E. coli* persisters that are not killed by ampicillin. The log phase *E. coli* culture (1.3 \times 10^7/mL) was subjected to ampicillin treatment at 50 mg/L for 1 h to kill any actively growing bacteria while leaving the persisters completely eliminated the cfu in combination with pyrazinamide.

Discussion

The activity of pyrazinamide against *M. tuberculosis* is influenced by many factors such as acid pH,5,6,10 age of the culture,6,12 oxygen tension,8 iron,7 albumin6 and energy inhibitors.9 The disconnection of the high *in vivo* sterilizing activity and poor *in vitro* activity against *M. tuberculosis* highlights the difference between *in vivo* and *in vitro* environments that affect pyrazinamide activity. As part of the effort to understand this disconnection, we examined the effect of weak acids, which can be present during the inflammation process, on the activity of pyrazinamide *in vitro*. Another reason behind the study of the effect of weak acids on pyrazinamide is derived from our current model of pyrazinamide, whose active component POA, a weak acid, could disrupt the membrane potential under acid pH.9 We reasoned that the presence of other weak acids might enhance the pyrazinamide activity. It was found that some weak acids such as benzoic acid, sorbic acid and propylhydroxybezoic acid could increase the activity of pyrazinamide mainly for old cultures with lower acidity.
membrane potential but had little enhancement activity for young cultures with more active metabolism and higher membrane potential (Tables 1 and 2). However, weak acids salicylic acid and lactic acid did not have significant effect on enhancing pyrazinamide activity for either young or old cultures of *M. tuberculosis*. The basis for such a difference between the weak acids in enhancing pyrazinamide activity is unclear but could reflect their different capacity to disrupt membrane potential. Overall, the enhancement effect of pyrazinamide activity by some weak acids is not as dramatic as that by the energy inhibitors. The degree of weak acid enhancement of pyrazinamide activity is only ~10-fold, which is indicative of an additive effect; whereas, the energy inhibitors tend to result in a higher enhancement of pyrazinamide activity, which is suggestive of a synergistic effect (Tables 4–6).

We have previously shown that energy inhibitors such as DCCD, azide and rotenone could increase pyrazinamide activity *in vitro*.9 The new TB drug candidate diarylquinoline, which inhibits proton-ATPase just like DCCD, could also synergize with pyrazinamide activity.14 In the present study, additional energy inhibitors CCCP, DNP, KCN and valinomycin were tested and CCCP and DNP were found to enhance pyrazinamide activity primarily for old cultures whereas KCN and valinomycin could enhance pyrazinamide activity for both young and old cultures (Tables 4–6). These findings reinforce the previous observation that energy inhibitors could enhance the activity of pyrazinamide.9 It is worth noting that in general the weak acids do not have as strong an effect on enhancing pyrazinamide activity as the energy inhibitors. This finding is probably due to the relatively poor ability of the weak acids to disrupt the membrane potential compared with the more powerful energy inhibitors such as KCN, valinomycin and CCCP. Both the weak acids and energy inhibitors are more effective in enhancing the pyrazinamide activity against old cultures than against young cultures. These observations are consistent with our model of pyrazinamide where pyrazinamide depletes the membrane energy.9 The significantly higher enhancement effect of weak acids and energy inhibitors on pyrazinamide activity for old cultures could be due to less energy reserves in old cells than the young cultures with more active metabolism and more energy reserves.

A somewhat surprising finding of this study is that UV had a significant enhancement effect of pyrazinamide activity against *M. tuberculosis* (Table 3). UV can generate free radicals causing damage to macromolecules such as DNA and also membrane. While we cannot rule out the possibility that the UV-mediated DNA damage is underlying the enhanced effect of pyrazinamide activity, we prefer the explanation of UV damage of the membrane as the more plausible explanation for the UV-mediated enhancement of pyrazinamide activity since UV has been shown to induce membrane damage.15 Thus it is quite likely that the UV enhancement of pyrazinamide activity could be due to UV damaging the membrane, which could in turn cause increased proton permeability and decreased membrane potential, which can synergize with pyrazinamide as proposed.9 This UV-mediated membrane damage leading to increased susceptibility to pyrazinamide action is also consistent with our current model of pyrazinamide action.9

The factors that influence the activity of pyrazinamide are best shown by drug exposure assays and not by conventional MIC experiments. This is due to the unusual nature of pyrazinamide, which is more active against non-growing tubercle bacilli than against growing bacilli,6 a property that is completely opposite to common antibiotics. Another unusual feature of pyrazinamide is the time dependence to show activity. That is, it takes a long time, i.e. several days or even weeks, to show any significant activity (data not shown). Exposure for a short time of less than 3–5 days hardly shows any activity of pyrazinamide. This presumably is a reflection of the time needed to convert the prodrug pyrazinamide into POA and also the time needed to deplete the energy reserve required to maintain bacterial viability.

Pyrazinamide has no activity against *E. coli* as shown by its resistance to over 2000 mg/L pyrazinamide at pH 5.5 in MIC-type experiments.13 However, when the growing *E. coli* bacteria were killed by ampicillin exposure, the residual persister bacteria with low metabolism were more susceptible to pyrazinamide (Table 7). It is interesting to note that the small number of *E. coli* persister cells that are susceptible to pyrazinamide as demonstrated by the drug exposure test cannot be demonstrated by conventional MIC testing in media that encourage the growth of bacteria. Similarly, when *E. coli* was subjected to acid citrate buffer with pyrazinamide, the non-growing starved bacteria could be killed to some extent by pyrazinamide. Despite the similarity of the above finding to pyrazinamide on *M. tuberculosis*, where pyrazinamide kills both persister bacteria not killed by other drugs, a significant difference is that energy inhibitors such as DCCD and azide did not enhance pyrazinamide activity in *E. coli* as they did in *M. tuberculosis*. This could be due to differences in the physiology of the two organisms such as those in energy production and maintenance and also possible differences in efflux activity of the drug.

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Transparency declarations

None to declare.

References

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