Pyrazinoic acid decreases the proton motive force, respiratory ATP synthesis activity and cellular ATP levels.
Lu, P.; Haagsma, A.C.; Pham, H.; Maaskant, J.J.; Mol, S.; Lill, H.; Bald, D.

published in
Antimicrobial Agents and Chemotherapy
2011

DOI (link to publisher)
10.1128/AAC.00507-11

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
vuresearchportal.ub@vu.nl

Download date: 25. Apr. 2022
Pyrazinoic Acid Decreases the Proton Motive Force, Respiratory ATP Synthesis Activity, and Cellular ATP Levels

Ping Lu, Anna C. Haagsma, Hoang Pham, Janneke J. Maaskant, Selena Mol, Holger Lill, and Dirk Bald

Pyrazinoic acid, the active form of the first-line antituberculosis drug pyrazinamide, decreased the proton motive force and respiratory ATP synthesis rates in subcellular mycobacterial membrane assays. Pyrazinoic acid also significantly lowered cellular ATP levels in Mycobacterium bovis BCG. These results indicate that the predominant mechanism of killing by this drug may operate by depletion of cellular ATP reserves.

Shortening tuberculosis treatment duration is a key objective in order to reduce noncompliance and to combat recently emerging multidrug-resistant strains of Mycobacterium tuberculosis (6, 19, 36). Pyrazinamide (PZA), an important first-line drug employed in tuberculosis chemotherapy, played a key role in shortening the duration of tuberculosis treatment from 9 months to 6 months (22). PZA is a sterilizing drug that efficiently kills populations of Mycobacterium tuberculosis residing in acidic environments, as found during active inflammation (1, 7, 10, 11, 20, 21, 28, 32). Despite the importance of PZA, no cellular target proteins have been clearly identified (4, 23, 30, 7, 10, 11, 20, 21, 28, 32). Despite the importance of PZA, no cellular target proteins have been clearly identified (4, 23, 30, 41), and its mechanism of action is probably the least understood among all first- and second-line antituberculosis drugs. A better understanding of PZA action may help in development of new drugs to further shorten tuberculosis treatment.

PZA constitutes a prodrug that is hydrolyzed in the mycobacterial cell by pyrazinamidase to yield the active entity pyrazinoic acid (POA) (15, 16). According to the hypothesis put forward by Zhang and colleagues, POA, a weak acid (pKa, 2.9), bacterial cell by pyrazinamidase to yield the active entity pyrazinoic acid (POA). Pyrazinamide (PZA), an important first-line drug employed in tuberculosis chemotherapy, played a key role in shortening the duration of tuberculosis treatment from 9 months to 6 months (22). PZA is a sterilizing drug that efficiently kills populations of Mycobacterium tuberculosis residing in acidic environments, as found during active inflammation (1, 7, 10, 11, 20, 21, 28, 32). Despite the importance of PZA, no cellular target proteins have been clearly identified (4, 23, 30, 41), and its mechanism of action is probably the least understood among all first- and second-line antituberculosis drugs. A better understanding of PZA action may help in development of new drugs to further shorten tuberculosis treatment.

POA decreases rates of respiratory ATP synthesis. Next, we investigated the extent to which POA, by decreasing the proton motive force, interferes with respiratory ATP production. Rates of ATP synthesis by the mycobacterial membranes were determined as described previously (8, 9). As depicted in Fig. 2, POA inhibited ATP synthesis in a dose-dependent manner. The affinity for POA significantly decreased at higher pH, with a 50% inhibitory concentration (IC50) value of 200 μg/ml at pH 5.5 compared with 850 μg/ml at pH 6.5 (Fig. 2, closed bars). The POA concentrations needed to significantly decrease the proton motive force were comparable to values reported earlier for Mycobacterium tuberculosis at the whole-cell level (40). This result shows that POA directly interferes with membrane energetics.

POA decreases rates of respiratory ATP synthesis. Next, we investigated the extent to which POA, by decreasing the proton motive force, interferes with respiratory ATP production. Rates of ATP synthesis by the mycobacterial membranes were determined as described previously (8, 9). As depicted in Fig. 2, POA inhibited ATP synthesis in a dose-dependent manner. The affinity for POA significantly decreased at higher pH, with a 50% inhibitory concentration (IC50) value of 200 μg/ml at pH 5.5 compared with 850 μg/ml at pH 6.5 (Fig. 2, closed bars). The POA concentrations needed to significantly decrease the proton motive force were comparable to values reported earlier for Mycobacterium tuberculosis at the whole-cell level (40). This result shows that POA directly interferes with membrane energetics.

POA decreases rates of respiratory ATP synthesis. Next, we investigated the extent to which POA, by decreasing the proton motive force, interferes with respiratory ATP production. Rates of ATP synthesis by the mycobacterial membranes were determined as described previously (8, 9). As depicted in Fig. 2, POA inhibited ATP synthesis in a dose-dependent manner. The affinity for POA significantly decreased at higher pH, with a 50% inhibitory concentration (IC50) value of 200 μg/ml at pH 5.5 compared with 850 μg/ml at pH 6.5 (Fig. 2, closed bars). The POA concentrations needed to significantly decrease the proton motive force were comparable to values reported earlier for Mycobacterium tuberculosis at the whole-cell level (40). This result shows that POA directly interferes with membrane energetics.

POA decreases rates of respiratory ATP synthesis. Next, we investigated the extent to which POA, by decreasing the proton motive force, interferes with respiratory ATP production. Rates of ATP synthesis by the mycobacterial membranes were determined as described previously (8, 9). As depicted in Fig. 2, POA inhibited ATP synthesis in a dose-dependent manner. The affinity for POA significantly decreased at higher pH, with a 50% inhibitory concentration (IC50) value of 200 μg/ml at pH 5.5 compared with 850 μg/ml at pH 6.5 (Fig. 2, closed bars). The POA concentrations needed to significantly decrease the proton motive force were comparable to values reported earlier for Mycobacterium tuberculosis at the whole-cell level (40). This result shows that POA directly interferes with membrane energetics.

POA decreases rates of respiratory ATP synthesis. Next, we investigated the extent to which POA, by decreasing the proton motive force, interferes with respiratory ATP production. Rates of ATP synthesis by the mycobacterial membranes were determined as described previously (8, 9). As depicted in Fig. 2, POA inhibited ATP synthesis in a dose-dependent manner. The affinity for POA significantly decreased at higher pH, with a 50% inhibitory concentration (IC50) value of 200 μg/ml at pH 5.5 compared with 850 μg/ml at pH 6.5 (Fig. 2, closed bars). The POA concentrations needed to significantly decrease the proton motive force were comparable to values reported earlier for Mycobacterium tuberculosis at the whole-cell level (40). This result shows that POA directly interferes with membrane energetics.

POA decreases rates of respiratory ATP synthesis. Next, we investigated the extent to which POA, by decreasing the proton motive force, interferes with respiratory ATP production. Rates of ATP synthesis by the mycobacterial membranes were determined as described previously (8, 9). As depicted in Fig. 2, POA inhibited ATP synthesis in a dose-dependent manner. The affinity for POA significantly decreased at higher pH, with a 50% inhibitory concentration (IC50) value of 200 μg/ml at pH 5.5 compared with 850 μg/ml at pH 6.5 (Fig. 2, closed bars). The POA concentrations needed to significantly decrease the proton motive force were comparable to values reported earlier for Mycobacterium tuberculosis at the whole-cell level (40). This result shows that POA directly interferes with membrane energetics.

POA decreases rates of respiratory ATP synthesis. Next, we investigated the extent to which POA, by decreasing the proton motive force, interferes with respiratory ATP production. Rates of ATP synthesis by the mycobacterial membranes were determined as described previously (8, 9). As depicted in Fig. 2, POA inhibited ATP synthesis in a dose-dependent manner. The affinity for POA significantly decreased at higher pH, with a 50% inhibitory concentration (IC50) value of 200 μg/ml at pH 5.5 compared with 850 μg/ml at pH 6.5 (Fig. 2, closed bars). The POA concentrations needed to significantly decrease the proton motive force were comparable to values reported earlier for Mycobacterium tuberculosis at the whole-cell level (40). This result shows that POA directly interferes with membrane energetics.
take of metabolites as reported earlier (40) and with respiratory ATP synthesis as shown here, POA thus exerts at least a dual action, which potentially renders it an exceptionally powerful drug. This unusual property of POA may hold for bacteria under conditions of a low energy supply in particular (12, 34) and may in part explain why pyrazinamide appears to constitute an essential component of antituberculosis drug regimens.

POA decreases cellular ATP levels. We investigated the impact of ATP synthesis inhibition by POA on cellular ATP levels. Addition of POA to M. bovis BCG grown in liquid culture significantly decreased cellular ATP levels in both a time-dependent and a dose-dependent manner (Fig. 3). The concentrations of POA needed to reduce cellular ATP levels correlate well with those required for ATP synthesis inhibition; e.g., 800 μg/ml POA (the IC50 in the ATP synthesis assay) decreased cellular ATP levels by ~40% after 1 day and >60% after 6 days. In control experiments, PZA did not significantly change ATP levels. These results suggest that POA interference with respiratory ATP synthesis has a significant impact on cellular ATP levels and may be the cause of bacterial killing.

PZA and POA share several interesting characteristics with a new series of ATP synthesis inhibitors, the diarylquinolines (2, 5, 9a), as follows. Both drugs show delayed action in vitro and in vivo, with time-dependent killing observed only from days 3 and 4 on (27, 40). Moreover, both drugs display a particularly strong effect on mycobacteria under (semi-) dormant conditions (10, 18, 26, 33, 38). Finally, in similarity to our data reported here for POA, TMC207 reduces cellular ATP levels (17, 18, 26). Based on the similarities in activity signatures, we suggest that the two drugs share the same predominant mechanism of killing, i.e., depleting the cellular ATP pool. It needs to be determined to what extent alternative targets are involved and to what extent they contribute to the pronounced bactericidal action of pyrazinamide.

The chain of events leading from (too) low ATP levels to

FIG. 1. Pyrazinoic acid decreases the proton motive force in a subcellular assay. The proton motive force in membrane vesicles from M. bovis BCG was monitored in the presence of pyrazinoic acid at the indicated concentrations (measured in micrograms per milliliter) by quenching of ACMA as a fluorescence indicator at pH 6.5 (A) and pH 5.5 (B). Succinate and the uncoupler SF6847 were added to establish and collapse the proton motive force at the time points indicated by the arrows. Each experiment was carried out in triplicate; representative results are shown.

FIG. 2. Pyrazinoic acid inhibits ATP synthesis. ATP synthesis activity by membrane vesicles was determined using the glucose-6-phosphate method for quantification of the ATP produced (9). The reaction was carried out in the presence of the indicated concentrations (Con) of pyrazinoic acid (closed bars) or pyrazinamide (open bars) at pH 6.5 (A) or pH 5.5 (B). Each graph shows the mean values and standard deviations of the results of three independent experiments.
bacterial killing is presumably complex (14, 24), and the factors involved need to be elucidated. Drugs interfering with cellular energy pools appear to be very powerful, especially against dormant bacteria. Membrane function and respiratory ATP synthesis may constitute examples of a new generation of antibiotic targets for treatment of persistent infections (3, 13, 19, 40). The subcellular membrane assay for characterization of pyrazinoic acid described here can be applied for screening and characterization of this new generation of compounds targeting respiratory ATP production.

P.L. gratefully acknowledges financial support from the Chinese Scholarship Council. A.C.H. and D.B. gratefully acknowledge financial support from the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO-ECHO grant 700.55.017).

We thank B. Appelmelk and W. Bitter (Medical Microbiology & Infection Control, VU University Medical Center) and M. Braster (Molecular Cell Physiology, VU University) for assistance with bacterial culture and luminescence measurements, respectively.

ADDENDUM IN PROOF

While this paper was in press, W. Shi et al. (Science 333: 1630–1632, 2011) reported the ribosomal protein RpsA as an additional target of pyrazinamide. The relative importance of the respective targets for bacterial killing needs to be investigated.

REFERENCES


35. Reference deleted.