Acetic Acid, the Active Component of Vinegar, Is an Effective Tuberculocidal Disinfectant

Claudia Cortesia,a Catherine Vilchèze,b Audrey Bernut,c Whendy Contreras,a Keyla Gómez,d Jacobus de Waard,d William R. Jacobs, Jr.,b Laurent Kremer,c,e Howard Takiffa

OBSERVATION

Mycobacteria are important agents of human infections. While the most well-known infections are tuberculosis and leprosy, infections with nontuberculous mycobacteria are an increasing problem in soft tissues after surgical or cosmetic procedures (1) or in the lungs of cystic fibrosis and immunosuppressed patients (2). Killing mycobacteria in health care settings is important because Mycobacterium tuberculosis strains can be multidrug resistant and therefore potentially fatal biohazards, and environmental mycobacteria must be thoroughly eliminated from surgical implements and respiratory equipment. Currently used mycobactericidal disinfectants can be toxic, unstable, and expensive. We fortuitously found that acetic acid kills mycobacteria and then showed that it is an effective mycobactericidal agent, even against the very resistant, clinically important Mycobacterium abscessus complex. Vinegar has been used for thousands of years as a common disinfectant, and if it can kill mycobacteria, the most disinfectant-resistant bacteria, it may prove to be a broadly effective, economical biocide with potential usefulness in health care settings and laboratories, especially in resource-poor countries.

ABSTRACT Effective and economical mycobactericidal disinfectants are needed to kill both Mycobacterium tuberculosis and non-M. tuberculosis mycobacteria. We found that acetic acid (vinegar) efficiently kills M. tuberculosis after 30 min of exposure to a 6% acetic acid solution. The activity is not due to pH alone, and propionic acid also appears to be bactericidal. M. bolletii and M. massiliense nontuberculous mycobacteria were more resistant, although a 30-min exposure to 10% acetic acid resulted in at least a 6-log10 reduction of viable bacteria. Acetic acid (vinegar) is an effective mycobactericidal disinfectant that should also be active against most other bacteria. These findings are consistent with and extend the results of studies performed in the early and mid-20th century on the disinfectant capacity of organic acids.

IMPORTANCE Mycobacteria are best known for causing tuberculosis and leprosy, but infections with nontuberculous mycobacteria are an increasing problem after surgical or cosmetic procedures or in the lungs of cystic fibrosis and immunosuppressed patients. Killing mycobacteria is important because Mycobacterium tuberculosis strains can be multidrug resistant and therefore potentially fatal biohazards, and environmental mycobacteria must be thoroughly eliminated from surgical implements and respiratory equipment. Currently used mycobactericidal disinfectants can be toxic, unstable, and expensive. We fortuitously found that acetic acid kills mycobacteria and then showed that it is an effective mycobactericidal agent, even against the very resistant, clinically important Mycobacterium abscessus complex. Vinegar has been used for thousands of years as a common disinfectant, and if it can kill mycobacteria, the most disinfectant-resistant bacteria, it may prove to be a broadly effective, economical biocide with potential usefulness in health care settings and laboratories, especially in resource-poor countries.

Received 8 January 2014 Accepted 21 January 2014 Published 25 February 2014

Citation Cortesia C, Vilchèze C, Bernut A, Contreras W, Gómez K, de Waard J, Jacobs WR Jr, Kremer L, Takiff H. 2014. Acetic acid, the active component of vinegar, is an effective tuberculocidal disinfectant. mBio 5(2):e00013-14. doi:10.1128/mBio.00013-14.

Editor Louis Weiss, Albert Einstein College of Medicine

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Address correspondence to Howard Takiff, htakiff@gmail.com.
tions were plated on LB agar plates for *Escherichia coli* and Middlebrook 7H10 plates for mycobacteria, except for *Mycobacterium smegmatis*, which was plated on either medium with the same results. All experiments were performed at least twice, and the results were the same whether the bacterial pellets were resuspended in Middlebrook 7H9 medium-OADC or in a sodium hydroxide solution to neutralize the acetic acid. Negative controls with sterile water were included to determine the original number of viable bacteria.

Exposure to acetic acid at a concentration of 5% for 20 min reduced viable *Escherichia coli* DH5α and *M. smegmatis* mc²155 bacteria by at least 7 log₁₀ (Table 1). In some experiments in which the initial culture had ~9 log₁₀ of bacteria/ml, there were rare surviving colonies of *E. coli* and *M. smegmatis*. When these colonies were grown in broth media and tested again, they showed no evidence of resistance or tolerance to acetic acid, and their exposure to 5% for 20 min again produced 7 log₁₀ of killing.

To test whether the low pH of acetic acid was responsible for the bactericidal effect, we exposed *M. smegmatis* and *E. coli* for 20 min to a dilute solution of HCl in water with pH 2.5, corresponding approximately to the pH of 5% acetic acid. No bactericidal effect was seen (data not shown), indicating that the bactericidal effect of acetic acid was caused by its carboxylic acid function.

We then tested for activity against *M. tuberculosis*. While exposure to 5% acetic acid for 20 min obtained only a 3- to 4-log₁₀ reduction of viable bacteria, exposure to a 6% solution for 30 min resulted in at least an 8-log₁₀ reduction. The same levels of mycobacterial activity were seen with virulent (H37Rv) and avirulent auxotrophic (mc²7000 [H37Rv ΔRDI ΔparCD]) (16) *M. tuberculosis* laboratory strains, as well as with multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis* clinical isolates.

We also tested acetic acid on the notoriously resistant *Mycobacterium abscessus* complex bacteria (17). Although *M. abscessus sensu stricto* was efficiently killed by exposure to 6% acetic acid for 30 min, *Mycobacterium bolletii* and *Mycobacterium massiliense* were more resistant. The levels of reduction in viable bacteria differed somewhat in different experiments, but exposure to 10% acetic acid for 30 min achieved a minimum of a 6-log₁₀ killing of *M. abscessus* (data not shown), indicating that the bactericidal effect of acetic acid was caused by its carboxylic acid function.

Finally, we demonstrated that the mycobacterial activity remained even under "dirty" conditions that were meant to simulate contamination with organic material in clinical samples, with the acetic acid solution containing 2 to 3.5% bovine serum albumin and 2 to 3.5% red blood cells (Table 1).

The results described above suggest that acetic acid can be an effective, economical bactericidal agent for *M. tuberculosis* and

### TABLE 1 Bactericidal effect of increasing exposure times and acid concentrations

| Acid and/or conditions | Solution characteristics* | Logₐ₁₀ reduction of viable bacteria* |
|------------------------|--------------------------|------------------------------------|
|                        | BSA and RBC concen (%) | pH with M. abscessus | Exposure time (min) | Final concen (%) | E. coli DH5α survivors | M. smegmatis | M. smegmatis mc²7000 | M. tuberculosis H37Rv | M. tuberculosis | MDR M. tuberculosis | XDR M. tuberculosis | M. abscessus | M. bolletii R | M. massiliense massiliense S |
| Acetic acid            |                          | 0.34 20 2.5 3 | 4 4 4 3 | 7 7 7 4 | 3 | 4 | 4 3 | 3 |
|                        |                          | 0.34 30 3 3 | 4 4 4 3 | 7 7 7 4 | 3 | 4 | 4 3 | 3 |
|                        |                          | 0.54 20 4 4 | 3 3 4 3 | 5 5 5 5 | 3 | 4 | 4 3 | 3 |
|                        |                          | 2.45 2.62 5 5 | 8.5 7 7 7 | 3 | 4.5 | 3 4 | 3 |
|                        |                          | 0.83 25 5 | 5 | 5 | 5 | 5 | 5 | 5 |
|                        |                          | 0.83 30 5 | 5 | 5 | 5 | 5 | 5 | 5 |
|                        |                          | 2.45 2.57 6 6 | 9 | 9 | 9 | 9 | 9 | 9 |
|                        |                          | 1.00 25 6 | 9 | 9 | 9 | 9 | 9 | 9 |
|                        |                          | 1.00 30 6 | 8 | 8 | 8 | 8 | 8 | 8 |
|                        |                          | 1.00 30 6 | 8 | 8 | 8 | 8 | 8 | 8 |
|                        |                          | 2.31 2.42 30 30 | 8 | 8 | 8 | 8 | 8 | 8 |
|                        |                          | 1.33 30 8 | 8 | 8 | 8 | 8 | 8 | 8 |
|                        |                          | 2.27 2.40 1.67 30 | 10 | 10 | 10 | 10 | 10 | 10 |
| Acetic acid, dirty conditions |                          | 2 | 3 | 3.5 | 2.5 | 20 6 6 6 | 6 6 6 | 6 6 6 |
|                        |                          | 1.00 25 6 | 9 | 9 | 9 | 9 | 9 | 9 |
|                        |                          | 1.00 30 6 | 8 | 8 | 8 | 8 | 8 | 8 |
|                        |                          | 1.00 30 6 | 8 | 8 | 8 | 8 | 8 | 8 |
|                        |                          | 2.27 2.40 1.67 30 | 10 | 10 | 10 | 10 | 10 | 10 |
| Propionic acid         |                          | 0.9 20 6.7 8 | 7 | 7 | 7 | 7 | 7 | 7 |
|                        |                          | 1.08 20 8.0 | 7 | 7 | 7 | 7 | 7 | 7 |
|                        |                          | 1.08 25 8.0 | 7 | 7 | 7 | 7 | 7 | 7 |
|                        |                          | 1.15 20 8.5 8 | 7 | 7 | 7 | 7 | 7 | 7 |

* High-level disinfectant activity is indicated in bold.
* The numbers represent the reductions, expressed in log₁₀, in the number of colonies recovered after the acid exposures, compared to that of controls exposed to sterile water alone under the same conditions.
* BSA, bovine serum albumin; RBCs, human red blood cells.
* M. abscessus with optical density at 600 nm of 1 was diluted 1:10 in the acetic acid solutions.
* Limit of detection (no colonies recovered from exposed bacteria).
* R and S refer to the rough and smooth morphotypes of *M. massiliense*.

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nontuberculous mycobacteria, although the 20- to 30-min exposure time required to obtain optimal killing is longer than the 5 min recommended for some commercial bactericides. Exposure to 6% acetic acid for 30 min resulted in a 8-log$_{10}$ reduction of viable M. tuberculosis bacteria, including XDR and MDR strains. In tests with the M. abscessus complex, which are emerging as the most pathogenic of the nontuberculous mycobacteria (17), the same conditions produced 8 log$_{10}$ of killing only with M. abscessus sensu stricto, while M. massiliense and M. bolletii were more resistant in some assays. However, the generally accepted definition of an effective mycobactericide is one that has the ability to reduce viable bacteria by 4 to 5 log$_{10}$ (18), while the 6-log$_{10}$ reduction of both these bacteria achieved with 10% acetic acid for 30 min would be classified as high-level mycobactericidal activity (19). In some assays in which 10$^{6}$ M. smegmatis bacteria were exposed to 6% acetic acid for 20 min and 3 $\times$ 10$^{6}$ M. massiliense bacteria were exposed to 10% acetic acid for 30 min, there were no survivors. Although we did not attempt carrier tests, the effective mycobactericidal activity was maintained even under “dirty” conditions that simulate contamination with organic material. While this level of killing may not be adequate for all critical uses, such as sterilizing surgical instruments, it is likely that higher levels of killing of these highly resistant strains could be achieved with higher concentrations of acetic acid and/or longer exposure times than the ones tested here. Preliminary studies, however, suggest that 10% acetic acid for 30 min does not kill Bacillus subtilis spores (data not shown), so it cannot be classified as a high-level general disinfectant (20). We have not assessed the activity of acetic acid against viruses and fungi.

Although the disinfectant properties of organic acids such as acetic acid, propionic acid, and butyric acid—a component of sweat (21)—are well known, they are not usually included in reviews of bactericides (4). However, in the early part of the 20th century, they were fairly extensively studied for disinfectant properties, as reviewed by Reid in 1932 (22), and their tuberculocidal activity was evaluated by Barker in 1964 (23). It was found that they had broad bactericidal activity that increased with increasing carbon chain length through caprylic acid (C = 8), although the longer-chain acids were less soluble. Bactericidal activity was also found to increase with decreasing surface tension of the organic acid solutions and appeared to be due to the undissociated acid rather than the hydrogen ion concentration. It was therefore suggested that the bactericidal effect might be related to the ability of the acids to pass through the bacterial membrane (23).

Acetic acid is not very toxic, although prolonged exposure will produce corrosive effects, both on skin and metals. In reports from nearly 100 years ago, it was found that the topical application of a 1% acetic acid solution in saline cured Pseudomonas aeruginosa (Bacillus pyocyanus) wound infections (24, 25). It might be worthwhile testing its effectiveness as a topical agent on mycobacterial ulcers (26, 27).

Acetic acid is relatively inexpensive—2.5 liters of 99% acetic acid costs less than US$100 and could effectively disinfected up to 20 liters of M. tuberculosis cultures or spuata. Commercial vinegar bought in supermarkets was used wherever possible in the experiments described here, but the concentrations vary from country to country. Commercial vinegar could be used at effective concentrations for M. smegmatis or M. tuberculosis in France, where it is sold as 8% acetic acid, but not in the United States or Venezuela, where vinegar is sold as 5% acetic acid. While longer-chain organic acids may have better bactericidal activity, acetic acid (vinegar) is relatively nontoxic, inexpensive, and available, which could make it an effective, economical biocide for disinfecting M. tuberculosis from clinical specimens, cultures, and laboratory surfaces, and it would be particularly useful in low-income countries. The high-level capacity of acetic acid in killing mycobacteria, regarded as the most disinfectant-resistant bacteria due to the structure of their lipid-rich cell walls (4), suggests that perhaps it should be revived as a broadly effective bactericide that can be used as a general sanitizer.

ACKNOWLEDGMENTS

This work was funded by FONACIT project G2005000393 to H.T., LOCTI project “Nuevos fármacos contra la tuberculosis” to H.T., “Vaincre La Mucroviscidose” grant RF2011 0600446 to A.B., the visiting investigator program of the Université Montpellier 2 to H.T., and NIH AI26170 and Center for AIDS Research award AI51519 to W.R.J., Jr.

We declare no conflicts of interest.

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