Influence of Vehicles Used for Oral Dosing of Test Molecules on the Progression of Mycobacterium tuberculosis Infection in Mice

Shubhra Singh, Richa Dwivedi, and Vinita Chaturvedi

Drug Target Discovery and Development Division, Central Drug Research Institute, Lucknow, India

Preclinical evaluation of drug-like molecules requires their oral administration to experimental animals using suitable vehicles. We studied the effect of oral dosing with corn oil, carboxymethyl cellulose, dimethyl sulfoxide, and polysorbate-80 on the progression of Mycobacterium tuberculosis infection in mice. Infection was monitored by physical (survival time and body weight) and bacteriological (viable counts in lungs) parameters. Compared with water, corn oil significantly improved both sets of parameters, whereas the other vehicles affected only physical parameters.

Every year, progressive infection with Mycobacterium tuberculosis leads to approximately 8 million new tuberculosis (TB) cases and 3 million deaths worldwide. Furthermore, the spread of multidrug-resistant (MDR) TB has become a major threat to global TB control programs. This alarming situation emphasizes the need for new drugs against TB (28, 31).

Evaluation of the in vivo efficacy of a new drug candidate in a suitable animal model is a critical step in determining whether it will enter the preclinical and clinical development phases (10). When infected with a high number of CFU of M. tuberculosis by the intravenous route, the lung of a mouse harbors a bacillary population that is similar in number and in metabolic state to that present in the lungs of TB patients (10). Thus, the mouse model can provide information on lead molecule activity that can be extrapolated to humans (8, 22). Outbred mice, particularly Swiss mice, are used for in vivo assays for anti-TB activity because of their heterogeneity, which is akin to that of the human population (3).

For in vivo evaluations, a lead molecule is preferably administered as a solution or suspension by the oral route (1), which is an important requirement for its successful development as a new drug. Water is the universally accepted “ideal” vehicle for oral dosing, having no chemical, biochemical, immunological, or pharmacological effects on the test molecule, the host, or the pathogen (2). However, if the molecule is highly hydrophobic, then edible oils (such as corn or peanut oil) are used as vehicles (21). For moderately hydrophobic molecules, certain solubilizing agents, such as carboxymethyl cellulose (CMC), dimethyl sulfoxide (DMSO), and polysorbate-80 (Tween 80), can be added to the aqueous dosing vehicle (23, 24).

The influence of dosing vehicles on the progression of disease in animal models, if any, is an important consideration for in vivo evaluation of test molecules. There is hardly any information available on whether these vehicles themselves could modulate the disease process, thereby influencing the outcome of the evaluation of the drug candidates. This is particularly important in cases of chronic infections, such as TB, where treatments are given to the animals for long periods of time (6, 12, 19). We therefore studied the effect that some of the vehicles themselves could exert on the progression of M. tuberculosis infection in mice. Five vehicles—water, corn oil, CMC, DMSO, and Tween 80—were comparatively studied. Study parameters were physical, i.e., mean survival time (MST) and body weight, as well as bacteriological, i.e., CFU recovered from the lung.

Corn oil was administered undiluted, whereas dilutions of CMC (0.5%), Tween 80 (0.05%) and DMSO (10%) were made in sterile water. Female outbred Swiss mice (16 to 18 g), obtained from the Laboratory Animal Division of the Central Drug Research Institute, Lucknow, India, were infected intravenously (2 × 10⁷ bacilli/mouse) with M. tuberculosis H37Rv (ATCC 27294) and caged in groups of 8 to 10 animals. Each group was given water or the other treatments separately (0.2 ml/mouse, by oral gavage, once daily, for 30 days). The progress of infection was monitored in the control (untreated) mice by determining numbers of viable bacilli (CFU) on day 1 postinfection and then at weekly intervals (Fig. 1). For this, serial dilutions of lung homogenates (1 g tissue/ml) in physiological saline were spread on Middlebrook 7H11 agar medium (containing OADC [oleic acid-albumin-dextrose-catalase] supplement). The plates were incubated at 37°C, and CFU were counted after 3 to 4 weeks (4). Numbers of CFU in the lungs of mice from each experimental (vehicle-treated) group were determined on day 30.

The corn oil-fed mice showed far less decline in body weight (weight on day 0 = weight on day 21) than the water-fed animals (50.0 ± 1.00 g and 4.67 ± 3.06 g, respectively [Table 1]). This difference was statistically significant (P = 0.047 [Table 2]). More importantly, oil-fed animals also exhibited a >1-log reduction in lung CFU compared with the water-fed animals (Table 1). This difference was also statistically significant (P = 0.0016 [Table 2]). However, a significant difference was not observed between MST (mean survival time of all animals in a group) of the water-fed (24.75 ± 4.50 days) and oil-fed (25.25 ± 3.69 days) animals (Table 1).

Among the three aqueous vehicles, administration of DMSO resulted in “healthier” physical parameters. The MST with DMSO was >30 days (no deaths up to day 30) compared with 28.00 ± 2.31 days with CMC and 28.75 ± 2.50 days with Tween 80 (Table 1). All three MSTs were higher than those observed with water and
oil. As none of the mice in DMSO group died, it was not possible to calculate statistical significance of the differences in MST. The decline in body weight with all three vehicles was also less than that with water (Table 1), though the differences were not significant (P > 0.05). With respect to oil, only CMC showed a significantly greater decline in the body weight (3.00 ± 1.15 g, P = 0.0176) (Table 2). Finally, the numbers of CFU in lungs with all aqueous vehicles (Table 1) were close to that obtained with water (P > 0.05) but significantly higher than that obtained with oil (P = 0.0007 versus CMC, 0.0021 versus DMSO, and 0.0060 versus Tween 80) (Table 2).

The apparent beneficial effects of corn oil could be due to the presence of 86% unsaturated fatty acids, of which two-thirds is linoleic acid and one-third is oleic acid (15). Linoleic acid has been found to inhibit mycobacteria in vitro (15, 29); thus, it could also produce the same effect in vivo. The three aqueous vehicles (DMSO, CMC, and Tween 80) caused some apparent, though not significant, modulation of only the physical parameters, particularly MST. Among the three, administration of DMSO resulted in much improved physical parameters. This may influence the general body physiology and/or metabolism, which may in turn modulate the handling of the pathogen by the infected host (5, 7, 9, 11, 13, 14, 17, 18, 25–27, 30).

Measurement of survival time or body weight of animals is an appropriate, cost- and time-effective criterion to assess the progression of infection and to evaluate new drugs against M. tuberculosis in vivo (10, 16, 20). Our results, however, show that the weight loss and MST may not be reliable criteria and that determination of bacterial load in the infected organs is a true measure of infection. Moreover, in a previous screening for TB-induced weight loss, some active compounds or drugs possessing anabolic properties could have affected the results (20). In conclusion, the vehicles used for oral dosing of test molecules can influence physical as well as bacteriological parameters in mice infected with M. tuberculosis.

ACKNOWLEDGMENT

We are grateful to Director, Central Drug Research Institute, Lucknow, India, for providing necessary facilities and support to carry out the work. This paper has CDRI communication number 8299.

REFERENCES

therapeutic potential in guinea-pig tumor model of deoxyribonucleic acid
from Mycobacterium bovis BCG complexed with poly-t-lysine and car-
15. Layton HW, Youmans GP. 1965. Effect of dietary factors upon the resis-
tance of albino mice to experimental infection with Mycobacterium tu-
screening of experimental drugs for tuberculosis using gamma interferon
reducing serum total cholesterol and low-density lipoprotein levels in hy-
Tween 80 on the growth of Mycobacterium avium complex. Microbiol.
Immunol. 34:653–663.
screen for drugs active against Mycobacterium tuberculosis. Antimicro-
21. O’Driscoll CM, Griffin BT. 2008. Biopharmaceutical challenges associ-
ated with drugs with low aqueous solubility—the potential impact of lip-
22. Orme IM. 2003. The mouse as a useful model of tuberculosis. Tubercu-
losis (Edinburgh) 83:112–115.
23. Sastry SV, Nyshadham JR, Joseph AF. 2000. Recent technological ad-
24. Strickley RG. 2004. Solubilizing excipients in oral and injectable formu-
25. Topolev VV, Krishtalik LI. 1999. Adsorption of dimethylsulphoxide on
26. van Boxtel RM, Lambrecht RS, Collins MT. 1990. Effect of polyoxyeth-
ylene sorbate compounds (Tw eens) on colonial morphology, growth, and
ultrastructure of Mycobacterium paratuberculosis. APMIS 98:901–908.
27. Wood DC, Wood J. 1975. Pharmacologic and biochemical consider-
28. World health organization. 2012. Guidelines for the programmatic man-
agement of drug resistant tuberculosis, 2011 update. World Health Orga-
nization, Geneva, Switzerland.
29. Youmans AS, Youmans GP. 1954. Studies on the metabolism of Myco-
bacterium tuberculosis. The effect of fatty acids on the growth of M. tu-
30. Yu ZW, Quinn PJ. 1998. The modulation of membrane structure and
ing the development of tuberculosis therapy. Nat. Rev. Drug Discov. 11:
171–172.
ERRATUM

Influence of Vehicles Used for Oral Dosing of Test Molecules on the Progression of *Mycobacterium tuberculosis* Infection in Mice

Shubhra Singh, a,b Richa Dwivedi, a,c Vinita Chaturvedi a

Drug Target Discovery and Development Division, Central Drug Research Institute, Lucknow, India a; IFTM University, Moradabad, India b; Academy of Scientific & Innovative Research (AcSIR), New Delhi, India c


Page 6027: The Acknowledgments section should include the following statement, “Shubhra Singh and Richa Dwivedi are senior research fellows of ICMR New Delhi and would like to thank IFTM University, Moradabad, and AcSIR, New Delhi, respectively, for registering them for the Ph.D. degree.”