Death and Intracellular Degradation of *Mycobacterium leprae* after Exposure *in vitro* to Enzymic Free-Radical Generators

J. DELVILLE and P. J. JACQUES

School of Public Health and Institute of Cellular Pathology, Université Catholique de Louvain, Brussels, Belgium-1200

An increase in the resistance to sulphones and the prohibitive cost of rifampicin necessitate the development of new chemotherapeutic agents against leprosy. The O₂-dependent myeloperoxidase-mediated bactericidal system of Klebanoff (1975) and analogous free-radical and/or singlet-O₂ generators are suitable as first-choice candidates for this purpose. Such generators proved highly and readily effective *in vitro* against a variety of infectious agents (Klebanoff, 1975) including, among intralysosomal parasites, the three mycobacteria causing tuberculosis in man (Demoulin-Brahy *et al.*, 1975) and *Mycobacterium lepraemurium* (R. J. W. Rees & P. J. Jacques, unpublished work; Avila *et al.*, 1976).

The approach and techniques were essentially those previously described (Delville *et al.*, 1976). Fresh suspensions of *M. leprae* were exposed *in vitro* at pH 5 and 36°C in aerobic conditions to a mixture of fungal glucose oxidase, glucose, plant peroxidase and iodide or to a similar mixture lacking peroxidase and iodide, for either a few seconds or 1 h. The controls were incubated for 1 h in the same conditions, except that iodide and both enzymes were omitted. After washing the microbial preparation in the cold and resuspension, hind foot-pads of normal mice were inoculated with this resuspended preparation. At various times, ranging from 180 to 424 days after inoculation, the foot-pads were collected and processed for enumeration of total acid-fast bacteria and for differentiation between granular and solid-stained bacteria (Shepard, 1960; Rees & Valentine, 1962).

As shown in Table 1, contact *in vitro* of *M. leprae* with the complete oxidase mixture for only a few seconds resulted in a considerable decrease of the number of solid-stained (live) and granular (dead) mycobacteria that could be observed in mouse foot-pads up to 14 months after the treated microbial suspension had been transferred to that growth system *in vivo*. Since not a single acid-fast bacterium could be detected when treatment with the complete oxidase mixture had been applied for 1 h, we conclude that a true bactericidal action of the oxidative 'cocktail' has been observed. In addition, tissue concentration of the dead mycobacterium was considerably decreased

<table>
<thead>
<tr>
<th>Type of staining</th>
<th>Granular (dead mycobacteria)</th>
<th>Solid (live mycobacteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95.7 ± 1.8</td>
<td>4.3 ± 1.8</td>
</tr>
<tr>
<td>Short-exposure-time</td>
<td>62.3 ± 20.2</td>
<td>2.7 ± 1.6</td>
</tr>
<tr>
<td>Long-exposure-time</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Control—short-exposure-</td>
<td>33.2 ± 20.7</td>
<td>1.6 ± 1.2</td>
</tr>
<tr>
<td>time group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Effect of exposure *in vitro* of *M. leprae* to complete oxidase mixture on the number of acid-fast bacteria and viable counts

Tabulated values are means ± S.D. for 12 individual results (ten for group with exposure for a few seconds only). The latter are mycobacterial counts expressed as percentage of total acid-fast bacteria in the corresponding control. The probability (P) was determined by Student's *t* test applied to the population of individual differences.
in the case of treated compared with control preparations, presumably through an acceleration of intralysosomal biodestruction of acid-fast bacteria.

Exposure for 1h to peroxidase- and iodide-depleted ‘cocktail’ produced the same effect as exposure for a few seconds to the complete oxidase ‘cocktail’. When the concentration of glucose oxidase was increased 100-fold in that depleted ‘cocktail’, results similar to those after exposure for 1h to the complete glucose oxidase mixture were obtained.

Finally, heat-killing of M. leprae (60 min at 60°C) led to a much slower decrease in vivo of acid-fast bacteria than death caused by exposure to complete or ‘depleted’ oxidase mixture as described above.

In conclusion, these O₂-dependent enzymic ‘cocktails’ are of interest for the chemotheraphy of leprosy, if their bactericidal and biodegradation-adjuvant actions can be induced to take effect in vivo within the intracellular vacuoles that are parasitized by M. leprae. That this hope is realistic is supported by positive results obtained after local administration to infected mice of an adapted ‘complete’ oxidase mixture when constituents of the mixture were in the free soluble form (Delville & Jacques, 1977) or when the two enzymes had been sequestered within phagocytosable liposomal microcapsules (J. Delville, P. J. Jacques & G. Gregoriadis, unpublished work).

The authors gratefully acknowledge financial support from Fonds de la Recherche Scientifique Médicale (Contract 3.4580.75) and Amis du Père Damien.


---

**Effect of Treatment in vivo with Triton WR-1339 and Macrocyclon on Infection of the Mouse Foot-Pad by *Mycobacterium leprae***

J. DELVILLE and P. J. JACQUES

*School of Public Health and Institute of Cellular Pathology, Université Catholique de Louvain, Brussels, Belgium-1200*

Among the various approaches of applied lysosomology to the therapy of parasitic diseases (Jacques et al., 1976), the use of lysosomotropic detergents such as Triton WR-1339 and Macrocyclon deserves special consideration. Although lacking direct antimicrobial activity at usual concentrations, these substances were found to be therapeutic in several parasitic diseases, e.g. experimental tuberculosis (Cornforth et al., 1955; Hart & Rees, 1955), mouse leprosy (Rees, 1957) and cancer (Franchi et al., 1971; Mantovani et al., 1977). Their host-mediated mode of action, whether it involves host lysosomes (Hart et al., 1969; Jacques & Demoulin-Brahy, 1974) or not, suggests that their therapeutic activity might be additive to that of directly acting antibiotics. Indeed, this ‘additive therapy’ has already been established in the case of experimental tuberculosis (Solotorovsky & Gregory, 1952).

These studies and the urgent need for new and more adequate therapeutic procedures against human leprosy prompted us to test the effect of Triton WR-1339 and of Macrocyclon on the experimental disease that follows the injection of human leprosy