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DDS Sensitivity of Mycobacteria

Antagonistic Effect of PABA for *M. ulcerans* and *M. kansasii*[1]

S. R. Pattyn and J. van Ermengem[2]

In the words of Shepard the great sensitivity of *Mycobacterium leprae* to 4-4′-diaminodiphenyl sulfone (DDS) was at first surprising ([13]). The DDS sensitivity of some other mycobacteria was shown by Karlson ([3]) to be much higher. Since many new species of mycobacteria have been described since 1963, we thought it would be useful to test these and to determine the minimal inhibitory concentration (MIC) for the most sensitive ones.

At the same time we made some observations on the antagonistic effect of para-aminobenzoic acid (PABA) on DDS, using *M. ulcerans*, *M. kansasii*, and *M. gastri* as test organisms.

MATERIALS AND METHODS

All sensitivity tests were performed on Loewenstein-Jensen (L-J) medium, applying the proportion method ([2]). Most strains were screened at 1, 3 and 10 μgm. DDS/ml. For strains showing sensitivity at the 1 μgm./ml. level, the MIC was determined, using DDS at concentrations of 1, 0.3, 0.1, 0.03 and 0.01 μgm./ml..

DDS was dissolved in alcohol at 10 μgm./ml. Dilutions were then made, first in 1/20 alcohol. Further dilutions were made in distilled water and added to the L-J medium before coagulation.

PABA was diluted in distilled water and also added before coagulation. The mycobacterial strains were used from our own collection. We took care to choose those strains that either had originated from the American Type Culture Collection (ATCC) or the National Culture Type Collection (NCTC), or had been included in earlier studies ([5, 7, 8, 9, 10]). Some strains belonging to species formerly tested by Karlson ([3]) were also included to make comparison possible.

RESULTS

Sensitivity of mycobacterial species (Table 1). Since Karlson ([3]) tested an appreciable number of strains of *M. tuberculosis*, we included only a recently isolated one of this species. It was resistant to 10 μgm. DDS/ml.

M. ulcerans was inhibited by 1 μgm./ml. The MIC was 0.3 μgm./ml for two strains, and 0.1 μgm./ml. for one strain.

Most strains of *M. kansasii* were rather sensitive to DDS. Four out of 17 were resistant to 3 μgm. DDS/ml. or more. For the others the MIC varied between 1 and 0.3. For one strain it was 0.1 μgm./ml.

Most scotochromogens, of which we described five provisional subgroups ([10]) (some of them corresponding with known species, e.g., *M. marianum* and *M. flavescens*), were inhibited by 3 μgm. DDS/ml.

Strains of *M. xenopei* tested were resistant to 3 and 10 μgm. DDS/ml.

Four strains of *M. avium*, the reference strain of *M. intracellulare*, and several strains of *M. terrae* ([15]), were resistant to 3 μgm. DDS/ml.

Strains of *M. gastri* were as sensitive to DDS as *M. kansasii*, the MIC being 0.3 and 0.1 μgm. DDS/ml. The rapidly growing strains were resistant to 3 μgm. DDS/ml.

Anatagonistic effect of PABA in the case of *M. ulcerans*. L-J media were prepared containing 3, 10 and 30 μgm. DDS/ml. respectively. To these batches, PABA was added at 10^{-5} M and two-fold dilutions of this from 1:2 to 1:4,096. These were tubed and coagulated and resistance tests were performed by the proportion method, with the two strains of *M. ulcerans* for which the

[1] Received for publication 15 May 1968.
[2] S. R. Pattyn, M.D., Professor of Bacteriology, and J. van Ermengem, Lic. Biol. Sci., Assistant in the Laboratory of Bacteriology, Prince Leopold Institute for Tropical Medicine, 155 Nationalestraat, Antwerp, Belgium.

TABLE 1. *Sensitivity of mycobacterial species to DDS.*

Species	Concentration of DDS (μgm./ml.)								
	10	3	1	Lower					
M. tuberculosis	R[a]	R							
M. ulcerans									
No. 206 & 456		S[b]							
273		S	S	R	0.3				
340		S	S	R	0.3				
454		S	S	S	0.3		R	0.1	
M. kansasii									
No. 244	S	S	S	S	0.3		R	0.1	
359	S	S	S	S	0.3		R	0.1	
433	S	S	S	R	0.3				
765	S	S	S	S	0.3		R	0.1	
811	R	R							
844		R	R						
862	S	S	S	S	0.3		R	0.1	
897	R	R							
904	S	S	S	R	0.3				
905	S	R							
906	S	S	S	R	0.3				
907	S	S	S	R	0.3				
911	S	S	S	S	0.3		R	0.1	
912	S	S	S	10% R	0.3;	50% R	0.1;	R	0.03
913	S	S	S	S	0.3		R	0.1	
914	S	S	S	S	0.3		R	0.1	
915	S	S	S	S	0.3		R	0.1	
Scotochromogens									
Subgroup 1 (tap water strains)									
2 strains	S	S	R						
Subgroup 2									
22	S	S	S	R	0.3				
31	S	S	R						
86	S	S	R						
Subgroup 3 (tap water strains)									
40	S	S	R						
387	S	S							
691	S	S	R						
Subgroup 4 (*M. marianum*)									
251	S	S	R						
692	S	R							
887	S	S	R						
Subgroup 5 (*M. flavescens*)									
331	S	S	15% R						
362	S	S	R						
395	S	S	R						
414	S	S	R						
415	R	R							
430	S	S	R						
M. xenopei									
Nos. 421, 848, 928	R	R							
M. avium									
4 strains		R							

TABLE 1. *Continued.*

Species	Concentration of DDS (μgm./ml.)			
	10	3	1	Lower
M. intracellulare				
699		R		
M. terrae				
6 strains	R	R		
M. gastri				
721	S	S	S	S 0.3 R 0.1
722	S	S	S	S 0.1 R 0.03
723	S	S	S	S 0.3 R 0.1
724	S	S	S	S 0.3 R 0.1
725	S	S	S	S 0.3; 10% R 0.1; R 0.03
M. fortuitum				
3 strains		R		
M. smegmatis				
5		R	R	
226		2%R	2%R	R 0.3
M. phlei				
158		R	R	
M. balnei				
3 strains		R		

[a] R = resistant.
[b] S = sensitive.

MIC was known (Nos. 340 and 454). Tubes showing 50 per cent of the number of colonies, as compared with the control tubes without drugs, were taken as end points. Results are shown in Table 2.

Calculation of the inhibition index (molar concentration of DDS: molar concentration of PABA) gives a value of 500-2,000. The surfaces of the control tubes containing 3 μgm. DDS/ml. were scraped off at the end of the experiments and subinoculated on fresh L-J media. Normal growth ensued, showing no bactericidal effect of DDS. DDS at 3 μgm./ml. was not antagonized by the addition of glutamic acid at 73.5 μgm./ml., nor by glutamine at 29.2 μgm./ml., or folic acid at 100 μgm./ml.

Antagonistic effect of PABA in the case of *M. kansasii* and *M. gastri*. The same experiments as for *M. ulcerans* were performed with one strain each of *M. kansasii* (No. 914) and *M. gastri* (No. 722) (Table 3). Calculation of the antibacterial index gave a figure of approximately 1-3 for this particular strain of *M. kansasii* and of 0.5-0.1 for *M. gastri*.

DISCUSSION

Although some mycobacterial species, especially *M. ulcerans*, and many strains of *M. kansasii* and *M. gastri* are rather sensitive to the action of DDS, the MIC in most cases was found to be 0.3 μgm./ml. and in a few cases 0.1 μgm./ml., a concentration which is still ten times higher than the MIC in mice for *M. leprae* from untreated patients. ([11, 13]). It may be concluded therefore that *M. leprae* is indeed exceptionally more sensitive to DDS than most other mycobacteria. Th action of DDS at 3 μgm./ml. on *M. ulcerans* is bacteriostatic. This is in accord with earlier results from this laboratory on the action of DDS in experimentally infected mice ([6]). The mode of action of DDS is generally supposed to be, at least in part, competition with PABA during the synthesis of folic

TABLE 2. *End points of antagonistic effect of PABA on DDS inhibition of* M. ulcerans.

Concentration of DDS (μgm./ml.)	Strain No.	Dilutions of 10^{-5} M PABA where antagonistic effect stops	
		Experiment 1	Experiment 2
3	340	1:256	1:512
	454	1:512	1:256
10	340	1:128	1:256
	454	1:256	1:128
30	340	1:32	1:64
	454	1:32	1:64

TABLE 3. *End points of antagonistic effects of PABA on DDS inhibition of* M. kansasii *and* M. gastri.

Concentration of DDS (μgm./mo.)	Strain No.	Dilutions at 10^{-4} PABA where antagonistic effect stops
3	*M. kansasii* (914)	1:16
	M. gastri (722)	1:2
10	*M. kansasii* (914)	1:8
	M. gastri (722)	not at 10^{-4} M
30	*M. kansasii* (914)	1:2
	M. gastri (722)	not at 10^{-4} M

acid. The antibacterial index found for *M. ulcerans* is comparable with the values found by McIlwain (4) and Rubbo and Gillespie (12) with other microorganisms for sulfonamide and PABA, where relatively small amounts of PABA are able to counteract the inhibition of sulfonamides. However, in the case of *M. kansasii* and *M. gastri,* the amount of PABA necessary to inhibit the action of DDS is substantially higher. This is comparable with the situation for *M. leprae* in mice ((13) and our own unpublished results), where PABA is also relatively ineffective in reversing the activity of sulfone. Whether this is due to difficulty of PABA in penetrating some mycobacterial cells, as was suggested by Brown (1), or to some other mechanism, remains to be investigated.

Another point illustrated by these observations is the relationship between *M. kansasii* and *M. gastri,* which have many other characteristics in common (Schröder, personal communication, and our own unpublished observations). Finally these results also show that DDS treatment of human infections with *M. kansasii* might be considered if the infecting strain is sufficiently sensitive to this drug.

SUMMARY

An extensive series of mycobacterial species was tested for DDS sensitivity. *M. ulcerans, M. kansasii* and *M. gastri* were among the most sensitive organisms, the minimal inhibitory concentration (MIC) being as low as 0.3 to 0.1 μgm. DDS/ml. for some strains. This is still ten times as high as the MIC of DDS for *M. leprae in vivo.* DDS at 3 μgm./ml. was bacteriostatic and not bactericidal for *M. ulcerans.* The inhibition index was 500-2,000 in the case of *M. ulcerans,* but only 1-3 for *M. kansasii* and 0.1-0.5 for *M. gastri.*

RESUMEN

Se probó la sensibilidad al DDS en una extensa serie de especies de micobacterias. *M. ulcerans, M. kansasii* y *M. gastri* fueron los organismos mas sensibles, y siendo la concentración mínima de inhibición (MIC) tan baja como 0.3 a 0.1 μgm. DDS/ml. para unas cepas. Esto es todavía diez veces tan alto como el MIC de DDS para el *M. leprae in vivo*. DDS a dosis de 3 μgm./ml. fué bacteriostático y no bactericida para el *M. ulcerans*. El índice de inhibición fué de 500-2,000 en el caso del *M. ulcerans*, pero solo de 1-3 en el *M. kansasii* y de 0.1-0.5 para *M. gastri*.

RÉSUMÉ

Une large gamme d'espèces mycobactériennes a été étudiée quant à leur sensibilité pour la DDS. *M. ulcerans, M. kansasii,* et *M. gastri* se sont révélés être parmi les organismes les plus sensibles; la concentration minimale entraînant leur inhibition (MIC) était, pour certaines souches, aussi faible que 0.3 à 0.1 μgm. de DDS par ml. Une telle concentration est encore dix fois plus élevée que la MIC de la DDS pour *M. leprae in vivo*. La DDS à la concentration de 3 μgm. par ml. était bactériostatique, mais non bactéricide, pour *M. ulcerans*. L'indice d'inhibition a été de 500 à 2,000 dans le cas de *M. ulcerans*, mais seulement de 1 à 3 pour *M. kansasii*, et de 0.1 à 0.5 pour *M. gastri*.

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