

SENSITIVITY *IN VITRO* OF LEPTOSPIRA TO OXAMICETIN

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Oxamicetin was tested against 12 strains of parasitic leptospira each representing a different serotype and serogroup, and 8 saprophytic strains in liquid media. The results showed general susceptibility of parasitic leptospira being MIC's from 0.1 to 0.5 $\mu\text{g/ml}$. The MIC's against saprophytic strains were 10~40 $\mu\text{g/ml}$.

In the course of search for new antibiotics KONISHI *et al.*¹⁾ extracted from the fermentation broth of *Arthrobacter oxamicetus* a substance active against a variety of microorganisms including Gram-positive, Gram-negative and acid-fast bacteria. The structure of this antibiotic was found to be closely related to ampicillin²⁾ with an additional hydroxylic group in the molecule, and therefore the name oxamicetin was given. The antibacterial spectrum was similar to ampicillin, though somewhat more active against Gram-negative bacteria.

Our aim is to test the *in vitro* sensitivity of leptospira to satisfy the requirement, suggested

Table 1. Inhibition of growth of strains by different concentrations of oxamicetin after 5* and 10* days of incubation at 30°C. †=growth similar to the control (without oxamicetin)
+=numbers of leptospira lower than the control, ±=scarce growth, -=absence of leptospira

Strains	Oxamicetin concentrations ($\mu\text{g/ml}$)									
	0.1	0.25	0.5	1	2.5	5	10	20	25	
Ictero N° 1	* ** † †	† +	+ ±	—	—	—	—	—	—	
Akiyami	† †	† —	—	—	—	—	—	—	—	
Alarik	± ±	—	—	—	—	—	—	—	—	
Ballico	† †	† †	† †	+ ±	—	—	—	—	—	
Veldrat Bat. 146	† +	± +	± +	—	—	—	—	—	—	
Mus 127	± ±	± —	—	—	—	—	—	—	—	
Salinem	+ +	+ +	+ —	—	—	—	—	—	—	
Pomona	± ±	—	—	—	—	—	—	—	—	
Moskva	± ±	—	—	—	—	—	—	—	—	
Hebd. H Pasteur	† +	+ +	+ ±	—	—	—	—	—	—	
Van Tienen	+ +	± —	± —	—	—	—	—	—	—	
Riccio 66	+ +	± —	± —	—	—	—	—	—	—	
Patoc	† †	† †	† †	† ±	± —	—	—	—	—	
S. Giusto	† †	† †	† †	† †	† †	† †	† +	+ +	—	
Basovizza	† †	† †	† †	† †	† †	† †	+ +	+ +	± ±	
CH 11	† †	† †	† †	† †	† †	† †	± ±	—	—	
Bovedo	† †	† †	† †	† †	† †	† †	† †	± ±	—	
Farneti	† †	† †	† †	† †	† †	† †	† †	± ±	—	
RPE	† †	† †	† †	† †	† †	† †	† †	± ±	—	
Muggia	† †	† †	† †	† †	† †	† †	+ +	± ±	± —	

Table 2. Growth of leptospira strains in antibiotic-free KORTHOFF medium. Inocula were taken from cultures after 5* and 10** days of incubation in presence of oxamicetin at the indicated concentrations

Symbols as in Table 1

Strains	Oxamicetin concentrations ($\mu\text{g/ml}$) Incubation at 30°C												
	0.1	0.25	0.5	1	2.5	5	10	20	40				
Ictero N° 1	* ** + +	+ +	+ -	-	/	/	/	/	/				
Akiyami	††	† -	-	/	/	/	/	/	/				
Alarik	± -	- -	/	/	/	/	/	/	/				
Ballico	††	††	††	+ ±	-	/	/	/	/				
Veldrat Bat. 146	††	††	+ +	-	/	/	/	/	/				
Mus 127	- -	-	/	/	/	/	/	/	/				
Salinem	††	††	+ +	- -	/	/	/	/	/				
Pomona	- -	/	/	/	/	/	/	/	/				
Moskva V	± -	-	/	/	/	/	/	/	/				
Hebd. H Pasteur	††	††	† +	- -	/	/	/	/	/				
Van Tienen	††	††	† +	- -	/	/	/	/	/				
Riccio 66	† +	† +	- -	/	/	/	/	/	/				
Patoc	††	††	††	††	† +	± -	/	/	/				
S. Giusto	††	††	††	††	††	††	+ ±	- -	- /				
Basovizza	††	††	††	††	††	††	††	††	+ +	± ±	± -		
CH 11	††	††	††	††	††	† +	+ ±	- -	/	/			
Bovedo	††	††	††	††	††	††	††	††	††	- -	/		
Farneti	††	††	††	††	††	††	††	††	††	† +	- /		
RPE	††	††	††	††	††	††	††	††	††	††	+ +	- /	
Muggia	††	††	††	††	††	††	††	††	††	††	††	††	/

/=Not done

by the WHO Expert Group on leptospira, to test new antibiotics against leptospira for possible therapeutic use.

Materials and Methods

The following 12 strains of parasitic leptospira each representing a different serogroup, and 8 saprophytic strains were employed: Ictero N° 1, Alarik, Akiyami, Veldrat Bataviae 146, Ballico, Mus 127, Salinem, Pomona, Moskva V, Hebdomadis H. Pasteur, Van Tienen, Riccio 66 and Patoc 1, Basovizza, Bovedo, Andaman CH 11, Farneti, RPE, S. Giusto, Muggia (halophilic strain).

Cultures of 2×10^8 cells/ml were obtained in KORTHOFF-BABUDIERI medium with 5% rabbit serum³⁾ and in the sea-water KORTHOFF medium⁴⁾ for the halophilic strain Muggia, after 5~6 days of incubation at 30°C. Oxamicetin was kindly supplied by Dr. KAWAGUCHI of Bristol Banyu Research Institute, Tokyo. The antibiotic assay was performed in KORTHOFF liquid medium (5 ml for each tube) to which different concentrations of oxamicetin were added. Each tube of KORTHOFF medium, supplemented with oxamicetin, was inoculated with 0.2 ml of 5~6 day old cultures of leptospira and incubated at 30°C. A drop of the cultures was examined by dark-field microscopy on day 5 and 10 for the presence of leptospira. This gave an estimate of the bacteriostatic effect of oxamicetin on leptospira. To evaluate the bactericidal effect on day 5 and 10, 0.1 ml of the content of each tube was transferred to 5 ml

of antibiotic-free KORTHOFF medium and examined for growth of leptospira by dark-field microscopy after 7 days of incubation at 30°C.

Results

The results reported in Table 1 show that oxamicetin is very active on pathogenic strains of leptospira, if compared to the results obtained with other sensitive bacteria¹⁾. The minimal inhibitory concentrations ranged from 0.1 µg to 0.5 µg/ml. Different values were observed with the different strains used, strain Ballico being viable up to 1 µg/ml. These oxamicetin concentrations were not sufficient to inhibit saprophytic strains for which the minimal inhibitory dose ranged between 10 and 40 µg/ml. Only strain Patoc 1 did not grow in presence of 5 µg/ml of oxamicetin. The different degree of sensitivity of saprophytic and parasitic leptospira was also found for vancomycin but not for penicillin and other antibiotics in general.

The sensitivity to oxamicetin can be used as an additional test in the differentiation of parasitic and saprophytic leptospira. It is remarkable that strain Muggia and particularly Basovizza are less sensitive than the other saprophytic strains tested, being viable up to 20 and 40 µg/ml of oxamicetin respectively. However, as indicated in Table 1, growth at these concentrations was slow. The resistance of Basovizza to other antibiotics is already known.

Results reported in Table 2 should suggest that oxamicetin is bactericidal. In fact growth of strains inoculated in antibiotic-free KORTHOFF medium after 5~10 days of contact with oxamicetin occurred only below the original MIC.

Conclusions

We can conclude that oxamicetin is active *in vitro* on pathogenic strains of leptospira. On the contrary saprophytic strains are resistant.

References

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