In Vitro Activity of Nonsteroidal Anti-Inflammatory Agents, Phenotiazines, and Antidepressants against *Brucella* Species

S. Muñoz-Criado, J.L. Muñoz-Bellido, J.A. García-Rodríguez*

The in vitro antimicrobial activity of streptomycin, rifampicin, tetracycline, seven nonsteroidal anti-inflammatory agents (acetyl-salicylic acid, piroxicam, indomethacin, ibuprofen, ketoprofen, sulindac, and diclofenac), and eight phenotiazine derivatives and antidepressant agents (clorpromazine, fluphenazine, amitryptiline, clomipramine, imipramine, maprotiline, sertraline, and diazepam) against 62 strains of *Brucella* spp. was tested. Diclofenac was the most active of the anti-inflammatory agents (MIC90 = 16 μg/ml). The activity of the phenotiazines and antidepressants was heterogeneous, with MIC90s ranging from 16 μg/ml for sertraline and 32 μg/ml for fluphenazine and clomipramine to > 512 μg/ml for diazepam. When the six most active anti-inflammatory agents and the six most active psychiatric drugs were tested at pH 5 and pH 4, the MICs remained unchanged except for those of fluphenazine; the MIC50 and MIC90 of this agent increased by one dilution.

Brucellosis is a health problem in certain regions, such as Mediterranean countries (1). Joint involvement as a complication of brucellosis has been found in 30% of patients in one study (2) and in 20 to 60% in another (3) and leads mainly to sacroiliitis (4, 5). Since nonsteroidal anti-inflammatory drugs (NSAIDs) are used frequently in these cases, we were prompted to study the antimicrobial activity of seven widely used NSAIDs against *Brucella* spp. In addition, some psychiatric drugs, such as clorpromazine and derivatives and some monoamine reuptake inhibitors, have been shown to be active against some microorganisms (6, 7). We therefore included two neuroleptics (clorpromazine and fluphenazine), four tricyclic and heterocyclic antidepressants (imipramine, clomipramine, amitryptiline, and maprotiline), an anxiolytic (diazepam), and a selective serotonin reuptake inhibitor (sertraline) in our study to investigate the possible activity of these agents against *Brucella* spp.

Materials and Methods. We compared the in vitro activity of seven NSAIDs (acetyl-salicylic acid, piroxicam, indomethacin, ibuprofen, ketoprofen, sulindac, and diclofenac), and eight psychiatric drugs (clorpromazine, fluphenazine, amitryptiline, clomipramine, imipramine, maprotiline, sertraline, and diazepam) with that of streptomycin, tetracycline, and rifampicin against 62 strains of *Brucella* spp. The substances used were kindly provided by their respective manufacturers (acetyl-salicylic acid, Bayer, Germany; piroxicam, Pfizer, USA; indomethacin, Merck, Sharp & Dohme, USA; ibuprofen, Boots, USA; ketoprofen, Rhône Poulenc, France; sulindac, Merck, Sharp & Dohme; diclofenac, Geigy; Switzerland; clorpromazine, Rhône Poulenc; fluphenazine, Schering Plough, USA; amitryptiline, Merck, Sharp & Dohme; clomipramine, Geigy; imipramine, Geigy; maprotiline, Ciba, Switzerland; sertraline, Pfizer; diazepam, Roche, Switzerland).

The 62 strains of *Brucella* spp. included 19 type strains (*Brucella melitensis* ATCC 23456, 23457, 23458; *Brucella abortus* ATCC 23448, 23449, 23450, 23451, 23452, 23453, 23455, NCTC 8038, 11363; *Brucella suis* ATCC 23444, 23445, 23446, 23447; *Brucella neotomae* ATCC 23459; *Brucella ovis* ATCC 25840; and *Brucella canis* ATCC 23365) and 43 clinical isolates of *Brucella melitensis* (biotype undetermined), all obtained from blood cultures of patients with acute brucellosis. The strains were stored in skim milk at −70°C and subcultured twice before the start of the study.

The in vitro activity of the substances tested was determined by the agar dilution method using methods described previously (8, 9). Antibiotics were tested at dilutions ranging from 0.008 μg/ml to 128 μg/ml. Nonantibiotic drugs were tested at dilutions ranging from 0.008 μg/ml to 512 μg/ml.

Results and Discussion. Results obtained are shown in Table 1. Most anti-inflammatory agents showed very low activity against *Brucella* spp., with MIC50s of ≥ 128 μg/ml and MIC90s of ≥ 256 μg/ml. Only indomethacin and, in particular, diclofenac showed higher activity. To evaluate the

Department of Microbiology, Hospital Clínico Universitario, Paseo de San Vicente 108, 37007 Salamanca, Spain.

Table 1: In vitro activity of various drugs tested against *Brucella* spp.

Drugs	MIC (μg/ml) 50	90	Range
Antibiotics			
Streptomycin	2	4	0.2–4
Tetracycline	0.2	0.2	0.06–0.2
Rifampin	0.2	0.5	0.1–2
Anti-inflammatory agents			
Acetyl-salicylic acid	256	512	64–512
Indomethacin	64	256	64–512
Piroxicam	>512	>512	256–>512
Sulindac	256	>512	128–>512
Diclofenac	16	16	8–16
Ibuprofen	256	256	64–256
Ketoprofen	128	512	128–512
Antidepressants			
Fluphenazine	32	32	32–64
Clorpromacin	256	512	128–>512
Imipramine	>512	>512	512–>512
Clomipramine	32	32	32–64
Amitryptiline	128	128	128–256
Maprotiline	128	128	64–128
Sertraline	16	16	8–16
Diazepam	>512	>512	>512

therapeutic importance of these results, pharmacokinetic parameters for these drugs must be taken into account. The Cmax (the highest serum concentration reached at therapeutic dosages) for all NSAIDs tested is at least tenfold lower than the respective MIC50 and MIC90 values for *Brucella* spp. The Cmax values for the most active NSAIDs, indomethacin (5 μg/ml) (10) and diclofenac (1–2 μg/ml) (11), are approximately tenfold lower than the MIC50 of these drugs for *Brucella*, and the Cmax for NSAIDs that reach higher plasma levels, such as ibuprofen (30 μg/ml) (12), is also approximately tenfold lower than the MIC50 (256 μg/ml). Therefore, since NSAIDs reach synovial concentrations similar to plasma levels (13), synovial concentrations in normal joints would be lower than MICs, as plasma levels are.

Nevertheless, several factors may influence the possible role of NSAIDs in the evolution of brucellosis in vivo. NSAIDs show a very high serum protein binding (13). Since synovial and joint protein concentrations increase significantly during inflammation (14), NSAID joint concentration should also increase up to concentrations near the MIC.

On the other hand, NSAIDs might have subinhibitory activity at therapeutic plasma levels, as has been shown for other non-antibiotic drugs (15). If this were the case, the evolution of the disease might be modified or the activity of antibiotics might be enhanced in vivo. We are now investigating these possibilities.

Psychiatric drugs have been previously reported as being active against some microorganisms. Tricyclic and heterocyclic antidepressants, neuroleptics, and monoamine reuptake inhibitors are schizonticidal by themselves (15), enhance the activity of chloroquine against both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* (16), and are capable of reversing chloroquine resistance in this microorganism (17). This is probably because they inhibit an efflux pump structurally similar to the cellular structures on which they act in the brain (18). Moreover, clorpromazine and derivatives have some activity against aerobic and anaerobic bacteria (19).

Among the psychiatric drugs tested, the phenotiazine derivative fluphenazine, the tricyclic antidepressant clomipramine, and particularly the selective serotonin reuptake inhibitor sertraline had the highest activities. The pharmacokinetic profile of these psychiatric drugs is a principal factor in evaluating the possibility of clinical usefulness, as has been commented previously for NSAIDs. Psychiatric drugs reach plasma levels ranging between 20 and 175 μg/l for clomipramine (20), around 100 μg/l for fluphenazine (21), and 20 to 55 μg/l for sertraline (22), lower than the MICs for all the strains. Nevertheless, sertraline reaches very high concentrations in cerebrospinal fluid and in the brain, more than 40-fold higher than plasma levels (22). This pharmacokinetic characteristic led to cerebrospinal fluid concentrations near the MIC and encourages new studies on the usefulness of sertraline and derivatives against the infrequent but severe neurological complications of brucellosis.

The treatment of brucellosis is decisively affected by its characteristic of being an intracellular infection. *Brucella* remains viable inside the phagosome, where the pH can be decisive for the activity of drugs. Some drugs, such as fluorinated quinolones, were shown to be very active in vitro against *Brucella* under standard conditions, but their activity became seriously impaired by pH modifications (23). This reduction in activity correlates with a high incidence of clinical relapses (24). The NSAIDs and most psychiatric drugs, excluding fluphenazine, exhibit, according to the present study, the same inhibitory activity at standard conditions (pH 7), at pH 5, and at pH 4, a factor which does not seem to impair the activity of these drugs.

Obviously, more studies on the activity of these drugs against *Brucella* (bactericidal activity, subinhibitory activity, and, eventually, animal testing) are required to assess the therapeutic properties of these drugs or their derivatives. The results reported here should encourage these studies.

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Prevention of Suicide Phenomenon in Aeromonads

M.S. Guimarães[1], M.S. Neves[2], M.P. Nunes[1]*

Clinical isolates of *Aeromonas* (13 *Aeromonas caviae*), 8 *Aeromonas hydrophila*, 3 *Aeromonas* spp., and 2 *Aeromonas media* recovered from diarrheal feces of children were submitted to the suicide phenomenon test and investigated at intervals

[1] Instituto de Microbiologia, and [2]Departamento de Higiene Social e Análises Clínicas, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Centro de Ciências de Saúde, Rio de Janeiro, 21941-590, Brazil.