LETTER TO THE EDITOR

EFFECT OF BETAMETHASONE IN COMBINATION WITH ANTIBIOTICS ON GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Betamethasone is an anti-inflammatory steroid drug used in cases of anaphylactic and allergic reactions, of Alzheimer and Addison diseases and in soft tissue injuries. It modulates gene expression for anti-inflammatory activity suppressing the immune system response. This latter effect might decrease the effectiveness of immune system response against microbial infections. Corticosteroids, in fact, mask some symptoms of infection and during their use superimposed infections may occur. Thus, the use of glucocorticoids in patients with sepsis remains extremely controversial. In this study we analyzed the *in vitro* effect of a commercial formulation of betamethasone (Bentelan) on several Gram positive and Gram negative bacteria of clinical relevance. It was found to be an inhibitor of the growth of most of the strains examined. Also the effect of betamethasone in combination with some classes of antibiotics was evaluated. Antibiotic-steroid combination therapy is, in such cases, superior to antibiotic-alone treatment to impair bacterial growths. Such effect was essentially not at all observable on *Staphylococcus aureus* or Coagulase Negative *Staphylococci* (CoNS).

Bentelan is the commercial name of a drug the active ingredient of which is betamethasone sodium phosphate, an anti-inflammatory steroid belonging to the class of glucocorticoids (1, 2). It is used in cases of severe anaphylactic and allergic reactions, for the treatment of Alzheimer and Addison diseases, in soft tissue injuries such as lateral epicondylitis or peri-arthritis of the shoulder. The administration of betamethasone may be by oral, intravenous, intramuscular or subcutaneous route.

Betamethasone binds to plasma transcortin, then crosses the cytoplasmic membrane. Subsequently it binds a specific glycoprotein situated in the soluble fraction of the cytoplasm. The betamethasone-glycoprotein complex passes the nuclear membrane and binds to the glucocorticoid response elements (GRE) where it stimulates the transcription of DNA and modulates gene expression for anti-inflammatory activity. The anti-inflammatory effect of glucocorticoids depends, at least in part, on the induction of two regulatory proteins, lipocortin and vasocortin, both preventing the release of inflammatory mediators (2). Lipocortin (also known as annexin 1) inhibits phospholipase A2 (PLA2) and therefore reduces arachidonic acid metabolite formation, such as prostaglandins, prostacyclins

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and leukotrienes (3-6). Vasocortin inhibits histamine release from mast cells (7). The immune system response is suppressed by corticosteroids for the decrease in the function of the lymphatic system, a reduction in immunoglobulin and complement concentrations, the precipitation of lymphocytopenia, and interference with antigen-antibody binding (5). The drug also inhibits accumulation of macrophages and leukocytes at the site of inflammation, reducing their migration, and inhibits phagocytosis and lysosomal enzyme release (8). This latter effect might decrease the effectiveness of immune system response against microbial infections. Corticosteroids, in fact, mask some symptoms of infection and during their use superimposed infections may occur.

Despite 30 years of investigation and more than a dozen of meta-analyses, the use of glucocorticoids in patients with sepsis remains extremely controversial and recommendations are conflicting (9). In literature it has been frequently observed that during corticosteroid therapy the organism seems to have a reduced resistance to infection (10, 11). By contrast, it has been recently demonstrated that long-term cortisol levels are not associated with nasal carriage of *Staphylococcus aureus* (12). Nasal carriage of this pathogen is the major risk in the development of various *S. aureus* infection (12). Emgård and co-workers showed that the topical steroid betamethasone dipropionate treatment without antibiotics was effective for external otitis caused by infection with *Pseudomonas aeruginosa* and *Candida albicans* (13). These findings could be explained considering that inflammation is a major mechanism in the development of external otitis, irrespective of the presence of either bacteria or fungi.

In this study we analysed the *in vitro* effect of a commercial formulation of betamethasone sodium phosphate on several Gram positive and Gram negative bacteria of clinical relevance. In particular we used staphylococci, enterococci, streptococci, *Pseudomonas* and *Enterobacteriaceae*. The effect of betamethasone in combination with some classes of antibiotics was also evaluated.

MATERIALS AND METHODS

Bacterial strains and culture conditions

One hundred clinical and reference bacterial strains were collected and used for the experiments (40 Gram-negative and 60 Gram-positive strains). Fifteen strains belonging to the *Pseudomonadaceae* family and 25 strains belonging to the *Enterobacteriaceae* family were included for the study on Gram-negative strains. For the Gram-positive strains, 15 *S. aureus*, 15 Coagulase-negative staphylococci (CoNS), 15 *Enterococci* and 15 *Streptococci* strains were included. The strains were collected either from clinical isolates or from DSMZ and ATCC collections (Table I). Each strain was validated using microbiological and biochemical techniques such as: bacterial morphology, Gram staining, selective culture media, biochemical reactions (catalase, oxidase, coagulase assays), Api™-tests and by Vitek™ instrumentation.

Cultures were grown in Brain Heart Infusion broth (BHI, Oxoid, UK) at 37°C under vigorous agitation (180 rpm). Streptococci were grown without agitation in 5% CO_2 atmosphere at 37°C.

Mueller-Hinton Agar (MHA, Oxoid, UK) was used for MIC assays. For streptococci MHA was supplemented with 5% sheep blood. All strains were maintained at -80°C in cryovials with 15 % of glycerol.

Chemicals

The injectable formulation of Bentelan (Sigma Tau - Rome, Italy) used in this work contains 2 mg/ml of betamethasone, and was stored at 4°C.

Erythromycin (Sigma Tau - Rome, Italy), was dissolved in 100 % ethanol at a concentration of 3 mg/ml and stored at -20°C. Ceftazidime (Santa Cruz Biotechnology, Heidelberg, Germany) was dissolved in distilled water at a concentration of 4 mg/ml and stored at -20°C. Ofloxacin (Sigma Tau, Rome, Italy) was dissolved in distilled water at a concentration of 10 mg/ml and stored at -20°C.

Single dose betamethasone growth curves

Bacterial growth curves were performed both in the presence and in the absence of Bentelan at a concentration of 1 mg/ml betamethasone. Overnight cultures were diluted 1/100 in BHI and grown at 37°C over 24 h, collecting at least 6 time points (0, 2, 4, 6, 8 and 24 h).

Antibiotic susceptibility testing and betamethasone effect

Minimum Inhibitory Concentration (MIC) assay was conducted by the standard method as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2004). The MICs of betamethasone, ceftazidime (cephalosporin class), ofloxacin (quinolone class) and erythromycin (macrolide class) were determined by agar dilution-testing on MHA. The plates were incubated at 37°C and, under microaerophilic conditions (i.e., 5% CO_2) for streptococci.

The effect between betamethasone and above tested

Table I. *Bacterial strains used in this study.*

Denomination	Species	Origin	Denomination	Species	Origin
6538P	*S. aureus*	ATCC	RP62A	*S.epidermidis*	ATCC
20372	*S. aureus*	DSMZ	15917	*S.epidermidis*	DSMZ
25923	*S. aureus*	ATCC	12228	*S.epidermidis*	DSMZ
Sa1448	*S. aureus*	Clinical	20501	*S. carnosus*	DSMZ
Sa1511	*S. aureus*	Clinical	O-47	*S.epidermidis*	Clinical
Sa1544	*S. aureus*	Clinical	Se1515	*S.epidermidis*	Clinical
Sa33	*S. aureus*	Clinical	Se1518	*S.epidermidis*	Clinical
Sa34	*S. aureus*	Clinical	Se1519	*S.epidermidis*	Clinical
Sa35	*S. aureus*	Clinical	Se1521	*S.epidermidis*	Clinical
Sa43	*S. aureus*	Clinical	Se1523	*S.epidermidis*	Clinical
MRSA 165	*S. aureus*	Clinical	Se1524	*S.epidermidis*	Clinical
MRSA166	*S. aureus*	Clinical	Se1526	*S.epidermidis*	Clinical
MRSA 200	*S. aureus*	Clinical	Se1527	*S.epidermidis*	Clinical
MRSA 201	*S. aureus*	Clinical	SAC1	*S.epidermidis*	Clinical
USA300	*S. aureus*	Clinical	She1	*S.hemoliticus*	Clinical
10556	*S. sanguinis*	ATCC	19434	*E. faecium*	ATCC
25175	*S. mutans*	ATCC	19433	*E. faecalis*	ATCC
Strepto1	*S. viridans*	Clinical	Ent1	*E. faecium*	Clinical
Strepto2	*S. viridans*	Clinical	Ent2	*E. faecium*	Clinical
Strepto3	*S. viridans*	Clinical	Ent3	*E. faecium*	Clinical
Strepto4	*S. viridans*	Clinical	Ent4	*E. faecium*	Clinical
Strepto165	*S. viridans*	Clinical	Ent5	*E. faecalis*	Clinical
Strepto202	*S. viridans*	Clinical	Ent190	*E. faecalis*	Clinical
Strepto203	*S. viridans*	Clinical	Ent222	*E. faecalis*	Clinical
Strepto223	*S. viridans*	Clinical	Ent233	*E. faecalis*	Clinical
Strepto265	*S. viridans*	Clinical	Ent259	*E. faecalis*	Clinical
Strepto274	*S. viridans*	Clinical	Ent268	*E. faecalis*	Clinical
Strepto256	*S. viridans*	Clinical	Ent275	*E. faecalis*	Clinical
StreptoCA1	*S. viridans*	Clinical	Ent6015	*E. faecalis*	Clinical
StreptoA1	*S. agalactiae*	Clinical	EfAC1	*E. faecalis*	Clinical
1128	*P. aeruginosa*	DSMZ	K12	*E.coli*	DSMZ
PaO1	*P. aeruginosa*	DSMZ	BAA-1705	*K. pneumoniae*	ATCC
PS1333	*P. aeruginosa*	Clinical	E64	*E.coli*	Clinical
PS226	*P. aeruginosa*	Clinical	E65	*E.coli*	Clinical
PS235V	*P. aeruginosa*	Clinical	E66	*E.coli*	Clinical
PS235B	*P. aeruginosa*	Clinical	E67	*E.coli*	Clinical
PS260	*P. aeruginosa*	Clinical	E69	*E.coli*	Clinical
PS1620	*P. aeruginosa*	Clinical	E70	*E.coli*	Clinical
PS292	*P. aeruginosa*	Clinical	E73	*E.coli*	Clinical
PS301	*P. aeruginosa*	Clinical	E77	*E.coli*	Clinical
PS391	*P. aeruginosa*	Clinical	E78	*E.coli*	Clinical
PS392	*P. aeruginosa*	Clinical	ECA1	*E.coli*	Clinical
PS393	*P. aeruginosa*	Clinical	E239	*E.coli*	Clinical
PS394	*P. aeruginosa*	Clinical	E241	*E.coli*	Clinical
PS395	*P. aeruginosa*	Clinical	E1589	*E.coli*	Clinical
			E1604	*E.coli*	Clinical
			K189	*K. pneumoniae*	Clinical
			K190	*K. pneumoniae*	Clinical
			K241	*K. pneumoniae*	Clinical
			K6515	*K. pneumoniae*	Clinical

antibiotic was evaluated using two different concentrations of betamethasone (0.5 mg/ml and 0.125 mg/ml).

RESULTS

Analysis of betamethasone effect on growth behaviour

Betamethasone was generally an inhibitor of the growth of most of the strains examined. In particular, almost all the Gram-negative strains analyzed resulted susceptible to its action. A complete inhibition of *Enterobacteriaceae* growth was observed when betamethasone was added to the medium (Fig. 1A). Betamethasone showed a strong inhibitory effect on growth of almost all *Pseudomonas* strains (8 out of 10). Two strains, in fact, showed a diminished growth without affecting bacterial viability (Fig. 1B). Betamethasone showed a mild inhibition effect on the growth of about half of the *Enterococcus* strains analyzed. This effect was highlighted during the Lag phase of growth but the exponential phase was not influenced. In fact, bacteria reached the same optical density after 8 h (Fig. 1C).

With regard to Gram positive, betamethasone showed a clear inhibition of the growth of all *S. aureus* strains and an inhibitory effect on CONS, not species specific but strain -ependent (Fig. 1D-E). Furthermore, an inhibition effect was observed on the growth of almost all the *Streptococci* analyzed, although two strains did not show inhibition (Fig. 1F).

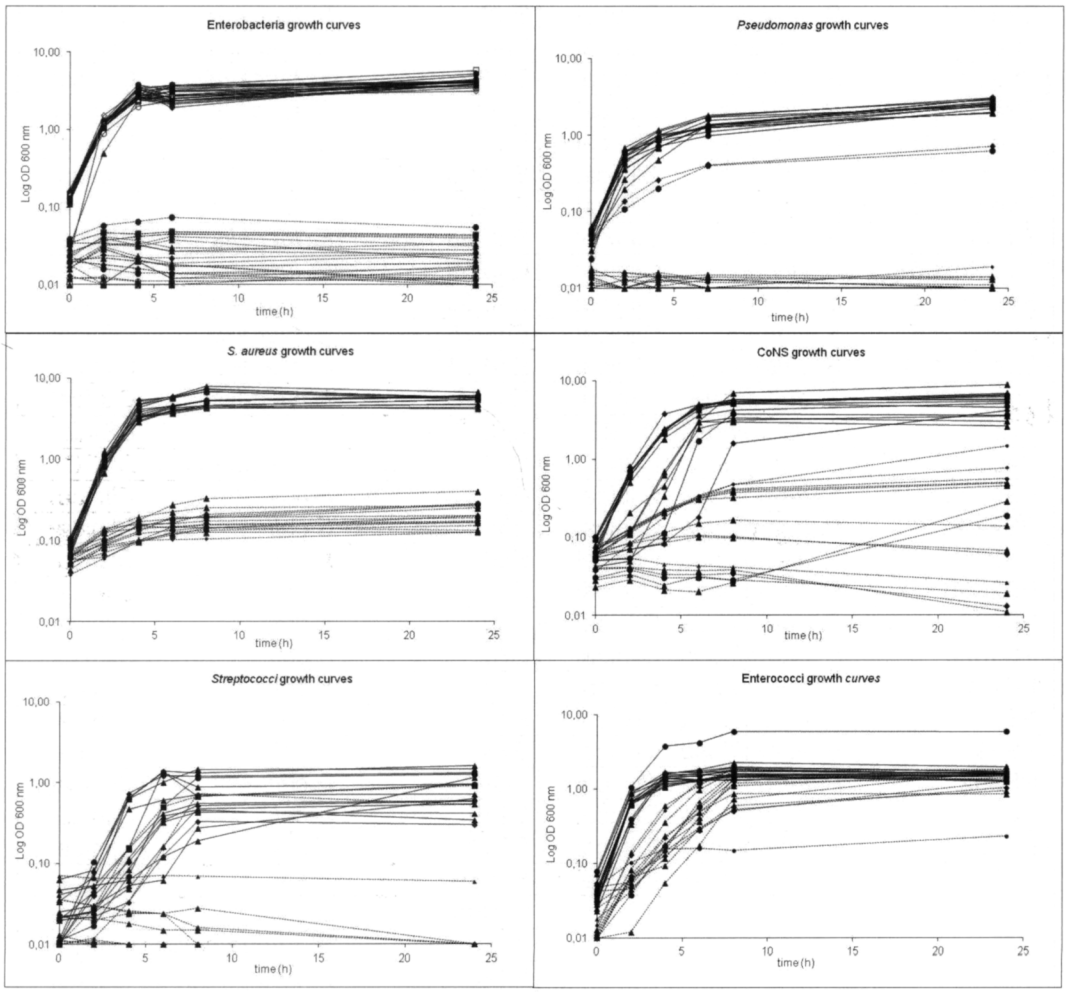

Fig. 1. *Growth curves of bacterial cultures. All bacterial cultures were grown in the absence (continued lines), and in the presence (dashed lines) of 1mg/ml betamethasone. Results are representative of three independent experiments.*

Betamethasone effect on the action of selected antibiotics against Gram positive and Gram negative

MIC assays were first performed using betamethasone. The range explored was from 1.0 mg/ml to 0.03 mg/ml starting from betamethasone injectable solution 2 mg/ml. These assays were performed on 30 selected strains belonging to the six genera previously described. Results are shown in Table II. For almost all *Enterobacteriaceae, S. aureus* and CoNS the MIC value was 0.5-1.0 mg/ml. While for all *Pseudomonas* strains MIC resulted between 0.25–0.5 mg/ml. *Enterococci* seemed to be resistant to betamethasone with MIC higher than 1 mg/ml for four out of five strains. By contrast, *Streptococci* seemed to have a behaviour not species-specific but strain-specific with MIC values between 0.25-0.5 mg/ml (one strain out of five), 0.5-1 mg/ml (two strains out of five) and > 1mg/ml (two strains out of five), respectively.

In order to have a reference scale for the experiments, MIC experiments on the selected antibiotics were performed. In Table II also MIC values obtained with the three different antibiotics on 30 selected strains of the bacterial species previously described are listed. Data obtained using the ceftazidime antibiotic showed that *Pseudomonas* and CoNS selected strains were clearly responsive, while among *Enterobacteriaceae* and *Streptococci* few strains did not show inhibition. On the contrary among *S. aureus* and *Enterococci* almost all the strains were resistant, with just a few susceptible strains.

Using erythromycin, all *Pseudomonas* and *Enterobacteriaceae* selected strains were clearly unsusceptible to this antibiotic. About half of the *Enterococci* and some CoNS also showed to be unsusceptible, while almost all *S. aureus*, and *Streptococci* were susceptible.

Using ofloxacin, only very few strains (among the *Streptococci*, *Pseudomonas* and *Enterobacteriaceae*) were unsusceptible to the highest dosage of 25.0 µg/ml of this antibiotic. All the remaining were susceptible.

Table III shows the variations of MIC values in

Table II. *Minimum inhibitory concentration (MIC) of three different classes of antibiotics on six different bacterial species.*

CEFTAZIDIME (µg/ml)	>50	50-25	25-12.5	12.5-6.2	6.2-3.1	3.1-1.6	1.6-0.8	0.8-0.4	<0.4	R	S	TOTAL
Enterobacteriaceae	2							1	12	≥ 32	≤8	15
Streptococci	2	1	1	1	2		1	2	5	≥ 4	≤1	15
Enterococci	15									UNSUSCEPT		15
Pseudomonas			1	4		7	1		2	≥ 32	≤8	15
S. aureus	7		2	5	1					≥ 32	≤8	15
CoNS			2	8	5					≥ 32	≤8	15
OFLOXACIN (µg/ml)	>12.5	12.5-6.2	6.2-3.1	3.1-1.6	1.6-0.8	0.8-0.4	<0.4					
Enterobacteriaceae	4						11			≥ 8	≤2	15
Streptococci	1		2	1	7	1	3			≥ 8	≤2	15
Enterococci	15									≥ 8	≤2	15
Pseudomonas	5	1	1		5	1	2			≥ 8	≤2	15
S. aureus		5			3	5	2			≥ 4	≤1	15
CoNS					2	10	3			≥ 4	≤1	15
ERYTHROMYCIN (µg/ml)	>12.5	12.5-6.2	6.2-3.1	3.1-1.6	1.6-0.8	0.8-0.4	0.4-0.2	0.2-0.1	<0.1			
Enterobacteriaceae	15									UNSUSCEPT		15
Streptococci	2	1					2		10	≥ 1	≤0.25	15
Enterococci		2	1			1				≥ 1	≤0.5	15
Pseudomonas	15									UNSUSCEPT		15
S. aureus	4		3		8					≥ 8	≤0.5	15
CoNS	3		1		1	10				≥ 8	≤0.5	15
BETAMETHASONE (mg/ml)	>1.0	1.0-0.5	0.5-0.25									
Enterobacteriaceae		5										5
Streptococci	2	2	1									5
Enterococci	4	1										5
Pseudomonas			5									5
S. aureus		5										5
CoNS		5										5

Unsuscept: Unsusceptible

Table III. *Effect of betamethasone on MIC assays.*

Enterococcus	**Ceft**	**Ceft Betam1/4**	**Eryth**	**Eryth Betam1/4**	**Ofl**	**Ofl Betam1/4**
Ent3	25 – 12.5	**12.5 – 6.25**	> 100	> 100	0.8 – 0.4	0.8 - 0.4
Ent4	> 200	> 200	100 - 50	**25 – 12.5**	6.25 - 3.12	**3.12 - 1.6**
Ent190	> 200	> 200	3.12 - 1.6	6.25 - 3.12	1.6 - 0.8	3.12 - 1.6
Ent275	> 200	> 200	> 100	**100 - 50**	3.12 - 1.6	3.12 - 1.6
EfAC1	> 200	> 200	25 - 12.5	**6.25 - 3.12**	3.12 - 1.6	3.12 - 1.6
Streptococcus	**Ceft**	**Ceft Betam1/4**	**Eryth**	**Eryth Betam1/4**	**Ofl**	**Ofl Betam1/4**
Strepto 1	50 - 25	100 - 50	3.12 - 1.6	3.12 - 1.6	3.12 - 1.6	3.12 - 1.6
Strepto203	100 - 50	100 - 50	25 - 12.5	**< 0.006**	0.4 - 0.2	1.6 - 0.8
strepto274	> 200	> 200	> 100	**< 0.006**	> 50	**1.6 - 0.8**
strepto256	50 - 25	50 - 25	12.5 - 6.25	**< 0.006**	0.4 - 0.2	**< 0.003**
Strepto A1	0.8 - 0.4	**0.4 - 0.2**	0.4 - 0.2	**< 0.006**	0.8 - 0.4	**< 0.003**
Pseudomonas	**Ceft**	**Ceft Betam1/4**	**Eryth**	**Eryth Betam1/4**	**Ofl**	**Ofl Betam1/4**
PaO1	1.6 - 3.2	1.6 - 3.2	> 100	**< 0.006**	6.25 - 3.12	**< 0.003**
PS226	25 - 12.5	**< 0.012**	> 100	**< 0.006**	> 50	**< 0.003**
PS1620	6.25 - 3.12	**< 0.012**	> 100	**< 0.006**	0.8 - 0.4	**< 0.003**
PS301	6.25 - 3.12	**< 0.012**	> 100	**< 0.006**	1.6 - 0.8	**< 0.003**
PS392	25 - 12.5	**< 0.012**	> 100	**< 0.006**	12.5 - 6.25	**< 0.003**
Enterobacteriaceae	**Ceft**	**Ceft Betam1/4**	**Eryth**	**Eryth Betam1/4**	**Ofl**	**Ofl Betam1/4**
E239	0.4 - 0.2	6.25 - 3.12	> 100	> 100	0.2 - 0.1	**0.05- 0.025**
E241	0.8 - 0.4	0.8 - 0.4	> 100	> 100	50 - 25	**25 - 12.5**
E1604	> 200	**0.8 - 0.4**	> 100	> 100	0.2 - 0.1	0.2 - 0.1
K189	> 200	> 200	> 100	> 100	25 - 12.5	25 - 12.5
E64	25 - 12.5	**1.6 - 0.8**	> 100	> 100	12.5 - 6.25	**0.1 - 0.05**
S. aureus	**Ceft**	**Ceft Betam1/4**	**Eryth**	**Eryth Betam1/4**	**Ofl**	**Ofl Betam1/4**
Sa1511	> 200	> 200	6.25 - 3.12	6.25 - 3.12	0.8 - 0.4	**0.1 - 0.05**
Sa1544	> 200	> 200	> 100	> 100	12.5 - 6.25	50 - 25
MRSA 200	200 - 100	> 200	6.25 - 3.12	25 - 12.5	12.5 - 6.25	50 - 25
Sa43	25 - 12.5	25 - 12.5	6.25 - 3.12	6.25 - 3.12	0.8 - 0.4	0.8 - 0.4
MRSA 165	> 200	> 200	6.25 - 3.12	6.25 - 3.12	0.4 - 0.2	0.8 - 0.4
CoNS	**Ceft**	**Ceft Betam1/4**	**Eryth**	**Eryth Betam1/4**	**Ofl**	**Ofl Betam1/4**
RP62A	100 - 50	100 - 50	> 100	> 100	0.4 - 0.2	0.8 - 0.4
DSM15917	12.5 - 6.25	12.5 - 6.25	6.25 - 3.12	12.5 - 6.25	0.4 - 0.2	0.8 - 0.4
DSM12228	6.25 - 3.12	**3.12 – 1.6**	6.25 - 3.12	12.5 - 6.25	0.4 - 0.2	0.8 - 0.4
DSM20501	25 - 12.5	25 - 12.5	6.25 - 3.12	6.25 - 3.12	0.4 - 0.2	0.8 - 0.4
She!1	25 - 12.5	25 - 12.5	> 100	> 100	0.4 - 0.2	0.8 - 0.4

Ceft: Ceftazidime; Eryth: Erythromycin; Ofl: Ofloxacin; Betam: Betamethasone. Injectable formulation of Bentelan was diluted 1:4 containing 0.5 mg/ml of active principle.

the presence of betamethasone for each bacterial species on three antibiotics. The lower dosage of betamethasone (0.125 mg/ml corresponding to 1/4 of the stock concentration) did not influence the MIC at all (thus data are not shown), while several appreciable differences were evident with betamethasone higher dosage (0.5 mg/ml corresponding to 1/4 of the stock concentration) for different bacterial species.

To schematize the effects of the combined administration of antibiotic and betamethasone, we could group the data into three main ensembles: Group A - bacterial species which were totally

unsusceptible to an antibiotic and became susceptible upon addition of the higher dosage of betamethasone; Group B – bacterial species which were susceptible to the antibiotic and became more susceptible for the combined administration of antibiotic and betamethasone, allowing the usage of a lower dose of antibiotic; Group C – bacterial species showing no variation in the response to the combined administration compared with the reference single administration of the antibiotic.

Notably *Pseudomonas* (PS226), *Streptococci* (Strepto274) and *Enterobacteriaceae* (E241), all of which could be classified as belonging to the Group A response because unsusceptible to ofloxacin, became susceptible upon addition of the higher dosage of betamethasone. Moreover, among the group B response to ofloxacin we found *Enterococcus* (Ent4), *Streptococcus* (Strepto256, StreptoA1), *Pseudomonas* (all), *Enterobacteriaceae* (E239, E64) and *S. aureus* (Sa1511).

Considering the strains unsusceptible to erythromycin we found a similar activating result for *Enterococcus* (Ent275), *Streptococcus* (Strepto274) and *Pseudomonas* (all) ascribing them to group A ensemble, while *Enterococcus* (Ent4, EfAC1) and *Streptococcus* (Strepto203, Strepto256, StreptoA1) to group B. On the contrary, no effect was observed on *S. aureus*, CoNS and *Enterobacteriaceae*.

Considering the strains unsusceptible to ceftazidime, we found an activating result only for the *Enterobacteriacea* (E1604) ascribing it to group A, while *Enterococcus* (Ent3), *Streptococcus* (StreptoA1), *Enterobacteriacea* (E64) and almost all *Pseudomonas* might be assigned to group B. The effect of betamethasone on ceftazidime MIC was notably appreciable on *Pseudomonas* and *Enterobacteriaceae*, where four and two strains showed a diminution in MIC range, respectively.

DISCUSSION

The objective of the study was to determine whether the addition of betamethasone increased the efficacy of some classes of antibiotics.

The strains were chosen to cover the main Gram-positive and Gram-negative genera responsible for the most common opportunistic infections. The strains were collected from clinical isolates, DSMZ or ATCC (Table I). Each strain was validated using microbiological and biochemical tests, as described in the material and methods section.

Firstly, we evaluated the growth behavior of all strains in the presence of betamethasone. As reported in the Results section, the effect was species-dependent in such cases but occasionally was strain-dependent. For subsequent experiments we chose 30 strains belonging to six classes with different behavior to betamethasone exposure, except for *Enterobacteriaceae* and *S. aureus*, where all the strains showed the same growth behavior. In the remaining cases we selected strains with different capability of growth in the presence of betamethasone.

We first evaluated the MIC values of selected antibiotics on these bacterial strains, and subsequently their combination with betamethasone. The antibiotics used belong to different classes and cover the majority of infections caused by Gram positive and Gram negative bacteria. Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class considered to be a second-generation. It is important to underline that ofloxacin is one of the major antimicrobials used in therapy against infections caused by these bacteria.

Erythromycin is a macrolide antibiotic that has an antimicrobial spectrum similar to or slightly wider than that of penicillin, and is often prescribed for people who have an allergy to penicillin. For this reason the results obtained are very promising.

Ceftazidime is a third-generation cephalosporin antibiotic and, like other third-generation cephalosporins, it has broad-spectrum activity against Gram-positive and Gram-negative bacteria. Furthermore, ceftazidime is usually reserved for the treatment of infections caused by *P. aeruginosa*.

According to the literature, bacteria were characterized as resistant or susceptible following the guidelines reported for each species (BSAC Methods for Antimicrobial Susceptibility Testing).

For each antibiotic two different dosages of betamethasone were used: the higher dosage being 0.5 mg/ml (1/4 of the stock concentration) and the lower dosage being 0.125 mg/ml (1/16 of the stock concentration). The rationale being to ensure that betamethasone was in sub-inhibiting condition. To note that for *Pseudomonas* only the lower dosage was actually in sub-inhibiting conditions.

We find an action of betamethasone administered in sub-inhibiting dose, in combination together with an antibiotic in at least 18 different cases. These cases are counted excluding the activation effect found on all the *Pseudomonas* strains, since this effect may be ascribed essentially to the action of the betamethasone alone at 0.5 mg/ml dose, as can be inferred from the MIC results of betamethasone shown in Table II. It should also be noted that by excluding the result of all the *Pseudomonas* we are being extremely conservative since for at least 2 strains out of 5 this dose is not sufficient to impair growth completely (Fig. 1B). All the remaining 18 cases should be regarded as true cases of positive effect where the presence of a sub-inhibiting dose of betamethasone enhances the effect of the antibiotic. Antibiotic–steroid combination therapy is, in such cases, superior to antibiotic-alone treatment to impair bacterial growths. Such effect was essentially not observable at all on *S. aureus* or CoNS.

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