

Pharmacodynamic Assessment of Amoxicillin-Sulbactam Against *Acinetobacter baumannii*: Searching the Optimal Dose and Infusion Time Through a Human *ex-vivo* Model

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Amoxicillin-sulbactam (AMX-SUL) is an aminopenicillin/ β -lactamase inhibitor combination currently available in 29 countries and may be a suitable option for treating infections caused by *Acinetobacter* spp. Thus, we sought to search the optimal dosing strategy for this formulation through an *ex vivo* pharmacodynamic human model against *Acinetobacter baumannii*. Four volunteers were randomized to receive alternatively a single dose AMX-SUL infused both either over 30 min or 3h at the following ratios (g/g): 1/0.5; 1/1, and 0/2. Time-kill studies were performed with the 0-, 0.5-, 2-, 4-, 6- and 8-h sera after dose against a clinical isolate of *A. baumannii* (sulbactam MIC, 4 μ g/mL). Bactericidal activity (i.e. a mean decrease $\geq 3 \log_{10}$ CFU/mL in the viable cell counts from the initial inoculum) was displayed by the 0.5- and the 2-h sera after dose for all formulations. The 4-h sera proved inhibitory with the AMX-SUL 1g/1g formulation, albeit a trend to regrowth was observed after 24-h incubation. With the AMX-SUL 0g/2g dose, the 4-h sera proved almost bactericidal activity (i.e. a mean decrease of 2.4 \log_{10} CFU/mL in the viable cell counts from the initial inoculum), whereas the 6-h sera was inhibitory, with a trend to regrowth after 24-h incubation. When infused over 3h, AMX-SUL 1g/0.5g and 1g/1g, bactericidal activity was displayed by the 0.5-, 2- and the 4-h sera after dose and the 6-h sera proved inhibitory with the AMX-SUL 1g/1g formulation. The present study, albeit preliminary, might give a rationale for the dosing strategy to treat infections caused by *A. baumannii* with sulbactam, either alone or combined with amoxicillin. A 2-g sulbactam dose seems to be optimal to be infused over 30 min with a 6-h dosing interval. When infused over 3h, AMX-SUL 1g/1g given every 6h or 8h seems a suitable dosing schedule.

Key-Words: Amoxicillin-sulbactam, *Acinetobacter*.

Acinetobacter baumannii is becoming an important pathogen associated with nosomial infections worldwide [1]. It is invariably multiresistant and one major concern is the increasing incidence of carbapenem-resistant isolates, since these antibiotics are frequently the therapeutic choice for these infections [2]. Carbapenem resistance in South America has already reached levels that significantly influence therapeutic choices [3,4] and this looks set to be a problem in several other regions [5,6]. *Acinetobacter* spp. has been found at the fourth rank in hospitalized patients with pneumonia in Latin America [6] and at the second one in patients with nosocomial pneumonia in Argentina [7], with carbapenem resistance increasing dramatically [4], especially in Argentina [4,7] and in Colombia [4,8]. This kind of resistance has also been reported at modest rates in Chile [4] and Brazil [4,9], albeit it seems to remain stable in some regions of this late country [10]. As a result, old antibiotics, such as colistin and ampicillin-sulbactam, have emerged as the obligate alternative for treating serious infections associated with *A. baumannii* [11].

Both early [12] and present [13] evidences suggest that sulbactam is active *in vitro* against *Acinetobacter* spp, whereas some reports showed that this drug, given as a combination of ampicillin-sulbactam, may yield acceptable clinical success when treating infections associated with this

pathogen [11,14]. However, neither large controlled clinical trials, nor pharmacodynamic studies have been undertaken to assess the efficacy of sulbactam against *Acinetobacter* spp [11,14]. Amoxicillin-sulbactam (AMX-SUL) is an aminopenicillin/ β -lactamase inhibitor combination marketed in Argentina since 1988 and is currently available in 29 countries. Indeed, more than two millions of patients with either urinary or respiratory tract infections have been treated by oral route with this formulation from 1988 to 2007 only in Argentina (data from files of Laboratorios Bagó S.A., Argentina). Although we have described the pharmacodynamics of AMX-SUL oral tablets thoroughly [15-18], the intravenous formulation of this drug have not been assessed, as the intravenous combination was allowed to be marketed on the basis of the pharmacokinetics and pharmacodynamics (pk/pd) tested for the oral formulation. As a result, neither pk/pd studies, nor clinical trials were required for the approval of intravenous AMX-SUL. Because of intravenous AMX-SUL may be a suitable option for treating infections caused by *Acinetobacter* spp, we sought to search the optimal dose of sulbactam to be used in this formulation through an *ex vivo* pharmacodynamic human model. In addition, the benefit of an extended infusion was assessed.

Material and Methods

Study Design

This was an experimental, randomized, single-blind, 4-way crossover intra-subject study. It was conducted following the principles of the Declaration of Helsinki on Good Clinical Practice and received approval of an independent Ethics Committee and the national health authorities (A.N.M.A.T,

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authorization N° 3203/06). Informed consent was obtained from the volunteers before enrollment.

Subjects, Antimicrobial Dose and Sampling

After an overnight fasting, 4 healthy subjects, 1 male and 3 female; mean age, 35.0 years (range 27 to 51 years) and mean weight, 60.5 kg (range 46.0 to 75.0 kg), were randomized to receive alternatively (i.e. separated by a 7-days washout period) the different doses. AMX-SUL (Trifamox IBL Inyectable Laboratorios Bagó SA, Buenos Aires, Argentina) was given diluted in 100 mL of sterile saline solution as a single dose infused over 30 min at the following amoxicillin/sulbactam ratios (g/g): 1/0.5; 1/1 and 0/2. In addition, after a 15-days period, AMX-SUL, both at 1g/0.5g and at 1g/1g formulation, was infused over 3h to the 4 volunteers separated by a 7-days washout period. Venous blood samples were obtained immediately before (time zero, control) and at 2, 4, 6 and 8h after dose. Serum harvested from blood was immediately fractionated and stored at -70°C until use.

Antibiotic Concentration in Serum

Levels of antibiotics were determined in all serum samples by a bioassay method as previously described [13]. The *Bacillus subtilis* ATCC 6633 strain and a clinical isolated of *A. baumannii* (strain 5355-81, sulbactam MIC, 2µg/mL) were used for testing amoxicillin and sulbactam, respectively. The lower limit of detection was 0.12 µg/mL for amoxicillin and 2.5 µg/mL for sulbactam. Percent coefficient of variation ranged between 9% and 15%. The linearity of every standard curve was assured by a regression coefficient (r) of at least 0.998.

Time-Kill Studies

Time-kill studies were performed as described previously [15] with the 0- (growth control), 2-, 4-, 6- and 8-h sera after dose against a clinical isolate of *A. baumannii* (sulbactam MIC, 4µg/mL). Briefly, serum samples were inoculated with equal volumes of Mueller-Hinton broth (Difco, Detroit, Mich.) containing the organism at a concentration of roughly 1×10^6 cfu/mL in log phase. All tubes were incubated at 35°C in room atmosphere. Viable cell counts were performed after 0, 2, 4, 6, 8 and 24 h of incubation to establish a 24-h time-kill curve. Bactericidal activity was defined as a $\geq 3\text{-log}_{10}$ cfu/mL decrease in the viable cell counts regarding the original inoculum. Inhibitory activity was defined as $< 3\text{-log}_{10}$ cfu/mL decrease in viable cell counts. Any viable cell count higher than the starting inoculum was considered as overgrowth. Carryover of antibiotics was avoided by 10-fold serial dilutions before plating. The lower limit of detection was 100 cfu/mL and this value was assigned to any count dropping below this limit.

Data Analysis and Statistics

Levels of antibiotic in serum are given as mean values and standard deviation (SD) calculated from the 4 volunteers. Likewise, for sera corresponding to the different times after dose from all volunteers, an average time-kill curve was

constructed for every AMX-SUL dose with the mean values of viable cell counts (\log_{10} cfu/mL) obtained at the different times of incubation (i.e. 0, 2, 4, 6, 8 and 24h).

Results

Table 1 shows the mean serum levels of amoxicillin and sulbactam after the infusion of AMX-SUL at different ratios between components, infused both over 30 min and 3h. When infused over 30 min, the formulation AMX-SUL 1g/1g yielded higher amoxicillin and sulbactam levels than AMX-SUL 1g/0.5g at every time after dose, whereas AMX-SUL 0g/2g reached the highest sulbactam concentrations. When infused over 3h, AMX-SUL (both 1g/0.5g and 1g/1g) yielded amoxicillin and sulbactam levels at 2h and 4h after dose higher than the respective values observed with any AMX-SUL formulation infused over 30 min, with the formulation 1g/1g showing the highest levels. Furthermore, sulbactam concentration obtained at 2h and 6h after dose with AMX-SUL 1g/1g infused over 3h was higher than the respective values observed with 2g of sulbactam alone infused over 30 min.

Average time-kill curves of serum from the 4 volunteers receiving the different AMX-SUL formulations over 30 min against *A. baumannii* 5355-81 are given in Figure 1. Bactericidal activity (i.e. a mean decrease $\geq 3 \log_{10}$ CFU/mL in the viable cell counts from the initial inoculum) was displayed by the 0.5- and the 2-h sera after dose for all formulations. The 4-h sera proved inhibitory with the AMX-SUL 1g/1g formulation (Figure 1B), albeit a trend to regrowth was observed after 24-h incubation. With the AMX-SUL 0g/2g dose, the 4-h sera proved almost bactericidal activity (i.e. a mean decrease of 2.4 \log_{10} CFU/mL in the viable cell counts from the initial inoculum), whereas the 6-h sera was inhibitory, with a trend to regrowth after 24-h incubation (Figure 1C). The remaining serum samples collected after dose were unable to exert significant antimicrobial effect with any formulation.

Average time-kill curves of serum from the 4 volunteers receiving the AMX-SUL 1g/0.5g and 1g/1g combinations infused over 3h against are given in Figure 2. Bactericidal activity was displayed by the 0.5-, 2- and the 4-h sera after dose. The 6-h sera proved inhibitory with the AMX-SUL 1g/1g formulation (fig. 2B), albeit a trend to regrowth was observed after 24-h incubation.

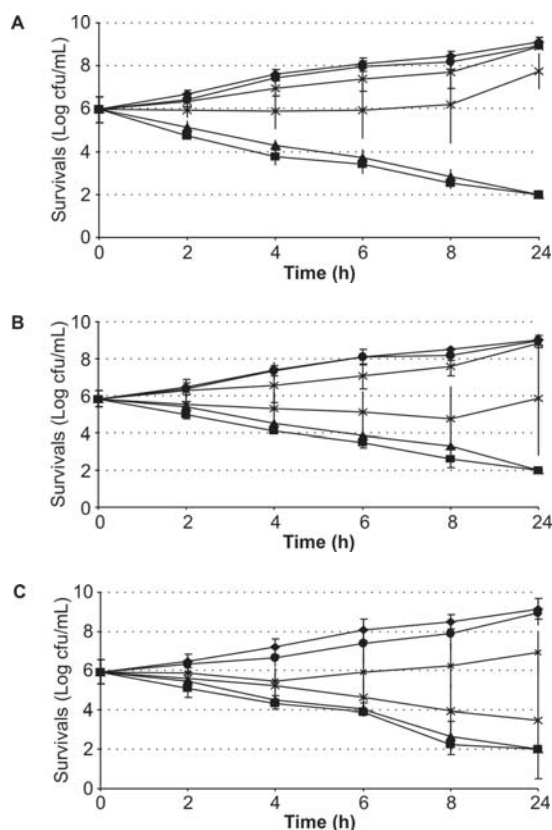
Discussion

Several efforts are being made to assess alternative options for treating infections associated with multidrug-resistant *A. baumannii* and both early and newer antibiotics have been tested [11]. Ampicillin-sulbactam is one of these early drugs proving *in vitro* activity [12] and two recent comparative, one retrospective [14] and one prospective [19], studies suggest that it may be effective against infections caused by this organism. In the retrospective study, more than 80 patients were either treated with colistin or ampicillin-sulbactam and colistin, but not ampicillin-sulbactam, proved independently associated with higher mortality rate [14]. In the prospective

Table 1. Serum levels of different amoxicillin-sulbactam (AMX-SUL) formulations infused to 4 volunteers by intravenous route over 30 min and 3h.

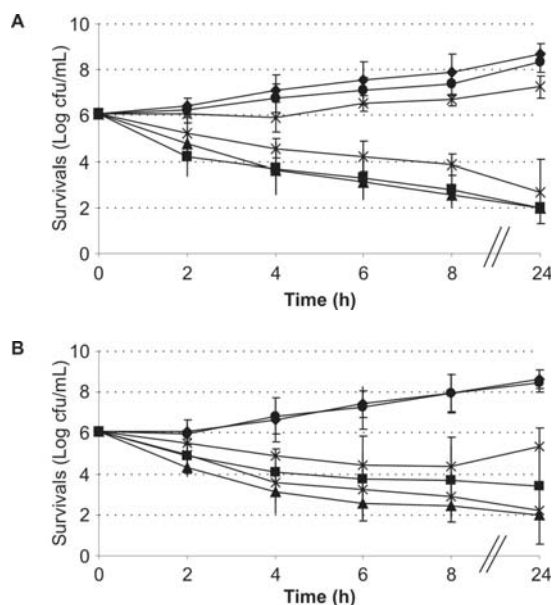
Component and time of infusion	Mean \pm SD concentration ($\mu\text{g/mL}$) at the following time after dose				
	0.5h	2h	4h	6h	8h
Over 30 min					
AMX-SUL 1g/0.5g					
AMX	42.7 \pm 28.9	4.6 \pm 3.0	0.76 \pm 0.44	0.25 \pm 0.13	<0.12
SUL	25.8 \pm 11.3	3.8 \pm 1.50	<2.5	<2.5	<2.5
AMX-SUL 1g/1g					
AMX	57.5 \pm 41.6	6.1 \pm 4.1	1.0 \pm 0.94	0.36 \pm 0.94	<0.12
SUL	50.8 \pm 22.9	8.63 \pm 3.81	2.54 \pm 0.14	<2.5	<2.5
AMX-SUL 0g/2g					
SUL	98.1 \pm 5.78	24.1 \pm 11.4	5.5 \pm 3.3	<2.5	<2.5
Over 3h					
AMX-SUL 1g/0.5g					
AMX	43.4 \pm 28.8	52.5 \pm 31.7	3.1 \pm 2.1	0.50 \pm 0.42	0.18 \pm 0.10
SUL	29.0 \pm 18.6	41.0 \pm 23.3	4.0 \pm 1.71	<2.5	<2.5
AMX-SUL 1g/1g					
AMX	50.4 \pm 53.5	75.8 \pm 54.2	5.2 \pm 1.3	0.60 \pm 0.50	0.3 \pm 0.23
SUL	30.4 \pm 29.4	59.0 \pm 33.5	5.1 \pm 0.14	3.1 \pm 0.25	<2.5

Figure 1. Average time-kill curve of serum from 4 volunteers receiving intravenous AMX-SUL; 1g/0.5g (A), 1g/1g (B) and 0g/2g (C) infused over 30 min, against a clinical isolate of *Acinetobacter baumannii* (sulbactam MIC, 4 $\mu\text{g/mL}$). Data are means \pm standard deviation (error bars) at the following times after dose (h): 0 (\blacklozenge , control), 0.50 (\blacksquare), 2 (*), 4 (\blacktriangle), 6 (x) and 8 (\bullet).



trial, 10 of 13 patients with ventilator-associated pneumonia by *Acinetobacter* spp displayed resolution or improvement of the symptoms and signs after treatment with high doses of ampicillin-sulbactam (i.e. 9g every 8h). In addition, some case series had previously been reported [20-23]. In most studies an ampicillin-sulbactam combination containing 1-g of sulbactam, given every 8h, was used; but 2g [20] and 3g [19] of sulbactam were also employed. This dose variability may be attributable to the lack of pharmacodynamic studies for supporting any dosing schedule. We believe that determining the optimal dose is a crucial issue to reach the maximum antimicrobial efficacy with the minimal rate of certain adverse events observed in some small clinical trial testing high sulbactam dosage (i.e. 3g), such as diarrhea, skin rash or nephrotoxicity [19]. Our data show that both 0.5g and 1g of sulbactam, infused as AMX-SUL over 30 min, were able to reach bactericidal serum levels at 2h after dose, but a net regrowth and trend to regrowth occurred at 4h after dose with the 0.5-g and 1-g sulbactam dose, respectively. Although these features might be enough for a 4-h dosing interval, they are insufficient to get the desirable time about MIC ($fT > MIC \geq 50\%$) of a 6-h schedule dosing described for a beta-lactam antibiotic [24]. By contrast, the 2-g sulbactam dose proved highly active at 4-h after dose (i.e. 67% of a 6-h dosing interval). These findings suggest that, if 1g of sulbactam is going to be used to treat an infection associated with *A. baumannii*, a dosing interval no longer than 4-h should be preferred, whereas using a 2-g dosage would allow a 6-h dosing schedule. Although we have used only one *A. baumannii* strain in the present study, one of us has recently demonstrated the bactericidal property of sulbactam against several clones of this organism at concentrations equal or higher than the MIC values [25].

Figure 2. Average time-kill curve of serum from 4 volunteers receiving intravenous AMX-SUL; 1g/0.5g (A) and 1g/1g (B) infused over 3h, against a clinical isolate of *Acinetobacter baumannii* (sulbactam MIC, 4 μ g/mL). Data are means \pm standard deviation (error bars) at the following times after dose (h): 0 (\blacklozenge , control), 0.50 (\blacksquare), 2 ($*$), 4 (\blacktriangle), 6 (\times) and 8 (\bullet).



Interestingly, the 3-h extended infusion enhanced the probability of reaching the desirable pharmacodynamic target. Indeed, both 0.5g and 1g of sulbactam proved bactericidal at 4h after dose with this infusion strategy. In addition, the 8-h after dose serum of volunteers receiving AMX-SUL 1g/1g displayed inhibitory activity. Although the benefit of the extended infusion has been described for some beta-lactams, such as piperacillin/tazobactam [24,26] and meropenem [24], it has not been reported, to our knowledge, for aminopenicillin-sulbactam combinations. It should be noted that this extended-infusion strategy may not be applicable to generic products, as stability of the drug over time is a crucial requirement to be achieved to perform extended infusion and all these experiences, including the present one, have been carried out with the original trades. In fact, significant potency decrease has been recently reported for a number of generic trades of piperacillin/tazobactam [27].

In summary, the present study, albeit preliminary, might give a rationale for the dosing strategy to treat infections caused by *A. baumannii* with sulbactam, either alone or combined with amoxicillin. A 2-g sulbactam dose seems to be optimal to be infused over 30 min with a 6-h dosing interval. When infused over 3h, AMX-SUL 1g/1g given every 6h or 8h seems a suitable dosing schedule. Further studies may be required to definitively confirm our findings.

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