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ORIGINAL ARTICLE

Efficacy of humanlike Augmentin SR (2000/125 mg) twice daily treatment on Haemophilus influenzae experimental pneumonia in rabbits

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Abstract

We investigated the efficacy of 2 formulations of Augmentin on experimental pneumonia due to Haemophilus influenzae (HI) in rabbits. Two strains were used (H128 and 401285) with amoxicillin/clavulanic acid MICs of 1/0.5 mg/l and 4/2 mg/l. Pneumonia was induced in immunocompetent rabbits by inoculation of 10 $\log_{10}$ CFU HI. The treatments were infused by using computer controlled pumps in order to mimic the human pharmacokinetic (PK) profile of either conventional Augmentin treatment (875/125 mg twice daily) or the sustained release formulation (SR: 2000/125 mg twice daily). After 2 d of treatment, the bacterial concentrations in the lungs were similar for both strains and both treatments: isolate H128, conventional Augmentin reduced bacterial numbers to 3.8 ± 2.1 $\log_{10}$ CFU/g and Augmentin SR to 3.1 ± 2.4 $\log_{10}$ CFU/g; isolate 401285, conventional Augmentin to 3.5 ± 2. Thus, both treatments demonstrated similar efficacy against H. influenzae pneumonia in this model, even when induced by a strain with an amoxicillin/clavulanic acid MIC of 4/2 mg/l. These results support current breakpoints for conventional Augmentin against H. influenzae and suggest that Augmentin SR is at least as effective against these isolates.

Introduction

Acute respiratory infections represent the third leading cause of death due to infections in the world and the leading cause in children [1]. In 2000, approximately 20% of deaths in children under 5 y of age were caused by acute respiratory infection, and mortality rates worldwide reach more than 2 million deaths per y. The incidence of pneumonia in children under 5 y of age is estimated to be 0.3 episodes per child per y, and the incidence of pneumonia in developing countries reaches 448 million cases annually [2].

Haemophilus influenzae is considered to be the second most prevalent organism (S. pneumoniae being the first) implicated in acute respiratory infections and is probably underestimated [3,4]. It remains a public health problem; approximately 300,000 to 700,000 children worldwide still die of H. influenzae type b disease each y [2,5] despite the sharp decrease in the incidence of invasive disease in the US and Europe associated with recent vaccination programmes [6]. This bacterium is also frequently involved in chronic bronchitis [7,8] and pneumonia in adults [9].

20–50% of all HI strains are beta-lactamase positive [10–12], which can partly explain the high failure rate of amoxicillin in the treatment of respiratory tract infections [13–17]. Thus, third generation cephalosporins or the combination amoxicillin-clavulanate (Augmentin) are proposed for treatment and are widely used.

The short half-life of amoxicillin can minimize the antibiotic exposure and lead to inadequate antibacterial efficacy. A new sustained release formulation of Augmentin has been developed which increases the time that serum amoxicillin levels remain elevated. This new formulation is associated with good clinical efficacy in respiratory tract infections [18,19]. However, little is known concerning the efficacy of this new formulation on pulmonary

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infections due to less susceptible Haemophilus influenzae.

The aim of this study was to investigate the efficacy of simulated human serum concentrations of either conventional Augmentin (875/125 mg twice daily) or Augmentin SR (2000/125 mg twice daily) against experimental Haemophilus influenzae pneumonia due to isolates with amoxicillin/clavulanic acid MICs of 1/0.5 mg/l or 4/2 mg/l.

Methods and materials

Study strains

H. influenzae H128 (beta-lactamase positive) and 401285 (beta-lactamase negative) were obtained from GlaxoSmithKline Pharmaceuticals, Collegeville, PA. The amoxicillin/clavulanic acid MICs were 1/0.5 and 4/2 mg/l, respectively, using CLSI methodology.

Preparation of the inoculum

Before each animal experiment, 1 aliquot of HI stock was inoculated into BHI broth, cultured on chocolate agar plates, and incubated for 24 h at 37°C in 5% CO_2. Colonies (10–15) were inoculated into 9 ml of BHI broth, incubated for 6 h at 37°C, and then cultured on chocolate agar plates for 18 h at 37°C in 5% CO_2. Colonies from this culture were diluted in physiological saline in order to obtain a final concentration of 10 $\log_{10}$ CFU/ml in 2% agar solution. These concentrations were confirmed by successive dilution cultures.

Animals

Male New Zealand white rabbits (body weight 2.5 to 3 kg) were obtained from CEGAV ssc, Saint Mars d'Egrenne, France. These animals were not immunosuppressed and had a sanitary status of virus antibody free and specific pathogen free. They were placed in individual cages and were nourished ad libitum with drinkable water and feed, according to current recommendations.

Experimental bacterial pneumonia in rabbits

This animal procedure has previously been described [20–24]. Briefly, the animals were anaesthetized intramuscularly with 1.5–2 ml of a mixture of ketamine (500 mg/ml) and xylazine (2.75 mg/ml). Two silicone catheters were introduced into the jugular vein (a short one with the extremity in the superior vena cava, a longer one with the extremity in the right auricula) through a lateral incision in the neck, and then subcutaneously tunnelled through the interscapular area. The short catheter was introduced in order to infuse antibiotics at rates designed to simulate the human pharmacokinetic profiles, and the other was placed to draw blood samples at timed intervals. Heparin serum was withdrawn and rinsed at each use to prevent clotting. 24 h later, the rabbits were anaesthetized intravenously by using 0.6–0.8 ml of the ketamine-xylazine mixture. Under view control, a silicone catheter (Sigma Medical, Nanterre, France) was introduced through the vocal cords into the trachea and pushed until it reached the bronchia. Freshly prepared inoculum (1.5 ml) was then gently flushed through this catheter. The endobronchial catheter was then immediately removed after the inoculum instillation, and the animals were placed upright for 15 s to facilitate distal alveolar migration by gravity. Treatment was started 5 h after this inoculation; at this time, a crude pneumonia exists and the mean bacterial concentration was 7.8 ± 1.4 log CFU for both strains.

Amoxicillin and clavulanate assay

The compounds were reconstituted from laboratory powder of known potency according to the manufacturer's instructions, just before each experiment. Concentrations in blood were determined by the disk plate bioassay method for these 2 drugs [25]. Amoxicillin concentrations were determined within 1 d from the experiment using Micrococcus luteus ATCC 9341 as the test organism (inter- and intra-d variations were 7% and 5%, respectively). Clavulanate concentrations were determined within 1 d from the experiment using Klebsiella pneumoniae 10031 as the test organism with 32 mg/l benzylpenicillin in the agar (the intra- and inter-assay coefficients of variation were 4% and 6%, respectively).

Simulation of human Augmentin pharmacokinetics in rabbits

The objectives were to simulate the human pharmacokinetic profiles following oral administration of either Augmentin SR (2000/125 mg twice daily) or conventional (875/125 mg twice daily) for 48 h [26]. The procedure used to compensate for the faster elimination of antibiotics in small animals compared with humans has been previously described [20]. Briefly, from the pharmacokinetic parameters of both amoxicillin and clavulanate, the timed interval compensatory dose can be calculated to obtain the desired (human) concentrations. A variable flow rate infusion with successive levels was used. For each

experiment, 2 computer-controlled pumps containing either amoxicillin or clavulanate were connected to a central venous catheter. This connection was protected and allowed the animals to maintain free circulation and free access to food and water. Infusion rates were controlled by programmable computer software.

Blood samples were obtained through the second central venous catheter. Amoxicillin and clavulanate concentrations were measured as described above. From these blood concentrations, Akaike criteria were used to determine the best fit and then, for each animal, individually-tailored simulations were obtained using Kinetica software.

Pharmacodynamic analysis

From the individual pharmacokinetic profile of each treated animal, the following pharmacodynamic (PD) parameters were calculated against the susceptibility (MIC) of each strain: peak concentration (Cmax), AUC (0–24 h) and the time over which concentrations exceeded the MIC (T > MIC) expressed as a percentage.

Evaluation of the HI pneumonia in rabbits

Bacterial content in lungs. Tissues were removed 2–3 h after the infusion was complete. Post mortem examination was performed after anaesthesia by using overdoses of thiopental. For each rabbit, the thorax was opened, and the existence of pleural effusion was noted. The lungs were then dissected aseptically and placed on sterile gauze for at least 5 min to allow residual pulmonary blood absorption. Each pulmonary lobe was weighed and homogenized in sterile saline. The spleen was prepared under the same conditions. Bacteria were counted in a sample of this crude homogenate by plating 10-fold dilutions on HI selective agar (BioMérieux, Craponne, France) and incubating the plates for 24 to 48 h at 37°C. Bacterial concentrations in each lobe or in the spleen were determined after adjusting for weight. The threshold value was 1 $\log_{10}$ CFU/ml (for low bacterial concentrations, 1 ml was plated). For statistical comparisons of the difference between bacterial densities in the lungs, culture-negative lobes were considered to contain 1 $\log_{10}$ CFU/g.

Statistics

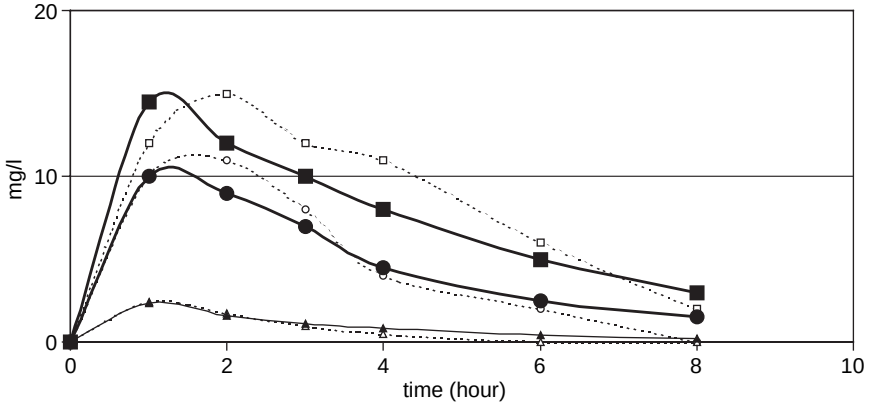
The results were expressed as the mean or percentage ± standard error. Differences between quantitative values were analysed by using the Mann-Whitney non- parametric test. Continuous variables were analysed with analysis of variance. In case of a significant test, post hoc analysis comparing results for each treated arm versus untreated arm were conducted with a Bonferoni adjustment. All calculations were carried out with SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Pharmacokinetics

Figure 1 shows the pharmacokinetic profiles of both formulations of amoxicillin along with that of clavulanate. The serum concentration curves of Augmentin SR (2000/125 mg) and conventional Augmentin (875/125 mg) measured in the rabbits were similar to those obtained in humans. These results were obtained from infected/treated animals (see Methods and materials).

The Cmax in the rabbit for amoxicillin SR was 15.5 ± 4.6 mg/l and 10.4 ± 3 mg/l for conventional amoxicillin ($p < 0.01$). The human Cmax targets were 16 mg/l and 11.8 mg/l, respectively).



Solid line: simulation in rabbits; dash line: human concentration.
Triangle: 125 mg clavulanate, circle: 875 mg amoxicillin; square: 2000 mg amoxicillin.

Figure 1. Serum concentration curves of Augmentin SR (2000/125 mg) and conventional Augmentin (875/125 mg) in the rabbit.

At the sixth h, the concentrations measured in the rabbit were 4.3±0.9 mg/l for amoxicillin SR and 2.4±0.9 mg/l for conventional amoxicillin ($p < 0.01$), and at 8 h the concentrations were 2.6±0.8 mg/l and 1.4±0.7 mg/l, respectively ($p < 0.01$). The human concentration targets at 8 h were 2 and 0 mg/l, respectively.

Finally, the 0–24 h AUCs measured in rabbit for amoxicillin SR and conventional were 115±30 and 70±18 mg.h/l, respectively ($p < 0.01$). The human targets for $AUC_{0\text{-}24h}$ were 140 and 80 mg.h/l, respectively.

For clavulanate, the dose of 125 mg was the same for both formulations. The maximal concentration achieved in rabbits was 2.5±1.0 mg/l. The human Cmax target was 2.4 mg/l.

Efficacy results

Bacterial contents in the lungs of the treated rabbits were compared after 48 h of infusion with those of infected controls receiving no treatment (Table I). Both simulated formulations of Augmentin significantly reduced bacterial load in the lungs compared to untreated animals ($p < 0.001$). There was no difference between the efficacy of the 2 formulations ($p > 0.5$) nor was there a statistical difference in efficacy ($p > 0.5$) against the 2 isolates (although there was a tendency towards higher bacterial content in untreated animals with isolate 401285). Of note, no Haemophilus influenzae with increased MIC were detected in any animals, regardless of the strain used.

Pharmacodynamic results

Since 2 strains with different amoxicillin susceptibilities and 2 different amoxicillin exposures were used, a PK/PD analysis was performed (Table II). For both groups of animals, i.e. infected by either HI strain, the 3 PK/PD parameters were all significantly different according to the treatment received (875/125 mg twice daily vs 2000/125 mg twice daily) ($p < 0.05$).

The antibacterial efficacy was found to be significantly associated with T > MIC ($p < 0.01$) but not with AUC/MIC or Cmax/MIC (not shown). To put into perspective the real difference between the 2 regimens, the proportions of treated animals in which T > MIC was found above 35% were calculated for both regimens. For the susceptible strain infection, this proportion was 83% for the conventional Augmentin (875/125 mg) treatment versus 100% for Augmentin SR (2000/125 mg); the corresponding bacterial reductions were 1.8 log CFU/g vs 2.5 log CFU/g. For the less susceptible strain, the percentage of animals with T > MIC was 40% and 80% for conventional Augmentin and Augmentin SR, respectively; the corresonding bacterial reduction was 1.6 log CFU/g vs 2 log CFU/g.

Discussion

This study shows that pneumonia caused by H. influenzae has been obtained in immunocompetent rabbits. The human PK profiles of both Augmentin formulations (875/125 mg and 2000/125 mg twice daily) were successfully reproduced, although the global amoxicillin exposures for both regimens were slightly lower than desired. Under these conditions, the efficacy of both formulations was excellent, and probably maximal in this model, achieving a bacterial reduction of at least 3 $\log_{10}$ CFU within 48 h of treatment. These results are similar to those obtained in a rat RTI model using simulated human PK for the isolate with an amoxicillin/clavulanic acid MIC of 1/0.5 mg/l [27]. In our model, when the animals were infected with the less susceptible Haemophilus influenzae strain with an MIC of 4/2 mg/l, the efficacy of the Augmentin SR formulation was slightly reduced (Table I), although this difference (0.9 $\log_{10}$ CFU) did not reach statistical

Table I. Bacterial content in lungs (CFU/g) of rabbits having Haemophilus influenzae pneumonia and treated with simulated Augmentin SR (2000/125 mg twice daily) or conventional Augmentin (875/125 mg twice daily).

	Mean±SE bacterial content in lungs at 48 h ($\log_{10}$ CFU/g)					
	H128			401285		
		Treated			Treated	
Strain	Control	Conv.	SR	Control	Conv.	SR
n	10	6	8	9	10	7
	6.5±0.9	3.8±2.1	3.1±2.4	7.2±1.1	3.5±2.3	4.0±2.5

Conv.: Conventional Augmentin 875/125 mg twice daily.
SR: Augmentin SR 2000/125 mg twice daily.
Note: controls vs treated: $p < 0.05$ and no significant difference between treated (H128 vs 401285).

Table II. Pharmacodynamics results obtained from animals having Haemophilus influenzae pneumonia and treated with simulated Augmentin SR (2000/125 mg twice daily) or conventional Augmentin (875/125 mg twice daily).

	PK/PD analysis			
	H. influenzae H128 (MIC 1/0.5 mg/l)		H. influenzae 401285 (MIC 4/2 mg/l)	
	Conv.	SR	Conv.	SR
T > MIC (%)	56 ± 18	90 ± 14	30 ± 8	51 ± 9
Cmax (mg/l)	10 ± 3	15 ± 3	10 ± 2	15 ± 5
Cmax/MIC	10 ± 3	15 ± 3	2.4 ± 0.5	3.6 ± 1.5
AUC (24 h) (mg h/l)	81 ± 38	128 ± 38	78 ± 15	123 ± 36
AUC/MIC	81 ± 38	128 ± 38	19.5 ± 3.7	30.6 ± 9

Conv.: Conventional Augmentin 875/125 mg twice daily.
Note: for each PKPD parameter a significant difference ($p < 0.05$) was observed between H128 vs 401285.

significance. This differs slightly from the published rat data which demonstrated a similar (1.1 $\log_{10}$ CFU) but significant difference between these formulations against a BLNAR strain of H. influenzae having an MIC of 4/2 mg/l [27]. This difference could be due to less variability of PK in the rat model in which pharmacokinetic evaluation was not carried out in each infected/treated animal. In contrast, in our model the PK data were obtained from infected animals. Thus, the PK variability observed in our study was very similar to that obtained in humans [26] and indicates that the exposure achieved with both formulations was similar enough between some animals to produce similar efficacy despite treatment group, particularly for the higher MIC isolate.

No mutants were recovered from treated animals in which the residual bacterial content was not null. This is concordant with the extremely low rate of selection of resistance obtained with Augmentin versus Haemophilus influenzae both in vitro [28] and in vivo [29–31].

From a PK/PD viewpoint, it could be suggested that for Haemophilus influenzae infections and Augmentin, a T > MIC of 40–50% is sufficient to reach significant efficacy [32,33]; this conclusion is similar to that obtained from in vitro experiments [34,35]. In our experiments, this cut-off value was reached with Augmentin SR even when pneumonia was induced with the strain 401285 (amoxicillin/clavulanic acid MIC 4/2 mg/l). Thus, our findings are in agreement with clinical efficacy of Augmentin in the treatment of respiratory tract infections [18,19,36–38], but add information for currently rare cases that are due to Haemophilus influenzae with MICs as high as 4 mg/l [12,39–42].

To further explore the pharmacodynamics of Augmentin against pneumonia caused by H. influenzae, additional studies should be conducted, but our findings are concordant with the current CLSI breakpoint of ≤4/2 mg/l. Indeed, the human-like Augmentin treatment of infection due to strain 401285 was associated with an at least 1000-fold reduction of the pulmonary H. influenzae biomass that leads to a very small probability of failure and selection of resistance.

We conclude that, in this model of H. influenzae pneumonia in immunocompetent animals, the new formulation of Augmentin SR is associated with good efficacy even for pneumonia due to Haemophilus influenzae with amoxicillin/clavulanic acid MICs of 4/2 mg/l.

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