Identical pattern of highly variable absorption of clavulanic acid from four different oral formulations of co-amoxiclav in healthy subjects

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The aims of this investigation were to calculate the pharmacokinetic parameters of amoxicillin and clavulanic acid, and to identify parameters that may affect their observed differences in absorption. Data were obtained from plasma concentration–time curves from four different open, randomized, two-treatment, two-period, two-sequence, crossover Phase I bioequivalence studies, with the following co-amoxiclav formulations: tablets 250/125, 500/125 and 875/125 mg, or 10 mL of an oral suspension 250/62.5 mg per 5 mL. Data from 144 subjects and 288 drug administrations were available for evaluation. After a 125 mg clavulanic acid dose (administered as potassium clavulanate) for all four different formulations, the clavulanic acid AUCt data ranged from 1.5 to 8 mg·h/L, varying by a factor of 5. The absorption of clavulanic acid was not related to the absorption of amoxicillin, or demographic factors, and we were unable to identify the reasons for the large variability in the absorption of clavulanic acid. We conclude that the absorption of clavulanic acid, after oral administration, is highly variable and may vary over a five-fold range between patients.

Keywords: amoxicillin-clavulanate, co-amoxiclav, amoxiclav, pharmacokinetics, variable absorption, AUC, amoxicillin/clavulanate ratio

Introduction

Clavulanic acid improves the antibacterial spectrum of amoxicillin by rendering strains that are resistant due to β-lactamase production susceptible to amoxicillin. The binding of β-lactamases to clavulanic acid is a complex physicochemical process. In general, a reversible complex is formed initially, followed by covalent binding, which leads to irreversible inactivation of both the β-lactamase and clavulanic acid. For this interaction, relatively low plasma concentrations of clavulanic acid are required.1-3 As both amoxicillin and clavulanic acid are absorbed after oral administration and possess similar pharmacokinetic properties (similar elimination kinetics with similar half-lives of 1 h), they offer a rational antimicrobial combination.4,5

The standard dose of co-amoxiclav (amoxicillin/clavulanic acid) for adults with lower respiratory tract infection has, for many years, been 500/125 mg orally three times a day. However, it is generally recognized that patient compliance improves with a reduced dosing frequency, and co-amoxiclav has now been licensed for use at a dose of 875/125 mg orally twice daily. The efficacy of this twice-daily dosage regimen is supported by pharmacokinetic, microbiological and clinical data.6-10

The dosage ratio of amoxicillin/clavulanic acid in oral co-amoxiclav formulations has changed since the combination has been in use, from 2:1 initially, to 4:1, and more recently to 7:1. The amoxicillin/clavulanic acid ratio was increased in order to reduce the incidence of side effects of clavulanic acid seen with the 2:1 ratio.11 As the efficacies of all of these combinations appear to be similar, this would suggest that
the amount of clavulanic acid necessary to inhibit bacterial β-lactamases may not be that critical.

The aims of this study were to calculate the pharmacokinetic parameters, and to identify parameters that may influence the observed differences in absorption of clavulanic acid and amoxicillin, following administration of four different dosage formulations.

Materials and methods

Experimental design

The evaluation was based on the data from plasma concentration–time curves obtained from four different open, randomized, two-treatment, two-period, two-sequence, crossover Phase I bioequivalence studies, each involving 36 male subjects. Subjects were treated with any of the following co-amoxiclav formulations, given as a single dose: tablets 250/125, 500/125 and 875/125 mg, or 10 mL of an oral suspension 250/62.5 mg per 5 mL, and data from 144 subjects and 288 drug administrations were available for evaluation. Treatments were separated by a 1 week washout period, and an additional period of 1 week allowed, except for 2 h before until 4 h after dosing. Smoking was not allowed, except for 2 h before until 4 h after dosing.

Trial course

For each study, the subjects were divided randomly into two groups. Randomization was carried out by following the procedure PLAN of the SAS Institute (Cary, NC, USA).

Group 1 was assigned to treatment sequence I–II (formulation I followed by formulation II). Group 2 was assigned to sequence II–I. Subjects received each of the following two formulations following an overnight fast.

Study 1. Formulation A1 = single oral dose of one co-amoxiclav 250/125 mg tablet [Losan Pharma; 574 mg amoxicillin trihydrate and 148.9 mg potassium salt of clavulanic acid], and formulation B1 = single oral dose of one Augmentin 375 mg film-coated tablet (Beecham Research, UK). Thirty-six healthy Caucasian male volunteers without any co-medication (age 26 ± 5 years, height 182.1 ± 5.8 cm, body weight 78.6 ± 8.4 kg).

Study 2. Formulation A2 = single oral dose of one co-amoxiclav 500/125 mg tablet (Losan Pharma; 574 mg amoxicillin trihydrate and 148.9 mg potassium salt of clavulanic acid), and formulation B2 = single oral dose of one Augmentin 625 mg film-coated tablet (Beecham Research). Thirty-six healthy Caucasian male volunteers without any co-medication (age 26 ± 5 years, height 183.3 ± 6.9 cm, body weight 78.9 ± 8.6 kg).

Study 3. Formulation A3 = one single oral dose of 10 mL co-amoxiclav 250/62.5 mg/5 mL oral suspension, equivalent to 500 mg amoxicillin and 125 mg clavulanic acid (Losan Pharma; 574 mg amoxicillin trihydrate and 148.9 mg potassium salt of clavulanic acid), and formulation B3 = 10 mL suspension of Augmentin 250/62 SF oral suspension (Beecham Research). Thirty-six healthy Caucasian male volunteers without any co-medication (age 28 ± 4 years, height 181.8 ± 7.1 cm, body weight 79.7 ± 8.2 kg).

Study 4. Formulation A4 = single oral dose of one co-amoxiclav 875/125 mg tablet [Cimex AG Pharmaceutika, Liesberg, Switzerland (1004 mg amoxicillin trihydrate and 148.9 mg potassium salt of clavulanic acid), and formulation B4 = single oral dose of one Augmentin 1 g tablet (SmithKline Beecham, Austria). Thirty-six healthy Caucasian male volunteers without any co-medication (age 28 ± 4 years, height 181.8 ± 7.1 cm, body weight 79.7 ± 8.2 kg).

Drug administration

Before drug administration, subjects fasted for at least 10 h. Fasting was continued until 4 h after dosing. Subjects were free to drink water, low-fat milk, apple juice, diluted orange juice, coffee and tea after the initial 4 h. All subjects received the same standardized low-fat lunch (6 h) and light snack (9 h) after drug administration.

Blood sampling

On the day of dosing, a physician inserted an indwelling Venflon 2 intravenous cannula in a forearm vein of each subject. The cannula was removed after withdrawal of the 12 h post-dosing sample. Blood samples (5 mL) were collected in heparinized glass tubes just before dosing and at 0.25, 0.50, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 4, 5, 6, 8, 10 and 12 h after dosing. The blood samples were centrifuged at 3280 g for 10 min and plasma was stored at −70°C until analysis.

Bioanalysis

Plasma amoxicillin and clavulanic acid concentrations were determined using validated methods such as LC/MS/MS analysis (GTF).12,13 Lower limit of quantification (LOQ) values were, respectively, 20.0 and 50.0 ng/mL.
In brief, mass spectrometric positive ion detection (selected reaction monitoring, SRM) was achieved by precursor [M+H]+→product ion for amoxicillin m/z 366→208, and for the internal standard m/z 350→160. For clavulanic acid, the precursor [M+H]+→product ions were m/z 198→108, and for the internal standard m/z 232→140.

The inter-day precision and accuracy (relative error) of the back-calculated calibration standards of amoxicillin, the inter-day precision ranged from 3.6% to 9.2% with an inter-day accuracy (relative error) between −4.3% and 6.7%. The intra-day precision and relative error of the amoxicillin assay ranged from 2.7% to 5.4%, with an inter-day accuracy (relative error) between −6.1% and 5.0%.

The inter-day precision and accuracy (relative error) of the back-calculated calibration standards of clavulanic acid ranged from 0.7% to 4.6% and from −3.1% to 6.5%, respectively. For the spiked quality control standards of amoxicillin, the inter-day precision ranged from 0.7% to 4.6% and between −3.1% and 6.5%, respectively. For the spiked quality control standards of clavulanic acid, the inter-day precision ranged from 2.1% to 9.1% and between −6.1% and 5.0%.

The inter-day precision and accuracy (relative error) of the back-calculated calibration standards of clavulanic acid ranged from 0.7% to 4.6% and from −3.1% to 6.5%, respectively. For the spiked quality control standards of amoxicillin, the inter-day precision ranged from 3.6% to 9.2% with an inter-day accuracy (relative error) between −4.3% and 6.7%. The intra-day precision and relative error of the amoxicillin assay ranged from 2.1% to 9.1% and between −6.1% and 5.0%.

The inter-day precision and accuracy (relative error) of the back-calculated calibration standards of clavulanic acid ranged from 0.7% to 4.6% and from −3.1% to 6.5%, respectively. For the spiked quality control standards of amoxicillin, the inter-day precision ranged from 3.6% to 9.2% with an inter-day accuracy (relative error) between −4.3% and 6.7%. The intra-day precision and relative error of the clavulanic acid assay ranged from 0.7% to 2.6% and between −0.5% and 6.8%.

**Pharmacokinetics**

Based on the plasma amoxicillin and clavulanic acid concentrations of individual subjects, the following pharmacokinetic parameters were determined by non-compartmental analysis using WinNonlin Professional (Version 2.0, Pharsight Corporation, copyright 1994–1998, Palo Alto, CA, USA): maximum plasma drug concentration (C_{max}, mg/L); time to reach C_{max} (T_{max}, h); area under the plasma concentration–time curve until the last measurable concentration (C_{t}), calculated by the linear trapezoidal method (AUC_{t}, mg·h/L); elimination half-life associated with the terminal slope of a semi-logarithmic concentration–time curve (t_{1/2}, h), calculated as ln2/λ_z, where λ is the elimination rate constant; means of the individual AUC_{t} data of clavulanic acid were calculated over the intervals of 1 AUC_{t} unit (mg·h/L) and correlated for variation in amoxicillin AUC_{t} and demographic data.

**Statistical analysis**

Analysis of variance (ANOVA) was carried out, and significance was defined at \( P \leq 0.05 \).

**Results**

The mean (± S.D.) plasma concentration–time curves of amoxicillin and clavulanic acid in 36 healthy male subjects after oral administration of 500/125 mg co-amoxiclav tablets (formulation A2) are shown in Figure 1. The other dose formulations gave similar-shaped concentration–time curves. The half-life of elimination of amoxicillin was ~1.46 h, and that of clavulanic acid was ~1.08 h for all dosages. Absorption of all formulations was fast, although the T_{max} of amoxicillin increased with the dose, from 1.14 ± 0.41 h at 250 mg to 2.04 ± 1.01 h (\( P < 0.0001 \)) at 875 mg (Table 1). The AUC_{t} values of

![Figure 1. Mean (± S.D.) plasma concentration–time curves of amoxicillin (Amoxi) and clavulanic acid (Clav) after an oral dose of 500/125 mg co-amoxiclav tablets in 36 healthy volunteers (formulation A2).](image)

**Table 1. Pharmacokinetic parameters of amoxicillin (mean ± S.D.)**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dose (mg)</th>
<th>t_{1/2} (h)</th>
<th>T_{max} (h)</th>
<th>C_{max} (mg/L)</th>
<th>AUC (mg·h/L)</th>
<th>AUC (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>250</td>
<td>1.43 ± 0.18</td>
<td>1.14 ± 0.41</td>
<td>4.33 ± 1.07</td>
<td>10.1 ± 1.59</td>
<td>7.26–14.3</td>
</tr>
<tr>
<td>A2</td>
<td>500</td>
<td>1.46 ± 0.16</td>
<td>1.46 ± 0.46</td>
<td>6.81 ± 1.94</td>
<td>18.3 ± 3.55</td>
<td>8.63–24.1</td>
</tr>
<tr>
<td>A3</td>
<td>500</td>
<td>1.54 ± 0.17</td>
<td>1.14 ± 0.37</td>
<td>7.59 ± 1.45</td>
<td>19.2 ± 3.29</td>
<td>10.9–25.4</td>
</tr>
<tr>
<td>A4</td>
<td>875</td>
<td>1.76 ± 0.36</td>
<td>2.02 ± 0.96</td>
<td>9.93 ± 3.14</td>
<td>32.8 ± 7.05</td>
<td>19.4–48.7</td>
</tr>
<tr>
<td>B1</td>
<td>250</td>
<td>1.40 ± 0.18</td>
<td>1.39 ± 0.60</td>
<td>3.88 ± 1.15</td>
<td>9.87 ± 1.68</td>
<td>6.31–13.7</td>
</tr>
<tr>
<td>B2</td>
<td>500</td>
<td>1.48 ± 0.23</td>
<td>1.70 ± 0.70</td>
<td>6.34 ± 1.63</td>
<td>18.3 ± 3.43</td>
<td>5.15–22.9</td>
</tr>
<tr>
<td>B3</td>
<td>500</td>
<td>1.51 ± 0.17</td>
<td>1.14 ± 0.31</td>
<td>7.19 ± 1.29</td>
<td>18.9 ± 2.83</td>
<td>10.9–24.1</td>
</tr>
<tr>
<td>B4</td>
<td>875</td>
<td>1.71 ± 0.34</td>
<td>2.04 ± 1.01</td>
<td>9.77 ± 3.17</td>
<td>32.9 ± 8.15</td>
<td>14.3–47.8</td>
</tr>
</tbody>
</table>
Amoxicillin increased proportionally with the dose, from 10 mg·h/L at 250 mg to 33 mg·h/L at 875 mg. Table 2 shows the means (± S.D.) of the pharmacokinetic parameters of clavulanic acid after the four oral dosages with the two formulations; no differences in the pharmacokinetic parameters were seen between the different formulations.

Figure 2 shows a plot of $AUC_t$ amoxicillin against $AUC_t$ clavulanic acid after oral administration of 500/125 mg co-amoxiclav tablets (formulations A2 and B2). Although there was a five-fold variation in the $AUC_t$ of clavulanic acid with both formulations, the $AUC_t$ of amoxicillin only varied by a factor of 1.2. The other formulations gave similar results (data not shown).

The plots of $AUC_t$ amoxicillin versus $AUC_t$ clavulanic acid after three oral administrations of 250/125, 500/125 and 875/125 mg co-amoxiclav tablets, and the 500/125 mg co-amoxiclav oral suspension (formulations A+B), are shown in Figure 3. For each of the four dosages studied there was relatively little variation in the mean AUCs observed for amoxicillin, yet in excess of a five-fold variation in those of clavulanic acid.

### Discussion

After intravenous administration, the $V_{ss}$ of amoxicillin has been reported as $0.26 ± 0.06$ L/kg, and the $V_{ss}$ of clavulanic acid as $0.22 ± 0.06$ L/kg, giving a ratio for the volume of distribution between clavulamic acid and amoxicillin of 0.85. Consequently, for a co-amoxiclav dose of 500/125, and a $V_{ss}$ ratio of 0.85, the ratio of amoxicillin to clavulamic acid $AUC$s should be 3.4. In this study, the lowest $AUC_t$ amoxicillin/clavulanic acid ratio observed was $2.7 ± 0.5$ at the

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**Table 2. Pharmacokinetic parameters of clavulanic acid (mean ± S.D.)**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dose (mg)</th>
<th>$t_{1/2}$ (h)</th>
<th>$T_{max}$ (h)</th>
<th>$C_{max}$ (mg/L)</th>
<th>$AUC$ (mg·h/L)</th>
<th>$AUC$ (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>125</td>
<td>1.00 ± 0.16</td>
<td>1.24 ± 0.48</td>
<td>2.63 ± 0.90</td>
<td>5.49 ± 1.48</td>
<td>2.32–8.25</td>
</tr>
<tr>
<td>A2</td>
<td>125</td>
<td>1.08 ± 0.16</td>
<td>1.17 ± 0.22</td>
<td>2.32 ± 0.83</td>
<td>5.13 ± 1.80</td>
<td>1.24–8.32</td>
</tr>
<tr>
<td>A3</td>
<td>125</td>
<td>1.19 ± 0.29</td>
<td>1.10 ± 0.40</td>
<td>2.99 ± 0.74</td>
<td>6.74 ± 1.43</td>
<td>3.68–9.74</td>
</tr>
<tr>
<td>A4</td>
<td>125</td>
<td>0.98 ± 0.11</td>
<td>1.18 ± 0.30</td>
<td>2.68 ± 0.96</td>
<td>5.85 ± 2.15</td>
<td>1.25–8.93</td>
</tr>
<tr>
<td>B1</td>
<td>125</td>
<td>1.01 ± 0.14</td>
<td>1.29 ± 0.59</td>
<td>2.31 ± 1.09</td>
<td>4.79 ± 1.83</td>
<td>1.02–8.79</td>
</tr>
<tr>
<td>B2</td>
<td>125</td>
<td>1.09 ± 0.17</td>
<td>1.24 ± 0.31</td>
<td>2.03 ± 0.88</td>
<td>4.28 ± 1.76</td>
<td>1.32–7.90</td>
</tr>
<tr>
<td>B3</td>
<td>125</td>
<td>1.15 ± 0.18</td>
<td>1.09 ± 0.37</td>
<td>2.80 ± 0.67</td>
<td>6.28 ± 1.26</td>
<td>2.79–8.69</td>
</tr>
<tr>
<td>B4</td>
<td>125</td>
<td>0.97 ± 0.08</td>
<td>1.20 ± 0.44</td>
<td>2.67 ± 1.12</td>
<td>5.81 ± 2.25</td>
<td>0.84–9.16</td>
</tr>
</tbody>
</table>

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**Figure 3.** Mean $AUC_t$s of amoxicillin plotted versus the mean $AUC_t$s of clavulanic acid for the oral administration (A/B formulations) of co-amoxiclav suspension (black circles), and co-amoxiclav oral tablets 500/125 mg (white circles), oral tablets 875/125 mg (white triangles) and oral tablets 250/125 mg (black triangles). Dotted lines represent the 95% confidence intervals.
Absorption of clavulanic acid from co-amoxiclav

lower doses used, suggesting equivalent absorption of both amoxicillin and clavulanic acid. Higher amoxicillin/clavulanic acid AUC ratios (in the present studies up to 12), as seen with the 875/125 formulation, would suggest that with a similar amoxicillin absorption, clavulanic acid absorption must have been reduced. It has been reported that the absolute bioavailability of clavulanic acid, when co-administered with amoxicillin, ranged from 31.4% to 98.8%. Similar findings have been reported by Witkowski et al., who found that although the mean AUC of 125 mg clavulanate was not affected by co-administration of 500 mg amoxicillin, the coefficient of variation for the AUC increased from 27.6% for clavulanic acid alone to 45.6% when given with amoxicillin. In contrast, in other studies where clavulanic acid was administered alone, the mean absorption was 97% with little inter-patient variability, suggesting an interaction between the absorption of amoxicillin and clavulanic acid. This is supported by data from studies where co-amoxiclav has been given as an intravenous infusion. In these studies, a dose ratio of 10:1 (50/5 mg/kg) has been used for intravenous administration, which results in an AUC ratio of 10 and an identical plasma clearance of both amoxicillin and clavulanic acid, with no evidence of a pharmacokinetic interaction between the two agents.

The AUC observed for amoxicillin in this study did not show significant variation either between subjects, based on demographic data, or between formulations, once corrected for the dose. In contrast, high variability was seen between subjects in the AUC of clavulanic acid, with values ranging over a five-fold difference. All subjects in this study were healthy males (with normal renal function), and it is difficult to explain the high variability seen in the clavulanic acid AUC on patient factors. However, similar findings to these have been reported in other studies.

With the highest dosage of amoxicillin (875 mg) used in this study, the T_max broadened, which would suggest a rate-limiting step in the absorption process, in line with other studies. Of interest, in Figure 3 the AUC_amoxicillin/AUC_clavulanic acid regression curve of amoxicillin 875 mg has a negative slope, which may indicate that the saturable absorption seen for amoxicillin is influenced by the presence of clavulanic acid. This effect is reported to be bigger with higher dosages of amoxicillin, of up to 3 g in humans, or at even higher doses of 25 mg/kg in cats and dogs. In contrast, the slopes of the regression lines of the 250/125 and 500/125 mg dosages were all positive, indicating no influence of clavulanic acid administration on the absorption of amoxicillin at these dosages.

In this study, we have been able to show that four different co-amoxiclav formulations each gave a five-fold variation in the absorption, or in the AUC, value, of clavulanic acid for the same 125 mg dose. This would suggest that in some patients only a 20:1 amoxicillin/clavulanic acid AUC ratio is achieved after the oral dose of 500/125 mg co-amoxiclav, yet they still appear to benefit from the presence of clavulanic acid (as therapy failures are rarely reported). For patients receiving the 875/125 mg dosage, our findings would indicate that the individual AUC ratio may vary up to 35:1. However, as the clinical efficacy of co-amoxiclav has been maintained for >10 years with an apparent excess of clavulanic acid in fixed-dose combination preparations, it is probable that the absolute amount of clavulanic acid administered to patients is more important than maintaining a minimal plasma concentration ratio of the two agents.

In conclusion, the observed variations in the AUC ratio of amoxicillin/clavulanic acid (2–10:1) highlight the variable nature of clavulanic acid absorption. However, clinical data would suggest that such variability does not affect the efficacy of co-amoxiclav and that the current dosage ratio of 4:1 may be considered as conservative.

References


