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The pharmacokinetics of amoxicillin in healthy adult Nigerians

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ABSTRACT

Using a two stage approach, the population pharmacokinetics of amoxicillin was determined in 17 healthy male volunteers of the Hausa tribe of north-western Nigeria. Subjects were given a single dose of 500mg of Amoxicillin capsule at 0800 hrs after overnight fasting and plasma samples were collected at 0.25 to 12 hrs after drug ingestion. Ampicillin concentration in plasma was determined using microbiological assay. Pharmacokinetic data analysis was done using PK solutions and GraphPad prism. The results were compared with published values. Multiple dose pharmacokinetic parameters were also simulated from the results of the single dose. The study revealed that the elimination half-life of amoxicillin in the studied population was 0.55hrs while the trough concentrations at steady state was predicted as zero, both of which are significantly lower than literature values. These results suggest that it may be important to increase the dose levels or reduce the dose intervals of amoxicillin in the Hausa tribe.

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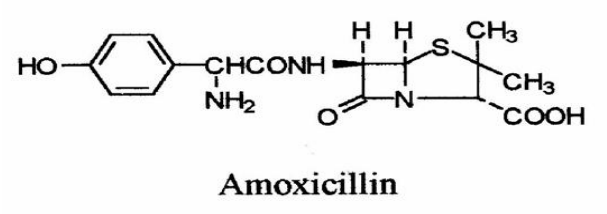
INTRODUCTION

Amoxicillin is a semi synthetic, amino penicillin type, ß -lactam antibiotic with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms.



Amoxicillin

It is used in a variety of infectious diseases including pneumonia, tonsillitis, otitis media, gonorrhoea, enteric fever [1] and Helicobacter pylori eradication [2]**.** Amoxicillin is presently the most commonly used antibiotic [3]. The recommended oral adult dose is 250mg to 500mg every 8 hrs [4]. This oral dose is accepted to give acceptable pharmacokinetic (PK) parameters to meet in vivo challenges [1]. Anecdotal evidence suggests high rate of treatment failure with amoxicillin in north-western Nigeria. This failure appears to remain high when quality amoxicillin is administered, suggesting that factors beyond adulterated drugs might be at play. Pharmacokinetic difference may be one such factor. Most PK studies on amoxicillin on which dose regimens have been based were conducted in Caucasians. The Hausa tribe constitute the majority of the inhabitants of northern Nigeria, especially north-western Nigeria [5]. It is known that population difference in PK could be large and clinically important. This study was therefore conducted to define the population pharmacokinetics of orally administered amoxicillin in Hausa tribe of north western Nigeria.

METHODS

Volunteers

Seventeen healthy non smoking male volunteers (age range, 18-32 years; mean body weight, 69.0 ±4.2 kg; mean body mass index± SD = 23.1 ± 4.1 kg/m^2 ; mean creatinine clearance, 123±13.5 ml/ min/1.73 m^2) participated in the study. All subjects declared both parents as Hausas and Nigerians. This claim was verified by one of the authors (HU) who has household knowledge of all the volunteers. Before entering the study, one of the authors (HU) assessed all volunteers for fitness by taking relevant history and performing a full physical examination. Volunteers with history of penicillin allergy or who take alcohol were excluded. Serum electrolyte, urea and creatinine were then evaluated in all subjects to confirm satisfactory renal function. Subjects were instructed to avoid antibiotics and beverages for 14 days before the study. Approval was obtained from the local ethics committee and all subjects gave written informed consent. The study was conducted in line with the Helsinki declarations on human subjects and good clinical and laboratory practice.

Study design

In this stand-alone study protocol**,** the full population pharmacokinetic design (also known as the experimental PK screen) was used. This design is considered to have the highest content of information [6]. At 0800 hrs after overnight fasting, each volunteer received a single oral 500 mg amoxicillin as the trihydrate (GlaxoSmithKline, UK). Food was allowed 4 hours after ingestion of amoxicillin.

Sample collection and processing

Blood samples (5ml) were taken from a peripheral vein before drug administration and then at 0.25, 0.5, 0.75,1, 1.5, 2, 4, 8, and 12 hours after dosing. All samples were collected into heparinised tubes and within 10 minutes centrifuged at 3000rpm for 15 min. Plasma samples were then collected and stored at -4^{0}C prior to analysis.

Microbiological assay

Amoxicillin standards were prepared in normal pooled serum. The concentrations of amoxicillin in plasma were measured by validated agar well-diffusion microbiological assay as described by Reeves and Bywater [7].The Zones of inhibition of the growth of St*reptococcus pneumonia* in six replicates per known concentrations of amoxicillin were read to the nearest 0.1 mm with a vernier calliper. These were then used to describe a linear regression equation for the assay.

Pharmacokinetic Analysis

Population pharmacokinetic was determined using the traditional two stage approach. Briefly, each subject's dense concentration-time data was used to estimate individual pharmacokinetic parameters. Individual parameters were then input for estimating descriptive summaries statistics of the study sample including means, variance and co-variance. Concentration-time data was analysed with PK Solutions 2.0 software (Summit Research Services, Montrose, Colorado, USA). The area under the curve described by concentration-time plot was determined using the linear trapezoidal rule. Volume of distribution (V_d), and clearance (CL) were also estimated. Other PK parameters were obtained by stripping the concentration-time plots and fitting exponential curves (the least square method).

To explore the implication of the single dose PKs, estimates of multiple dose PKs were calculated by imputing 8hrs dosing interval (tau) into the PK solution software. This multiple dose simulation assumes linear pharmacokinetics, consistent absorption and systemic clearance, and that the repeated doses are given during the elimination phase. Though simplistic, these assumptions appear tenable with Amoxicillin. Alternative dosing intervals of 4hrs and 6hrs were also simulated. Craig and Andes [8] reported bacteriologic cure rates of 80% to 85% when the time (T) the plasma concentration is above minimum inhibitory concentration (MIC) (i.e. T > MIC) for beta lactams and macrolides was above 40% to 50% of the dosing interval. Also, several studies have shown that plasma concentration levels present for 40% to 50% of the dosing interval may be used to determine a break point for a dosing regimen [9, 10]. Others have recommended T >MIC 60% [11]. We, therefore, sought

to explore the serum concentrations at steady state at 50%, 60%, 90% and 100% when tau was 8, 6 and 4 hrs.

Statistical Analysis

GraphPad Prism version 5.00 for Windows (GraphPad Software, California, USA) was used for all descriptive statistical analyses. The confidence intervals about the geometric mean of the Pk parameters were also determined using GraphPad Prism..

RESULTS

Accuracy of the microbiological assay used

Figure 1 shows the log concentration-response relationship and the best straight line predicted from data obtained by microbiological assay for standard preparations of amoxicillin. The representative linear equation was: y=0.7028x +1.662 where y = zone diameter (mm) and x = log of concentration (µg/mL). The R-square was 0.978, standard error of the estimate (Sy.x) was 0.092.The slope and intercept were statistically significant ($P < 0.0001$). The 95% confidence interval of the slope and intercept were 0.6093-0.7963 and 1.581 to 1.727 respectively, which are reassuringly narrow. The relationship between the diameters of the zone of inhibition of bacterial growth and the logarithm of drug concentration was linear over the concentration range of 0.06 to 15.6µg/mL. Coefficient of variation for the high standards was 6.9% while that for the low standards was 4.2%.

Pharmacokinetic parameters

The plasma decay curve after a single dose of amoxicillin is shown in Figure 2, with values presented as mean plus 95% confidence interval. The population pharmacokinetic data are summarized in Table 1, with values expressed as mean±SD rather than SEM to display biologic variability within group rather than assay precision. The predicted multiple dose pharmacokinetic parameters are shown in Table 2 and the predicted curve is shown in Figures 3 to 5. From the simulation, at steady state, a tau of 8hrs gives plasma concentration of 1.5µg/mL at 4hrs (50% of tau), 0.8µg/mL at 4.8hrs (60% of tau) 0.1 µg/mL at 7.2hrs (90% of tau) and 0 at 8hrs (100% of tau) (Figure 3). Also**,** a tau of 6hrs gives plasma concentration of 3.3µg/mL at 3hrs (50% of the tau), 2.1µg/mL at 3.6hrs (60% of tau), 0.5 µg/mL at 5.4 hrs (90% of tau) and 0.3 µg/mL at 6hrs (100% of tau)(figure 4). However, a tau of 4hrs gives plasma concentration of 6.1µg/mL at 2hrs (50% of the tau) , 4.9 µg/mL at 2.4hrs (60% of tau) , 2.1 µg/mL at 3.6 hrs (90% of tau) and 1.5µg/mL at 4hrs (100% of tau)(Figure 5)

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Table 1. Single dose population pharmacokinetic parameters of a 500mg oral dose of amoxicillin

Pharmacokinetic parameters	Amoxicillin in this study (Mean±SD)	95% CI	Amoxicillin Literature values	95% CI	Reference
AUC_{0-t} (µg-hr/mL)	17.9 ±1.15	16-21	21.7	14.1-33.8	de Abreu[3]
$AUC_{0-\infty}$(µg-hr/mL)	17.9±1.15	16-21	24.3	15.6-38.8	de Abreu[3]
$t_{1/2\ A\ phase}$ (hr)	0.550±0.12	0.5-0.8	0.72 ± 0.12	NA	Arancibia[12]
$t_{1/2\ D/A\ phase}$ (hr)	0.551±0.10	0.49-0.72	0.72	NA	Arancibia[12]
$t_{1/2\ E\ phase}$ (hr)	0.553±0.16	0.44-0.73	1.3	0.8-3.1	de Abreu[3]
C_{max} (µg/mL)	8.4±2.58	5.1-11.5	8.1	5.1-12.1	de Abreu[3]
T_{max}	1.50±0.0	NE	1.7	1.0-3.0	de Abreu[3]
V_d (ml/kg)	319.1±66.29	290-396	460	NA	Spyker[13]
CL (ml/hr/kg)	399.6±43.02	335-401	245	240-320	Zarowny.[14]

AUC_{0-t}, area under the concentration–time curve from zero up to a definite time t; $AUC_{0-\infty}$, area under the concentration–time curve from zero up to infinite time; $t_{1/2\ A\ phase}$, half-life of absorption phase;$t_{1/2\ D/A\ phase}$, half-life of distribution phase; $t_{1/2\ E\ phase}$, half-life of elimination phase; C_{max} ,maximum plasma concentration; t_{max}, time to reach maximum plasma concentration; V_d, volume of distribution; CL, total clearance; NA, Not available; SD, standard deviation.

Table 2. Predicted multiple dose pharmacokinetic parameters of amoxicillin at 8 hrs dose interval

Steady state parameters:	Amoxicillin (Mean±SD)	95% CI
$Css_{(max)}$ µg/mL	7.2±2.12	5.5-7.76
$Css_{(min)}$ µg/mL	0	NE
$Css_{(ave)}$ µg/mL	1.6±0.63	1.12-2.14
T_{max}(ss) (hr)	0.80±0.22	0.61-1.11

$Css_{(max)}$, Maximum concentration during dosing interval at steady state; $Css_{(min)}$, Minimum concentration during dosing interval at steady state; $Css_{(ave)}$, Average concentration at steady state based on exponentials; T_{max}(ss), Calculated Tmax at steady state; NE= Not estimated; SD, standard deviation.

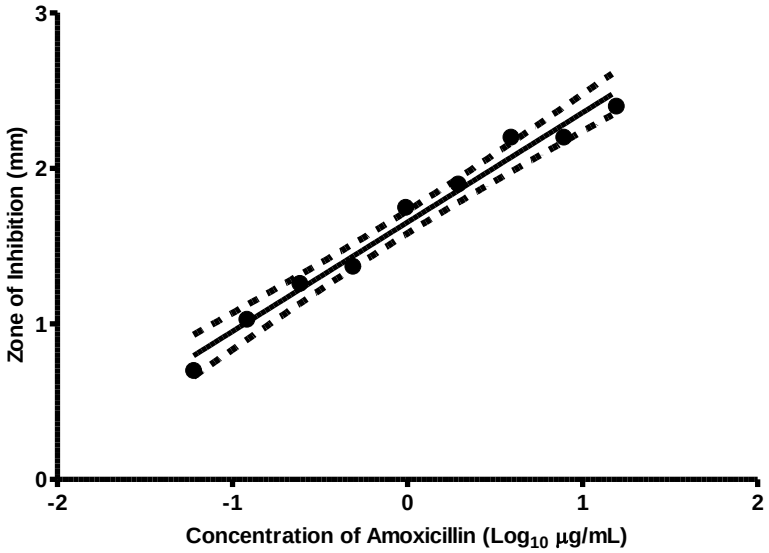

Figure 1. Calibration curve of inhibition of the growth of *Streptococcus pneumonia* (indicator) by amoxicillin
The 95% confidence band has been included as broken lines.

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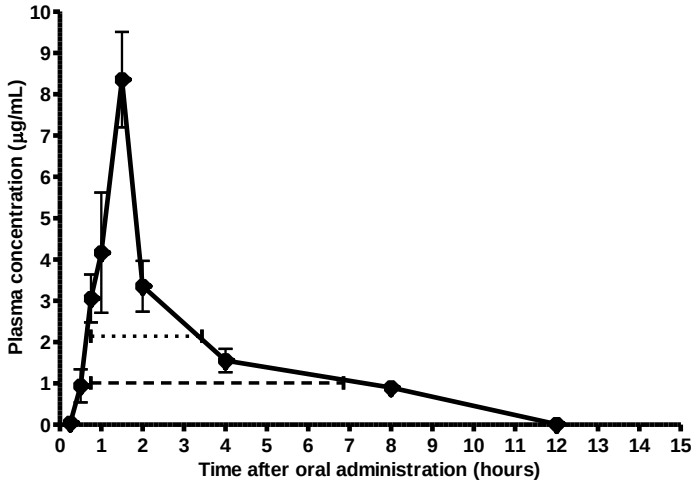

Figure 1. Mean plasma concentrations of amoxicillin versus time

The error bars represent 95% confidence interval. In this curve, the plasma concentration is above 2µg/mL for 3.5 hrs (dotted caped line) or 43.8% of the dosing interval and above 1µg/mL for 7hrs (broken capped line) or 87.5%of the dosing interval.

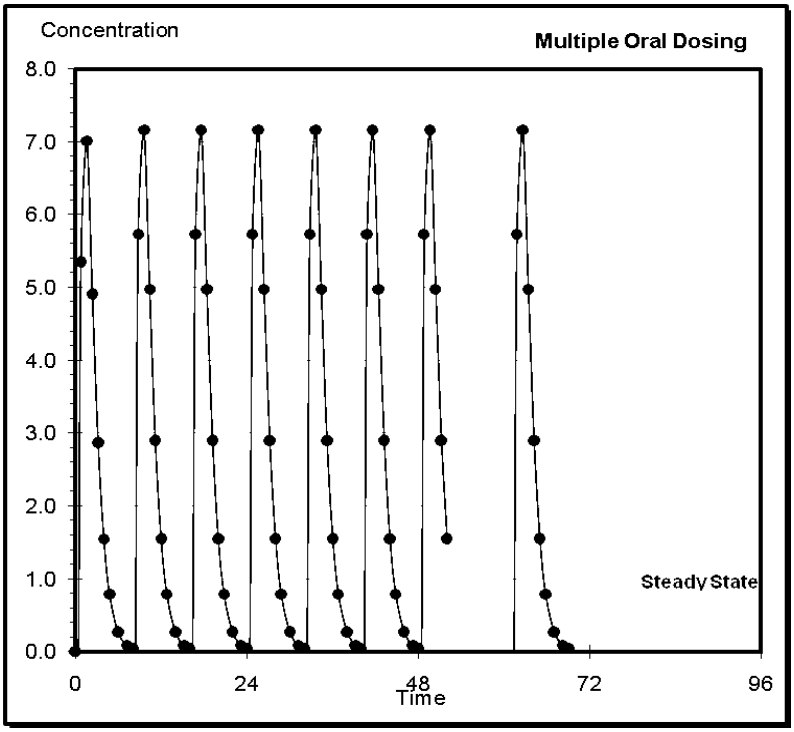

Figure 3. Predicted multiple dose decay curves of Amoxicillin when administered at the standard 8 hrs dose interval

Given the population pharmacokinetic profile determined in this study, a dose interval (tau) of 8hrs gives a Css(min) of 0 or undetectable level and Css(max) of 7.2 µg/mL respectively . The Css(min) is well below the MIC of most susceptible organism. Steady state is reached in 72 hrs.

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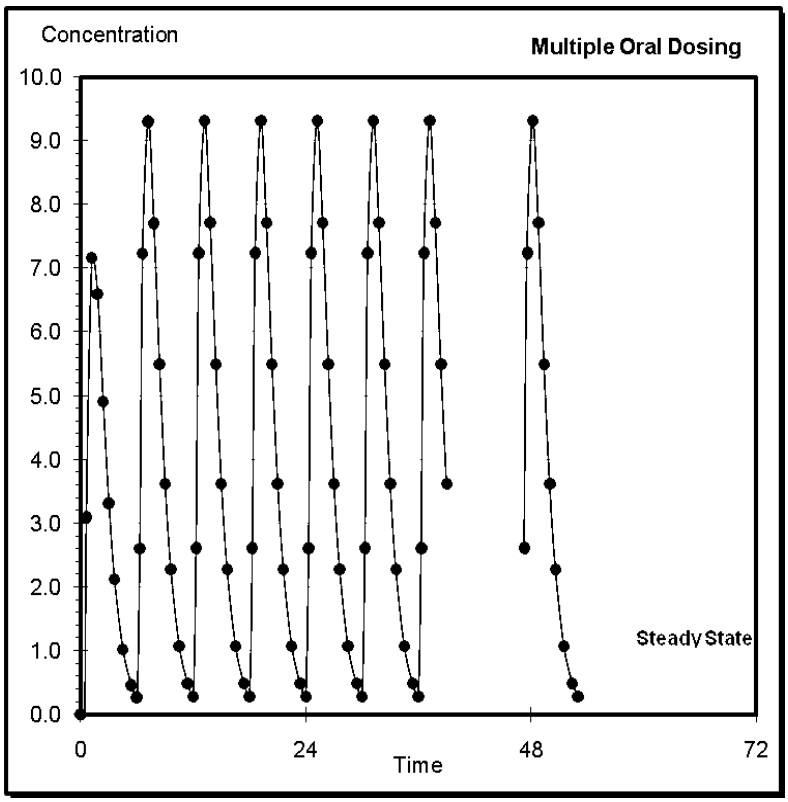

Figure 4. Predicted multiple dose decay curves of Amoxicillin when administered at 6 hrs dose interval (tau)
Given the population pharmacokinetic profile determined in this study, a dose interval(tau) of 6hrs appears superior to 8hrs because it gives a Css(min) of 0.3 µg/mL and Css(max) of 9.3 µg/mL respectively which are multiples of the MIC of 1.0 µg/mL shared by most sensitive micro-organisms and are still within toxicologically safe margins. Steady state is reached in 53 hrs.

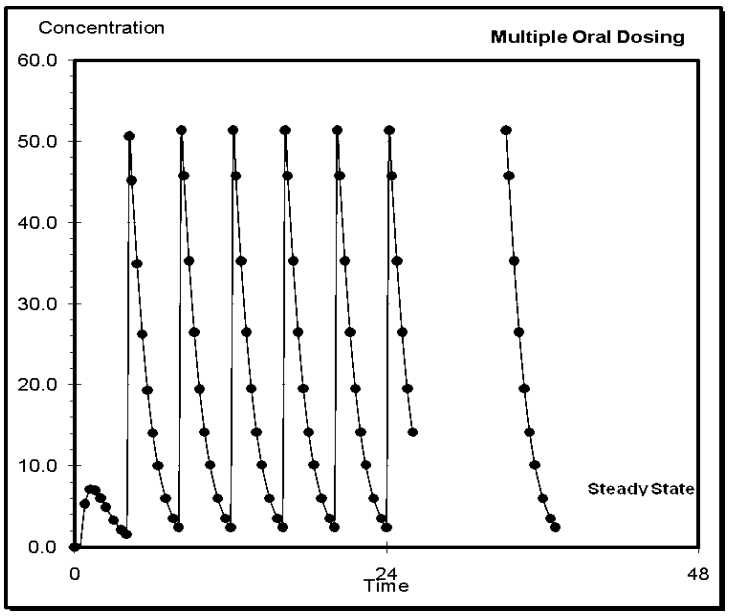

Figure 5. Predicted multiple dose decay curves of Amoxicillin when administered at 4 hrs dose interval (tau).
Given the population pharmacokinetic profile determined in this study, a dose interval(tau) of 4hrs appears to be optimal because it gives a Css(min) of 2.5 µg/mL and Css(max) of 51.2µg/mL respectively which are multiples of the MIC of 1.0 µg/mL shared by most sensitive micro-organisms and are still within toxicologically safe margins. Steady state is reached in just 37 hrs.

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DISCUSSION

The results of this study demonstrate that the pharmacokinetic profile of oral 500mg amoxicillin in the studied population is within the mean and 95% confidence interval of literature values except the elimination half life which is half of literature values. Also, the predicted steady state through concentration at the standard tau of 8 hrs is zero. These have important clinical implication because the elimination half-life and trough plasma concentrations dictate the dosing interval. The MIC for amoxicillin of most isolates of sensitive *Streptococcus pyogenes* ranges from 0.015–0.12 µg/mL [15] and that of moderately resistant *Streptococcus pneumoniae* is 0.1–1.0 µg/mL [16]. These suggest that a target plasma concentration above 1.0 µg/mL for 60% of the dosing interval would be desirable for bacteria exposed directly in the plasma, like in bacteraemia, but a higher value would be desirable for tissue infections. From the result of this study, this is only achievable with a 6hrs dosing interval in the population under consideration. Furthermore, this study predicts that a 4hrs dosing interval is what is required to cover for most gram positive enterococcuss whose MIC breakpoints are 8 µg/mL or less [17]. In fact, for gram negative enterobacteriacea where MIC of 8-16 µg/mL is considered the break point for intermediate susceptibility [18], more frequent dosing may be required. However, ß-lactam antibiotics run linear PKs [3] whereby all the parameters are directly proportional to the dose. Dose increase is therefore an option and may be a preferred option due to the known preference of patients for less frequent dosing and the unlikely situation of dose intervals less than 4hrs. The result of this study may have exposed one of the reasons for amoxicillin treatment failures in north-western Nigeria.

CONCLUSION

Clinically important difference in the elimination half life and trough plasma concentration of oral 500mg amoxicillin appears to exist in adult north-western Nigerian men of Hausa tribe. A dose interval of 6hrs appears to be more appropriate in this population but higher dose levels may be preferred.

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Author contribution

Both Bello S.O and Hayyatu Umar designed the research. Hayyatu Umar carried out the work while Bello S.O analysed the data and wrote the manuscript. Hayyatu Umar reviewed the manuscript.

Conflicts of interest

We declare no conflicts of interest

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