Bioequivalence of Two Formulations of Amoxicillin in Human Healthy Volunteers on (HPLC) Technique

Alaa K. Jabbar Alhamd

Abstract

Amoxicillin is commercially available in the form of capsules and containing 250mg or 500mg for oral administration. It is also available in the form of suspension containing "25mg/ml". Amoxicillin is presently used as the most common antibiotics. Ten healthy human volunteers were characterized respected to their pharmacokinetic and bioavailability of two formulations of Amoxicillin from two sources of industrial companies after a single dose administration was given orally. A procedure is described for determination the concentration levels of Amoxicillin in human plasma of healthy volunteers using high performance liquid chromatography (HPLC) with reversed-phase isocratic column at low wave length of UV-visible detection "230nm". An efficient drug extraction procedure was used for the separation of Amoxicillin after simple extraction with cold methanol using ODS-C18-DB column. The pharmacokinetic 500mg of Amoxicillin capsule orally administrated treatment through 10 hours has been examined. The Amoxicillin was eluted for "10.0 minutes" at flow rate "1.5mL/min." and Temperature equal to 298 K. The retention time of Amoxicillin was observed at 7.0 minutes. The mean absolute recovery of Amoxicillin in blood plasma of all healthy volunteers were 94.1% at 1.0ppm, 102% at 5.0ppm, 103% at 10.0ppm and 102% at 20ppm, 99.3% at 40ppm and 104% at 50ppm respectively. The assay showed excellent relationships between area under the curve ratios and drug concentration levels (P>0.002). Oral Amoxicillin administration in ten healthy volunteers gave maximum concentration peak plasma at two hours and decline through ten hours. Treatment with Iraqi formulation Amoxicillin produced higher area under the curve (“AUC”) and maximum concentration (“C (max)”) of Amoxicillin than Indian formulation.

Key word: Amoxicillin, Bioequivalence, ODS -DB column.

Introduction

Amoxicillin capsules and Amoxicillin suspensions were analyzed for their drug content as described in the united state pharmacopeia (1). Amoxicillin is the most commonly prescribed antibiotic for the treatment of pharyngitis in the US. (2,3) Pharmacokinetic and bioavailability may be vital to ensure successful protocol in the clinic as well as in researches. Often the clinical evaluation of drugs is carried out on the basis of some secondary response because of the non existence of directly measurable parameter which is related to the treatment of disease by the drugs (4). Many times no response is measured at all, and the clinician attempts to make objective and subjective assessment of the patients general welfare. A dosage regimen for a new drug may in fact be based on such an evaluation and may not include a comparison with a standard drug or analog, a dosage regimen based upon such studies can be only a rough approximation at best. This point is well illustrated if one compares drug dosage regimens (e.g. sulfonamides) calculated

1 Corresponding author E-mail: prof_alaaalahamd@yahoo.com
Received: 11/3/2009
Accepted: 28/12/2009
from pharmacokinetic data to those commonly used in the clinic. The same importance from this point of view does not concern only medicines but all other compounds including pharmaceuticals which are introduced to the organism for the diagnostic purposes. There is a through pharmacokinetic examination of a diagnostic agent which is extremely important from the point of view of decreasing of the possible risk. Pharmacokinetics are the studies of the movement of drugs in the body through the time course; it must not be hidden in mathematics itself. It is extremely important for everybody who is interested in pharmacotherapeutics in drug researches and in drugs productions to understand what the pharmacokinetics really means. Pharmacokinetic study of children that assessed the single-dose administration of an investigational oral Amoxicillin sprinkle designed to sequentially deliver an immediate-release and multiple delayed-release pulses of Amoxicillin to provide prolonged plasma concentrations of Amoxicillin. A new pharmacokinetically enhanced formulation of Amoxicillin has recently become available. The new formulation can maintain the main Amoxicillin serum concentration about 49% of the dosing interval in contrast with only 34% for three times daily regimen. The pharmacodynamic and pharmacokinetic properties of the new formulation predict high rates of success against respiratory tract pathogens. Amoxicillin (AMO) is oral semi-synthetic penicillin structurally related to Ampicillin as shown below:

The present of benzyl ring in the side chain extends the antibacterial activity to gram-negative bacteria. Amoxicillin presents as a highly absorption after oral administration and is not altered by the concomitant ingestion with food. Amoxicillin exhibits low binding with plasma proteins and is quickly distributed through the body. It has an elimination half life of one hour. Amoxicillin pharmacokinetics was obtained across pregnancy states. Oral clearance and renal clearance were higher while the half life was shorter during pregnancy, these changes suggest that the Amoxicillin exposure will be less while pregnancy that maintenance of trough concentrations will be difficult. A new per oral Amoxicillin \ Clavulanate therapeutic system was developed and evaluated by in vivo bioavailability study. Amoxicillin was effective in reducing oral micro organism level up to 12 hour post-dose. The treatment with Amoxicillin for 3 to 7 days had similar clinical efficiency and also similar selection of oral streptococci with reduced susceptibility to Amoxicillin. Amoxicillin is commercially available in the form of capsules and tablets (250 or 500 mg) for oral administration and also available in the form of suspensions containing 25 or 50 mg / ml. Amoxicillin is one as the most commonly used as antibiotic. To understand the bioavailability and pharmacokinetic behavior of this drug in human which is needed reliable qualitative and quantitative methods. There are several high performance liquid chromatography (HPLC) methods were used for separation and determination of Amoxicillin in body fluids. Some of these methods were developed to use direct UV-visible detection at low wave length (225-229nm). The bioavailability of two brands of melixican (7.5mg and 15mg) tablets and to obtained pharmacokinetic parameters of this molecules on Mexican population using modified and validated high performance liquid chromatography "HPLC" technique pharmacokinetic parameters AUC, C(max), and T(max) were determined from plasma concentration levels of both formulations that the results indicated in a C(max) 120% larger and T(max) 65% faster than those reported. Others were used fluorimetric detection. Some others special techniques have been used to enhance the sensitivity and selectivity such as ion-pairing reagents and post column derivatization. There are several different methods used to preparation the sample that have been applied prior to chromatographic analysis mostly based on...
Bioequivalence of two formulations of amoxicillin

Experimental Materials and methods

Chemicals and drugs
All chemicals used in this study were the highest analytical grade purchased from commercial sources and used without any further purification. The deionized distilled-water was used for all preparation. Methanol (Absolute MeOH) and acetonitrile (Absolute ACN) “HPLC grade” were purchased from (FLUKA). Amoxicillin capsules from two sources one from Iraq (S D I, Iraq ) and the other from India(MICRO LABS LIMITED ,Bangalore ,India) Potassium dihydrogen phosphate (KH$_2$PO$_4$), Dipotassium hydrogen phosphate (K$_2$HPO$_4$), Phosphoric acid and 1-octane sulphonic acid sodium salt were purchased from (BDH, England). Sepelco-ODS-C$_18$-DB column (250 X 4.6mm I.D.) was purchased from (sepelco, United Kingdom).

Standard solutions
Amoxicillin (1.0 mg) was dissolved in 100ml of freshly prepared mixture of water: methanol (95:5) “10000 ppm”. The standard solution was filtered, degassed and stored at 253 K for further use. The standards were prepared freshly every month. Stock solution of Amoxicillin (1mg/ml) was prepared in a mixture of (water: methanol) (95: 5). The applied standard solutions were prepared from stock solution by sequential dilution with the same mixture to produce final concentrations (1, 5, 10, 20, 40, 50ppm). The stock and applied solutions were protected from light and stored at 253 K. Calibration standard curve were performed to achieve the concentration of (0.1, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0ppm) Figure-1.

Figure 1: Linearity of different concentration levels of Amoxicillin using HPLC technique on ODS-DB column

Extraction of Amoxicillin
Blood sample (3–5ml) were drawn from vein by syringes in to hirparginized blood tubes, then transferred immediately into polypropene tubes and centrifuged within 5 min. at 500G for 15 min. One milliliter of sodium metabisulphate, (pH equal to 8.0) was added for each one milliliter of plasma. Amoxicillin was extracted from human plasma samples by deproteinization using precipitation process. A 500µl aliquot form each plasma sample was transferred to a 5.0ml polypropene tube. One milliliter of cold methanol was added. After slightly vortex mixing, the tubes were centrifuged for 15min. at 500G. A 100µl aliquot of the supernatant was transferred to the injection vials and 10µl were injected into chromatographic system. All samples from volunteers were analyzed on the same day in order to avoid inter-assay variation. Plasma solutions were protected from the light and stored in a deep freezer at (203 K).

HPLC Instrumentation
This study was performed on Shimadzu instruments model LC-6A HPLC system. The unit was operated in the isocratic model using solvent reservoirs fitted with 0.22 µm stainless steel filter at the end of polytrifluoroethylene (PTFE) tubes, transferring the mobile phase from reservoirs to the pump, the system also involved an injector with 50µL sample loop
model (Ressadyre 7125), Column in type of ODS-C18-DB "250 X 4.6mm I.D.", Thermostatic oven model CTO-6A Shimadzu, UV-visible detector model "SPD" and chromatopac unit model R-6A Shimadzu.

**HPLC Operation Condition**

For routine HPLC analysis of Amoxicillin use the following estimation condition. The mobile-phase was phosphate buffer conc. 10mM that containing 0.1mM 1-octane Sulphonic acid sodium salt: methanol (95:5) (v/v), pH buffer equal to six, column temperature 298 K, flow rate equal to (1.5ml/min.) and UV-visible detection at 230nm. The typical chromatograms of standard solution and blood plasma samples of Amoxicillin are shown in fig-2 and fig-3 respectively.

**Pharmacokinetics and statistical analysis**

The observation of maximum plasma concentration levels (C(max)) and time consuming to reach it (T(max)) were obtained from drug concentration versus time curves. The area under the curve "AUC" of the Amoxicillin concentration levels versus time from 5.0 minute to ten hours were estimated from “figure-4”.

![Figure 2: Typical chromatogram of HPLC analysis of Amoxicillin on ODS-DB column](image1)

**Figure 2: Typical chromatogram of HPLC analysis of Amoxicillin on ODS-DB column**

![Figure 3: Typical chromatogram of HPLC analysis of plasma Amoxicillin on ODS-DB column](image2)

**Figure 3: Typical chromatogram of HPLC analysis of plasma Amoxicillin on ODS-DB column**

**Results**

The isocratic reverse-phase HPLC technique described and used here for estimation of drug provides the appropriated sensitivity, specificity and high sample accuracy for bioavailability and pharmacokinetic studies. Fig.1 shows the retention time of Amoxicillin standard solution that under described chromatographic condition. The retention time of Amoxicillin was 7.0 minutes. The optimal chromatogram of analysis was given an ideal shape, symmetrical, and good resolution of peak. Fig.-2 shows the typical chromatogram of Amoxicillin in blood plasma samples of healthy volunteers which was appeared no endogenous Interfering peaks at the retention time of interest compound. The mean absolute recovery of Amoxicillin in blood plasma was 94.1% at 1.0ppm, 102% at 5.0ppm, 103% at 10.0ppm 102% at 20ppm, 99.3% at 40ppm and 104% at 50ppm respectively. The calibration curve was linear with regression coefficient $R^2 =0.989$ (Table-1). The analytical precision and accuracy values was obtained from assays of six quality control (1, 5.0, 10.0, 20.0, 40.0, and 50.0 ppm) are shown in table-1. The accuracy were 94.1%, 102%, 103%,
102%, 99.3% and 100% respectively and there is not significant degradation of Amoxicillin was observed during the period of storage.

Table 1: the linearity, precision and accuracy of blood plasma Amoxicillin samples.

<table>
<thead>
<tr>
<th>Spiked concentration (ppm)</th>
<th>1.0</th>
<th>5.0</th>
<th>10.0</th>
<th>20.0</th>
<th>40.0</th>
<th>50.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovered average con. (ppm)</td>
<td>0.94</td>
<td>5.1</td>
<td>10.3</td>
<td>20.4</td>
<td>39.7</td>
<td>50.2</td>
</tr>
<tr>
<td>Slope</td>
<td>1134</td>
<td>0.989</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion
The HPLC technique presented in this study decreases the lower limit of quantitation of Amoxicillin to about 0.1ppm. It was appeared that is more sensitive than many other assays. The low limit of the estimation of the plasma concentration of Amoxicillin was sufficient to perform the pharmacokinetics study of drug. Amoxicillin plasma concentration levels were measured by several methods in combination with UV-visible detection. The lowest plasma concentration levels of Amoxicillin was obtained by UV-visible detection which was 0.05ppm but the process was consumed long time that was 30min. Charles et al were described a procedure for determination of Amoxicillin in urine. Other complicated procedures for extraction and estimation of plasma concentration levels of Amoxicillin by using Solid-phase extraction has been also reported. Nevertheless, Solid-phase extraction (SPE) procedures are laborious and require SPE cartridges, increasing the cost of analysis. In order to improve the sensitivity, a column ion-pair HPLC with post column derivatization has been used. Where the low limit of quatitation was 0.01ppm. this procedure was more complicated due to the more step of post column derivatization and their retention time that will be longer than 10min. Also these procedures cannot be used in pharmacokinetics studies in human where a large number of samples were analyzed. The pharmacokinetics study was done in "10 hours" and the results indicate that the Iraqi formulation has higher bioavailability compared to the Indian formulation depending on the area under the curve AUC and C(max). Our technique was evaluated and produced the best results in terms of selectivity and sensitivity consideration the fact that the present technique involves a shorter running time and a simple sample preparation process.

Conclusion
Our HPLC technique was employed here proved to be fast, simple, precise, selective and sensitive enough to be used in clinical pharmacokinetic and bioavailability study for Amoxicillin in plasma human. The AUC and C(max) of Iraqi formulation are higher than Indian formulation of Amoxicillin and the T(max) of both two formulations are similar which is shown in table-2 and the relative bioavailability of Indian to Iraqi formulation was estimated equal to 64.11%.

Table 2 : The pharmacokinetic parameters “ Cmax , Tmax and AUC” for the Iraqi and Indian formulations of Amoxicillin.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Indian formulation &quot;A&quot;</th>
<th>Iraqi formulation &quot;B&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(max) &quot;ppm&quot;</td>
<td>8.9</td>
<td>11.5</td>
</tr>
<tr>
<td>T(max) &quot;hour&quot;</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>AUC</td>
<td>30.25</td>
<td>47.18</td>
</tr>
</tbody>
</table>

Acknowledgment
I wish to thank Miss. Kafa Q. Al-Obydee for carrying out the experiments that associated with preparation of samples and Kolood I. Mohamad for their technical assistance.

References
1. US pharmacopeial (2005), USP 28/NF 23 .The united states pharmacopeial convention, Inc, ROCKVILLE, MD , USA.
6. M. E. Pichichero J. R. Casey, S. L. Block et al , Pharmacodynamic analysis and clinical trials of Amoxicillin sprinkle


25. Hoizy G., lamiable D., Frances C., Trenque T., Kaltenbach M., Denis J., Millart H., Simultaneous determination of Amoxicillin and Clavulanic acid in human plasma by HPLC with UV.


