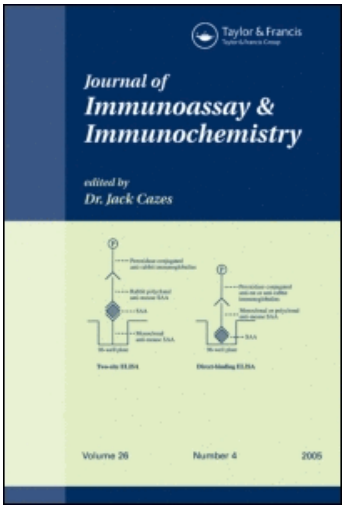

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Comparative Bioavailability Study of Two Tablet Formulations of Digoxin

Hassan Y. Aboul-Enein[a]; Laila I. Abou-Basha[b]; Lobna F. Wahman[b]
[a] Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03-65), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia [b] National Organization for Drug Control and Research, Cairo, Egypt

Online publication date: 04 July 2004

To cite this Article Aboul-Enein, Hassan Y. , Abou-Basha, Laila I. and Wahman, Lobna F.(2005) 'Comparative Bioavailability Study of Two Tablet Formulations of Digoxin', Journal of Immunoassay and Immunochemistry, 25: 2, 125 — 133
To link to this Article: DOI: 10.1081/IAS-120030522
URL: http://dx.doi.org/10.1081/IAS-120030522

JOURNAL OF IMMUNOASSAY & IMMUNOCHEMISTRY
Vol. 25, No. 2, pp. 125–133, 2004

Comparative Bioavailability Study of Two Tablet Formulations of Digoxin

Hassan Y. Aboul-Enein,[1,*] Laila I. Abou-Basha,[2] and Lobna F. Wahman[2]

[1]Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03-65), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia
[2]National Organization for Drug Control and Research, Cairo, Egypt

ABSTRACT

This investigation was carried out to evaluate the bioavailability of the generic product of digoxin 0.25 mg (cardixin) relative to a reference product, lanoxin (0.25 mg) tablets. The two formulations were found to be similar in in vitro assay (dissolution) as stipulated by USP XXIII. The comparison is carried out on 12 healthy male volunteers, who received a single dose (0.25 mg) of cardixin (product A) lanoxin

*Correspondence: Hassan Y. Aboul-Enein, Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03-65), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia; E-mail: enein@kfshrc.edu.sa.

DOI: 10.1081/IAS-120030522 1532-1819 (Print); 1532-4230 (Online)

(product B) as a reference product orally in the fasting state, in a randomized balanced two-way crossover design. After dosing, serial blood samples were collected for a period of 12 hr. Plasma samples were analyzed for digoxin by a sensitive and validated enzyme immunoassay method (ELISA). The maximum plasma concentration curve, up to the last measurable concentration (AUC_{0-24}), was analyzed under the assumption of a multiplicative model. The time to maximum concentration (T_{max}) was analyzed, assuming an additive model. The parametric confidence intervals (90%) of the mean values of the pharmacokinetic characteristics (AUC_{0-12} and C_{max}) for A : B ratio were, in each case, well within the bioequivalence acceptable range of 80–125%.

Key Words: Bioequivalence; Digoxin-specific antibodies; Bioavailability; Cardixin; Lanoxin.

INTRODUCTION

Digoxin is a cardiac glycoside, which increases the force of myocardial contractions and reduction in the conduction through the A.V. node.[1] It is basically used to improve the circulation in patients with congestive heart failure, and to slow the ventricular rate in the presence of arterial fibrillation and flutter.[2]

Studies describing the pharmacokinetics and disposition of digoxin in humans indicate that absorption of the drug after oral administration is variable and subject to bioavailability differences. Absorption occurs mainly in the small intestine, but the administration of food has been reported to affect the rate of absorption of digoxin.[3]

Digoxin is rapidly distributed throughout the body and its high concentrations are found in the heart, kidneys, and the skeletal muscles. The therapeutic serum levels for digoxin range from 0.5 to 2 ng/mL.[4],a

Digoxin is eliminated primarily by the kidneys; up to 80% of the dose is excreted in the urine with 27% of the dose in the first 24 hr. The remainder is eliminated in the feces via the bile. Plasma half-life time of digoxin is about 20–25 hr, but it is prolonged in subjects with renal impairment.[5]

The present study describes the determination of the bioavailability of cardixin relative to a reference formulation (lanoxin). Bioequivalence of the two products were assessed, based on the plasma concentration data obtained

[a]Product Information: Lanoxin®, Burroughs Welcome, 1984.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

following their administration to 12 healthy male volunteers in a balanced two-way crossover design.

EXPERIMENTAL

Preparations

Two marketed formulations containing digoxin were studied and designed as: product A, which is Cardixin® tablets 0.25 mg (Alexandria Co. for Pharmaceutical and Chemical Industries, Alexandria, Egypt), batch no. 5763011. Product B was lanoxin tablets 0.25 mg (Welcome Foundation Ltd., London, UK), batch no. A2512A.

Chemicals

Immunoassay kits for quantitative determination of digoxin was used. The kit was purchased from DRG Diagnostics (Marburg, Germany), Cat. No. EIA-3769, batch no. 23165.

Bioavailability Study

Protocol

The products were subjected to a comparative bioavailability study. The study design was a single-dose, fasting, two-treatment, two period, two-sequence crossover, comparing equal doses of the test and reference products, with a two-week washout period between the two phases of the study. An equal number was randomly assigned to the two dosing sequences. The healthy volunteers were selected and the study was conducted according to the internationally accepted guidelines and recommendations,[6,7] and in the spirit of the revised Helsinki Declaration.[8] The protocol was reviewed by National Organization for Drug Control and Research (NODCAR) Ethics committee.

Subjects

Twelve healthy adult male volunteers participated in the study. The subjects signed a consent form. Their mean age ($\pm$S.D.) was 27.5 $\pm$ 8.3 years, with a range of 20–35 years, body weights of 71 $\pm$ 7.33 kg with a range of 60–80 kg, and height of 166 $\pm$ 6.7 cm with a range of 155–

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

173 cm. On the basis of medical history, clinical examinations and laboratory investigations (HIV, HCV, hematology, blood biochemistry, and urine analysis) were performed. No subject who had a history or evidence of hepatic, renal, or gastrointestinal diseases participated in the study. Subjects fasted for a night before the administration of the drugs.

Treatment

Cardixin® tablets 1×0.25 mg (product A) or Lanoxin® tablets 1×0.25 mg (product B) were administered. On the first day, six subjects received 1×0.25 mg cardixin tablets, the other six received 1×0.25 mg lanoxin tablets. After a washout period of 2 weeks the alternative formulations were given. The subjects were given the treatment orally with 240 mL water, and then continued fasting for 4 hr followed by a standard breakfast.

Blood Sampling

Before drug administration, 2 mL venous blood samples were withdrawn as control. After drug intake, 2 mL venous blood samples were collected for the determination of digoxin at the following time intervals: 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3, 4, 5, 6, 8, 10, 12, and 24 hr. Subjects received no food or drink until 4 hr of blood samples were obtained. The serum samples were immediately frozen and stored at -20°C until assayed

Analytical Assay

The method is based on a competitive enzyme immunoassay using 96 plate wells. The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme–antigen conjugate, and native antigen. Upon mixing immobilized antibody, enzyme–antigen conjugate, and a serum containing the native antigen, a competition reaction results between the antigen and the enzyme–antigen conjugate for a limited number of insoluble binding sites. After equilibrium is obtained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated, from which the antigen concentration of an unknown can be ascertained.

The test samples from the dosed volunteers were analyzed along with standard and quality control samples. Standard curves were constructed and digoxin concentrations in serum samples were interpolated.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Pharmacokinetic Analysis

The pharmacokinetic characteristics for digoxin were determined from the plasma concentration–time data. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were obtained directly from the plasma concentration–time data, and used as measures of rate of absorption. The area under the plasma concentration (AUC)–time curve was determined by using the linear trapezoidal rule. The apparent elimination rate constant (K_{el}) was calculated by the technique of least-squares regression from the data of the last 4–5 points of each plasma concentration–time curve.

The $AUC_{(0-\infty)}$ values (express the magnitude of absorption) were determined by adding the quotient of $\hat{C}_1$ and the appropriate (K_{el}) to the corresponding $AUC_{(0-t)}$, that is:

$$AUC_{(0-\infty)} = AUC_{(0-t)} + \frac{\hat{C}_1}{K_{el}}$$

where $\hat{C}_1$ is the estimated last plasma concentration.

The apparent elimination half-life ($t_{1/2}$) for digoxin in plasma was calculated by using the following equation:

$$t_{1/2} = \frac{\ln 2}{K_{el}}$$

Statistical Analysis

The two-way analysis of variance (ANOVA) for crossover design was used to assess the effect of formulations, periods, sequences, and subjects within sequence, on logarithmically transformed data of $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, (C_{max}), K_{el} and $t_{1/2}$. The ANOVA of T_{max} was carried out on the untransformed data. Sequence effects were tested against the mean square term for subjects within sequence. All other main effects were tested against the mean square error term. Parametric 90% confidence intervals based on the ANOVA of the mean ratios of AUC parameters obtained by the analysis products A and B and C_{max} was computed under the assumption of a multiplicative model.

Non-parametric confidence interval was also performed.[9] In addition, the bioequivalence between the two formulations was also assessed by Schliemann's two one-sided *t*-tests.[9] All analyses of the data were performed with the statistical software package designed by NODCAR (Cairo, Egypt).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Mean dissolution profile of digoxin from cardixin and lanoxin tablets (0.25 mg; average 12 tablets for each product).

	Digoxin dissolved (%)	
Time (min)	Cardixin tablets	Lanoxin tablets
5	80.7	87.3
15	91.89	99.98
30	99.1	101.9
60	101.8	103.5

RESULTS AND DISCUSSION

The two products, cardixin tablets (product A) and lanoxin (product B) tablets, were found to be similar for in vitro-assay (dissolution as stipulated by USP XXIII as shown in Table 1 (Fig. 1). The mean serum concentration vs. time profile of the two products are presented in Table 2 (Fig. 2).

Following administration of cardixin (product A), the mean maximum serum concentration (C_{max}) was 1.91 ng/mL. Following the administration of lanoxin (product B), the values were significantly the same ($p < 0.05$) with a mean C_{max} of 1.79 ng/mL.

The relative bioavailability based on C_{max} was found to be 106.7%. There was no significant difference in time to maximum concentration median T_{max}, which was 0.5 and 1.0 hr for cardixin and lanoxin, respectively. The overall bioavailability judged from AUC_{0-24} was found to be 100.4%. The mean residue time (MRT) was 4.3 hr for both products as shown in Table 3.

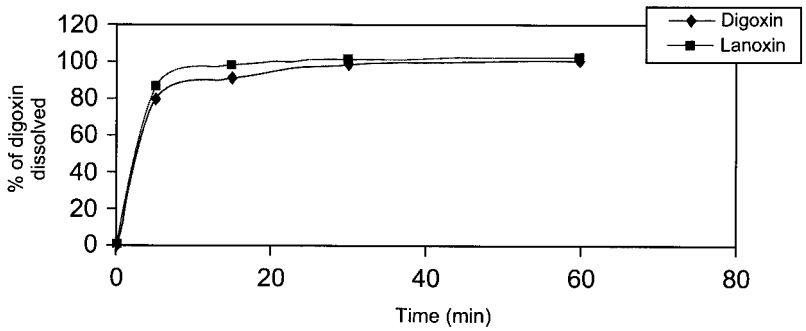

Figure 1. Dissolution profile of digoxin dissolved from cardixin and lanoxin tablets.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 2. Mean serum digoxin concentrations (ng/mL) after oral administration of products A and B.

Time	Product A (cardixin)	Product B (lanoxin)
0.0	0.0	0.0
0.25	1.2 ± 0.4	0.66 ± 0.28
0.5	1.81 ± 0.6	1.12 ± 0.58
1.0	1.49 ± 0.49	1.66 ± 0.66
1.5	1.2 ± 0.43	1.52 ± 0.49
2.0	1.06 ± 0.38	1.18 ± 0.37
2.5	0.95 ± 0.4	0.98 ± 0.33
3.0	0.85 ± 0.32	0.88 ± 0.26
4.0	0.77 ± 0.29	0.78 ± 0.26
5.0	0.73 ± 0.32	0.73 ± 0.25
6.0	0.70 ± 0.31	0.64 ± 0.25
8.0	0.67 ± 0.31	0.62 ± 0.22
10.0	0.64 ± 0.29	0.6 ± 0.25
12.0	N.D.	N.D.
24.0	N.D.	N.D.

Note: N.D., not detected.

The result of this study demonstrated that both formulations, cardixin tablets 0.25 mg batch no. (5763011) and lanoxin tablets 0.25 mg batch no. (A2512 A) are bioequivalent, since they deliver equivalent amounts of digoxin to the systematic circulation at equivalent rates.

The present work underlines the necessity for evaluation of pharmacokinetics and bioequivalence studies of the generic drugs compared to the

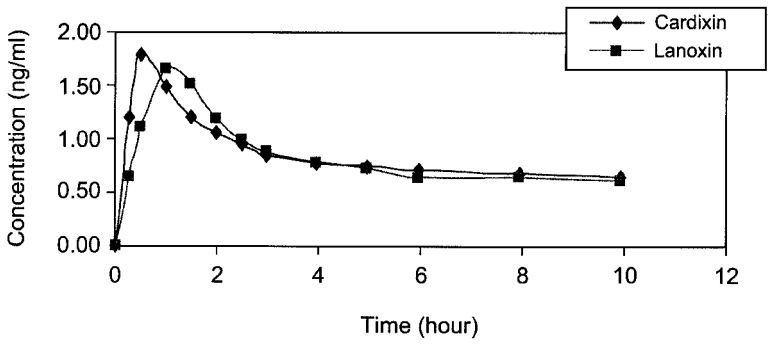

Figure 2. Mean digoxin serum concentration (ng/nL) following oral administration of 0.25 mg tablets for cardixin and lanoxin.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Table 3. Pharmacokinetic parameters of digoxin after oral administration of products A and B.

Parameter	Product A (cardixin)	Product B (lanoxin)
Dose (mg)	0.25 mg	0.25 mg
C_{max} (ng/mL)	1.91 ± 0.51	1.79 ± 0.62
T_{max} (hr)	0.5	1.0
AUC_{0-24} (ng mL/hr)	8.11 ± 2.58	8.08 ± 2.48
$AUC_{(0-\infty)}$ (ng/mL/hr)	20.29 ± 6.99	19.7 ± 5.66
MRT (hr)	4.3 ± 0.2	4.3 ± 0.2
$t_{1/2}$ (hr)	15.4 ± 6.8	15.4 ± 7.2

innovator brands, to ensure safe and effective drugs delivery in developing countries.[10,11]

ACKNOWLEDGMENTS

The authors acknowledge the technical assistant of Hossam Gharieb and Alla El-din Hamza. One of the authors (HYA-E) wish to thank the administration of the Research Centre at King Faisal Specialist Hospital and Research Centre for their support to the Pharmaceutical Analysis Research Program.

REFERENCES

1. Reynolds, J.E.F. *Martindale. The Extrapharmacopaeia,* 29th Ed.; Pharmaceuticals Press: London, 1989; 828.
2. Goodman & Gilman. *The Pharmacological Basis of Therapeutics*; Hardman, J.G., Gilman, A.G., Limbird, L.E., Eds.; McGraw-Hill Co.: New York, NY, 2001.
3. Cuena Boy, R.; Ortiz de Apodaca Ruiz, M.A.; Macia Martinez, M.A. Best result of digoxin dosing in the aged by taking into account that both the elimination as well as the volume of distribution of the drug decrease when the kidney function deteriorates. Anales de Medicina Interna **2002**, *19*, 331–335.
4. Aronson, J.K. Clinical pharmacokinetics of digoxin. Clin. Pharmacokin. **1980**, *5*, 137–149.
5. Moffat, A.C.; Jackson, J.V.; Moss, M.S.; Widdop, B. *Clarke's Isolation and Identification of Drugs in Pharmaceuticals Body Fluids and Post Mortem Materials,* 2nd Ed.; Pharmaceutical Press: London, 1986; 425.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

6. The United States of Pharmacopoeia 23/National Formulary 18, U.S. Pharmacopial Conversion, Rockville, MD, 1995.
7. WHO: *Drug Information*; 1994; Vol. 8, No. 2.
8. WHO: *Drug Information*; *WHO: Helsinki Declaration*; 1992; Vol. 6, No. 4.
9. Hauschke, D.; Steinijans, V.W.; Dilenl, E. A distribution-free procedure for the statistical analysis of bioequivalence studies. Int. J. Clin. Pharmacol. Therap. Toxicol. **1990**, *28*, 72–78.
10. Schulz, H.U.; Steinijans, V.W. Striving for standards in bioequivalence assessment. A review. Int. J. Clin. Pharmacol. Therapy & Toxicol. **1992**, *30* (Suppl. 1), S1–S6.
11. Henderson, J.D.; Esham, R.H. Generic issues for problematic drugs. Southern Med. J. **2001**, *94*, 16–21.

Received October 31, 2003
Accepted November 26, 2003
Manuscript 3122

Request Permission or Order Reprints Instantly!