Bioequivalence Study of Clindamycin Phosphate Injection (Clinott-P) in Thai Healthy Volunteers

Amorn Leelarasamee MD*,
Wallapa Tatong PhD**, Narat Kasattut MS***,
Tatta Sriboonruang BSc***, Duangchit Panomvana Na Ayudhya PhD***

** Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University*
*** Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University*
**** Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University*

Background and Objective: *Generic clindamycin given intramuscularly, should have identical active ingredient(s), strength, and demonstrable bioequivalence to those of original product. The aim of this investigation was to compare the bioavailability of a single, intramuscular injection, of 2 ml. of 300 mg. of a generic clindamycin (Clinott-P) and the original preparation (Dalacin C).*
Material and Method: *A randomized, double-blinded, crossover study was conducted. Twenty-four healthy males were recruited at Siriraj Hospital and randomized to receive a single intramuscular injection of either Clinott-P or Dalacin C . Treatment was followed by a two-week washout period. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hours after the injection. Plasma samples were analysed for clindamycin by a validated HPLC method at the Faculty of Pharmaceutical Sciences, Chulalongkorn University.*
Results: *Twenty-four volunteers enrolled in and completed the study. They exhibited an average height of 167.92 cm (SD = 5.82), weight of 60.10 kg (SD = 7.36), body mass index of 21.27 (SD = 1.73) and normal blood chemistries. The C_{max} of Clinott-P was 3.94225 μg/ml at T_{max} 1.75 hours and of Dalacin C , 3.6847 μg/ml at T_{max} 2.09 hours. The AUC_{0-24} of Clinott-P was 16.32 ± 6.13 μg.hr/ml and Dalacin C was 17.24 ± 7.46 μg.hr/ml. Ninety percent confidence intervals of the mean ratios (test/reference) of log transformed of C_{max} (93.07-123.43%), $AUC_{(0-24)}$ (82.58-112.31%) and $AUC_{(0-inf)}$ (81.54-110.06%) were all within the standard range (80-125 %) for bioequivalence study. Tenderness after injection around the deltoid area was assessed blindly and was found to be slight (visual basic score < 5) and presented for one or two days after the injection.*
Conclusion: *The two brands of clindamycin exhibit comparable pharmacokinetic parameters and volunteers exhibited slight and tolerable tenderness at the injection site.*

Keywords: *Clindamycin phosphate, Bioequivalence study*

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Clindamycin is a semisynthetic antibiotic produced by a 7(S)-chloro-substitution of the 7(R)-hydroxyl group of the parent lincomycin. Despite 40 years of widespread clinical use since its introduction in 1966, clindamycin retains potent activity against many aerobic and anaerobic gram-positive and gram-negative pathogens causing skin and subcutaneous tissue infections, respiratory tract infections, septicemia, abdominal infections and gynecologic infections[1]. Clindamycin binds to 50S subunit bacterial ribosome and inhibits protein synthesis. The range of minimum inhibitory concentrations (MIC) for various bacteria is 0.04-4.0 g/ml[1] and time above the MIC ($T_{>MIC}$) is the parameter that best correlates with its efficacy[2-3].

When clindamycin is administered intravascularly, biologically inactive clindamycin phosphate is

Correspondence to : Leelarasamee A, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Phone: 0-2419-7785, E-mail: siall@mahidol.ac.th

rapidly converted to active clindamycin with an elimination half life of 6 minutes. Plasma concentrations of a single dose of 300, 450 and 600 milligrams of clindamycin given to healthy volunteers produce the values of C_{max} at 3.8-4.9, 5.3, 6.2-6.3 g/milliliter and 12 hours later it would be at 1.2-1.6, 2.0, 2.5-2.8 g/milliliter, respectively[4,5]. Clindamycin is widely distributed in body fluids and tissues and 40-90% will bind with protein. It is largely metabolized (>90%) in liver to be both active and inactive metabolite. The important active metabolites are clindamycin sulfoxide and N-demethyl-clindamycin and will be excreted in both unchanged and metabolite forms via urine (10%) and feces (3.6%). The remainder is inactive metabolite excreted in bile and feces. Half-life of the drug is 4.5-5.3 hours and will be longer in patients with liver and renal disease. Absorption of clindamycin phosphate via intramuscular injection is almost complete and produces a peak serum level of active clindamycin within 3 hours in adults and 1 hour in pediatric patients that is slightly less than that obtained via the intravenous route.

In Thailand, original clindamycin phosphate is imported by Pfizer (Thailand) Limited with the trademark of "Dalacin C injection " (1 ampoule contains 300 mg/2 ml). Recently, raw material of clindamycin is available and has prompted a local pharmaceutical company to produce generic clindamycin for intravenous and intramuscular injection. With the exception of the intravenous form, administration of a generic clindamycin with oral preparation or intramuscular injection needs *in vivo* bioequivalence study before physicians are convinced with the quality and efficacy of a generic product[6]. The MacroPhar Co., Ltd. is a distributor of generic clindamycin phosphate with 150 mg/ml potency to be an alternative commercial product in Thailand. Hence, the aim of the present study was to compare the bioequivalence in healthy volunteers between the two products of clindamycin, Clinott-P and Dalacin C given by a single intramuscular injection of 300 mg or 150 mg/ml, 2 ml at the deltoid area of the forearm.

Material and Method

Test samples

Each ampoule of generic clindamycin phosphate for intramuscular injection contained 2 milliliters of 300 milligrams or 150 mg/ml (Clinott-P from the MacroPhar Co., Ltd.). Twenty sample ampoules were randomly collected from a pool of at least 400 productions at the factory. Twenty ampoules of the original product (Dalacin C) from Pfizer (Thailand) Limited with similar concentration and volume, were randomly bought from drug stores and hospitals in Bangkok. The drugs were given intramuscularly at the deltoid area of the forearm.

Volunteers

Twenty-four adult male volunteers were recruited at Siriraj Hospital. All volunteers gave informed consent after the study had been explained. They were non-drug addicted, non-smoking or stopped smoking for at least one month before and during the entire study. No history of chronic gastrointestinal disease, liver disease, renal disease, cardiovascular disease, endocrine and immunologic disease, respiratory tract disease or allergy, psychic and mental disease or alcoholism was elicited. Consumption of alcoholic beverage or alcohol-containing food, any drugs were also prohibited for at least 14 days before and throughout the study. All volunteers had a medical examination before the study started and had normal values of laboratory tests for complete blood count, BUN, serum creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase and negative hepatitis viral profile.

Study design

Randomized, double-blind, two-period cross-over study with a two-week washout period were conducted. Volunteers were randomly assigned into two groups with 12 individuals in each group by block randomization. Each volunteer received 150 mg/ml, 2 ml of either Dalacin C or Clinott-P as his first intramuscular administration. After two-weeks of washout period, the volunteer was switched to the other product for a similar administration.

Sample collection and clindamycin analysis[7]

Six to ten ml of venous blood samples were drawn from each subject at 13 different time points i.e., immediately after injection, at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hours after the injection. Blood samples were collected in heparinized tubes and plasma samples were separated and kept under -20 C. Analysis was performed by deproteinizing the plasma samples and clindamycin was extracted and dissolved into diethyl ether before drying under nitrogen gas at 40 C. The residue was redissolved and injected into HPLC (Dionex, Germany). Clindamycin level was measured and calculated by a data processing software, Chromeleon version 6.0 installed in HPLC system.

Data analysis[8]

Pharmacokinetic parameters, i.e., C_{max}, T_{max}, $AUC_{(0-24)}$, $AUC_{(o-inf)}$ were determined by non-compartmental analysis. Statistical comparison between pharmacokinetic parameters of the two products was analyzed using two-way ANOVA in order to assess the effect of formulation, periods, sequences, subjects (within sequence) and standard errors. The data were transformed into log form and 90% confidence interval (CI) of the mean ratios (generic/original) of log transformed of C_{max} and AUC of the two products were calculated. The bioequivalence of the two products was accepted if 90% CI of C_{max} and AUC of test fell within 80-125% of the original product.

The protocol has been approved by the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (No. 119/2547) on September 7, 2004.

Results

Twenty-four volunteers exhibited an average height of 167.92 cm (SD, 5.82), weight of 60.10 (SD, 7.36) and body mass index of 21.27 (SD, 1.73). All volunteers completed the entire study with two separate periods without presenting any severe adverse reactions except for mild local pain around the deltoid area (visual basic scale < 5). The volunteers, therefore, tolerated well the intramuscular injection of both Clinott-P and

Table 1. Plasma concentrations of single intramuscular injection of 300 mg of clindamycin at different time points in 24 healthy volunteers

Product	Time (hr)	Plasma concentrations of clindamycin (μg/ml)					
		Mean	SD	%CV	Max	Min	Max-Min
Dalacin C	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.50	1.8286	0.8391	0.0459	3.7806	0.0000	3.7806
	1.00	2.9413	1.2838	0.0436	5.8526	0.9041	4.9485
	1.50	3.1907	1.4324	0.0449	6.3317	1.2058	5.1259
	2.00	3.0331	1.2742	0.0420	6.3688	1.1028	5.2659
	2.50	2.9411	1.3593	0.0462	6.8890	0.8743	6.0147
	3.00	2.8313	1.3331	0.0471	7.0661	0.6377	6.4284
	4.00	2.3985	1.0760	0.0449	4.8142	0.5743	4.2400
	6.00	1.4999	0.9244	0.0616	4.4169	0.0000	4.4169
	8.00	0.8997	0.6584	0.0732	2.3050	0.0000	2.3050
	10.00	0.2699	0.4525	0.1677	1.3598	0.0000	1.3598
	12.00	0.1049	0.2890	0.2754	0.9759	0.0000	0.9759
	24.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Product	Time (hr)	Plasma concentrations of clindamycin (μg/ml)					
		Mean	SD	%CV	Max	Min	Max-Min
Clinott-P	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	0.50	2.0385	1.2713	0.0624	5.6521	0.0000	5.6521
	1.00	2.4071	0.9099	0.0378	4.6350	1.0257	3.6093
	1.50	3.1360	1.1678	0.0372	7.2497	1.1734	6.0763
	2.00	3.2386	1.3288	0.0410	6.1636	0.9087	5.2549
	2.50	2.7855	0.9666	0.0347	4.3397	0.8758	3.4639
	3.00	2.5877	0.8744	0.0338	4.1851	0.7840	3.4011
	4.00	2.1787	0.8037	0.0369	3.5973	0.6122	2.9851
	6.00	1.4016	0.6556	0.0468	2.4288	0.0000	2.4288
	8.00	0.7526	0.6164	0.0819	2.0293	0.0000	2.0293
	10.00	0.3150	0.4168	0.1323	1.4177	0.0000	1.4177
	12.00	0.0298	0.1461	0.4899	0.7156	0.0000	0.7156
	24.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Dalacin C . Plasma concentrations of each subject at different time points were reported as arithmetic mean, SD, percentage of Coefficient of Variation (CV), minimum and maximum as shown in Table 1. The results demonstrated that the rate and extent of absorption were nearly the same for both products. Peak concentrations were reached within two hours after injection. The C_{max} of the generic product was 3.94225 μg/ml at T_{max} 1.75 hours and of original product was 3.6847 μg/ml at T_{max} 2.09 hours. The concentrations after administration with either product steadily declined to undetectable level within 24 hours (Fig. 1). Two-way ANOVA analysis was carried out to assess the other factors that could affect the bioequivalence evaluation. The results showed no effect of period, sequence and subject on the study. The test power of C_{max}, was 0.9338, $AUC_{(0-24)}$ was 0.8952, and $AUC_{(o-inf)}$ was 0.9077.

Parameters for bioequivalence evaluation were C_{max}, $AUC_{(0-24)}$ and $AUC_{(o-inf)}$ and the results are presented in Table 2. Ninety percent CI of the mean ratios (generic/original) of log transformed of the three parameters were as follows: C_{max} 93.07-123.43%, $AUC_{(0-last)}$ 82.58-112.31% and $AUC_{(o-inf)}$ 81.54-110.06%. Since 90% confidence intervals for AUC and C_{max} were within the predefined bioequivalence acceptance limits (80-125% of the originator), bioequivalence of the generic and originator could be demonstrated. Hence Clinott-P could be concluded as having comparable pharmacokinetic profiles with Dalacin C and these two brands were bioequivalent. Single doses of intramuscular injections of 300 milligrams of clindamycin were well tolerated without relevant differences between both preparations.

Discussion

The debate over brand-to-generic drug substitution has always been a hot issue especially, in developing countries where financial resources of most people for expensive drugs are limited. Under the tight budget allocated from the universal health coverage scheme and social security health insurance system, many administrators of community hospitals seek less expensive generic drugs as alternatives for the original drugs if the former are proven in humans, to be equally efficacious or bioequivalent to the latter. The proven method for efficacy could be achieved by

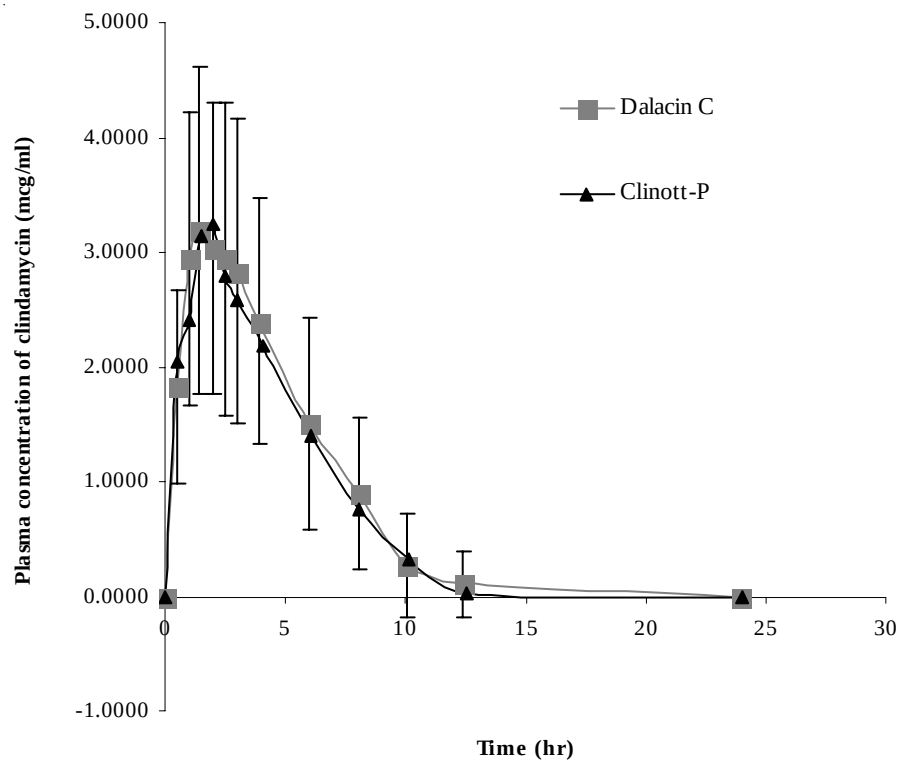

Fig. 1 The average clindamycin plasma concentrations at different time points after intramuscular injection of 300 mg of Clinott-P or Dalacin C in 24 healthy volunteers

Table 2. Pharmacokinetic parameters of Clinott-P and Dalacin C with 90% confidence interval (CI) of the mean ratios (generic/original) of log transformed values

Pharmacokinetic parameters	Product (Mean ± SD)		90% confident interval (CI) of the mean ratios (generic/original) of log transformed values
	Clinott-P (generic)	Dalacin C (original)	
T_{max} (hr)	1.75	2.09	-
C_{max} (μg/ml)	3.94 ± 1.40	3.68 ± 1.35	93.07-123.43
AUC_{0-24} (μg.hr/ml)	16.32 ± 6.13	17.24 ± 7.46	82.58-112.31
AUC_{0-inf} (μg.hr/ml)	17.34 ± 6.39	18.74 ± 8.19	81.54-110.06

conducting a randomized controlled trial that is time-consuming and costly. However, the study is designed for non-inferiority trial. Hence, bioequivalence study is an attractive alternative since it costs much less in terms of time and expense.

The present study was sponsored by the distributor of the generic product and therefore was potentially susceptible to certain biases that investigators took serious precautions to avoid. The ampoules of generic product were randomly picked up from a large pool of finished products at the manufacturer's factory. The original products were obtained from drug stores and hospitals. The present study employed a blind, randomized, two-period crossover design to study the bioequivalence in 24 healthy volunteers. The study design and sample size are considered most appropriate and standard for this type of study[9,10]. In addition, investigators and volunteers were blind to the products used for intramuscular injection and blood samples were drawn and encoded to ensure neutrality in laboratory determination of serum clindamycin. The plasma samples were assayed for clindamycin base by a validated gas-liquid chromatographic method with quality control[7]. After laboratory determination, the samples were unblind and pharmacokinetic values for plasma clindamycin were calculated and compared according to recommendation of the Thai Food Drug and Administration (FDA), the Ministry of Public Health, Thailand for this type of study[11]. Hence, the present study was performed with great effort to ensure accuracy and reliability and avoid bias. Data from the study were also treated with the highest standard for an unbiased analysis according to FDA guidance for statistical procedures for bioequivalence studies using a standard two treatment cross over design[12,13]. The study revealed that the two products were bioequivalent though absorption from the injection site seems to be slightly more rapid with the generic preparation. This small difference is not a matter of concern nor clinical relevance since antibacterial activity of clindamycin is classified as time above the MIC ($T_{>MIC}$). Therefore, peak concentration is not clinically as important as the duration above the MIC in case of clindamycin[14].

From the present study, it could be concluded that the two brands exhibit comparable pharmacokinetics profiles and that Clinott-P is bioequivalent to Dalacin C . The availability of a bioequivalent, generic clindamycin opens up an alternative to many administrators of community hospitals and patients who need an effective antibiotic with more affordable price.

Acknowledgment

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ชีวสมมูลของยาฉีด clindamycin phosphate

อมร ลีลารัศมี, วัลลภา ทาทอง, นารัต เกษตรทัต, ทัดตา ศรีบุญเรือง, ดวงจิต พนมวัน ณ อยุธยา

วัตถุประสงค์: *เพื่อศึกษาชีวสมมูลของยาฉีดเข้ากล้ามเนื้อ clindamycin phosphate ขนาดความแรง 150 มิลลิกรัม/มิลลิลิตร ปริมาณ 2 มิลลิลิตร ระหว่างยา Clinott-P กับยาต้นแบบ Dalacin C ในอาสาสมัครชาย*

วัสดุและวิธีการ: *อาสาสมัครเป็นเพศชาย สุขภาพดีจำนวน 24 คน อายุระหว่าง 18-45 ปี รูปแบบการศึกษาที่ใช้คือ randomized, single dose, double-blinded, two-way crossover study โดยมีระยะเวลา washout period นาน 2 สัปดาห์ อาสาสมัครถูกแบ่งออกเป็นสองกลุ่มแบบสุ่มและฉีดยา Clinott-P หรือ Dalacin C เข้ากล้ามเนื้อที่ต้นแขนปริมาณ 2 มิลลิลิตรในขนาด 300 มิลลิกรัม เจาะเลือดทันทีและเก็บตัวอย่างเลือดอีก ณ เวลา 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 และ 24 ชั่วโมง หลังฉีดยา ทำการตรวจวิเคราะห์หาระดับยา clindamycin โดยใช้เครื่อง HPLC ที่ได้ผ่านการตรวจสอบความถูกต้องแล้ว*

ผลการศึกษา: *อาสาสมัคร 24 รายเข้าร่วมการศึกษาทั้ง 2 ระยะ ไม่พบความผิดปกติใด ๆ นอกจากอาการเจ็บเล็กน้อยเหมือนกันที่ต้นแขนทั้งสองกลุ่มนาน 1-2 วัน (ค่า visual basic scale น้อยกว่า 5) ค่า C_{max} ของ Clinott-P เท่ากับ 3.94225 ไมโครกรัมต่อ มล. ที่เวลา T_{max} เท่ากับ 1.75 ชั่วโมงและของ Dalacin C เท่ากับ 3.6847 ไมโครกรัมต่อ มล. ที่เวลา T_{max} เท่ากับ 2.09 ชั่วโมง ค่า C_{max}, AUC_{0-24} และ AUC_{0-inf} ของยา Clinott-P เท่ากับ 3.94 $\pm$ 1.40 ไมโครกรัมต่อมิลลิลิตร, 16.32 $\pm$ 6.13 และ 17.34 $\pm$ 6.39 ไมโครกรัม.ชั่วโมงต่อมิลลิลิตรตามลำดับ และของยา Dalacin C เท่ากับ 3.68 $\pm$ 1.35 ไมโครกรัมต่อมิลลิลิตร, 17.24 $\pm$ 7.46 และ 18.74 $\pm$ 8.19 ไมโครกรัม.ชั่วโมงต่อมิลลิลิตรตามลำดับ โดยมีค่า 90% ความเชื่อมั่นของค่า log ของค่าเฉลี่ยทั้ง C_{max}, AUC_{0-24} และ AUC_{0-inf} ของยา Clinott-P ต่อ Dalacin C เท่ากับร้อยละ 93.07-123.43, 82.58-112.31และ 81.54-110.06 ตามลำดับ ค่าต่าง ๆ เหล่านี้อยู่ในช่วงที่ยอมรับได้ตามมาตรฐาน คือร้อยละ 80-125*

สรุป: *ชีวสมมูลของยาฉีดเข้ากล้ามเนื้อของยา Clinott - P ไม่แตกต่างจากยา Dalacin C ในอาสาสมัครที่มีสุขภาพดี*