Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691**
Sch. J. App. Med. Sci., 2013; 1(3):172-176

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Evaluation of Hematology and Biochemistry at Baseline and at End of study in Normal Healthy Post Menopausal Women administered with Combined Progesterone and Estradiol capsules in Bioequivalence studies

Ganesan M[1*], Ashok P[1], Ragunath M P[1], Elangovan N[1], Venkatarao K[1], Sailakshmi L[2], Methavi[3], Kathiravan S[1], Sudha A[1], Gopinath A[1], Kandida S[1],Sharmila S[1],Vasugidevi A[1].
[1]Department of Clinical Trial Quality Assurance, [2]Department of Biostatistics, [3]Department of Diagnostics, Micro Therapeutic Research Labs, Pvt. Ltd., Chennai-600 059, Tamil Nadu, India.

Corresponding author
Ganesan M
Email: **info@microtheraps.com**

Abstract: Current practices of bioequivalence studies perform safety assessment by visibly comparing screening and post study laboratory values. Safety assessment (hematology and biochemical parameters) not actually compared for generic drug with brand drug. Our objective is to establish a statistical method to compare and evaluate blood hematology and biochemistry parameters at baseline and at end of the study. In this research analysis, the safety of the combined progesterone and estradiol capsules evaluated in three cross over design, normal and healthy post menopausal women bioequivalence studies conducted by the Contract research organization, Micro Therapeutic Research Labs Pvt Ltd.
Keywords: Estradiol, Progesterone, Bioequivalence, Safety, Hematology and Biochemistry, Post menopausal women.

INTRODUCTION

In recent years, the use of generic drugs has invited due to cost-effective in Health domain. Generics are considered (by the FDA - Food and Drug Administration) identical in dose, strength, route of administration, safety, efficacy, and intended use with Brand drug [1, 2]. The development of a brand-name formulation requires the demonstration of its pharmacokinetics, efficacy, and tolerability in normal and healthy subjects and in the target patient population. However, the development of a generic equivalent requires only the demonstration of its bioequivalence with the brand-name product in normal and healthy subjects [3]. The historical reviews recommends [3] that generic drugs need to provide efficacy and tolerability data. Data sets of values of blood hematology and biochemistry parameters obtained are only shared to the regulatory. Abnormal values are reported as adverse events at the discretion of the investigator. But an overall post administration effect of the drug was rigorously not monitored to determine the health status of the population who had participated the BA/BE (Bioequivalence/Bioavailablity) study. The safety is the foremost basic ethical requirement of clinical trials [4]. The volunteers are professionals who participate in bioequivalence studies, have tendency of potentially but dangerous blood donation frequency nearly above the allowable limit [5] of blood donation within a year. In this article a statistical approach was attempted to determine any clinical significant difference on hematology and biochemistry safety parameters between baseline and at the end of the study of combined progesterone and estradiol capsules in healthy postmenopausal women. This current attempt has been made assuming that we would be able to determine a true effect of the drug on hematology and biochemistry values between the baseline and post administration of combined progesterone and estradiol capsules in healthy postmenopausal women both due to blood loss and the drug effect.

METHODS

An open labeled randomized, controlled, crossover, two period, two sequence single dose, bioequivalence study of the combined Progesterone 200 mg and Estradiol 2 mg Capsules under fasting and fed conditions were conducted in postmenopausal women. Studies conducted between 27 Sep 12 to 14 Oct 12 for Fasting study and 06 Oct 12 to 23 Oct 12 for Fed study. Another open labeled randomized, controlled, crossover, three period, three sequence single dose, bioequivalence study of the combined Progesterone 200 mg and Estradiol 2 mg Capsules under Fed condition was conducted in postmenopausal women between 07 Nov 12 to 22 Dec 12, as Multi-batch. All the three bioequivalence studies were conducted by Micro Therapeutic Research Labs Pvt Ltd, Chennai. Data's were selected from all three studies to analyze statistically, the effect of combined Progesterone 200 mg and Estradiol 2 mg Capsules on hematological and biochemical parameters at baseline and at post study. The studies were approved by an Independent Ethics Committee (CEC- Chennai Ethics Committee; Registered No. 11952; DCGI (Drugs Controller General of India) prior to start of the study. Written informed consent was obtained from all participants. Procedures followed were in accordance with ICH – GCP

(International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use), Schedule Y (Drugs and Cosmetics act 1940 India) and the applicable regulatory guidelines.

Subjects and Sample Size

66 subjects in the three - period Fed study, 24 subjects each in fast and fed two - period crossover studies were participated. Subject selection was based on the following criteria: age between 45 and 65 years; Body mass index between 18.50 and 29.99 kg/m^2. Normal hematology and biochemistry laboratory values, no evidence of underlying disease during screening and check-in and whose screening is performed within 29 days of check-in. In these studies the subjects who had completed the respective studies were taken to statistically analyze the effect on hematological and biochemical parameters at baseline and post administration of the combined Progesterone 200 mg and Estradiol 2 mg Capsules.

Data Selection

The Baseline safety hematological and biochemical parameters were obtained at least 29 days prior to administration of the combined Progesterone and Estradiol capsules. Progesterone 200 mg and Estradiol 2 mg Capsule was administered with an interval of 14 days between the successive treatments of generic formulation and Brand drug. Post study safety sample had been taken at 48.00 hrs after drug administration in the last period. Hematological parameters included Hemoglobin, RBC, Total Leukocyte count and Platelet count. Biochemical parameters included Plasma Glucose, Blood Urea, Serum Creatinine, Total Cholesterol, Bilirubin (Total), Bilirubin (Direct), SGOT and SGPT. Hematological parameters such as Hemoglobin, RBC, Total Leukocyte count and Platelet count were estimated using SYSMEX XS 800i. Biochemical parameters such as Glucose by GOD-POD method, Blood Urea by UREASE-GLDH (Glutamate Dehydrogenase) /UV Kinetic Method, Serum Creatinine and SGPT by Modified Jaffes method (Initial rate or fixed time method), SGOT by UV Kinetic Modified IFCC without PLP method, Total Cholesterol by CHOD-PAP (Cholesterol Oxidase - Peroxidase) (Enzymatic Photometric method) and Billirubin Total and Billirubin Direct by Jendrassik-Grof Method using SELECTRA-Pro M. In house derived range values were used as reference range values for both hematology and biochemistry laboratory values.

Data Analysis

Analysis was done for the subjects who had completed the study so as to facilitate analysis of uniformly paired data. For evaluation of changes versus baseline, both paired *t*-tests and the Wilcoxon matched-paired signed-ranks tests were applied; usage of both these tests allows the test with the lower (stricter) *P* value to be chosen if results are different. Analyses of the data were performed with the SPSS statistical package, version 10.0 (SPSS for windows, SPSS Inc., Chicago, IL).

RESULTS

66 from the three-period fed bioequivalence study, 24 subjects each from two period fasting and fed bioequivalence study were considered for this current study. Out of the 66 subjects from the three period fed study only 63 subjects completed the study. And out of the 24 subjects from the two period fasting study 20 subjects completed the study. And out of 24 subjects from the two period fed study, all the 24 subjects completed the study. The hematology and biochemistry data from 63 subjects of the three period fed study, 20 subjects of the two period fasting study and 24 subjects of the two period fed studies were considered for statistical analysis. It was not possible to obtain specimens for laboratory testing on subjects who had dropped out from the study hence only those subjects who were able to provide specimens at both baseline and at the end of the study were included in this current study.

Three period - Fed study

The biochemistry and hematology parameters obtained are shown in Table 1. The statistical data compares the baseline with post study evaluation. There were significant fall in serum creatinine values, hemoglobin and platelet count. There were no evidence to support any change in urea, cholesterol, total billirubin, direct billirubin, SGOT and SGPT levels between the baseline and post study evaluations. There were significant raise in blood glucose levels.

Two period - Fast Study

The paired data of biochemistry and hematology parameters at baseline and the post study evaluation are shown in Table 2. Significant decreases occurred in Serum Creatinine, urea and T. Billi levels and Hemoglobin and RBC count at the post study evaluation. There was no evidence to support difference in Lecuocyte, Platelet, glucose, SGOT, SGPT, D.Billi between the baseline and post study evaluation.

Two period - Fed study

The baseline and the end of study paired values of biochemistry and hematology parameters are shown in Table 3. Changes in Serum Creatinine, SGOT, and SGPT were variable between baseline and at the end of the study. There were statistically significant decrease in serum creatinine and SGPT and there was statistically increased SGOT at poststudy evaluations compared with baseline values. There was statistically increased platelet count. There was no evidence to support any change in HB, RBC, Lecuocyte, Blood glucose, Urea, Cholesterol, T.Billi, D.Billi levels between baseline and at the end of the study.

Table 1: Paired mean (S.E.M) hematology and Biochemistry data obtained from three period Fed Bioequivalence study.

Laboratory Evaluations		(n=63)		
		Baseline	End of study	*P* value
Biochemistry	Glucose	104.13±2.72	121.50±4.51	<0.001
	Urea	22.29±0.67	21.68±0.61	0.371
	Creatinine	0.68±0.01	0.59±0.01	<0.001
	Cholesterol	209.06±4.76	214.38±4.60	0.147
	T.Billi	0.55±0.02	0.58±0.02	0.23
	D.Billi	<0.4	<0.4	
	SGOT	28.08±1.37	29.86±1.27	0.12
	SGPT	28.14±1.68	26.90±1.79	0.427
Hematology	HB	12.26±0.12	11.70±0.15	<0.001
	RBC	4.52±0.05	4.45±0.06	0.196
	Lecuocyte	8.66±0.27	8.15±0.26	<0.05
	Platelet	2.68±0.08	2.87±0.08	0.08

Plasma Glucose (mg/dL); Serum Urea (mg/dL); Serum Creatinine (mg/dL); Serum cholesterol (mg/dL); Serum Total Bilirubin (T.Billi) (mg/dL); Serum Direct Bilirubin (D.Billi) (mg/dL) Serum SGOT (AST) (U/L); Serum SGPT (ALT) (U/L)

Table 2: Paired mean (S.E.M) hematology and Biochemistry data obtained from two period fasting Bioequivalence study.

Laboratory Evaluations		(n=20)		
		Baseline	End of study	*P* value
Biochemistry	Glucose	118.83±10.73	109.92±5.03	0.261
	Urea	21.20±0.90	19.28±0.10	0.083
	Creatinine	0.70±0.01	0.63±0.03	<0.05
	Cholesterol	227.71±8.90	234.21±10.01	0.336
	T.Billi	0.62±0.03	0.57±0.03	0.192
	D.Billi	<0.4	<0.4	
	SGOT	26.71±1.80	34.88±3.53	<0.05
	SGPT	28.17±2.40	20.17±1.80	<0.05
Hematology	HB	12.31±0.19	12.54±0.23	0.103
	RBC	4.41±0.09	4.40±0.09	0.739
	Lecuocyte	8.49±0.50	8.30±0.54	0.62
	Platelet	2.52±0.20	2.83±0.15	<0.05

Plasma Glucose (mg/dL); Serum Urea (mg/dL); Serum Creatinine (mg/dL); Serum cholesterol (mg/dL); Serum Total Bilirubin (T.Billi) (mg/dL); Serum Direct Bilirubin (D.Billi) (mg/dL) Serum SGOT (AST) (U/L); Serum SGPT (ALT) (U/L)

Table 3: Paired mean (S.E.M) hematology and Biochemistry data obtained from two period fed Bioequivalence study

Laboratory Evaluations		(n=24)		
		Baseline	End of study	*P* value
Biochemistry	Glucose	106.50±6.80	114.37±7.07	0.241
	Urea	25.74±1.40	18.02±1.08	<0.001
	Creatinine	0.77±0.02	0.70±0.02	<0.05
	Cholesterol	206.90±8.81	212.90±9.52	0.302
	T.Billi	0.58±0.07	0.37±0.05	0.012
	D.Billi	<0.4	<0.4	
	SGOT	32.90±4.90	32.79±2.50	0.976
	SGPT	33.32±3.95	32.95±2.50	0.923
Hematology	HB	12.30±0.21	11.65±0.26	<0.05
	RBC	4.52±0.08	4.27±0.09	<0.05
	Lecuocyte	8.35±0.47	7.68±0.46	0.244
	Platelet	2.66±0.12	2.85±0.15	0.106

Plasma Glucose (mg/dL); Serum Urea (mg/dL); Serum Creatinine (mg/dL); Serum cholesterol (mg/dL); Serum Total Bilirubin (T.Billi) (mg/dL); Serum Direct Bilirubin (D.Billi) (mg/dL) Serum SGOT (AST) (U/L); Serum SGPT (ALT) (U/L)

DISCUSSION

This study reveals the Pre-dose and post-dose effect of the combined progesterone and estradiol capsules on the haematology and biochemistry parameters of post menopausal women from three different cross over bioequivalence studies. The creatinine levels from all the three BE studies was significantly different from the baseline and at post study evaluation. There was decrease in creatinine and urea levels significantly from which we can conclude estradiol[10] possess nephroprotectivity. To our knowledge this is the first study outcome to be analyzed in healthy postmenopausal women to register that combined progesterone (a precursor of estradiol [12]) and estradiol combination to show nephroprotective activity. There are many animal studies supporting the nephroprotective [6-9] activity of estradiol. However there were reports in contraindication with these findings[10]. Even though there are studies to support estradiol for nephroprotectivity, contarindications co-exists but the current human study outcome favors nephroprotectivity of estradiol and this is evidenced by decrease of urea and creatinine levels in blood significantly in healthy adult postmenopausal women. The findings of the current study is in favour of Szekacs et al. [11] were they had demonstrated, in a small number of diabetic and hypertensive postmenopausal women, that hormone replacement therapy reduced proteinuria and improved creatinine clearance.

CONCLUSION

Progesterone is the precursor of estradiol hence the combined progesterone and estradiol capsules probably produced a synergetic effect in decreasing urea and creatinine in the blood levels of the current BE studies undertaken. The other parameters such as HB, RBC, Lecuocyte, platelet count, SGOT, SGPT which were found significantly different from the baseline and at the post study, does not have any clinical significant correlation with the administered combined progesterone and estradiol capsules. But could be explained by the fact that the studies involved blood loss which in turn attributed for the temporary changes in hematology and biochemical parameters.

In a regulatory set up individual adverse events occurring during the study and or during the post study laboratory evaluation are documented and clinical significance is analyzed and provided in the appropriate sections in the ICH-E3 clinical report. The individual clinical significance of abnormal laboratory values is analyzed to determine the comparative safety of the drug. The screening baseline laboratory values are individually correlated however significance test of individual laboratory parameters of both normal and clinically abnormal laboratory values between the baseline and post study laboratory values of all the volunteers through provided in the report as appendix are not compared statistically to determine the true effect of drug. This statistical analysis is required due to one or more of the following reasons; the volunteers

who report to CRO (Contract Research organization) mostly are professional volunteers who participate in clinical trials on a comparable but potentially dangerous blood donation frequency more than the allowable limit within a year. Considering the volunteer's status of health and their benefit the current practice of reporting individual laboratory abnormalities would not be sufficient to monitor the drug effect on volunteers on a long term basis. Certainly no abnormal laboratory results were detected in this current statistical analysis of the screening and post study evaluation in the selected BE trials of progesterone however we hope that the statistical analysis would be essential in the future for regulatory authorities as a predictive tool to evaluate the safety of the effect of drug in volunteers in BA/BE trials. This type of monitoring would be an ongoing process until we find to report with evidence the necessity of statistical analysis in comparing screening laboratory parameters with the post study values in BA/BE trials.

REFERENCE

1. Portoles A, Terleira A, Almeida S, García-Arenillas M, Mari-Cruz C, Filipe A, Vargas E; Bioequivalence study of two formulations of enalapril, at a single oral dose of 20 mg (capsules): A randomized, two-way, open label, crossover study in healthy volunteers. Curr Ther Res Clin Exp., 2004; 65 (1): 34-46.
2. Jing R, Xue HJ, Kejia Li, Chuanchuan Zhang, Chenrui Li, Ling Wang; Bioequivalence of Two Formulations of a Single Oral Dose of 500-mg Azithromycin Granules: A Randomized, Open-Label, Two-Period Crossover Study in Healthy Han Chinese Volunteers. Curr Ther Res, 2007; 68 (5): 369-377.
3. Ascione FJ, Kirking DM, Gaither CA, Welage LS; Historical overview of generic med- ication policy. J Am Pharm Assoc (Wash), 2001; 41(4): 567–577.
4. European Medicines Agency. ICH-Efficacy E6(R1) CPMP/ICH/135/95. (Jan 1997) http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002874.pdf (March 2013)
5. Ethical Guidelines for Biomedical Research on Human Participants. ICMR 2006. http://icmr.nic.in/ethical_guidelines.pdf
6. Gades MD, Stern JS, van Goor H, Nguyen D, Johnson PR, Kaysen GA.; Estrogen accelerates the development of renal disease in female Zucker rats. Kidney Int., 1998; 53 (1): 130–135.
7. Joles JA, Van Goor H and Koomans HA; Estrogen induces glomerulosclerosis in analbuminemic rats. Kidney Int., 1998; 53 (4): 862–868.
8. Sakemi T, Toyoshima H, Shouno Y, Morito F; Estrogen attenuates progressive glomerular injury in hypercholesterolemic male Imai rats. Nephron, 1995; 69 (2): 159–165.
9. Muller V, Szabo A, Viklicky O, Gaul I, Portl S, Philipp T; Sex hormones and gender related differences: their influence on chronic renal allograft rejection. Kidney Int., 1999; 55 (5): 2011–2020.
10. Baylis C; Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy, Male gender as a primer risk factor. J Clin Invest., 1994; 94 (5): 1823–1829.
11. Szekacs B, Vajo Z, Varbiro S, Kakucs R, Vaslaki L, Acs N, Mucsi I, Brinton EA; Postmenopausal hormone replacement improves proteinuria and impaired creatinine clearance in type 2 diabetes mellitus and hypertension. BJOG, 2000; 107 (8): 1017–1021.
12. Schumacher M, Guennoun R, Ghoumari A, Massaad C, Robert F, El-Etr M, Akwa Y, Rajkowski K, Baulieu EE; Novel perspectives for progesterone in hormone replacement therapy, with special reference to the nervous system. Endocr Rev., 2007; 28 (4): 387--439.