A comparative bioavailability study of two ibuprofen formulations after single-dose administration in healthy volunteers

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Abstrak

Penelitian ini dilakukan untuk mengetahui apakah bioavailabilitas formulasi ibuprofen suppositoria 125 mg yang diproduksi oleh PT Kalbe Farma,Tbk. (Ibukal®) bioekivalen dengan produk yang sama dari komparatornya (Proris®). Parameter farmakokinetik yang dinilai dalam studi ini ialah luas daerah di bawah kurva kadar - waktu selama 10 jam (AUC_{0-t}), luas daerah di bawah kurva kadar - waktu sampai waktu tak terhingga (AUC_{0-inf}), kadar puncak (C_{max}), dan waktu untuk mencapai kadar puncak (t_{max}). Penelitian ini menggunakan rancangan menyilang acak, tersamar tunggal yang mengikutsertakan 12 sukarelawan dewasa sehat. Sukarelawan dipuasakan semalam dan keesokan harinya diberi 1 suppositoria obat uji (produk PT.Kalbe-Farma) atau 1 suppositoria obat pembanding (produk komparatornya). Contoh darah diambil pada jam ke 0 (kontrol), 20 min; 40 min; 1; 1,5; 2; 2,5; 3; 4; 6; 8; dan 10 jam setelah pemberian obat. Setelah 1 minggu periode washout, prosedur ini diulang dengan memberikan obat pembandingnya. Kadar obat ditentukan dengan kromatografi cair kinerja tinggi dengan detektor ultraviolet. Pada penelitian bioavailabilitas ini, rerata (SD) AUC_{0-t}, AUC_{0-inf}, C_{max} dan t_{max} dari obat uji masing-masing adalah 28,59(3,37) µg.jam.mL^{-1}, 30,47(3,56) µg.jam.mL^{-1},8,24(1,44)µg/mL, dan 1,33(0,44) jam. Rerata (SD) AUC_{0-t}, AUC_{0-inf}, C_{max} dan t_{max} dari obat pembanding masing-masing adalah 28,13(8,14) µg.jam.mL^{-1}, 30,56(8,05) µg.jam.mL^{-1}, 8,27(2,88) µg/mL, dan 1,79(0,33) jam. Rasio rerata geometrik obat uji terhadap obat pembandingnya ialah 104,38% untuk AUC_{0-t}, 101,97% untuk AUC_{0-inf}, dan 104,02% untuk C_{max}, Nilai 90% confidence intervals(CI) nya ialah 90,38-120,54% untuk $AUC_{0-t,}$ 89,51-116,16% untuk $AUC_{0-inf,}$ dan 85,73-126,16% untuk C_{max}. Tidak ada efek samping yang dijumpai dalam penelitian ini. Dari hasil penelitian ini disimpulkan bahwa Ibuprofen suppositoria 125 mg produksi PT. Kalbe Farma,Tbk. (Ibukal®) bioekuivalen dengan produk yang sama dari komparatornya (Proris®). ***(Med J Indones 2007; 16:181-6)***

Abstract

This study was aimed to investigate the bioequivalence of ibuprofen 125 mg suppository formulation (Ibukal®, test formulation from PT. Kalbe Farma, Tbk., Jakarta) and the ibuprofen suppository comparative formulation (Proris®, from PT. Pharos Indonesia, Jakarta) in 12 healthy volunteers. The pharmacokinetic parameters used in this study were the area under the concentration-time curve from time zero to hour 10 (AUC_{0-t}), the area under the concentration-time curve from time zero to infinite (AUC_{0-inf}), the maximum concentration (C_{max}), and the time needed to reach the maximum concentration (t_{max}). The study was designed as a random cross-over fashion, single-blinded which included 12 healthy adult volunteers. The volunteers were fasted overnight and in the morning they received a suppository of the test drug (Ibukal®) or a suppository of the comparative drug (Proris®). Blood samples were withdrawn on hour 0 (control), 20 min; 40 min; 1; 1,5; 2; 2,5; 3; 4; 6; 8; and 10 time points after the administration of the drug. Following a wash-out period of 1 week, this procedure was repeated using the other drug. The serum concentration of the drug was determined by means of high-performance liquid chromatography with ultraviolet detection. The results of the study showed that, the mean (SD) of AUC_{0-t}, AUC_{0-inf}, C_{max} and t_{max} of the test drug were, respectively, 28.59(3.37) µg.h.mL^{-1}, 30.47(3.56) µg.h.mL^{-1}, 8.24(1.44) µg/mL, and 1.33(0.44) h. The mean (SD) of AUC_{0-t}, AUC_{0-inf}, C_{max} and t_{max} of the comparative drug were, respectively, 28.13(8.14) µg.h.mL^{-1}, 30.56(8.05) µg.h.mL^{-1}, 8.27(2.88) µg/mL, and 1.79(0.33) h. The geometric means ratio of the test to the comparative drug were 104.38% (CI 90%: 90.38-120.54%) for AUC_{0-t}, 101.97% (CI 90%: 89.51-116.16%) for AUC_{0-inf}, and 104.02% (CI 90%: 85.73-126.16%) for C_{max}. There was no side effect of the drug detected in this study. From the results we can conclude that the 125 mg of ibuprofen suppository of PT Kalbe Farma, Tbk. (Ibukal®) is bioequivalent to that of the comparative drug (Proris®). ***(Med J Indones 2007; 16:181-6)***

Keywords: ibuprofen suppository, high-performance liquid chromatography, bioequivalence

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Ibuprofen (2-(4-isobutylphenyl)-propionic acid, $C_{13}H_{18}O_2$) is a non-steroidal antiinflamatory drug. It is a racemate of (S)-(+) dan (R)-(-) enantiomer with the ratio 1:1, but only the S-enantiomer is active. Both enantiomers posses the same pharmacokinetic (concentration vs. time) profile. The analgesic and antiinflamatory effect of the drug have been used clinically to treat post operative pain in young adults and children with minor side effect. The main side effect of ibuprofen is gastro-intestinal tract irritation e.g., gastritis. The mechanism of antiinflamation of ibuprofen is through the inhibition of the prostaglandin and leukotriene biosynthesis.

Ibuprofen is rapidly absorbed after oral administration in men, and peak plasma concentration is observed after 1 to 2 hours. The half-life in plasma is about 2 hours. Although the rectal preparation may have some delay in reaching the peak plasma concentration, ibuprofen suppositories are absorbed efficiently. The peak plasma concentration (C_{max}) is about 5-20 mg/L (oral, single dose of 200 mg) or about 12.4-30.1 mg/L (suppository, doses of 20 mg/kg BB). The bioavailability of suppository formulation is about 73%.

Ibuprofen is extensively bound to plasma proteins (90-99%), penetrates slowly from plasma to synovial spaces and may accumulate there in higher con-centration. The drug is metabolized by hydroxylation and carboxylation to form two inactive metabolites, which are eliminated after conjugation to glucuronic acid. The excretion of ibuprofen is rapid and complete; more than 90% of an ingested dose is excreted in the urine as metabolites or their conjugates, and no ibuprofen is found in the urine.[1,2,5]

The bioavailability of two (or more) formulations of the same active ingredient could differ from one to the other; therefore bioequivalence studies become the important part of registration dossiers. If they are equivalent, then one may subsequently claim that the therapeutic efficacy of both formulations is similar. It may also mean that the beneficial and side effects of the two drugs are identical, and hence the formulations are interchangeable.

The aim of this study was to compare, the pharmacokinetic profiles and to evaluate the bioequivalence of two formulation of 125 mg ibuprofen suppositories (Ibukal® from PT.Kalbe Farma Tbk., Jakarta and Proris® from PT. Pharos Indonesia, Jakarta), after single dose rectal administration in healthy volunteers of both sexes.

METHODS

Clinical protocol

Twelve healthy subjects were recruited in this study. They were of both sexes between 18-55 years of age and with a normal body weight (Body Mass Index =18-25). Physical examination and laboratory check up (hemoblobin, hematocrite, total and differential white cell count, creatinine, alkaline phosphatase, Alanin Trans Aminase /ALT, total bilirubin, albumin and total protein, and routine urinalysis) were performed. Pregnant or nursing women, individual with a history of hypersensitive to ibuprofen or other anti-inflamatory Non Steroidal Drugs (NSAIDs), and those who had any gastrointestinal tract problem(s), were excluded from the study.

All subjects gave written informed consent and the protocol had been approved by Medical Research Ethics of the Medical Faculty, University of Indonesia. The study was conducted in accordance with the Good Clinical Practice (GCP) standard.

The study was a single-dose, 2-way randomized crossover design with a one week washout period between the doses. The subjects were instructed to abstain from taking any medication for at least 1 week before and during the study period. The subjects were also not permitted to consume alcohol, or beverage or food containing xanthines, such as tea, coffee and cola, or fruit juice.

The subjects were fasted for 10 hours prior to drug administration. On the morning of study phase as scheduled, the volunteers were given a single dose of either formulation (reference or test) of ibuprofen suppository. No food was permitted for 4 hours after dosing. Breakfast, lunch, and dinner were given to all the volunteers according to the time schedule. The volunteers were instructed to stay in bed for 4 hours after drug administration, afterwards they were allowed to walk but were prohibited from strenuous activity; they were under direct supervision at the study site. Blood pressure, pulse, and adverse events, which might occur, were monitored and recorded in the Case Report Form (CRF).

Blood samples (approximately 5 mL) from a suitable antecubital vein were collected into tube before (0 h) and at 0; 20 min; 40 min; 1; 1,5; 2; 2,5; 3; 4; 6; 8, and 10 hours after dosing. Blood samples were centrifuged at 3000 rpm for 10 min, serum were separated and stored frozen at -20^0 C until assayed.

After a washout period of 7 days, the study was repeated in the same manner to complete the crossover design.

Formulations

The following formulations were employed: Ibukal® 125 mg suppository (Test formulation from PT. Kalbe Farma,Tbk., batch No. 821001A, expiration date January 2008) and Proris®125 mg suppository (Reference formulation from PT. Pharos Indonesia, batch No. B5L44D, expiration date November 2008).

Drug analysis

Serum samples were analyzed for ibuprofen according to the HPLC method developed at the PT. Pharma Metric labs laboratory, and validated following international guideline.

To a tube of 0.5 mL serum containing ibuprofen was added 40 µL of internal standard (mefenamic acid, 125 µg/mL); the mixture was shaken for 30s by mean of a vortex mixer. 250 µL of 1.0 M phosphate buffer pH 2.0 and 4.0 mL diethylether was then added and the mixture was again vigorously shaken for 1 min. The mixture was then centrifuged for 10 min at 3000 rpm.The supernatant was transferred to a clean glass tube and evaporated to dryness under nitrogen stream in a waterbath 40^0C. The residue was redissolved in 200 µL methanol, and vortex-mixed for 30s. The solutions were then transferred to the auto-injector microvials.

An aliquot (20 µL) of each serum extract was injected into a Waters Symmetry C18 analytical column, (150 mm x 4.6 mm i.d.). The compounds were eluted by pumping the mobile phase (acetonitrile – 0.01M potassium dihydrogen phosphate, pH 7.0 (35:65; v/v)) at a flow rate of 1.0 mL/min. The effluent was monitored using an ultraviolet detector at 225 nm wavelength. Under this condition, typical retention times were 3.15 and 5.60 minutes for ibuprofen and mefenamic acid, respectively.

Accuracy, Precision and Recovery

Accuracy, precision and recovery of quality control serum samples were measured at the concentration of 1.5, 15.0, and 30.0 µg/mL of ibuprofen.

Stability

Stability of quality control serum samples (1.5, and 30.0 µg/mL) were subjected to 7, 14, 28 days storage, and 2 freeze/thaw (-20 to 25^0C) cycles test. Subsequently, the ibuprofen concentrations were measured and compared to freshly prepared samples.

Pharmacokinetics and statistical analysis

The bioequivalence between the 2 formulations was assessed by calculating individual test/reference ratio for:

1. Area under the curve (AUC) of serum concentration until last concentration (C_t) observed (AUC_{0-t}), AUC_t were calculated by applying the linear trapezoid rule
2. Area under the curve (AUC) between the first sample (pre-dosage) and infinity (AUC_{0-inf}), AUC_{0-inf} were calculated by the following equation: $AUC_{0-inf} = AUC_t + C_{0-t} / Ke$; $Ke = 0{,}693 / t_{½}$; $t_{½} = 0{,}693 / (2{,}303 \times slope)$
3. Peak serum concentration (C_{max})
4. Time to peak serum concentration (t_{max})
   The mean maximum serum concentration and the mean time to peak serum concentration obtain directly from observed data.

The AUC and C_{max} data for the 2 formulations were analyzed by ANOVA using Equiv Test® versi 2.0 programme (*Statistical Solution Ltd., Saugus. MA, USA*) to establish whether the 90% CI of the ratios was within 80-125% interval (for AUC) and 70-143% interval for C_{max}, indicating bioequivalence as proposed by US Food and Drug Administration.

Parametric and non parametric analyses of ln-transformed arithmetic means and individual t_{max} difference between the test and reference formulations were also performed.

RESULTS

The volunteer involved in the study had the following characteristic (expressed as mean ± SD (range)): age: 20.0±5.0 years old, (19-36) years old, height: 164.8±6.6 cm (150-176 cm), body weight: 61.2±7.1 kg (51-75 kg).

The HPLC chromatograms of the sample is shown in Figure 1, in which the retention times of ibuprofen and mefenamic acid are 3.15 and 5.60 minutes, respectively. The lower limit of quantification (LOQ) of our method was 0.5 µg/mL. Ibuprofen concentrations were quantified using linear regression of area under the curve (AUC) ratios (Ibuprofen/IS) versus concentration. The calibration curves were linear over the concentrations range of 0.5 to 40.0 µg/mL. Table 1 shows the precision, accuracy, recovery and stability data for QCs.

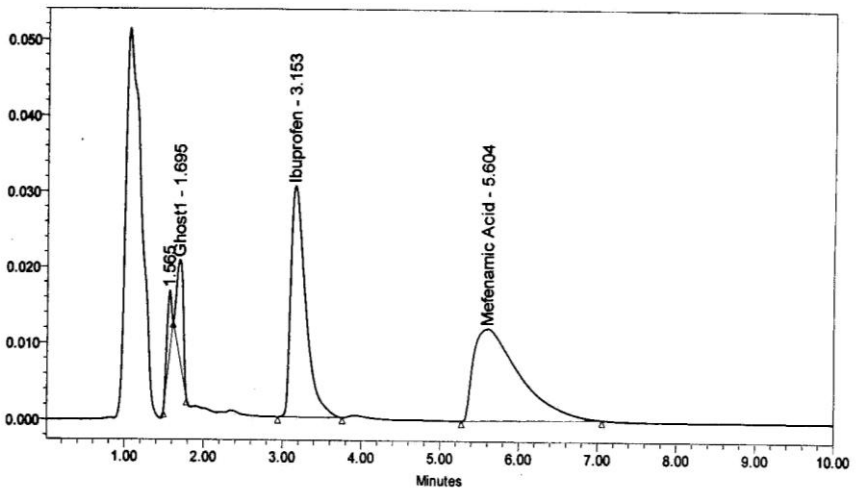

Figure 1. The HPLC chromatogram of serum sample after suppository administration of ibuprofen 125 mg suppository formulation in healthy volunteer

Table 1. The precision, accuracy, recovery, and stability data for ibuprofen from the pre-study validation in human serum

Intra-day validation			
Nominal concentration	1.5	15.0	30.0
Precision (%)	7.94	6.58	7.95
Accuracy (%)	15.73	9.24	5.52
Inter-day validation			
Nominal concentration	1.5	15.0	30.0
Precision (%)	8.06	4.85	6.64
Accuracy (%)	7.78	2.74	3.78
Recovery data			
Nominal concentration	1.5	15.0	30.0
Range (%)	114.5-118.4	103.1-104.7	104.3-110.7
Mean (%)	116.3	103.7	107.1

Stability on storage	Concentration measured at:			
Actual concentration	Day-0	Day-7	Day-14	Day-28
1.5 (µg/mL)	1.67(11.33)	1.43 (6.89)	1.59 (5.78)	1.45(6.67)
30.0 (µg/mL)	30.71(2.49)	28.79(5.38)	29.28(2.40)	30.96(11.41)

Freeze/thaw cycle	Cycle-1	Cycle-2	Cycle-3
1.5 (µg/mL)	1.67(11.33)	1.43 (6.89)	1.60 (6.44)
30.0 (µg/mL)	30.71(2.49)	28.79(5.38)	31.12(3.74)

Ibuprofen was found to be well tolerated and no significant adverse reaction was encountered during the trial.

The ibuprofen serum mean concentration vs. time profile obtained after the single suppositopies administration of each 125 mg suppository formulation is shown in Figure 2.

The mean pharmacokinetic parameters obtained from 12 volunteers after administration of a 125 mg suppository formulation is shown in Table 2.

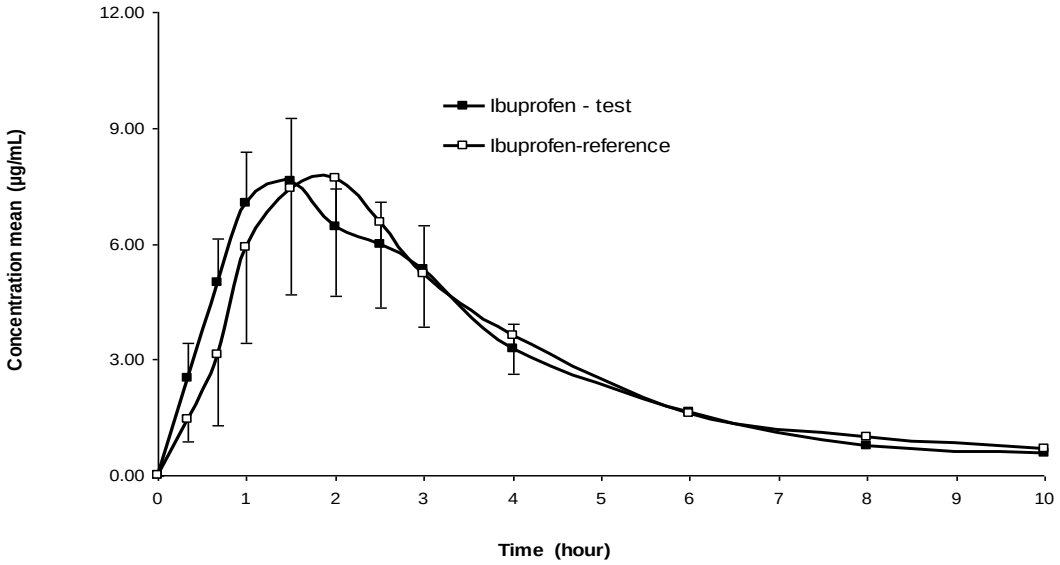

Figure 2. Ibuprofen serum mean concentration vs. time profile obtained after the single suppository administration of 125 mg of ibuprofen suppository formulation in 12 volunteers

Table 2. Mean pharmacokinetic parameters obtained from 12 volunteers after administration of each of 125 mg ibuprofen formulation

	Proris® (Reference drug)				Ibukal® (Test Drug)			
	Mean	SD (±)	Min	Max	Mean	SD (±)	Min	Max
t_{max} (h)	1.79	0.33	1.50	2.50	1.33	0.44	1.00	2.50
t_{last} (h)	8.17	1.80	6.00	10.00	8.33	1.44	6.00	10.00
$t_{1/2}$ (h)	1.90	0.45	1.36	2.66	1.85	0.29	1.50	2.42
K_e (1/h)	0.35	0.10	0.27	0.49	0.38	0.06	0.29	0.46
C_{max} (µg/mL)	8.27	2.88	4.18	13.49	8.24	1.44	6.62	11.42
C_t (h)	0.86	0.33	0.56	1.48	0.73	0.28	0.52	1.32
AUC_{0-t} (µg.h/mL)	28.13	8.14	8.29	45.79	28.59	3.73	23.36	36.43
AUC_{0-inf} (µg.h/mL)	30.56	8.05	21.46	48.42	30.47	3.56	25.36	38.35

Table 3 shows the geometric mean of the individual C_{max}, AUC_{0-t}, AUC_{0-inf}, (test/reference formulation), the respective 90% confidence intervals (CI), and Intrasubject CV for 12 volunteers. The calculated 90% CI for mean AUC_{0-t} and AUC_{0-inf}, individual ratios were within the 80-125%, and for C_{max} were within 75-143% interval defined by the US Food and Drug Administration(US FDA) and Indonesian FDA, thus establishing the bioequivalence of the two formulations

There is not statistically significant difference of t_{max} value between the test and the reference drug.

Table 3. Geometric mean of individual AUC_{0-t}, AUC_{0-inf}, and C_{max} ratios (test/reference formulation), the respective 90% confidence interval (CI) and inter-subject CV

	Geometric Mean	90% CI	Intra Subject CV%
AUC_{0-t}	104.39	90.38 – 120.54	19.44
AUC_{0-inf}	101.97	89.51 – 116.16	17.61
C_{max}	104.02	85.73 – 126.16	26.01

DISCUSSION

Ibuprofen has been determined in plasma or serum and other biological fluids by several methods. The method used in this study is a modification of that describes previously by Rustam AM et al.[4] The assay was performed using C18 analytical column, and mefenamic acid as the internal standard (IS). The HPLC chromatograms of the sample gave a good resolution and selectivity between ibuprofen and the internal standard. From the pre-study validation data (precision, accuracy, recovery and stability) obtained, it can be shown that the developed method is sufficient for ibuprofen pharmacokinetic study.

After rectal administration of the ibuprofen suppositories, the observed ibuprofen mean peak serum concentrationin (C_{max}) value were similar to those reported in the literature (Kyllonem M. et al.).

CONCLUSION

From this study, it can be concluded that ibuprofen 125 mg suppository produce by PT. Kalbe Farma, Tbk. (Ibukal®) is bioequivalence to its comparative drug (Proris®) for both rate and the extent of absorption.

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