Bioequivalence Study and Pharmacokinetic Evaluation of Two Brands of Rofecoxib 25 mg Tablets in a Lebanese Population

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ABSTRACT

Objective : To examine the in vivo average bioequivalence of a locally-manufactured rofecoxib brand (Rofib) and foreign brand (Vioxx acute), both in 25 mg tablet form and to evaluate the drug's pharmacokinetics in Lebanese male volunteers.

Design and Participants: Forty healthy male adults completed the study. Inclusion and exclusion criteria are detailed in the paper. The protocol was approved by the ethical committee of the Hospital Hôtel-Dieu de France and of the Saint-Joseph University and the study was conducted according to the Helsinky declaration standards. This was an open-label, randomized, double blind, single dose, two-way, non-replicated cross over design, with 19 days wash-out. The extraction procedure and HPLC analysis were adapted from previously published methods.

Results: The non-compartmental pharmacokinetic analysis was done using winNon Lin software. The bioequivalence statistical model used is a two-period crossover analysis of variance with the factors sequence, period, treatment, and subject-within-the sequence. The primary pharmacokinetic parameters compared were Cmax and AUC0- and were Ln transformed. The 90 and 95 % CI were calculated for both parameters.

Conclusion: The non-transformed pharmacokinetic parameters (Cmax, AUC0- , tmax, t1/2) and the geometric least square means are calculated for both brands. The confidence intervals for Cmax and AUC0- are fully contained within the interval 80-125%, thus establishing bioequivalence.

INTRODUCTION

Rofecoxib, a diaryl substituted furanone, is a selective COX-2 inhibitor. The pharmacokinetics of rofecoxib is well-documented in the literature;[1-4] it is readily absorbed following oral administration with a mean oral bioavailability of approximately 93 % for doses between 12.5 and 50 mg. It is highly bound to plasma proteins, primarily to albumin (87%). Metabolism occurs pri-

Table 1. Age, Height, and Weight of Selected Volunteers

	Average	SD
Age (years)	23.6	4.19
Height (cm)	176.4	7.06
Weight (kg)	75.9	8.63

marily by cytosolic reductases producing dihydro derivatives. Most of the drug is excreted in the urine as metabolites and 14 % is excreted in the feces unchanged. The elimination half-life of rofecoxib is reported to be around 9 hours for the 25 mg tablet form.[3,5]

To date, there is no documented evaluation of rofecoxib pharmacokinetics in the Lebanese population. Furthermore, the general pharmacokinetic characteristics of this population are poorly known. This study was designed to evaluate the pharmacokinetics of rofecoxib in Lebanese healthy male volunteers and to assess in vivo average bioequivalence of locally manufactured rofecoxib (Rofib, lot number 36271, expiry date 12/2005, Pharmaline, Lebanon) and imported UK brand (Vioxx acute, lot number 231539, expiry date 02/2005, MSD). The 25 mg tablet form was used for both brands.

The HPLC method adapted and used for rofecoxib assay in serum is sensitive, simpler, and less time consuming than previously published methods.[6-9] It has also the advantage of using only 200 µL of serum for determination of rofecoxib concentration.

SUBJECTS AND METHODS

Subjects

Forty healthy white male adults completed the study. The demographics are shown in Table 1. All subjects are Lebanese, within -15 % to +10 % of normal body weight (according to Broca's formula), non-smokers, and consume alcohol occasionally or not at all. Subjects were excluded if they had clinically or biologically significant abnormalities, history of allergy to rofecoxib or to any of the excipients in the two brands, history of gastro-intestinal ulcer or gastro-intestinal bleeding, history of asthma, allergic rhinitis, nasal polyps, urticaria induced by aspirin or any other NSAID, inflammatory disease of the intestine, addiction or history of addiction to drugs, any chronic disease or any chronic intake of medicine and heavy caffeine consumption.

Table 2. Validation of the HPLC Method Used in the Assay

Intra-day (n =4) Actual ng/mL	Observed ng/mL	CV %
20	19.325	8.375
50	52.5	6.25
100	99.115	1.3625
200	200.6625	0.73125
400	399.87	0.6737
800	800.125	0.3906
Inter-day (n =6) Actual ng/mL	Observed ng/mL	CV %
20	21.088	8.38
50	50.048	4.43
100	100.55	4.2167
200	198.155	2.95
400	402.3	2.408
800	795.58	1.218

The protocol was approved by the ethical committee of Hôtel-Dieu de France Hospital and Saint-Joseph University and the study was conducted in accordance with the guidelines on good clinical practice and with ethical standards for human experimentation established by the declaration of Helsinki. Every subject provided a written informed consent and was free to withdraw from the study at any time without any obligation.

Study Protocol

This was an open-label, randomized,

Table 3. Pharmacokinetic Parameters of Both Rofecoxib Brands

	Rofib 25 mg tablet mean (SD)	Vioxx acute 25 mg tablet mean (SD)
Cmax (ng/mL)	221.95 (69.92)	234.87 (60.39)
AUC 0-∞ (ngxh/mL)	4185.21 (2367.52)	4187.33 (2202.88)
Tmax (h)	3.37 (1.03)	3.24 (0.80)
T1/2ß* (h)	19.55 (11.42)	19.54 (13.13)

*Apparent terminal half-life.

double blind, single dose, two-way, non-replicated cross-over investigation, with a wash-out period of 19 days between the doses. Subjects were randomized to one of the rofecoxib brands during phase I of the study. After a wash out period of 19 days, each subject received the other brand. Subjects participating in the study were not allowed to take any medicine 2 weeks prior to the study and until the completion of the second phase of the protocol.

For occasional smokers, no smoking was allowed 48 hours before the study and until the completion of the second phase. Alcohol, caffeinated beverages, and grapefruit juice were also forbidden 48 hours before the study and until the completion of the second phase.

After fasting overnight, the subjects reported to the study unit at the Hotel Dieu hospital. Each subject took the tablet with 210 cc water and fasted for 4 hours post-dose. Preservative-free clear apple juice (200 cc) was served at 4 hours post-dose and a standardized lunch was served at 4.5 hours post-dose and a standardized snack given at 7 hours post-dose. All subjects ate standardized dinner at 10 hours post-dose.

Blood (3 cc) was drawn, via an indwelling intravenous catheter for determination of rofecoxib concentrations, at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 24, and 72 hours post-dose. Blood was immediately centrifuged and serum was stored at –20˚C until analysis (around 1 month later).

The assay was performed using high-performance liquid chromatography (column and precolumn Zorbax Eclipse XDB-C8, 4.5 x 150 mm, 5 µ, Agilent) with UV detection at 273 nm and flow rate of 0.5 mL/min. The mobile phase consisted of 5 % acetonitrile (HPLC grade-Romil LTD-batch H531462) and 45% water (HPLC grade-Romil LTD-batch F543462) containing 5.714 mL/L of glacial acetic acid.

Rofecoxib and internal standard (methylparaben) stock solutions were prepared in acetonitrile and stored at –20˚C in the dark (they remained stable for minimum 3 months). Working standards were prepared by diluting the stock in drug-free serum over various concentrations (20, 50, 100, 200, 400, and 800 ng/mL). Twenty µL of internal standard was added to 100 µ of serum and 50 µL 0.1N NaOH were added dropwise while vortexing (speed 15). The sample was further vortexed for 10 seconds at maximum speed and 1 mL ethylacetate was added and the mixture was vortexed for an additional 30 seconds before centrifugation at 4000 rpm for 10 minutes. Ethylacetate was then transferred to glass tubes and evaporated under a gentle stream of nitrogen at 37˚C and the dried residue reconstituted with 35 µL acetonitrile. Fifteen µL was injected in the HPLC apparatus.

The mean recovery of rofecoxib from serum was around 85%. The limit of detection was 8.6 ng/mL and the standard curve showed perfect linearity up to 1000 ng/mL (which was the highest concentration tested). The intra-day and inter-day variability is represented in Table 2.

Table 4. Cmax Bioeqivalence*

Cmax (ng/mL)		
Reference Geo LSM	227.561	CI 90%: 86.35-100.85
Test Geo LSM	212.348	CI 95%: 85.01-102.43
Ratio	0.9331	Power: 0.9985

*Reference is Vioxx acute and test is Rofib. Geo LSM is the geometric least square mean.

Table 5. AUC Bioequivalence*

AUC 0-∞ (ngxh/mL)		
Reference Geo LSM	3716.91	CI 90%: 88.97-104.55
Test Geo LSM	3584.95	CI 95%: 87.54-106.26
Ratio	0.9645	Power: 0.9975

Clinical examination and laboratory analysis were performed 1 week before the study and clinical examination was conducted after completion of the first phase and second phase. Adverse events were monitored throughout the study and kept track of in a special register. A physician was available throughout the study to examine and evaluate any adverse events and patient complaince.

RESULTS

Pharmacokinetic analysis using the untransformed data was performed using WinNonlin software and a non-compartmental approach. The untransformed pharmacokinetic parameters for both brands are represented in Table 3 (the values less than the limit of detection of the method were not considered in the analysis).

Log (natural) transformation of Cmax and AUC0- were performed prior to the statistical analysis.

The parametric general linear model used for statistical analysis includes factors accounting for sequence effect, subjects nested in sequences, period and treatment. The equivalence criteria range is 80 to 125% according to FDA guidelines.

The 90 and 95% confidence intervals for the ratio (Rofib/Vioxx acute) of the geometric least square means were calculated for each parameter. The significance level was set to 0.05. Results are presented in Tables 4 and 5. No sequence effect, period effect or treatment effect were detected.

DISCUSSION

We studied the pharmacokinetics of rofecoxib in Lebanese healthy white male adults and the results obtained are close to previously published rofecoxib pharmacokinetic data.[3,5] However, our data seem to indicate that the terminal half-life of the drug is more prolonged, with an average of 19.5 hours for both brands. A recent study[10] has demonstrated the involvement of uridine diphosphate-glucuronosyl transferase (UGT), specifically UGT2B7 and 2B15 in glucufluronidation of 5-hydroxyrefecoxib in rofecoxib metabolism in human liver microsomes. Since genetic and ethnic polymorphisms have been identified in these transferase genes, it may be possible that the longer terminal half-life observed in our volunteers compared to other studies, is related to polymorphisms in O-glucuronidation of the drug.

As for the comparison of the two rofecoxib brands, the 90 and 95% confidence intervals of Cmax and AUC0- are fully included within the 80 to 125% interval, thus establishing bioequivalence.

CONCLUSION

The locally manufactured brand of rofecoxib 25 mg tablet form is bioequivalent to the imported brand and both were well tolerated. No subject developed any adverse experience.

Further studies of the ethnic related specificities of rofecoxib pharmacokinetics are needed, particularly concerning the metabolism of the drug as well as the relation between efficacy of rofecoxib and its pharmacokinetics.

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