Bioequivalency of Two Piroxicam Products in Plasma by High Performance Liquid Chromatography

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Abstract

To determine piroxicam in human plasma and to compare the pharmacokinetic profile of the test drug (Piroxicam Tablet) and the reference drug (Piroxicam Capsule) for bioequivalence study. This study represents the result of a randomized, single dose, two period crossover studies in 15 healthy volunteers to assess the bioequivalence of two formulations of piroxicam 20 mg. The blood samples (5 ml) were drawn concomitantly from 1-72 hours after oral administration of a single dose of 20 mg piroxicam capsules (Piroxisam)® (Samarra Drug Industry) as reference drug to 15 volunteers then after 15 days wash out period, the same volunteers were given 20 mg of piroxisam tablets (Samarra Drug Industry) as test drug. The pharmacokinetic parameters were obtained from the mean plasma concentration measured at various sampling times. C_{max} and T_{max} of both products were similar**.** K_a and K_{elm} values of were very close between test and reference formulations. The AUC value of piroxicam tablet was lower than that of capsule. The test formulation was found to be bioequivalent to reference formulation based on the pharmacokinetic parameters.

الخلاصة

لتحديد البيروكسيكام في البلازما و مقارنة المؤشرات الحركية لكل الدواء الاختباري و المرجعي لدراسة التكافؤ الحيوي. ه ذه الدراسة هي نتيجة لتناول جرعة واحدة عن طريق الفم لاثنين من الصيغ الصيدلانية لدواء البيروكسيكام20 ملغم ل 15 متطوعا من الأصحاء لغرض تقييم التكافؤ الحيوي. تم سحب عينات الدم (5ملليلتر) للفترة من 1-72 ساعة بعد تناول كل من (بيروكسيكام كبسول (Piroxisam)® (معمل أدوية سامراء) وأقراص Piroxisam (معمل أدوية سامراء) لهؤلاء المتطوعين على فترتين تتخللها فترة 15 يوم من عدم تعاطي أي من المستحضرين، ثم تم الحصول على موشرات حركية الدواء من تركيز الدواء في البلازما المقاسة في أوقات مختلفة. كانت كل من C_{max} و T_{max} متماثلة لكلا المستحضرين كذلك قيم K_a و K_{elm} كانت متقاربة. أما قيمة AUC للأقراص فقد كانت اقل منها للكبسول. وجد بان الصيغة الجديدة (أقراص البيروكسيكام) مكافئ بيولوجيا للصيغة القياسية بالاعتماد على المؤشرات الحركية لهذا الدواء.

Introduction

Piroxicam [4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2- benzothiazine-3-carboxamide 1,1-dioxide] is a member of the oxicam group of nonsteroidal anti-inflammatory drugs (NSAIDs)[1,2], is well established in the treatment of rheumatoid arthritis and osteoarthritis [3] and offers an alternative to other analgesics in various pain states.[4] It has the advantage that once-daily dosage is sufficient to provide efficacy equal to or better than other NSAIDs.[5] The mechanism of action of piroxicam, like that of other NSAIDs, is not completely understood but may be related to prostaglandin synthetase inhibition [6] . It is well absorbed following oral administration [7], the C_{max} values were reported as 1.5 to 2 µg/mL when the healthy volunteers were administered 20 mg piorxicam as single dose [2,8] . Piroxicam has a small volume of distribution and a low plasma clearance[4], it is highly bound (approximately 99%) to plasma proteins[7] and it has been detected in breast milk.[9] It metabolized in the liver by hydroxylation and conjugation with glucuronic acid, and is excreted mainly in the urine with smaller amounts in the feces. [9] Enterohepatic recycling occurs and less than 5% of the dose is excreted unchanged in the urine and feces. [9] Because of the long half-life (about 50 hours) of piroxicam, steady-state blood levels are not reached for 7-12 days [7] . Age of the patient and renal or hepatic dysfunction do not seem to have any major effect on the pharmacokinetics of piroxicam [4] . The aim of the present study was to determine the pharmacokinetic parameters of the two formulation of piroxicam (Capsule and Tablet) in healthy volunteers then compare these parameters statistically to evaluate the bioavailability between the two brands.

Materials & Methods

Chemicals & Reagents

Piroxicam was prepared as aqueous solution in 0.1N NaOH at a concentration of 0.1mg/ml and stored in the dark at 4°C. No degradation was noted for at least one month. Appropriate dilutions were made with water to produce working standard containing 10µg/ml for piroxicam. HPLC grade acetonitrile, diethyl ether, acetic acid, sulfuric acid and tris (hydroxymethyl) aminoethane were of analytical grade were used without further purification. [10]

Method

The study was a randomized, single dose, cross-over complete two period of treatment dosing. Fifteen healthy volunteers were participating; their average age was 45 ±12 years and a body weight of 77 ± 5 kg. The volunteers were instructed to abstain from taking any other medication for at least one week prior to and during the study period. After an overnight fasting (10 hr) subjects were given a single dose of 20 mg piroxicam capsules as reference drug then after 15 days washout period, the same volunteers were have 20 mg of piroxicam tablets as test drug with 240 ml of water The subjects were maintained in the fasting state for 4 hour after administering the drug. A blood sample was collected before drug was given (Zero time) and then at 1, 2, 4, 6, 8, 10, 12, 24, 48, 72 hours after oral administration piroxicam. A 5 ml blood sample was collected each time. Plasma was gathered and analyzed for piroxicam by a HPLC method [10, 11]. The amount of food and water intake and physical activity for each individual subject were standardized during the sampling days. Xanthine-containing food or beverages and fruit juices were not allowed for 24 hours before and during the entire sampling days. Blood pressure, heart rate and adverse events were monitored during the blood sampling and also on follow-up study. [2, 11]

Sample preparation

Plasma were obtained from blood samples using heparinized tube (25 µg), were added to 5ml of blood before centrifugation at 3500 rpm for 10 min. A disposable culture tubes were used, in each tube 0.1 ml of plasma was fortified with 10µg of

piroxicam, then mixed with 0.5 ml of 0.1N sulfuric acid, extracted with 4ml of diethyl ether on vortex mixture for 30 sec. and centrifuged. [10, 12] The solvent layer was transferred to another tube and evaporated to dryness. The residue was reconstituted in 1ml of 0.05M tris (hydroxymethyl) aminemethane and aliquots subjected HPLC. Sample at this stage could be held for at least seven days at room temperature as demonstrated by the same peak area ratio of piroxicam to measure on day 1, 2, 4 and 8. [10, 12]

Chromatographic apparatus

A Shimadzu 10 AVP (Japan) analytical chromatography equipped with a (300mm x 3.9mm I.D), 5 µm µ-Bondapak cyano—propyl column was fitted with Shimadzu UV Detecter using low-dead volume hardware. A mobile phase of acetonitric: water: acetic acid (24:71:5) was filtered, degassed and used. The detection wavelength was a 355 nm. and the flow rate of mobile phase was 1.8 mL/min. The concentrations of piroxicam were calculated by comparison the peak area of standard with that of authentic standard under the same separated conditions. [12]

Pharmacokinetic analysis

The actual blood sampling times were used, and the maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were observed values. The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule. The elimination rate constant (K_{elm}) was obtained as the slope of the linear regression of the log-transformed concentration-time curve data in the terminal phase. The absorption rate constant (K_a) was obtained by using the residual method.

Statistical analysis

Paired *t*-test used to calculate the difference whether significant or insignificant between the values of the two formulations of piroxicam 20 mg (Piroxisam® Capsule and Piroxisam Tablet) that were manufactured by Samarra Drug Industry/ Iraq. [13, 14]

Results

The mean plasma concentration-time for reference drug piroxisam capsule from (Samarra Drug Industry) and test drug piroxicam tablet from (Samarra Drug Industry), after the administration of a single oral dose of 20 mg of both drugs to fifteen healthy volunteers were measured at different time from 1-72 hours, are shown in table 1 and 2 and the mean plasma concentration-time curves for both formulations are shown in figure1. Various pharmacokinetics parameters include K_a, K_{elm}, C_{max}, T_{max} and AUC were determined form plasma concentration for both formulations as shown in table 3.There was significant difference ($p < 0.05$) found in the value of C_{max} as well as AUC ($p < 0.01$) between the two products.

Table (1):- Concentration (µg/ml) versus times (hr) after the administration of 20 mg piroxicam capsules (piroxisam)®

Subject	Time (hr)										
	0	1	2	4	6	8	10	12	24	48	72
1	0	1.27	1.40	2.31	2.54	1.97	1.87	1.79	1.38	1.04	0.69
2	0	1.25	1.37	2.33	2.60	1.98	1.89	1.80	1.39	1.05	0.70
3	0	1.11	1.48	2.24	2.59	2.10	1.85	1.78	1.35	1.00	0.74
4	0	1.27	1.50	2.33	2.67	2.12	1.91	1.70	1.38	1.03	0.68
5	0	1.20	1.55	2.47	2.55	1.95	1.85	1.69	1.33	0.98	0.67
6	0	1.13	1.57	2.25	2.56	2.05	1.92	1.77	1.44	1.05	0.73
7	0	1.17	1.40	2.22	2.67	1.96	1.89	1.77	1.33	1.15	0.68
8	0	1.33	1.48	2.20	2.75	1.99	1.78	1.70	1.31	1.10	0.75

9	0	1.27	1.41	2.31	2.74	2.31	1.96	1.81	1.29	0.97	0.68
10	0	1.40	1.44	2.39	2.69	2.27	1.84	1.75	1.23	0.94	0.62
11	0	1.28	1.51	2.27	2.63	2.07	1.90	1.80	1.26	1.04	0.75
12	0	1.34	1.50	2.27	2.78	2.10	1.90	1.79	1.39	1.12	0.72
13	0	1.32	1.45	2.3	2.69	2.15	1.88	1.8	1.38	1.08	0.7
14	0	1.26	1.43	2.23	2.65	2.2	1.85	1.78	1.24	0.96	0.67
15	0	1.25	1.51	2.27	2.77	2.12	1.91	1.79	1.35	1.03	0.68
Mean	**0**	**1.25**	**1.46**	**2.29**	**2.65**	**2.08**	**1.88**	**1.76**	**1.33**	**1.03**	**0.69**
± SD	**0**	**0.07**	**0.05**	**0.06**	**0.07**	**0.11**	**0.04**	**0.03**	**0.06**	**0.05**	**0.03**

Table(2):- Concentration (µg⁄ml) versus times (hr) after the administration of 20 mg piroxicam tablets

Subject	**Time (hr)**										
	0	1	2	4	6	8	10	12	24	48	72
1	0	1.18	1.50	2.10	2.62	1.87	1.79	1.72	1.32	0.95	0.68
2	0	1.05	1.37	1.89	2.53	1.80	1.83	1.75	1.22	1.03	0.57
3	0	0.97	1.40	1.90	2.48	1.90	1.75	1.66	1.37	0.99	0.63
4	0	0.99	1.39	1.88	2.46	1.87	1.80	1.65	1.41	0.88	0.60
5	0	1.56	1.32	2.20	2.45	1.85	1.75	1.71	1.28	1.10	0.58
6	0	1.39	1.46	2.16	2.38	1.69	1.80	1.72	1.28	0.97	0.64
7	0	1.17	1.54	2.15	2.51	1.82	1.72	1.60	1.35	0.96	0.67
8	0	1.11	1.23	2.24	2.27	1.94	1.75	1.62	1.28	1.04	0.56
9	0	1.27	1.39	2.35	2.79	2.41	1.98	1.80	1.20	0.96	0.65
10	0	1.39	1.40	2.33	2.59	2.27	1.94	1.86	1.27	0.94	0.67
11	0	1.28	1.51	2.27	2.63	2.07	1.90	1.82	1.26	1.08	0.77
12	0	1.33	1.45	2.35	2.87	2.10	1.84	1.75	1.40	1.11	0.69
13	0	1.29	1.52	2.27	2.38	1.86	1.75	1.72	1.31	0.96	0.71
14	0	1.33	1.42	2.35	2.78	2.40	1.98	1.81	1.25	1.1	0.57
15	0	0.99	1.38	2.34	2.60	2.05	1.91	1.83	1.27	1.27	0.75
Mean	**0**	**1.22**	**1.41**	**2.18**	**2.55**	**1.99**	**1.83**	**1.73**	**1.29**	**1**	**0.64**
± SD	**0**	**0.17**	**0.08**	**0.17**	**0.16**	**0.21**	**0.08**	**0.07**	**0.06**	**0.06**	**0.06**

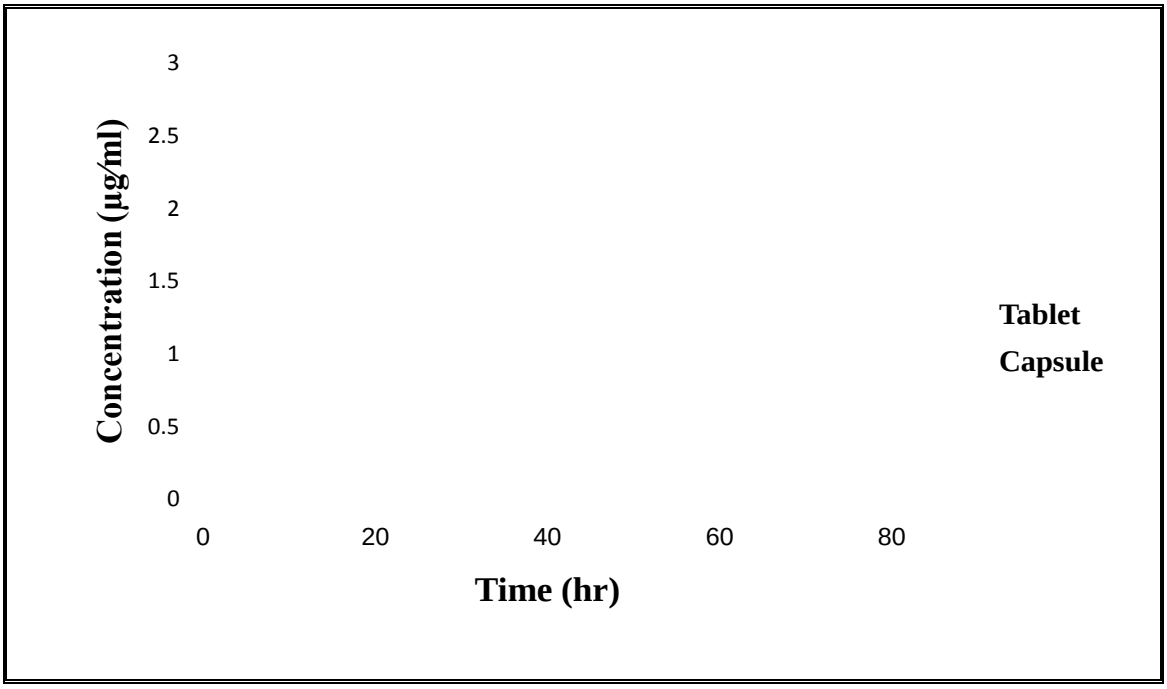

Fig. (1):- Mean plasma concentration-time curve following a single oral administration of a 20 mg of piroxicam tablet and capsule to 15 healthy volunteers

Table (3):- Pharmacokinetics parameters of two formulation of Piroxicam 20 mg

Pharmacokinetics Parameter	Piroxicam 20 mg tablets (test)	Piroxicam 20 mg capsules (reference)
AUC	87.842 ± 2.6998	90.958 ±2.3468**
C_{max}	2.556 ± 0.1666	2.658 ± 0.0797*
T_{max}	6	6
K_a	0.163 ± 0.0496	0.146 ± 0.0461
K_{elm}	0.018 ± 0.0013	0.017 ± 0.0035

***** $p < 0.05$ ****** $p < 0.01$

Discussion

This randomized, single dose, cross-over bioequivalence study of the two formulations of piroxicam 20 mg was performed to demonstrate that test and reference drugs are statistically bioequivalent. Piroxicam tablets have a comparable C_{max} and T_{max} to capsules. Statistically there was significant difference ($p < 0.05$) found in the value of C_{max} between the two brands but they still bioequivalent and T_{max} was the same in both formulations. K_a and K_{elm} values of piroxicam 20 mg were very close between test and reference formulations. The AUC value of piroxicam tablet was lower than that of capsule, this is due to the type of oral dosage form since tablet shows slowest bioavailability than capsule[15] , in spite of that they were generally considered bioequivalent according to the FDA guidelines. The mean and SD of both parameters for the two products were found to be very close suggesting that the plasma profile generated by piroxicam tablets are comparable to those produced by piroxicam capsules. The procedure adopted for characterization of piroxicam assay can be used for work in clinical laboratories. This HPLC analysis method can be useful method applied to monitor the concentration of piroxicam. [2]

Conclusion

On the basis of the values of pharmacokinetic and bioavailability parameters, it can be concluded that the

test formulation was bioequivalent to reference formulation.

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