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ORIGINAL ARTICLE

Pharmacokinetic study of an oral piroxicam formulation containing different molar ratios of β-cyclodextrins

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Abstract The objective of this work was to compare the pharmacokinetic parameters of piroxicam-β-cyclodextrin (PIX-CD) complex at molar ratio of 1:1, 1:2.5, 1:3, and 1:4 after an oral administration in rabbits and either to prove or not the Haborn et al. theory which states that the peak plasma concentration (C_{max}) of piroxicam increases with an increase of β-cyclodextrin concentration. The results showed an increase in C_{max} from 11 ± 1.7, 13.3 ± 6.17 to 17 ± 2.03 μg/ml for piroxicam alone, 1:1 (PIX-CD) and 1:2.5 (PIX-CD), respectively, and declined starting at molar ratio of 1:3 (PIX-CD). However, more rapid drug absorption was observed where the time of peak plasma concentration (T_{max}) became shorter and changed from 2 h (Piroxicam alone) to 0.5 h in the presence of cyclodextrin.

Keywords Piroxicam · Inclusion complex · Bioavailability · Molar ratio

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Introduction

All drugs must possess some degree of aqueous solubility to be pharmacologically active, and most drugs need to be lipophilic to be able to permeate biological membranes via passive diffusion. Oral absorption of drugs with solubilities <0.1 mg/ml is likely to be dissolution limited [1]. Various techniques have been used in attempt to improve solubility and dissolution rates of poorly soluble drugs which include complexation, particle size reduction, salt formation, solid dispersion, and liposomes [2, 3]. The use of cyclodextrins is an important subject in pharmaceutical field since this system can bind drug compounds which modify the undesirable physicochemical properties, including low aqueous solubility, poor dissolution rate and limited drug stability [4].

Piroxicam (PIX) is a potent non-steroidal anti-inflammatory drug (NSAID) of the oxicam family, carboxamic *N*-heterocycles derived from the benzothiazine-1,2-dioxyde-1,1, endowed with more rapid onset of analgesia activity (Fig. 1) [5, 6].

PIX is practically insoluble in water (0.003 % at pH 5, 37 °C) [7]. After single-dose oral administration, PIX is well absorbed (100 %) [8], with a peak plasma concentration (C_{max}) usually attained within 1–6 h and a plasma half-life of 54 h [9]. The long life time of PIX in the organism is due to an intense enterohepatic recycling and to strong plasma proteins binding (99.3 %) [10–12]. PIX is almost entirely metabolized and the main metabolites in human are 5-hydroxypiroxicam and its glucuronide [13]. This drug is associated with gastrointestinal side effects during the therapy [14, 15].

Cyclodextrins are group of structurally related natural products formed during bacterial digestion of cellulose. These cyclic oligosaccharides consist of (α-1,4)-linked

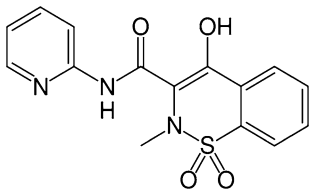

Fig. 1 Chemical structure of PIX

α-D-glucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface. The natural cyclodextrins improved the solubility of poorly water soluble active pharmaceutical ingredients and hence might increase their bioavailability [16–21]. Some papers demonstrated the interaction of PIX with β-CD [22–26]. According to Acerbi [27], the binding of PIX with β-CD molecules accelerates absorption and increases the drug's bioavailability.

Piroxicam is formulated in many dosage forms such as tablets, capsules and suppositories. Additionally it is marketed in many countries as Brexin® or Cycladol® under inclusion complex with β-CD [17, 28] where the later led to bioavailability and water solubility improvement as compared to PIX alone [27].

In this short note, we tried to study the effect of increasing concentration of β-CD on the absorption rate and extent to which the PIX is absorbed from the inclusion complex formulation and becomes available at the site of action by changing the molar ratios of PIX-β-CD as follows: 1:1, 1:2.5, 1:3, and 1:4 after an oral administration in rabbits and either to prove or not the Haborn et al. [29] theory which states that the peak plasma concentration (C_{max}) of PIX increases with an increase of β-cyclodextrin concentration.

Experimental

Material

β-CD was obtained from Roquette, France. PIX was purchased from Molecula, UK and Saidal (Algeria). Sodium heptanesulfonate monohydrate was purchased from Fluka. Acetonitrile, Ortho-phosphoric acid 85 %, sodium hydroxide and diethyl ether were obtained from Merck. Pure water was produced by UHQ system (Millipore).

Preparation of PIX-β-CD inclusion complexes

Piroxicam and β-CD at different molar ratios (1:1, 1:2.5, 1:3, and 1:4) were suspended in 10 ml of distilled water and 0.1 N NaOH solution was added to solubilize the drug where a yellow solution of pH above 10 was obtained. Solutions were freeze-dried at −50 °C for 3 h at least. Lyophilization parameters were validated by preliminary works and the parameters were as follows: vacuum <200 m Torr, condenser <−40 °C, shelf at +30 °C.

Animal study

New Zealand white male rabbits were used (2.5–2.8 kg body weight). The protocol for a cross-over study was approved by the IAEC. Rabbits were fasted overnight but water was allowed ad libitum. The PIX and PIX-β-CD were given in suspension form (2 % methylcellulose). The drug PIX (10 mg/Kg) and molar equivalent of PIX/β-CD (equivalent to 10 mg/Kg PIX) were administered orally via Ryle's tube (intubation tube). After drug administration, 2 ml of blood sample were collected from marginal ear vein at time intervals (0, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h) in the test tube containing heparin. The samples were centrifuged at 3,000 rpm for 15 min and plasma of 0.12–0.15 ml was collected.

Determination of PIX in rabbit plasma by HPLC

The concentration of PIX in plasma was determined using Hewlett-Packard 1050 as HPLC system. The mobile phase was acetonitrile/Phosphate buffer (35/65 %, V/V) at pH 3.5. Column was NUCLEOSIL 120-10 (C18, 10-mm) from Merck. The flow rate, retention time, wavelength, and temperature of the column were 0.8 ml/min, 3.5 min, 356 nm, and 40 °C, respectively. 200 µl of methanol containing 0.2 % perchloric acid was added to 100 µl of plasma cooled in an ice-bath. The mixture was mixed for 1 min and centrifuged at 3,000 rpm for 5 min then 50 µl of the supernatant was injected into the HPLC system.

Static or Statistical analysis

Pharmacokinetic analysis of plasma concentration data was performed using model independent methods. The area under the plasma concentration–time curve (AUC) and the cumulative AUC were calculated. The peak plasma concentration (C_{max}) and the time taken for attaining the peak concentration (T_{max}) were determined from individual plasma concentration–time curves. Statistical comparisons of pharmacokinetic parameters were made using one-way analysis of variance, and when significant differences were found, Scheffe's *F* test was applied.

Results and discussion

The oral bioavailability of complex inclusion formulations of PIX with β-CD was studied on rabbits at different molar

ratios (1:1, 1:2.5, 1:3, and 1:4). The following pharmacokinetic parameters were determined: C_{max}, T_{max}, and AUC; they are shown in Table 1. Figures (2, 3) illustrate the mean plasma concentration of PIX obtained after the oral administration of the different formulations.

The C_{max} values were improved with increase of the cyclodextrin concentration loading in the inclusion complex up to molar ratios (1:2.5) and declined thereafter. The C_{max} values were 11 ± 1.7, 13.3 ± and 17.2 ± 2.03 μg/ml for PIX alone, 1:1 (PIX-CD) and 1:2.5 (PIX-CD), respectively. However, C_{max} values for 1:3 (PIX-CD) and 1:4 (PIX-CD) were 15.5 ± 2.4 and 11.2 ± 2.3, respectively. In regards to T_{max}, more rapid drug absorption was observed where T_{max} became shorter and changed from 2 h (PIX alone) to 0.5 h in the presence of cyclodextrin.

Levels of PIX in the plasma (Figs. 2, 3) showed no significant in C_{max} values between the group treated with PIX-β-CD at 1:4 molar ratio and that treated with free PIX. However, in the complex group, C_{max} was reached sooner. No significant differences among AUC values were found for complexes compared with free drug. Furthermore, the highest value of AUC was obtained for the complex PIX-β-CD 1: 2.5 which also represents the optimum for C_{max} (Table 1).

The pharmacokinetic evaluation of PIX carried on rabbits in the present study shows that complexing PIX with β-CD modifies some parameters related to phases of absorption and elimination. β-CD improved the bioavailability of PIX up to molar ratio of 1: 2.5 but the excess of cyclodextrin has a negative impact on the permeability of the drug through the biologic membrane [20]. With 1:1 and 1:2.5 molar ratios, β-CD affected the permeability of the gastro-intestinal membrane increasing the drug partitioning process across intestinal barrier [30]. For the others molar ratio (1:3 and 1:4), the negative effect is presumably due to the decrease in aqueous solubility of PIX in gastric medium allowing its precipitation [31]. Similar results were found with oral administration of tolbutamide to rabbit [32] where mean C_{max} was higher with powder of pure drug with solution, solid dispersion, and CD complexes. Our results are not in agreement with those reported from Haborn et al. [29] which states that C_{max} of PIX increases with an increase of β-CD concentration.

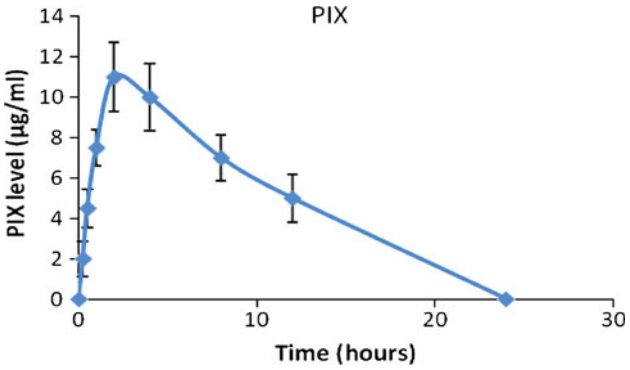

Fig. 2 Mean plasma concentration time profile of PIX. Each value represents the mean ± SD of three rabbits

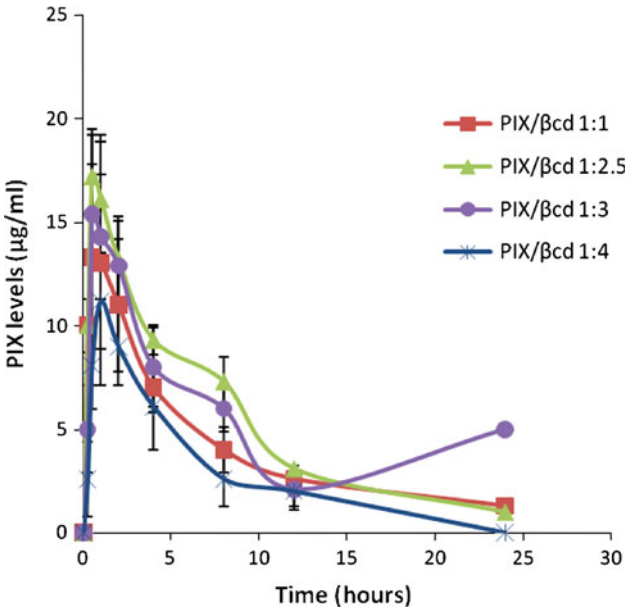

Fig. 3 Mean plasma concentration time profile of PIX/β-CD at different molar ratios (1:1, 1:2.5, 1:3, and 1:4). Each value represents the mean ± SD of three rabbits

Conclusion

In summary, it was concluded that complex inclusion formulations in different ratios (1:1, 1:2.5, 1:3, and 1:4) of PIX with β-CD showed better C_{max} as compared to pure drug exceptionally for 1:4 molar ratio which did not vary

Table 1 PIX pharmacokinetic parameters in the rabbit after oral administration of equal doses (10 mg/Kg as PIX) of PIX and PIX-β-CD at different molar ratios

Samples administered	C_{max} (μg/ml)	T_{max} (h)	AUC (μg h/ml)
PIX	11 ± 1.7	2	122
PIX/β-CD 1 : 1	13.3 ± 6.17	0.5	99
PIX/β-CD 1 : 2.5	17.2 ± 2.03	0.5	128
PIX/β-CD 1 : 3	15.5 ± 2.4	0.5	102
PIX/β-CD 1 : 4	11.2 ± 2.3	1	70

significantly with PIX alone. The bioavailability results show that PIX-β-CD complex had faster absorption than the free drug for all molar ratio studied. The highest values of C_{max} and T_{max} were obtained with a molar ratio of 1:2.5 (PIX- β-CD). Haborn I et al. [29] theory is not valid since the increase of C_{max} value is not linear with an increase of β-CD concentration.

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