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Der Pharmacia Lettre, 2010, 2(4): 307-314
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

In vivo evaluation of directly compressible spherical crystals of Celecoxib

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ABSTRACT

Celecoxib spherical agglomerates were prepared with polyvinylpyrrolidone (PVP) using acetone, water and chloroform as solvent, non-solvent and bridging liquid, respectively.The agglomerates were characterized by differential scanning calorimetry (DSC), X-ray diffraction (XRD), IR spectroscopic studies and scanning electron microscopy (SEM). The IR spectroscopy and DSC results indicated the absence of any interactions between drug and additives. XRD studies showed a decrease in crystallinity in agglomerates. The crystals exhibited significantly improved micromeritic properties compared to pure drug. The loading efficiency (% or mg drug per 100 mg crystals) was in the range of 93.9 ± 2.3 and 97.3 ± 1.3% (n = 3) with all formulations. The aqueous solubility and dissolution rate of the drug from crystals was significantly ($p < 0.05$) increased (nearly two times). The solubility and in vitro drug release rates increased with an increase in PVP concentration (from 2.5 to 10%). The SEM studies showed that the crystal posseses a good spherical shape with smooth and regular surface. Based on these results, formulation prepared using 10% w/v PVP K 30 selected as optimized formulation. In vivo studies carried out on human volunteers with optimized formulation and the pharmacokinetic parameters were compared with marketed formulation. The celecoxib spherical crystals have shown enhanced therapeutic activity than the marketed formulation.

Key Words: Celecoxib, spherical crystallization, solubility, dissolution rate, micromeritic properties, In vivo evaluation

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INTRODUCTION

Direct tabletting of pharmaceutical materials is desirable to reduce the cost of production [1]. However, compressing a high-dosed drug directly requires good micromeritic properties, such as flowability, and good and reproducible compression behavior. Spherical crystallization technique transforms directly the fine particles produced in crystallization or in the reaction process into a spherical shape. Agglomerates exhibit improved secondary characteristics, like flowability and compressibility, so that direct tabletting or coating is possible without further processing (mixing, agglomeration, sieving, *etc*.) [2, 3]. Celecoxib, a non-steroidal anti-inflammatory drug (NSAID), is the first selective cyclooxygenase-2 inhibitor used in the treatment of osteoarthritis and rheumatoid arthritis in adult patients [4]. Celecoxib exhibits poor flow and compression characteristics and is hence a suitable candidate for spherical crystallization process to improve the flow properties and compressibility. Also, celecoxib shows incomplete and poor oral bioavailability due to low aqueous solubility [5]. Hence, the improvement of aqueous solubility in such a case is a valuable goal to improve therapeutic efficacy [6]. Apart from particle enlargement, this technique has also been applied for various purposes such as taste masking and particle size enlargement [7–10]. Spherical crystallization of celecoxib with Hydroxypropyl methyl cellulose has been investigated to improve the micromeritic properties of celecoxib but the aqueous solubility of spherical crystallized drug was not satisfactorily improved [11]. There are no reports available on the spherical crystallization of celecoxib by using a more hydrophilic polymer, polyvinylpyrrolidone K-30 (PVP), to improve aqueous solubility and dissolution rates there by to enhance the bioavailability, in addition to improving its micrometric properties, and hence the present work has been undertaken with this objective.

MATERIALS AND METHODS

Materials

Celecoxib was a gift sample from Aurobindo Pharma, India. PVP was purchased from s.d. Fine Chemicals, India. All other chemicals used were of analytical reagent grade.

Methods

Preparation of celecoxib spherical crystals

A solution of celecoxib (2 g) in acetone (3 mL) was added to a solution of hydrophilicpolymer (PVP, 2.5–10%, *m*/*V*) in 100 mL distilled water. The mixture was stirredcontinuously using a mechanical stirrer (Remi Motors, India) at 500 rpm to obtain spherical agglomerates. The bridging liquid (chloroform, 0.5 mL) was added dropwise. The agglomerates were separated by filtration using Whatman filter paper (No. 1) and dried for 24 h at room temperature. The physical mixture of drug and polymer (celecoxib+ PVP) was prepared by triturating 1:1 ratio of celecoxib and PVP using mortar and pestle.

Among all formulations, formulations prepared using 10% w/v PVP-K30 selected as optimized formulation [12] and invivo studies were carried out using optimized formulation on human volunteers.

Characterization

Bioavailability and pharmacokinetic studies of the developed formulation became an integral part of drug development. Hence the aim of researchers is to study the bioavailability of newly developed formulations. Though *invitro* dissolution tests seem to be sensitive and reliable predictors of bioavailability, they cannot always predict *invivo* performance. Poor correlation between *invitro* and *invivo* has been observed due to various unpredictable physiological factors. These factors can affect the rate and / or extent of drug absorption for any oral dosage form. Therefore, validation of final product accomplished by *invivo* testing in human subjects remains the ultimate test of any dosage form.

The objective of pharmacokinetics is to describe the time course of drug concentrations in blood in mathematical terms so that (1) the performance pharmaceutical dosage forms can be evaluated in terms of the rate and amount of drug they deliver to the blood and (2) the dosage regimen of the drug can be adjusted produce and maintain therapeutically effective blood concentrations with little or no toxicity.

The applicability of the prepared spherical crystals as directly compressible materials was thoroughly evaluated and characterized by the *invitro* tests. The objective of present investigation is to evaluate the *invivo* performance of these spherical crystals in comparison with commercial product.

Pharmacokinetic evaluation of celecoxib dense compacts

Dense compacts prepared with spherical crystals of 10 % w/v PVP K 30 shown the required micromeritc properties, solubility and drug release, hence these formulations were selected for *invivo* evaluation in healthy human volunteers. The *invivo* performance of commercially available celecoxib capsules was also carried out for comparision.

Invivo experiments were carried out in healthy human volunteers as per the following experimental design and protocol. All subjects were presented with full details of the investigation, both verbally and in written form, Prior to providing written informed consent. An independent ethics committee of Navodaya medical college hospital and Research center, Raichur, Karnataka state approved this study.

Experimental design:

A crossover, non-blinded, open label randomized block design (RBD) (n=6) in which 6 volunteers received one treatment (product) each every 15 days such that all products are tested in all the six volunteers during the study.

In-vivo study protocol

Six healthy male subjects with a mean age of 25.3 ± 1.8 years (ranging from 23 to 27 years), a mean body weight of 65.3 ± 8.4 kg (ranging from 60 to 70 kg) and a mean height of 165.1 ± 5.8 cm (ranging from 160 to 171 cm) participated in this study. The volunteers were judged healthy on the basis of their previous medical history, physical examination and routine laboratory tests. None of the subjects used alcohol or tobacco. All subjects were free from drugs 15 days before and during the study.

The subjects were fasted overnight at least 10 hours prior to dose. After collecting the zero hour blood sample (blank). Selected formulation and marketed product were given with 200ml of water. Blood samples were collected at 0.5, 1.5, 2.5, 3.5, 4, 8, 12 and 24 hours. Plasma was separated by centrifugation at 4000 rpm for 10 minutes from the blood samples. All the samples were stored in properly labeled tubes at -20°C prior to analysis. The plasma concentration of celecoxib from the selected formulation and marketed product were measured by HPLC method.

The amount of celecoxib concentration in plasma following the oral administration of selected formulation and marketed product are given in Tables (1), (2) and shown in Fig. 2.

Determination of Pharmacokinetic Parameters:
Various pharmacokinetic parameters such as peak plasma concentration (C_{max}), time at which C_{max} occurred (T_{max}), area under the curve (AUC), elimination rate-constant (K_{el}), biological half-life ($t_{1/2}$), Mean residence Time (MRT) in plasma were calculated in each case using the data.

Determination of elimination rate constant and biological half-life:
The graphs of plasma concentration versus time data were plotted on a semi logarithmic graph. The elimination rate constant, K_{el} was calculated from the slope of the linear line in the elimination phase. The "best-fit" linear regression line for the points in the elimination phase was drawn by the method of least squares. The corresponding $t_{1/2}$ was calculated using the following equation.

$$t_{1/2} = \frac{0.693}{K_{el}} \qquad \text{equation ---- (1)}$$

Determination of area under curve (AUC):
The area under the plasma concentration versus time curve from 0 to t hrs AUC_{0-t} was measured by applying trapezoidal rule. The area under the curve from 0 to ∝ hrs $AUC_{0-\infty}$ was calculated using the equation as given below.

$$AUC_{0-t} = \int_0^t C(t)\, dt \qquad \text{equation ---- (2)}$$

$$AUC_{0-\infty} = AUC_{0-t} + C_t/K_{el} \qquad \text{equation ---- (3)}$$

Where, C_t is plasma cocentration of drug at t hrs. The values of AUC_{0-24} and $AUC_{0-\infty}$ are given in Table - 3.

Determination of Mean residence time (MRT)
The tendency of drugs and metabolites to remain in the body can be assessed by measuring the mean residence time (MRT). Assuming that the drug in the organs of elimination is always in equilibrium with drug in plasma, the mean residence time can be defined as the average amount of time spent by drug molecules in the body before being eliminated (under constant clearance conditions). The MRT is considered as the statistical movement analogy to the half-life ($t_{1/2}$).

Mean residence time upto t hr (MRT_{0-t}) is calculated from plasma drug concentration by following equation.

$$MRT_{0\text{-}t} = AUMC_{0\text{-}t} / AUC_{0\text{-}t} \qquad \text{equation ---- (4)}$$

Where, $AUC_{0\text{-}t}$ is the area under the time versus plasma concentration curve from 0-t hrs and measured by applying trapezoidal rule. $AUMC_{0\text{-}t}$ is the area under the first movement curve and is obtained from a plot of the product of drug concentration in plasma and time versus time from zero to t hrs.

$$AUMC_{0\text{-}t} = \int_0^t t\,C\,(t)\,dt \qquad \text{equation ---- (5)}$$

Plots of product of plasma concentration and time versus time [C(t) vs t] were plotted and area under the corresponding curve i.e. $AUMC_{0\text{-}t}$ was computed.

Statistical analysis:
The data obtained from each experiment were subjected to statistical analysis by Student t-test and one-way analysis of variance (ANOVA) using Graph Pad Instat software. $P<0.05$ was considered to be indicative of significance.

RESULTS

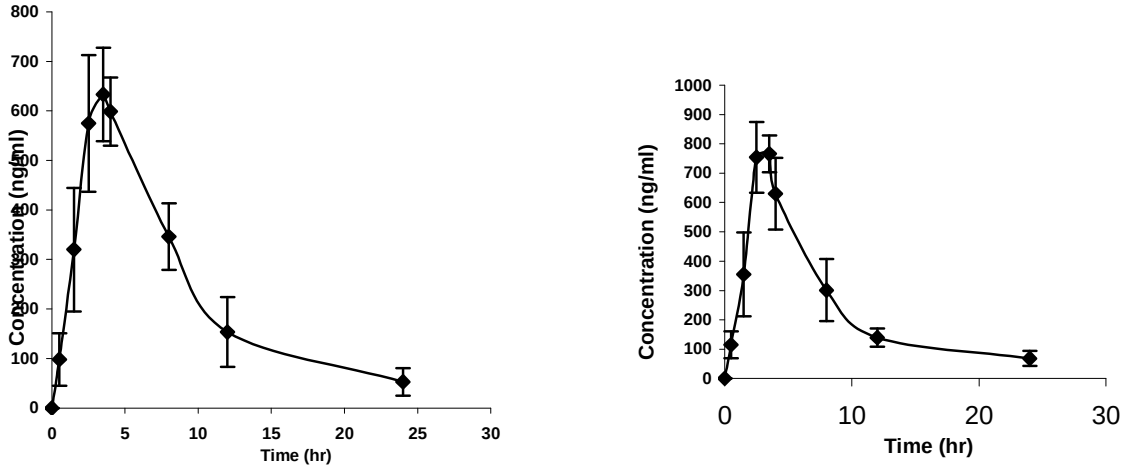
The concentration of celecoxib in plasma and the respective average values at different time intervals following the oral administration of selected formulations and commercial product are given in tables (1), (2) and the average plasma concentration versus time were shown in figure 1 & 2. The mean pharmacokinetic parameters ($AUC_{0\text{-}24}$, $AUC_{0\text{-}\infty}$, T_{max}, C_{max}, K_{el}, $t_{1/2}$ and $MRT_{0\text{-}24}$) calculated from the average serum concentrations from the invivo experiments are in shown in table - 3.

Table - 1:- Plasma celecoxib concentration (ng/ml)-time profile following an oral administration of 200 mg celecoxib (Marketed product) to six healthy male volunteers

Time (hrs)	Plasma concentration (ng/ml)							
	A	**B**	**C**	**D**	**E**	**F**	**Average**	**S.D.**
0	0	0	0	0	0	0	0	0.00
0.5	49.85	62.89	195.30	87.30	117.96	73.87	97.86	53.13
1.5	312.27	240.84	180.77	501.57	245.78	438.14	319.89	125.02
2.5	636.93	556.30	415.99	673.81	414.99	749.75	574.62	138.11
3.5	648.14	516.03	713.04	739.16	523.19	661.23	633.46	94.28
4	489.16	593.17	581.28	701.91	624.18	601.39	598.51	68.76
8	318.14	341.21	255.16	449.63	321.79	390.85	346.13	66.91
12	203.78	177.04	169.38	234.10	50.60	86.74	153.60	70.54
24	48.97	56.84	103.42	38.38	21.43	48.23	52.87	27.59

Table – 2:- Plasma celecoxib concentration (ng/ml)-time profile following an oral administration of 200 mg celecoxib dense compacts (selected formulation) to six healthy male volunteers.

Time (hrs)	Plasma concentration (ng/ml)							
	A	B	C	D	E	F	Average	S.D.
0	0	0	0	0	0	0	0	0.00
0.5	57.63	121.85	93.61	89.95	189.61	135.89	114.75	45.66
1.5	273.68	581.61	181.19	299.01	461.17	331.27	354.65	143.63
2.5	787.15	698.61	914.33	556.94	819.13	749.75	754.31	120.80
3.5	716.58	761.32	818.91	681.16	769.75	851.16	766.48	62.81
4	402.94	643.45	660.97	611.34	754.10	709.34	630.35	122.26
8	142.62	442.11	249.92	347.31	366.73	258.68	301.22	105.70
12	97.90	184.74	158.22	115.99	132.94	143.28	138.84	30.77
24	51.79	70.12	103.42	84.48	65.99	30.43	67.70	25.33



(a) **(b)**

Figure 2: Average plasma concentration- time curves of celecoxib following oral administration of (a) marketed product (b) Spherical Crystals of Optimized formulation.

Table - 3: Pharmacokinetic parameters (mean ± SE) of celecoxib after oral administration of selected formulation (200 mg) and marketed product (200 mg filled in capsules) to six healthy male volunteers.

Pharmacokinetic parameter	Selected Formulation	Marketed product
C_{max} (ng/ml)	802.37 ± 79.75	677.90 ± 64.96
T_{max} (hours)	3 ± 0.547	3.5 ± 0.5477
AUC_{0-24} (ng · hr/ml)	5910.10 ± 985.7	5720.10 ± 925.45
$AUC_{0-\infty}$ (ng · hr/ml)	6420.08 ± 1066.114	5890.01 ± 914.30
MRT_{0-24}(hrs)	7.25 ± 0.15	7.41 ± 0.23
K_{el} (hrs^{-1})	-0.047 ± 0.0106	-0.0545 ± 0.0135
$t_{1/2}$ (hrs)	15.317 ± 3.158	13.351 ± 3.84

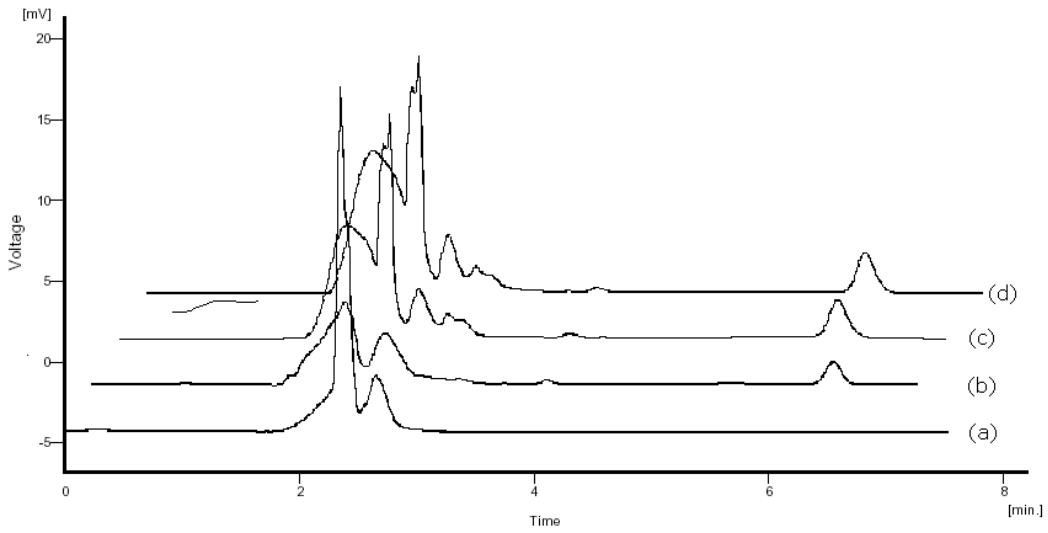


Figure 1: Typical HPLC chromatogram of (a) blank plasma (b) plasma spiked with 800 ng /ml of celecoxib (c) celecoxib of a volunteer administered with formulation and (d) celecoxib of a volunteer administered with marketed product.

The C_{max} of celecoxib from selected formulation and marketed product were found to be 802.37 ± 79.75 and 677.90 ± 64.96 ng/ml respectively and the values of the marketed product are below the selected formulations. The t_{max} values from selected formulation and marketed product were found to be 3 ± 0.547 and 3.5 ± 0.5477 hr respectively and the values of the marketed product are below the selected formulations. The K_{el} values were found to be -0.047 ± 0.0106 and -0.0545 ± 0.0135 hr^{-1} after oral administration of selected formulation and marketed product respectively calculated from the slope of the terminal portion of log plasma concentration of celecoxib versus time. The corresponding $t_{1/2}$ values were found to be 15.317 ± 3.158 and 13.351 ± 3.84 hrs respectively table - 3. The $t_{1/2}$ values are almost similar to the reported values.

The values of AUC_{0-24}, and MRT_{0-24} of selected formulation and marketed product were 5910.10 ± 985.7, 7.25 ± 0.15 and 5720.10 ± 925.45, 7.41 ± 0.23 respectively.

DISCUSSION

The major problems associated with the drug Celecoxib, its poor solubility and poor micromeritic properties. However the micromeritic properties were improved to certain extent by using polymers like HPMC by making Celecoxib agglomerates. But the above phenomenon does not increase its poor solubility to desirable extent [5, 11].

The objective of the present study is to enhance the solubility of celecoxib, their by to enhance the bioavailability of the celecoxib by making spherical agglomerates using different concentrations of hydrophilic polymer PVP-K30 and we selected formulation prepared by

using10%w/v PVP-K30 as optimized formulation [11]. The results of In-vivo studies carried out on human volunteers using optimized formulation were compared with marketed formulation.

The AUC values of selected formulation were higher compared to the commercial product indicating the higher bioavailability of the selected formulation. Thus the results of present study indicated the applicability of spherical crystals of celecoxib as directly compressible material in direct tableting.

CONCLUSION

Thus the results of present study indicated the applicability of spherical crystals of celecoxib as directly compressible material in direct tableting, the technique which reduces the cost of production and improves the micromeritic properties and bioavailability of celecoxib.

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