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PHARMACOKINETIC, PHARMACODYNAMIC AND INVITRO EVALUATION OF THE PREPARED MICROSPHERES OF INDOMETHACIN

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Abstract

The main objective of any drug therapy is to achieve a desired concentration of the drug in blood or tissue which is therapeutically effective and non-toxic for an extended period of time. The aim of present work is to increase the biological half life and patient compliance of Indomethacin. For that purpose Indomethacin Microspheres were prepared using different polymers by Emulsion Solvent Evaporation Technique.

The cumulative percentage drug release for all formulations of Indomethacin microspheres follow first order kinetics and they show increased dissolution rates. The result showed that the cumulative percentage release of Indomethacin from the formulations D:EC:HPMC(1:1:1), D:CE:EuRS100(1:1:1) D:EC:EuRL100(1:1:1), D:CA:EuRL100(1:1:1), D:CA:EuRS100(1:1:1) were 77.4%,78.2%,80.2%,88.74%and 83.5% respectively. Among these formulations D: CA: EuRL100 (1:1:1) shows similar percentage release as that of marketed drug (Indocide SR). Pharmacokinetic studies show that Cmax,tmax,AUC,t1/2 and MRT values of D:EC:EuRS100 and D:CA:EuRL100 formulations were increased compared to the marketed drugs The pharmacodynamic study revealed that anti inflammatory activity was well noticed in the microsphere formulation D:CA:EuRL100(1:1:1) than the marketed sample of Indomethacin and other microspheres formulation. So thereby these formulations have several advantages over the conventional form that the prolongation of drug action, improvement of bioavailability, patient compliance and reduction of gastro intestinal and centrally mediated side effects .In future the invitro-invivo correlation of the prepared formulation has to be done. Clinical evaluation is also under consideration.

Keywords: Indomethacin, microspheres, pharmacokinetic, pharmacodynamic.

Introduction

The concept of controlled release system has begun to play its part in the treatment of illness through the delivery of drugs, as the success of therapy is determined by the selection of the appropriate drug and its delivery system[1]. The controlled release dosage forms ensure safety and it improves the efficacy of the drugs as well as patient compliance. If the drug has to be fully effective and safe, it should reach the site of action or receptor in the optimum concentration, should remain there for the desired time without spreading itself to other sites and must be rapidly removed from this site when a steady state blood level that is therapeutically effective and non toxic is achieved.

Numbers of techniques have been used to produce sustained or controlled drug delivery system. They include the use of barrier coating embedding in slowly erodible matrix, skeleton type matrix, ion exchange resin etc[2]. Microspheres have been widely accepted as a means to achieve oral and parental controlled release. The microspheres require a polymeric substance as a coat material or carrier. Microspheres are free flowing powders consisting of spherical particles of size ideally less than 125 microns that can be suspended in a suitable aqueous vehicle and injected by an 18 or 20 number needle. Each particle is basically a matrix drug dispersed in a polymer from which release occurs as a first - order process.

Aims and Objective

The aim of the present work is to increase the biological half life of Indomethacin and to improve patient compliance. The biological half - life of Indomethacin, tcan be increased by incorporating the drug with polymers such as Eudragit RL 100, Eudragit RS 100, Ethyl cellulose, HPMC and Cellulose acetate and prepared in the form of microspheres, using Emulsion solvent evaporation technique.

Materials and Methods

Indomethacin was gift sample from Reddy's laboratories and Eudragit RL 100 and RS 100 were purchased from Orchid R& D center Chennai. Ethyl cellulose and other analytical grade chemicals were purchased from S.D.Fine Chemical (P) Ltd., Mumbai.

Preparation of indomethacin microspheres

Microsheres of indomethacin were prepared by Emulsion solvent evaporation technique[3]. Required amount of the polymer was accurately weighed and it was added to a solvent mixture of acetone: ethanol (1:1). Then the required amount of Indomethacin was uniformly dispersed in this mixture. This mixture was taken in a syringe and then it was dropped into a non - solvent (liquid paraffin) containing a tensio - active agent (Tween 80) through a 20 G needle. Then the emulsion was stirred under a constant speed of 300 rpm for 5 hours, using a mechanical stirrer. After 5 hours microspheres were formed by the evaporation of solvent at room temperature. The microspheres were separated from the oil phase by vacuum filtration. After filtration the microspheres were washed with cyclohexane to remove the adhering liquid paraffin.

Table no 1: Composition of prepared formulations.

Batch No.	Indomethacin	Composition of Polymers				
		Eudragit RL 100	Eudragit RS 100	Ethyl Cellulose	HPMC	Cellulose Acetate
I	75 mg	-	-	75 mg	75 mg	-
II	75 mg	75 mg	-	75 mg	-	-
III	75 mg	-	75 mg	75 mg	-	-
IV	75 mg	75 mg	-	-	-	75 mg
V	75 mg	-	75 mg	-	-	75 mg
VI	75 mg	-	-	150 mg	150 mg	-
VII	75 mg	150 mg	-	150 mg	-	-
VIII	75 mg	-	150 mg	150 mg	-	-
IX	75 mg	150 mg	-	-	-	150 mg
X	75 mg	-	150 mg	-	-	150 mg

Table no 2: Estimation of Indomethacin in microspheres.

S.No	Drug : Polymer	Amount of drug Loaded (mg)	% drug loaded
1	D : EC : HPMC[1:2:2]	51.68	68.90
2	D : EC : EuRS [1:2:2]	51.06	68.08
3	D : EC : EuRL [1:2:2]	54.12	72.16
4	D : CA : EuRS [1:2:2]	50.62	67.49

5	D : CA : EuRL [1:2:2]	52.24	69.65
6	D : EC : HPMC[1:1:1]	61.86	82.48
7	D : EC : EuRS [1:1:1]	60.56	80.74
8	D : EC : EuRL [1:1:1]	63.62	84.82
9	D : CA : EuRS [1:1:1]	62.82	83.76
10	D : CA : EuRL [1:1:1]	65.04	86.72

Study of pharmacodynamic activity

Indomethacin is a non-steroidal anti-inflammatory drug. Hence anti-inflammatory activity was carried out on different formulation of Indomethacin microspheres by carrageenin induced paw oedema method in albino rats[4].

Albino rats of either sex weighing about 150-200 gms were divided into 12 groups of 6 animals each. 0.1ml of carrageenin (1% w/v) in normal saline was injected into the sub planter region of the left hind paw of all groups of animals.

Rats in each group were administered orally the following preparations.

Group I. Tween 80 5% solution was used as control.

Group II. Tween 80 5% containing 10mg/kg body, weight of Indomethacin marketed sample (Indosid SR Cap)

Group III. Tween 80 5% containing 10mg/Kg body weight of prepared D:EC:HPMC (1:2:2) Indomethacin microspheres.

Group IV. Tween 80 5% containing 10mg/Kg body weight of prepared D: EC:EuRS[1:2:2] Indomethacin microspheres.

Group V. Tween 80 5% containing 10mg/Kg body weight of prepared D:EC:EuRL(1:2:2) Indomethacin microspheres.

Group VI. Tween 80 5% containing 10mg/Kg body weight of prepared D:CA:EuRS(1:2:2) Indomethacin microspheres.

Group VII. Tween 80 5% containing 10mg/Kg body weight of prepared D:CA:EuRL (1:2:2) Indomethacin microspheres.

Group VIII. Tween 80 5% containing 10mg/Kg body weight of prepared D:EC:HPMC (1:1:1) Indomethacin microspheres.

Group-IX. Tween 80 5% containing 10mg/kg body weight of prepared D:EC:EuRS(1:1:1) Indomethacin microspheres.

Group X. Tween 80 5% containing 10mg/kg body weight of prepared D:EC :EuRL (1:1:1) Indomethacin microspheres.

Group XI. Tween 80 5% containing 10mg/kg body weight of prepared D:EA:EuRS(1:1:1) Indomethacin microspheres.

Group XII. Tween 80 5% containing 10mg/kg body weight of prepared D:CA:EuRL (1:1:1) Indomethacin microspheres.

The volume of hind paw oedema was measured by Vernier at 0 hour and at the interval of 1, 2, 3, 4, 6, 12 and 24 hours[5].

The mean increase in paw volume and the percentage inhibition of inflammatory swelling were calculated.

The percentage inhibition of paw volume produced by the different formulation of Indomethacin microspheres was compared with that of the control. Percentage inhibition was calculated by using the formula,

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc is average increase in Paw volume of control

Vt is average increase in paw of volume of drug treated animals.

The anti-inflammatory activity of the microsphere of Indomethacin, control and marketed sample are represented in table no.3.

Table no 3: Anti-inflammatory activity of Indomethacin microspheres.

S.No	Drug given in different groups of animals.	Paw volume (mm) Mean ± SEM after 12 hours	Reduction level of oedema (% inhibition) at 10 mg/kg dose
1	Control (10%Tween80)	0.85 ± 0.18	-
2	Marketed sample (Indocid 75 mg SR)	0.62 ± 0.22 *	27.05 %
3	D : EC : HPMC[1:2:2]	0.68 ± 0.190 *	20 %
4	D : EC : EuRS [1:2:2]	0.71 ± 0.185 *	16.4 %
5	D : EC : EuRL [1:2:2]	0.70 ± 0.187 *	17.6 %
6	D : CA : EuRS [1:2:2]	0.69 ± 0.188 **	23 %
7	D : CA : EuRL [1:2:2]	0.68 ± 0.240 **	20 %
8	D : EC : HPMC[1:1:1]	0.64 ± 0.22 **	24.7 %
9	D : EC : EuRS [1:1:1]	0.65 ± 0.19 **	23.5 %
10	D : EC : EuRL [1:1:1]	0.63 ± 0.186 *	25.8 %

11	D : CA : EuRS [1:1:1]	0.62 ± 0.221*	27 %
12	D : CA : EuRL [1:1:1]	0.60 ± 0.240 ***	29.41 %

SEM = Standard Error Mean.

n = 3 per group results are expressed as mean ± SD significant differences compared from control by student's 't' test on 12 hrs are indicated as below.

* $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$

Table no 4: Pharmacokinetic Parameters of Different Batches of Indomethacin Microspheres.

Parameters	Marketed drug (IndocidSR)	D:EC:HPMC 1:1:1	D:EC:EuRS 1:1:1	D:EC:EuRL 1:1:1	D:CA:EuRS 1:1:1	D:CA:EuRL 1:1:1
Peak Plasma Concentration (C_{max}) µg/ml	2.26 ± 0.62	2.06 ± 0.6	2.42 ± 0.75	2.32 ± 0.70	2.60 ± 0.85	2.06 ± 0.44
Time required to reach C_{max} (t_{max}) hrs	4 ±0.12	3±0.24	5 ± 0.22	4 ± 0.14	4 ± 0.16	4 ± 0.12
Elimination rate constant (kel) hr^{-1}	0.07 ± 0.01	0.09 ± 0.02	0.06 ± 0.01	0.12 ± 0.08	0.045 ± 0.07	0.08 ± 0.01
Elimination half life (t ½) hrs	9 ± 0.12	7.7 ± 0.18	11.5 ± 0.41	8.66 ± 0.24	15.4 ± 0.42	8.66 ± 0.28
[AUC] 0→24 µg hrs / ml	23.6 ± 2.8	23.13 ± 2.03	23.37 ± 3.18	27.03 ± 2.42	24.5 ± 2.41	33.63 ± 3.42
[AUMC] 0→24 µg hrs / ml	197.24 ± 12.2	193.94± 10.5	287.94± 20.4	211.92 ± 15.8	198.88 ± 12.9	236.72 ± 14.2
[AUC] 0→∞ µg hr^2 / ml	25.7 ± 0.68	25.13 ± 0.42	25.7 ± 0.86	28.13 ± 0.62	24.5 ± 0.48	36.63 ± 1.20
[AUMC] 0→∞ µg hr^2 / ml	229.8 ± 15.4	253.94 ± 18.2	326.8 ± 20.3	238 ± 18.3	288.88 ± 12.1	308.34 ± 25.8
MRT hrs	10.33 ± 0.52	8.65 ± 0.33	12.71 ± 0.49	8.43 ± 0.32	12.09 ± 0.47	8.64 ± 0.32

The above table values are expressed by Mean ± SD.

Results and Discussion

The prepared microspheres were found to small size and free flowing, non-hygroscopic and easy to prepare.

Estimation of drug content

The results showed the percentage of Indomethacin was ranging from 67% to 88% at all the ten microspheres formulation. Drug incorporated efficiency was best in 1:1:1 formulation than 1:2:2 formulation[4].

Flow property of microspheres

By comparing and compiling the results obtained from the angle of repose and compressibility index of the prepared microspheres the following discussion can be made[6].

Angle of repose

D:CE:EuRL100(1:1:1), D:CE:EuRS100(1:1:1), D:CA:EuRL100(1:1:1), D:CA:EuRS100(1:1:1), D:EC:HPMC(1:1:1) were found to give the values 23.42^0, 24.26^0, 20.52^0, 20.12^0, and 23.18^0 respectively. υ Value less than 30 is considered to give good flow property. The ratio of 1:2:2 formulations exhibit poor flow property with υ values more than 30^0. The pure drug had a value of about 45.59^0.

Compressibility index

Compressibility index value of less than or equal to 15% is considered to give good flow property D:EC:HPMC(1:1:1), D:CE:EuRS100(1:1:1), D:CA:EuRL100(1:1:1), D:CA:EuRS100(1:1:1), D:CA:EuRL100(1:1:1)were found to have the values of about 14.6%,15.2%,14.8%,11.0%and 12.5%respectively.The other ratio of 1:2:2 was found to have values of about 22.6%,25.2%,20.1%,24.7%.24.7%and 26.3%. The pure drug had a value of about 27.4%

Drug polymer interaction study

FTIR spectral Analysis

FTIR spectral analysis was carried out to study the interaction between drug and polymer used. The FTIR spectrum of pure Indomethacin and different polymers using prepared Indomethacin microspheres were all identical. The results of FTIR spectral analysis showed the peaks and pattern of the spectra were similar in all cases. This indicates that there was no chemical interaction or bonding decomposition of Indomethacin employed in the microspheres[7].

Determination of particle size and shape

Particle shape and its morphology were determined by SEM analysis. SEM photographs indicated D:CE:HPMC microspheres are spherical with smooth surface.D:EC:Eudragit microspheres and D:CA:Eudragit microspheres are grossly spheroid forms. Particle size was determined by sieving method. Particle size of Indomethacin Microspheres 1:1:1 range was smaller than Indomethacin Microspheres 1:2:2.

In-vitro release studies

The dissolution rate studies[3] are performed to evaluate the dissolution character of Indomethacin from prepared microspheres with marketed drug (Indocide SR). The in-vitro dissolution studies were carried out in

pH1.2 acid buffer for 2 hours, which was replaced with pH 7.2 phosphate buffer up to 12 hours. The formulation D:CA:EuRL100(1:1:1) shows similar percentage release as that of marketed drug (Indocide SR) at the end of 12 hours in-vitro release study. The cumulative percentage drug release for all formulations of Indomethacin microspheres followed first order kinetics.

Pharmacodynamic studies

The percentage inhibition of inflammatory oedema produced by marketed sample of Indomethacin (Indocide SR) was compared with prepared microspheres of Indomethacin. D:CA:EuRL100 exhibited the highly significant anti-inflammatory activity among the different test groups. Significant reduction was observed in the case of test formulation compared to the control ($P<0.001$).

The microspheres of Indomethacin showed increased rate of dissolution. The result is in conformity with the concept of any increase in the in-vitro dissolution would enhance the absorption and bioavailability of the drug and hence the anti-inflammatory activity of Indomethacin microspheres, D:CA:EuRL100 (1:1:1), was more than the marketed sample[8].

Pharmacokinetic Evaluation

All batches of prepared Indomethacin microspheres were compared with marketed drug (Indocide SR).The results showed that the C_{max} of the Indomethacin microspheres was found to be the highest followed by D: CA: EuRS100 (1:1:1), which indicate a good sustaining effect. The increase in AUC represents better absorption and bioavailability of drug from the formulated microspheres, which may be due to the retarded release of drug from the dosage form. $C_{max,}$ $t_{max,}$ AUC, $t_{max,}$ MRT values of D: EC: EuRS100 (1:1:1) and D: CA: EuRS100 (1:1:1) were higher than marketed drug. So these formulations were confirmed to be suitable for sustained release dosage form[9,10].

Conclusion

Microspheres of Indomethacin were prepared by Emulsion solvent evaporation technique, using polymers such as HPMC, Ethyl cellulose, Eudragit RL100, Eudragit RS100 and Cellulose acetate in different ratios. These formulations were easy to prepare and free flowing. From the TLC studies and FTIR spectral analysis, it was observed that there was no interaction between Indomethacin and the polymers. Dissolution studies showed that

all the polymer combinations gave sustained release. Maximum drug release was from the formulation of D:CA:EuRL100 1:1:1. Pharmacodynamic study revealed that anti-inflammatory activity was well noticed in the microspheres formulation D:CA:EuRL100 1:1:1 than the marketed sample of Indomethacin and other microspheres formulations. Results of pharmacokinetic study showed that D:EC:EuRS100 and D:CA:EuRL100 formulation C_{max}, $t_{max,}$ AUC, $t_{½}$, MRT values were increased compared to the marketed drug.

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