IN VITRO-IN VIVO CORRELATION (IVIVC) STUDY OF LEFLUNOMIDE LOADED MICROSPHERES.

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ABSTRACT

This study is to establish a correlation between in vitro dissolution and in vivo absorption data of prepared sustained release Leflunomide microcapsules and compare with conventional tablets of Leflunomide(Erava10mg).We took New-Zealand white rabbit species for performing this study. The correlation ship was established according to Drewe and Guitard basing on (degree A). Comparison of cumulative in vitro dissolution profile, in vitro dissolution constant (K) Vs AUC, Mean dissolution time Vs mean residence time. The plasma drug concentration was measured with standard curve equation and compared with the standard tablet data which showed all the formulations have1hr to 4 hr extended T-max value confirming their sustained action. All formulated micro spheres show identical pharmacological effect in comparison to standard Leflunomide tablet. On the basis of the plasma concentration data analysis the formulations B1,B2, B3 were selected for in vitro - in vivo correlation which was established by comparing the in vitro in vivo correlation of the same formulation and marketed tablets. The parameters like dissolved fraction absorbed, MDT Vs MRT and$T_{85\%}$ revealed a significant in vitro in vivo correlation which substantiate the success of correlation study.

Keywords : Leflunomide, Microspheres, In Vitro Evaluation, In Vitro In Vivo Correlation.

INTRODUCTION

Microparticulate delivery systems are reliable means of delivering the drug to the desired concentration at the site of interest without untoward effect. It has added advantages over the conventional delivery system which include increased bioavailability, subject variability and drug induced toxicity and side effect[1-3] .IVIVC plays an exceedingly prominent role in the formulation of extended release products. Leflunomide is one of the new drug of choice in the treatment of arthritis[4] .It works by suppressing immune system since rheumatoid arthritis is caused by damage from overacting immune system. Hence the present study proceeds with an objective of finding out a significant in vitro - in vivo correlation after administering orally to New-Zealand white rabbit species.

MATERIAL AND METHODS

Materials

Leflunomide was received as a gift sample from Aventis Pharma Limited, Mumbai. Eudragit RS 100 and Eudragit RL 100 were received from Albert Devid Limited, Kolkata as a gift sample. All others chemicals and solvents used were of analytical grade procured from an authorized dealer, USP XXI Paddle type dissolution apparatus and UV-Visible spectrophotometer (UV-1700,Shimadzu, Japan) were the instruments employed in the current study.

Methods

Preparation of micro spheres[5]

This is the method widely used in the micro capsulation process where the polymers (Polymetacrylate in this case) were dissolved in acetone to get a clear solution. The drug was added and dissolved in this polymer

solution. The resultant mixture was then stirred at 800 rpm for 1 hr to evaporate the volatile substance. The formed micro spheres were collected and air dried for 3 hr and stored in desiccators for further use.

In vitro drug release profile[6].

In vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using phosphate buffer (pH 7.2) as dissolution medium. The volume of dissolution medium was 900 ml and temperature was maintained $37\pm1°$ through out study. Paddle speed was adjusted to 50 rpm. An interval of 1hr ,5 ml of sample was withdrawn with replacement of 5 ml fresh medium and analyzed for Leflunomide concentration by UV-Visible spectrophotometer at 260nm[7].All the experimental units were analyzed in triplicate (n= 3) .

In vitro drug release kinetics

In order to study the exact mechanism of drug release from microcapsules, drug release data was analyzed according to Zero Order Kinetics[8], First order kinetiscs[9].Higuchi square root equation[10], Hixon –Crowell equation[9].The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test .All the experimental unit were analyzed in triplicate (n = 3).

In vivo experimentation[11-16].

4 groups containing 6 animals in each group were used for performing the experiment. The animals were kept fasting for over night. Water was given *adlibitum* during fasting and throughout experiment. Microsphers were swallowed easily without any difficulties. The procedures employed in this study were approved by Institutional Ethical Committee, North Bengal University, Darjeeling by using registration No: HPI/07/60/iaec/0008.

One group was fed with standard Leflunomide tablet (Erava) at a dose of 2 mg/ kg .Other three groups were fed with prepared Leflunomide microspheres (B1, B2,B3 having the drug polymer ratio 1:1, 1:1.5, 1:2) and marked as test A,B and C. One animal of each group was kept as control. Blood samples (3ml) were collected from marginal ear vein of control animals using xylene into centrifuge tubes containing 0.4 ml of 2.5 % (m/v) sodium citrate solution. The same method was followed in all cases at an interval of 30 min, 1 hr, 2, 4, 6, 12, 18 and 24th hr during study.

In vitro- iv vivo Correlation (*IVIVC*)

According to FDA guidance four levels of IVIVC have been described which are levels A, B, C, and multiple C[17].Here the correlation was established according to Drewe and Grewe (Drgee A)[18].The parameters compared were cumulative absorption profile to that of in vitro dissolution i.e.correlation of the amount of drug dissolved to that of respective fraction of dose absorbed , time taken for 50% dissolution to that of 50% absorbed (T_{50}), In vitro dissolution rate constant (K) Vs Area Under Curve (AUC) and Mean dissolution time (MDT) versus mean residence time (MRT).

RESULTS AND DISCUSSION

The in vitro Leflunomide release from micro spheres prepared by solvent evaporation technique and with different drug : polymer (Eudragit RL and Eudragit RS, 9:1) ratio shown good sustained release property. Results were condensed in table 1.

Table 1: It shows In vitro dissolution profile of B1,B2,B3 and B4(Marketed tablet,Erava)

Time in hr.	B1	B2	B3	B4
		Concentration		
0	0	0	0	0
1	24.5	22.8	21.0	39.5
2	43.1	40.1	36.6	80.1
3	57.8	53.2	48.7	95.1
4	74.3	68.4	60.1	97.7
6	87.4	79.9	73.3	99.7
12	93.1	87.1	86.2	99.9
18	95.6	95.2	94.1	99.9
24	95.6	95.8	94.4	99.9

All the formulations were found to release Leflunomide in a controlled manner for a prolonged period over 10 hr. The comparative drug release profiles of different formulations were presented in figure 1.

Fig.1: It shows comparatives cumulative % of drug release with respect to time
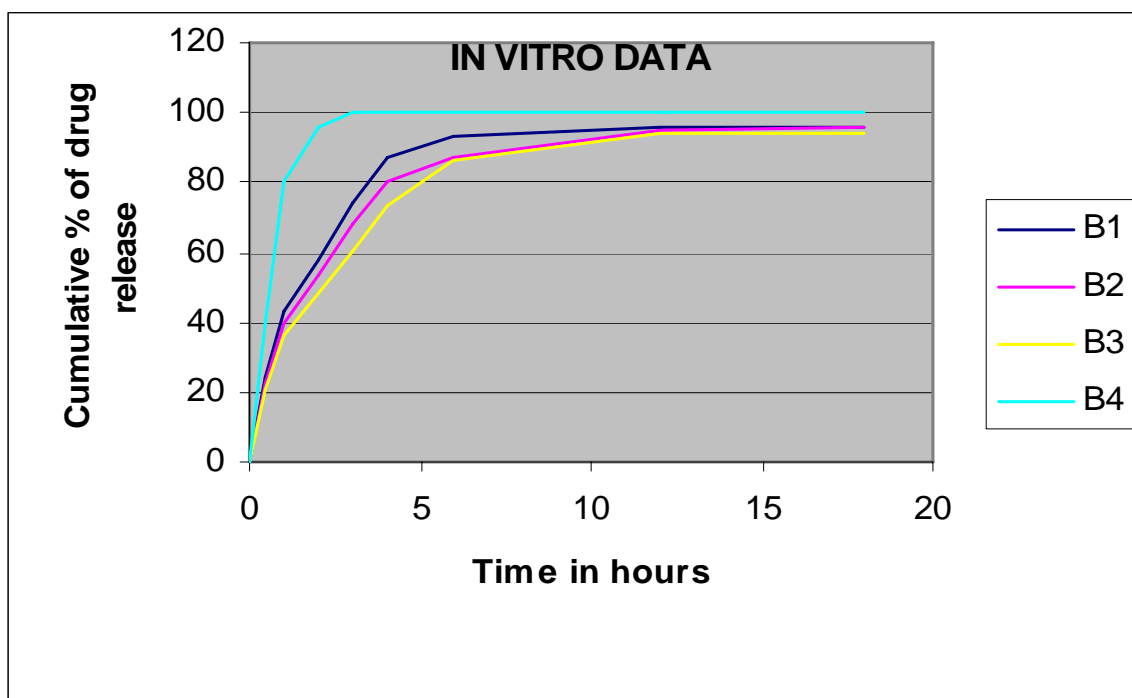
The release data of B1, B2, B3 and marketed tablet were done according to different kinetics equation depicted in the text. The release data of B3 obeys Zero order kinetics where as B1 seems to best fit in Higuchi Square root kinetics model and B2 releases drug following Hixon – Crowell cube root equation kinetic optimized in table 2.

Table 2: It shows release order kinetics of different formulation Regression analysis

Products	Zero Order Diffusion	Higuchi Matrix Diffusion	Hixon – Crowell Diffusion
B1	0.926	0.993	0.853
B2	0.897	0.914	0.967
B3	0.993	0.932	0.883
B4	0.786	0.876	0.895

Then selected formulations were examined in in vivo rabbit model. In comparison to Leflunomide releases, T max of all the microspheres were increased from 1 hr to 4 hr confirms its sustaining property. In the figure 1 indicates that the B3 formulation has lowest concentration among all and after 4 hr it was sustained over 12 hr which facilitates its sustained activity in body. On the basis of extended releaseB1,B2, B3 microspheres and marketed tablet were selected for in vitro- in vivo correlation study. The cumulative percentage of drug dissolved and cumulative fraction of drug absorbed were compared and data are provided in Table 2,3 and 4.

**Table 3: It shows cumulative percentage of drug dissolved of different formulations In vivo data**

Time in hr.	B1	B2	B3	B4
	(Concentration in µg /ml)			
0	0	0	0	0
1	1.9	1.8	1.5	2.0
2	2.8	2.7	2.5	3.9
3	4.0	3.5	3.1	6.5
4	5.2	4.1	3.8	6.0
6	6.0	5.5	5.1	5.0
12	3.0	2.8	2.7	2.9
18	2.1	2.2	2.1	1.0
24	0.8	0.7	0.9	0.5

Table 4: It shows cumulative % F.D absorbed of different formulations

Time in hr.	B1	B2	B3	B4
	(Concentration)			
0	0	0	0	0
1	22.1	20.2	18.8	40.4
2	40.3	37.7	36.6	54.7
3	54.7	50.3	46 7	71.1
4	71.2	68.8	54 8	80.9
6	90.2	82.3	80.6	90.3
12	93.4	92.1	90.8	93.0
18	93.5	92.3	91.5	94.1
24	94.6	93.1	92.2	95.5

The graphical analysis in Figure 2,3 and 4 which confirm a good degree of correlation (r^2 =0.9760) and fulfill our objective.
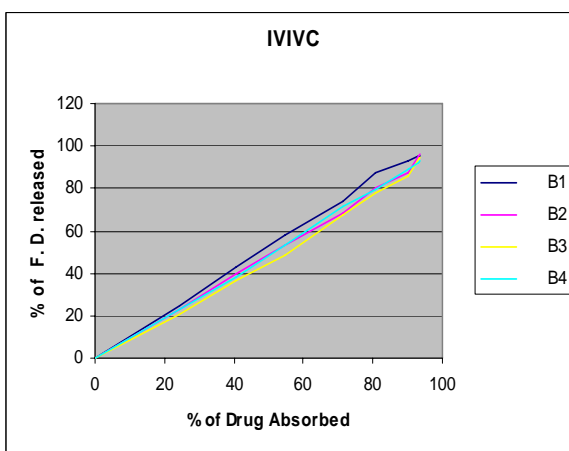
Fig. 2: It shows *invivo* data of different formulation
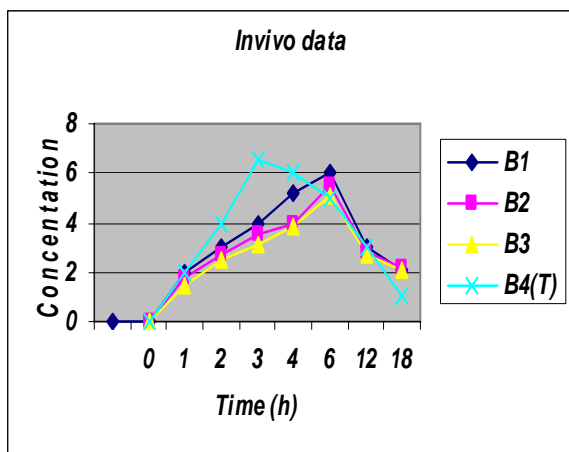
Fig. 3: It shows % FD absorbed in different time of all formualtion
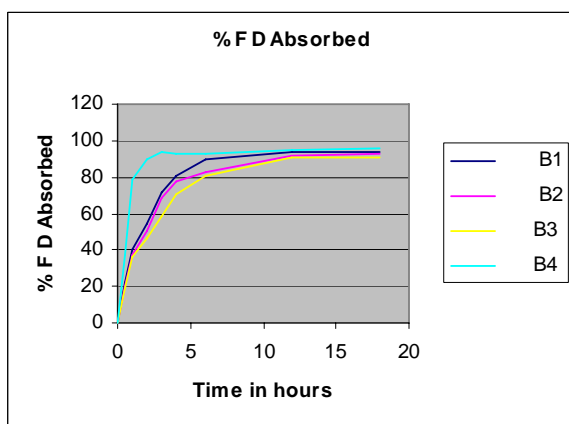
Fig. 4: It shows % FD absorbed vs. % F.D. released of all formulation

Significant level of correlation was observed between the release rate constant and AUC of the formulation .Degree A level of correlation was established from the results. Thus the formulation has the potential to liberate Leflunomide following Fickian diffusion mechanism having good degree of *in vitro- in vivo* correlation.

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