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Evaluation of Anti inflammatory activity and pharmacokinetic profile of Nimesulide incorporated Solid Lipid Nanoparticles.

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ABSTRACT

To evaluate the anti inflammatory activity and pharmacokinetics of Nimesulide (NIM) incorporated solid lipid nanoparticles (SLN), using Albino rat model. NIM 25 mg/kg and NIM-SLN (25 mg/kg in NIM) was given intraperitoneally to the rats. Edema, body weight and clinical observations were recorded. Finally, the edema reduction rate was calculated. For the pharmacokinetic research, the rats were administered with intraperitoneal injection of NIM (25mg/kg) and NIM-SLN (25mg/kg in NIM). Blood samples were collected at different time to determine the NIM concentration in plasma by HPLC. Blood plasma drug level-time curve was made and pharmacokinetic parameters were calculated. As a result of drug administration, the edema level and weight of the rat injected with NIM-SLN were significantly restrained compared with rats treated with NIM or negative control. The edema reduction rate of 90.09% showed a significant anti-inflammatory activity of NIM-SLN. At the same time, the increased body weight gain of the rat injected with NIM-SLN suggested a reduced toxicity of NIM in SLN. Pharmacokinetics study displayed a higher blood concentration, a prolonged circulation time, and an increased bioavailability of NIM-SLN compared with that of NIM. The results demonstrated that NIM-SLN could optimize pharmacokinetics, enhance anti-inflammatory activity, so it has a promising prospect for the application in anti inflammatory treatment.

Key words: Nimesulide, SLN and Inflammation.

1. INTRODUCTION

Nanoparticles made from solid lipids are widely used as colloidal drug carriers for intravenous application[1-4]. The nanoparticles are in the submicron size range (50–1000 nm) and they are composed of physiological lipids. At room temperature the particles are in the solid state. Therefore, the mobility of incorporated drugs is reduced, which is a prerequisite for controlled drug release. They are stabilized with non-toxic surfactants like sodium taurocholate and lecithin[5]. Due to the production by high pressure homogenization they can be produced on large industrial scale. In addition, this production method avoids the use of organic solvents.

Conventional preparation like solution, suspension or emulsion is suffer from following limitation. High dose and low availability, first pass effect, exhibit fluctuations in plasma drug levels and they don't provide sustained effect therefore there is a need for some novel carriers which could meet ideal requirement of parenteral delivery system. Recently nanoparticles delivery system have been proposed like liposome nanoemulsion, microemulsion, nanosuspension, microparticles, polymeric nanoparticles, nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN). But all systems except last two suffer from various limitations. Nanoparticles made from solid lipids is gaining increasing attentions as colloidal drug carriers for intravenous application[1,4,6,7]. In future NIM-SLN can be used as anticancer. SLN not change their organoleptic as well as pharmaceutical feature on aging for almost one year. They are based on biocompatible lipid and provide sustained effect by either diffusion or dissolution. NIM was introduced in 1985 and it is one of the most potent NSAIDs advocated for use in various inflammatory conditions. It is official in British Pharmacopoeia[8]. Nimesulide is an acidic non-steroidal anti-inflammatory agent, which differs from many similar compounds in that it is acidic by virtue of a sulfonanilide rather than a carboxyl group. Chemically it is [4-nitro-2-(phenoxy) methane sulphoanilide] and has a structure potentially capable of accessing the COX-2 side pocket when the two COX isoforms became

organized[9-10]. It is an inhibitor of cyclo-oxygenase 2, hence inhibits the synthesis of destructive prostaglandins and spares cytoprotective prostaglandins. Other than Prostaglandins inhibition it is also inhibit the platelets aggregation. Clinical studies have shown that NIM to be analgesic, anti-inflammatory and antipyretic in a wide range of conditions[11]. Unfortunately, the use nimesulide in clinics is restricted by various countries, which is caused by the accumulation of this drug colitis and oral ulcerations[11]. To avoid treatment-limiting side effects and to get better efficacy, modern therapy requires that the drug reaches the site of action in the most efficient way, which can be achieved, by using colloidal drug carriers as delivery system[12-14].

In this study, work was carried out to investigate the anti inflammatory effect and pharmacokinetics of NIM-SLN in vivo.

2. EXPERIMENTAL

2.1. Materials

Pure drug sample of Nimesulide was kind gifted by Sigma pharma, Kanpur, India. NIM-SLN was prepared by a procedure reported elsewhere and was dissolved in normal saline before use. All reagents and solvents were of analytical or HPLC grade and were used without further purification. For the quantitative determination of NIM, a HPLC was used. (Agilent 1100 system).

2.2. Rats

Albino rats weighing 120-170g were collected from Central Animal house, RMMCH, Annamalai University, Chidambaram, India and housed in stainless steel cages in a ventilated animal room. Room temperature was maintained at 24+_ c, relative humidity was 50 +_ 10 %, and light cycle and dark cycle alternate every 12 hour. Distilled water and sterilized food for rat were available *adlibitum.*

2.3. In vivo studies in inflammation bearing rats

2.3.1. Anti inflammatory activity

Anti-inflammatory activity was determined by carrageenan-induced acute paw edema models in rats. Animals were divided I to 4 groups comprising 5 animals each. In all groups, acute inflammation was produced by sub-plantar injection of 0.02 ml freshly prepared 1% Carrageenan in normal saline in the right hind paw of rat. One group injected with saline served as Negative control. One group injected with diclofenac sodium 10mg/kg body weight served as positive control. One group was administered with NIM-SLN 25mg/kg body weight and one group was administered with NIM 25mg/kg body weight. The paw thickness was measured using Vernier calipers before and 3 hr after carrageenan injected[15-16]. Increase in paw thickness was calculated using the formula Pt-Po, where as Pt is the thickness of paw at time t (ie, 3 hr after carrageenan injection) and Po is the paw thickness at 0 times.

Percent inhibition was calculated using the formula:

$$\text{Inhibition } (\%) = (C\text{-}T/C)\times 100$$

where, C is the increase in paw thickness of the control and T is that of treatments.

2.3.2. Statistical Analysis

The significance of difference between means was determined by students t test values of $p < 0.05$ were considered.

2.4. Pharmacokinetic Research

2.4.1. Administration of NIM-SLN and NIM to rat

NIM-SLN and NIM formulation were injected intraperitoneally at the NIM dose of 25 mg/kg body weight and each group consist of five animals. Blood samples were collected in heparin containing tubes at the designated time (0, 30 min and 1, 1.30, 1.45, 2, 2.15, 2.30 and 3 hour) via quickly removing the eyeball from the socket with a pair of tissue forceps. Plasma was isolated by centrifugation (10 min at 5000 r/minutes) and stored at -20c.

2.4.2. Plasma sample treatment

I ml plasma was mixed with 0.4 ml ethyl acetate. After vortex for 110min and centrifuging for 10min (13000 r/min) the organic phase was separated and evaporated and the residue was then reconstituted with 200 Ul mobile phase and was mixed in a vortex mixer for 10 min. After centrifugation at 13000 r/min for 10mts, a portion (20UL) of the reconstituted sample was injected on to the chromatography column

2.4.3. HPLC determination of Nimesulide

Nimesulide concentration was analyzed by HPLC using a CLC C18 (5 UM, 25cmx4.6 mm id) column using a mobile phase consisting of an acetonitrile 0.05 M KH 2P04 buffer mixture of PH 7.00 (55:45, v/v). The detection was carried out at 230 nm and the linearity range was found to be 0.5- 100 Ug/Ml

2.4.4 Statistics and PK analysis

Pharmacokinetic parameters including area under the curve (AUC), total body clearance (CL) and plasma half life for the distribution and

elimination phase were assessed using a software program (3P87).

3. RESULTS AND DISCUSSION

3.1. Therapeutic efficacy of NIM- SLN in inflammation bearing rat

Mean inflammation levels are shown in Figure 1. Rapid inflammation growth was observed in saline treated rats (– ve control). NIM-SLN exhibited a higher anti inflammatory effect compared with NIM and Diclofenac sodium, probably due to their long circulation. Thus NIM-SLN was demonstrated to be more effective than free NIM in inhibiting inflammation growth. Inflammation suppression rates were calculated and are shown in table 1. In the same way rats injected with NIM-SLN displayed higher suppression rate than that of free NIM. Body weights of the rats were monitored throughout the experiment as an indication of adverse effects of the drugs. As shown in table 1, loss of body weight in rat injected with NIM-SLN was less than Diclofenac sodium (positive control) and frees NIM, which proved the better biocompatibility of NIM-SLN.

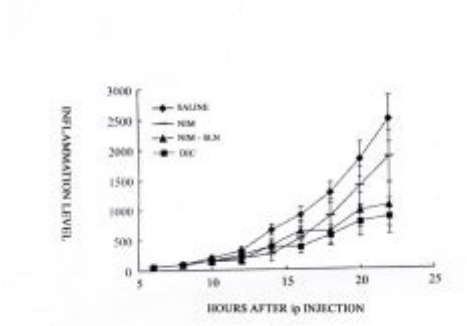

Figure.1. In vivo anti inflammatory effect by intraperitoneal injection of NIM and NIM-SJN at a dose of 25 mg NIM/kg in inflammation bearing rats evaluated by carrageenan induced paw edema method.

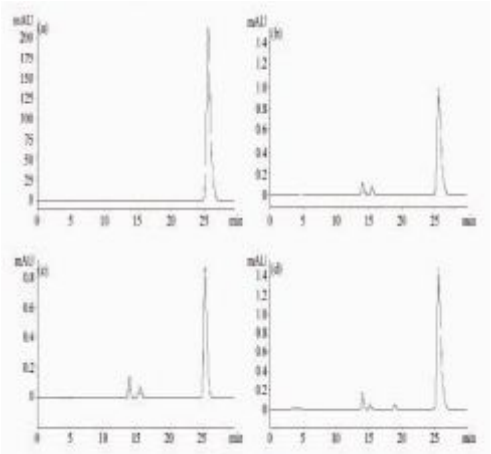

Figure .2. Representative chromatographs of (a) standard NIM, (b) plasma spiked with NIM, (c) plasma sample from the rat added with NIM, (d) plasma sample from the rat added with NIM-SLN.

3.2. Pharmacokinetic Studies

3.2.1. Chromatographic performance

Figure 2 shows HPLC chromatograms for extracts from standard NIM, plasma spiked with NIM, and plasma sample from rat treated with NIM. No interference from serum constituents was observed for either assay system.

3.2.2. The calibration curve of NIM in serum samples

The linearity of the method was conducted using drug free plasma with NIM added to yield concentration of 0.1, 0.5, 1.0, 2.0, 5.0, 10.0 and 50.0 Ug/Ml. Data were obtained through linear regression analysis of peak height ration of NIM (A) Vs NIM concentration (Ug/ML) in spiked plasma samples (C). A typical calibration curve presented the regression equation of A= 4.618C-8.243 (r=0.09997, n=5). The calibration curve for the determination of NIM in plasma was linear over the range of 0.1-50 Ug/Ml.

3.2.3. PK properties

The plasma NIM concentration time curves are illustrated in Figure 3. At all times, NIM plasma concentration was higher in NIM-SLN treated group than NIM treated group. The curves meet the two compartment model according to 3P87 program.

4. CONCLUSION

The research on Nano drug delivery system has become the hot spot in recent years. It was reported that the anti-inflammatory activity of NIM when mixed with Nano liposome could be significantly enhanced[17]. The SLN synthesized is a novel drug delivery system different from Nano liposome. It has been proved that SLN could depress side effect, improve drug solubility and achieve delayed release[17-34].

In this study, Diclofenac sodium, a frequently used anti-inflammatory drug, was adopted as the positive control. The results of the efficacy study with inflammation showed a significant difference. Between positive control and negative control ($p<0.01$), indicating the reliable data and reasonable error. The remarkable therapeutic effect of NIM-SLN was demonstrated by the statistical significance inflammation suppression and volume between rats injected with NIM SLN and saline ($p<0.01$). While the free NIM when administered at the molar equivalent dose, was less effective than NIM-SLN. Results from this inflammation study

Table .1. Anti-inflammatory effect of different drug formulations reflected by body weight, edema level and edema inhibition rate:

Drug Formulation	Dose (mg/kg)	Body weight before administration	Body weight before administration	Change in paw edema mean (mm)	% edema inhibition relative to control at 3rd hour
Saline	0.3ml	130.45	130.45	1.1 +-0.05	-------
NIM-SLN	25	132.00	138.23	0.1+-0.004	90.9%
NIM	25	140.24	144.12	0.2+-0.007	81.81%
Diclofenac sodium	10	138.17	142.26	0.3+-0.01	72.72%

Statistical significances are a) $p<0.01$ and b) $p<0.05$.

also showed the inhibition of weight gain in rat injected with NIM or Diclofenac sodium. In addition it was found NIM-SLN is less toxic, viewed from the body weight increasing in rat administered with NIM SLN which represents a reduced side effect of NIM.

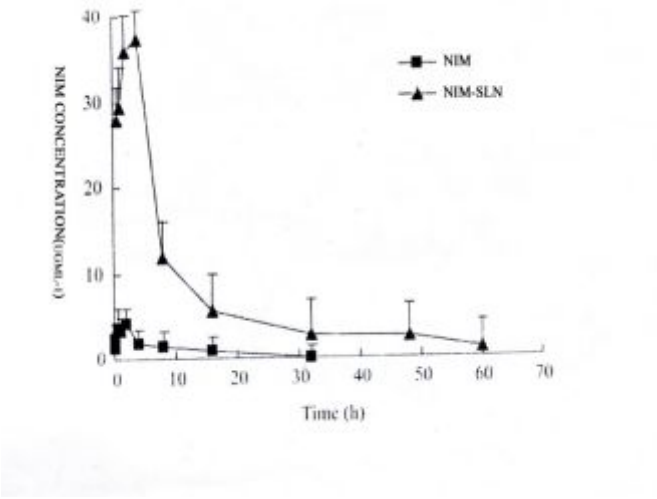

Figure .3. Plasma concentration vs time curves for NIM and NIM-SLN in rat. NIM and NIM-SLN formulations were administered via intraperitoneal injection at a dose of 25 mg/kg in NIM. Each data point is the average of three to five animals and error bar equals one standard deviation.

Plasma clearance profile of NIM-SLN was compared with those of free NIM at the molar equivalent dose. NIM-SLN displayed a longer systemic circulation time relative to free NIM, suggesting its greater in vivo stability. Serum concentration time data of rat administrated with NIM and NIM SLN were calculated using 3P87 software. The results showed that both groups exhibit two compartment models, which have an initial redistribution phase with a short half life followed by an elimination phase with a longer half life. Encapsulation of NIM in SLN obtained marked differences in terms of the PK parameters. Using SLN as carriers, a seven fold enhancement of AUC was acquired, with an eight fold reduction of CL, which suggests the increase of NIM entering body, and the decrease of NIM eliminated by organism, respectively. Furthermore, animals administered with NIM SLN exhibit an increased peak serum concentration and a delayed time to peak concentration of NIM compared with those treated with free NIM conceivably. This sustained NIM, release mechanism is favorable to the suppression of inflammation and much account for the in vivo efficacy experiments. In conclusion there is a potential for a broad range of new therapeutic applications using SLN as drug carriers.

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