Acta Pharmaceutica Turcica
XLI (1) (15-18) 1999

DEVELOPMENT OF MULTIPLE W/O/W EMULSIONS SHOWING PROLONGED ANTIINFLAMMATORYY ACTIVITY OF NIMESULIDE

TANDON, P. and MISHRA, B.*

Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005, India

Three multiple w/o/w emulsions containing nimesulide were prepared using three different formulae and were evaluated in vitro in pH 7.4 phosphate buffer and in vivo in carregeenin induced rat paw oedema model to investigate the drug release characteristics and prolonged release potential of multiple w/o/w emulsions. The results were compared with marketed suspension of nimesulide. Multiple emulsions provided slow and controlled release of nimesulide with prolonged antiinflammatory activity in rats in comparison to drug suspension. The in vitro and in vivo data of formulations showed good correlation between in vitro and in vivo results.

Keywords: *w/o/w emulsion; Multiple emulsion; Nimesulide; Antiinflammatory activity; Prolonged action*

Introduction

Multiple water-in-oil-in-water (w/o/w) emulsions were first suggested as a method of producing a prolonged antibody response (1). Later, some other novel uses of w/o/w emulsions in medicine were also investigated such as: to prolong drug release (2-4), to mask the unpleasant taste of drug (5) and to protect drugs, like insulin which are normally degraded when given perorally (6).

Nimesulide, a newer NSAID, still suffers from the disadvantage of frequent administration and gastrointestinal discomfort, when given as conventional tablet/ suspension. Controlled and prolonged release dosage forms of this drug were expected to overcome the above problems to a great extent. Based on the above facts, present study was conducted to see the *in vitro* and *in vivo* prolonged release potential of w/o/w multiple emulsion containing nimesulide.

Table. Formula of different multiple emulsions.

Notation	Internal Aqueous Phase	Middle Oil Phase	External Aqueous Phase	PPVR*	SPVR*
ME_2	Nm+20%v/vPG+1%w/v ascorbic acid +PB(pH 7.4)	HLP+15%w/w Span 80	5%v/v PG+PB(pH 7.4)+ 1%v/v Tween 80	0.6	0.6
ME_3	Nm+50%v/vGly+PB (pH 7.4)		10%v/v Gly+PB(pH 7.4)+ 1%v/v Tween 80	0.6	0.6
ME_4	Nm+10%v/v PG+2%w/v NaCl+PB (pH 7.4)		5%v/v PG+1%v/v Tween 80 + PB (pH 7.4)	0.6	0.6

*PPVR&SPVR are primary and secondary phase volume ratios respectively

Nm = Nimesulide

PB = Phosphate buffer

PG = Propylene glycol

HLP = Heavy liquid paraffin

Gly = Glycerol

Materials and Methods

Nimesulide (Panacea Biotech.), heavy liquid paraffin I.P. (Reidel Chem.) nimesulide suspension (Nimulid® Panacea Biotech.), Span 80, Tween 80 and all the other chemicals were obtained commercially from India and were used as received. Instruments like magnetic stirrer, emulsifier and U.V. spectrophotometer (Japan Spectroscopic Co. Ltd., Japan) were used as and when required.

Preparation of multiple w/o/w emulsion

Each multiple w/o/w emulsion was prepared freshly on the day of evaluation in 50 ml total volume and contained 150 mg (5 mg/ml) of drug in the internal aqueous phase only. The multiple emulsions using the formula shown in Table 1 were prepared by two step emulsification procedure. 18 ml of internal aqueous phase containing the drug was emulsified with 12 ml of the oil phase containing 15% w/w Span 80 by stirring at 2000 emulsification procedure. 18 ml of internal aqueous phase containing the drug was emulsified with 12 ml of the oil phase containing 15% w/w Span 80 by stirring at 2000 rpm for 5 min at 70°C to produce the primary emulsion. This primary (w/o) emulsion was reemulsified with 20 ml of external aqueous phase containing 1% v/v Tween 80 by stirring at 1000 rpm for 3 min at room temperature.

In vivo Studies

The *in vivo* studies to determine the antiinflammatory activity of nimesulide were conducted on healthy albino rats of either sex weighing 200-250 g. The rats were fed on Hind Lever diet and were acclimatized with the housing environment for a week before use. Eight groups of 5 rats each, fasted overnight with water adlibitum were used for the studies. The antiinflammatory activity was determined in terms of decrease in experimentally induced rat paw oedema. The oedema was produced by injecting 0.1 ml of 1% carrageenin suspension in normal saline, into plantar surface of the rats left hind paw below the plantar aponeurosis. For each formulation two groups of rats were used out of which, one group was orally intubated with drug formulation at 20 mg/kg dose level, and the other group received the same volume of formulation without drug. Each intubation was done 45 min before carrageenin injection. The paw volume was measured plethismographically before and at one hour interval upto 6 hours, at 2 hours interval upto 12 hours and finally at 24 hours after the injection of carrageenin. The percentage inhibition of rat paw oedema was calculated for each formulation and plotted versus time.

In vitro Studies

The *in vitro* drug release studies were carried out over times upto 24 hrs, in triplicate, in a standardized glass diffusion apparatus (7) using phosphate buffer pH 7.4 as diffusion medium.

Results and Discussion

The *in vitro* release profile of these formulations are shown in Fig. 1 and the *in vivo* results calculated as percentage change in oedema with respect to control oedema at the corresponding hour of

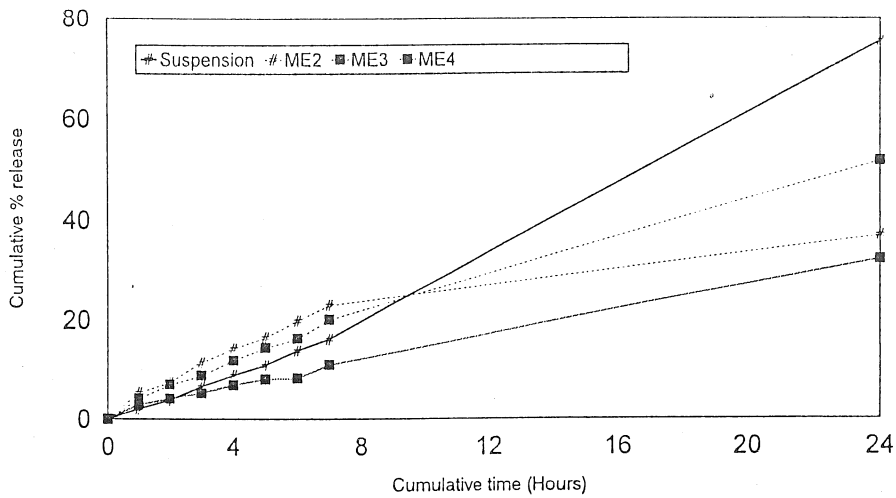

Fig.1. In-vitro release profile of different types of formulations

paw volume measurement are shown in Fig.2. The results obtained from four different formulations, at same time intervals are worth observing. All the three multiple emulsions decreased the rat paw oedema for much longer duration in comparison to marketed suspension. All the formulations gave a peak oedema inhibition response between 3 to 5 hours and after that there was a decline in the inhibition activity. This oedema inhibition activity decreased sharply in case of marketed suspension and this was effective for approximately 8-10 hours. In contrast, multiple emulsions exhibited slow decline in the antiinflammatory activity of nimesulide and provided almost constant and controlled inhibition of oedema even beyond 10 hours and were found effective till 24 hours. This greater and prolonged inhibition observed from the multiple emulsion was attribited to controlled, slow and continuous release of drug from the innermost aqueous phase through the two interfacial barriers and immiscible oil layer into ex ternal aqueous phase. The two interfacial barriers played an important role in controlling the release of drug from internal to external aqueous phase.

The reason for achieving a higher inhibition from marketed suspension during initial hours could be attributed to the fact of absence of any barrier in formulation. Though the drug release from suspension was limited by dissolution of drug particles in gastrointestinal fluid, all the solubilized drug molecules were absorbed rapidly and might result into a disproportionate increase in free drug levels in blood with subsequent faster elimination and this may be the possible reason of faster decline of antiinflammatory activity of drug in case of suspension.

The multiple emulsion ME_4, was able to provide a maximum inhibition of 39.3% only, but was most effective till 24^{th} hour with 18.5% inhibition in oedema. On the other hand multiple emulsion ME_3 provided a much higher peak inhibition as 58.1% but showed a faster decline in inhibition activity till 12 hours

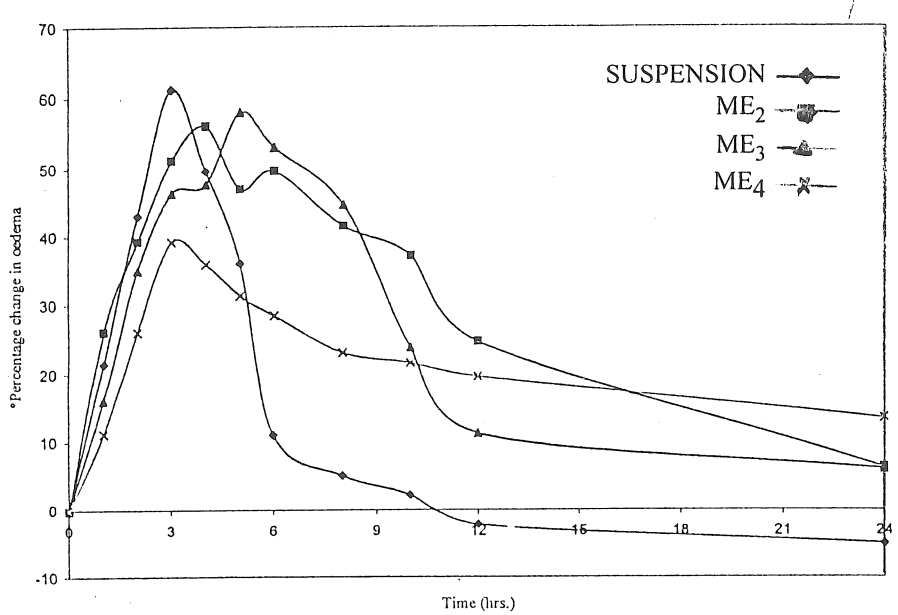

Fig.2. Profile VII: Profile showing effect of orally administered (20 mg/kg) different formulations of Nm on carragenin induced pedal oedema in rats.

*%change in oedema is calculated with respect to control oedema at corresponding hours.

and almost constantly maintained the inhibition activity between 12 to 24 hours.

Prior to subjecting the above formulations for *in vivo* investigation, they were tested *in vitro*. The cumulative percent of drug release observed in 24 hours in phosphate buffer pH 7.4 were 36.7%, 51.6% and 32.1% from the formulations ME_2, ME_3, ME_4 respectively and 75% from the marketed drug suspension. The *in vivo* antiinflammatory response of nimesulide from different formulations appears to be in good agreement with *in vitro* results.

Based on the above findings it was concluded that multiple w/o/w emulsion system can be utilized as a potential prolonged release dosage form of nimesulide. Moreover, further *in vivo* investigations are needed in human volunteers to correlate our findings to human usage for this type of dosage form.

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Accepted: 04.06.1998