
Enhanced Brain Uptake of Rifampicin from W/O/W Multiple Emulsions via Nasal Route

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Multiple emulsions with oily liquid membrane containing rifampicin were prepared and characterized for droplet size, encapsulation efficiency, *in vitro* drug release, and pharmacodynamic properties. A comparative *in vivo* study from oral and nasal routes was made with a special reference to brain uptake. Results showed - i) average drop size as 32.4 μm ; ii) a very high encapsulation efficiency; iii) prolonged drug release *in vitro*; iv) prolonged brain and plasma levels as compared to the control plain drug from both routes, and v) substantial accumulation of drug in brain when administered through multiple emulsion via nasal route compared to plain drug.

ACCUMULATED experimental evidence support the presence of transport pathway for drug molecules from submucosal layer of nose to the perivascular and subarchnoid space of olfactory lobes of the cerebrum^{1,2}. Higher levels are achieved in cerebrospinal fluid after intranasal application of lipid soluble drugs, progesterone and estradiol as compared to i.v. injection^{3,4}. Reports on systemic investigation with an appropriate drug delivery system are however lacking.

Multiple emulsions are studied as prolonged drug delivery system through various routes, Ohwaki *et al*⁵ have shown that it is feasible to develop multiple emulsion (W/O/W) as nasal dosage form. Pandit *et al*⁶ have shown enhanced brain and plasma levels of indomethacin from O/W/O multiple emulsion after oral administration. Rifampicin is a first line drug in the treatment of mycobacterial diseases. The comparatively high dose of rifampicin required for achieving high and prolonged levels in the brain for the treatment of mycobacterial meningitis is associated with various side effects such as GIT disturbances and neurological problems. Keeping this in view, we attempted to develop W/O/W multiple emulsion system through nasal route for efficacious brain delivery.

Two step emulsification procedure was adopted for the preparation of W/O/W emulsion⁸. Internal aqueous phase (12.5 ml) bearing drug (20 mg/ml) was emulsified in 20 ml of liquid paraffin containing 10% Span-80 (Burogyne, Bombay) using a mechanical stirrer at 2500 rpm for 30 min. The primary emulsion was then emulsified in outer aqueous phase containing 1.0% Tween-80 (Burogyne, Bombay) on a magnetic stirrer. The volume fraction of W/O emulsion to W/O/W emulsion was 0.5.

Droplet size of multiple emulsion was determined photomicrographically from the negatives. The photographs were taken on Nikon microscope (Magnification X 450) (Fig. 1).

The mean droplet size was 32.4 μm and the maximum size was about 75.0 μm . The maximum size of internal droplets was < 2.4 μm . The maximum number of droplets were in < 1.0 μm range which could not be measured hence, mean was not calculated.

For the determination of encapsulation efficiency, freshly prepared W/O/W emulsion (10 ml) was centrifuged at 2500 rpm for 15 min. The lower aqueous phase (2 ml) was withdrawn carefully and filtered

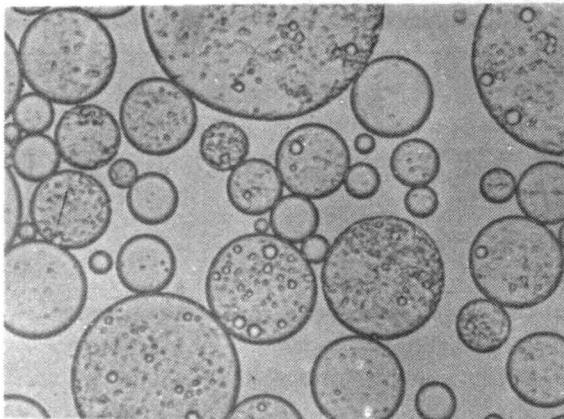


Fig. 1

through millipore filter ($< 1 \mu\text{m}$ pore size). The sample was then analysed by HPLC method⁹ after suitable dilution. A very high encapsulation efficiency of $97.0 \pm 2.7\%$ was obtained.

Drug permeation test was done in a locally fabricated diffusion cell. Fresh W/O/W multiple emulsion (20 ml) was kept in the donor compartment which was continuously stirred at 300 rpm with a vertical stirrer. The solution used for the outer aqueous phase for multiple emulsion was introduced in the receptor compartment separated from donor by treated cellophane membrane (Sigma, USA). The receiving medium was maintained at $37 \pm 0.1^\circ\text{C}$ and kept continuously stirred on magnetic stirrer. The samples were withdrawn at appropriate time intervals and analysed by HPLC. The permeation study showed a first order release with $78.3 \pm 6.43\%$ drug leakage at the end of 24 h.

In vivo Studies were performed on male Albino rats weighing between 250- 300g (Animal Breeding Centre B.J. Medical College, Pune). Two Rifampicin formulations viz. plain drug solution and multiple emulsion were used in 5.0 mg/kg dose for both oral and nasal routes. Rats were anaesthetized by 20 mg/kg i.p. injection of phenobarbitone. An incision was made on the neck, the trachea was cannulated with polyethylene tube and the oesophagus was cannulated towards the nasal end. Nasopalatine duct

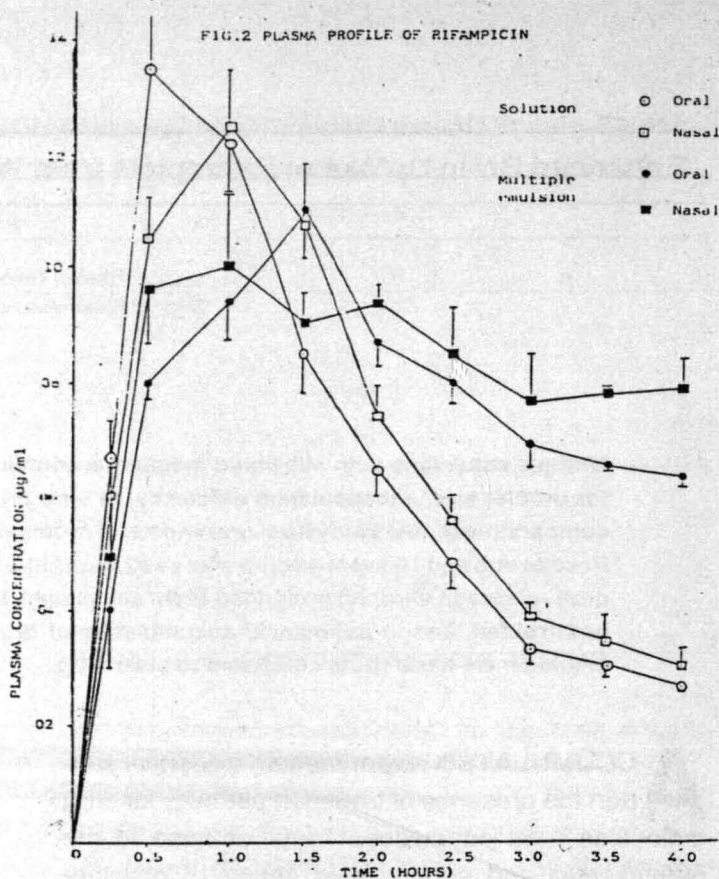


Fig. 2

was closed to prevent drainage of drug into the mouth¹⁰. In case of early recovery from anaesthesia, tracheal tube was connected to anaesthetic ether chamber to maintain the rat in anaesthetized state. At appropriate time intervals after dosing through nose, rats were sacrificed by excessive anaesthetic ether inhalation and blood was collected immediately by cardiac puncture. The brain tissue was removed by cranial fracture, washed, dried on a filter paper, weighed, sliced and macerated with saline. The macerate was subjected to assay by HPLC. The reported HPLC procedure was found to hold equally good in accuracy and precision for macerate.

For oral administration, rats were treated similarly as for intranasal administration. Rifampicin formulations were administered through the polyethylene tube which cannulated oesophagus towards the stomach end. The samples were collected as mentioned above.

In vivo studies showed prolonged plasma drug levels from W/O/W multiple emulsion from both the

Table - 1: Brain levels of Rifampicin from the two formulations from nasal and oral route

Time	Brain Levels*			
	Solution		Emulsion	
	Oral	Nasal	Oral	Nasal
0.5	2.20 ± 0.11	10.90 ± 1.54	1.95 ± 1.04	8.93 ± 1.06
1.0	5.66 ± 0.52	14.30 ± 1.70	4.98 ± 1.00	13.72 ± 0.58
2.0	16.95 ± 0.47	29.53 ± 0.35	11.46 ± 0.22	25.54 ± 0.36
3.0	9.18 ± 0.36	20.05 ± 1.20	13.35 ± 1.24	28.61 ± 1.43
4.0	7.16 ± 0.72	7.85 ± 1.32	15.68 ± 0.46	30.39 ± 1.18

*Mean ± S.D.; n = 3

routes (**Fig. 2**). For drug solution, almost the same plasma profile was obtained from respective routes. In the brain, through nasal route, higher rifampicin concentrations are reached than simultaneously recorded in blood. Through oral route, rifampicin similarly attains high concentrations, which on the whole are roughly at par with those measured in the blood at the same point in time. The brain concentration from solution rises more slowly than in blood, reaches their peak values later, and also declines more gradually (**Table-1** and **Fig. 2**).

In the case of multiple emulsion, significantly greater brain drug levels are achieved in 4 h. probably because of the greater clearance of drug from the brain for solution than for multiple emulsion which in fact is due to the slow release of drug from multiple emulsion and transport via direct pathway to the brain from nasal mucosa. On the basis of above discussion it was concluded that W/O/W multiple emulsion system can be successfully developed for brain infections, to be administered through the nasal route.

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