RESEARCH PAPER

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Resealed Erythrocytes as Carriers for Salbutamol Sulphate

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Resealed erythrocytes as carriers for salbutamol sulphate were prepared as a novel drug delivery system for controlled release therepy by hypotonic haemolysis technique. *In vitro* drug release study was carried out by dialysis technique. The product was lyophillised. *In vivo* studies in guinea pigs using histamine chamber showed promising results.

NOVEL drug delivery systems are important to overcome the drawbacks of conventional medications. They have better patient complaince and therapeutic efficacy. An ideal drug delivery system should take the active principle to the site of action and release the drug at a slow and steady rate. Erythrocytes are biodegradable, noninmmunogenic, circulate nearly as long as normal erythrocytes, entrap large quantity of drug and patients own blood can be used to encapsulate the drug[1]. Propranolol and its prodrugs were encapsulated to serve as slow release system[2]. Urogastrone has been encapsulated to increase stability[3]. Encapsulation of B-galactosidase is used for enzyme replacement therapy[4]. In this study an attempt is made to evaluate the potential use of resealed erthyrocytes as a means for controlled drug delivery system.

Salbutamol sulphate I.P (M/s Cipla Ltd, Bangalore) was selected as the drug. It is a selective Beta-2 receptor agonist which shows a shorter half life of 2-7 hours[5].

EXPERIMENTAL

DRUG ENCAPSULATION PROCEDURE

The laboratory procedure was based on hypotonic haemolysis technique[6]. Erythrocytes were seperated from fresh whole human blood by centrifugation at 1000 rpm for 15 minutes. Cells were washed with isotonic saline. Twenty five ml of 50% v/v cell suspension was made. This was placed in ice mixture (Ice + Ammonium chloride. 0°C ± 1°C) along with the drug (5 ml of 10% w/v Salbutamol sulphate solution) and 20 ml of 0.25% w/v sodium chloride hypotonic solution. This was stirred slowly using a magnetic stirrer. Haemolysis takes place within 4 minutes.

Drugs gets entrapped. At the end of equilibrium isotonicity was restored by the addition of hypertonic saline solution. (5% w/v of sodium chloride solution). The contents were warmed slowly to 37° C and then incubated at 37° C for 45 minutes (resealing period). These cells were collected by centrifugation at 3000 rpm for 15 minutes, washed with saline and were lyophilised.

Similarly a blank was prepared without drug and lyophilised.

DRUG RELEASE STUDIES

In vitro drug release studies were carried out by dialysis membrane and isotonic saline as dialysis medium, at refrigerated condition (4° C + 1° C). Twenty five ml of the suspension of resealed erythrocytes containing 15 mg of the drug and 1 g of the lyophilised product equivalent to 16 mg of the drug,

Table - I: In Vitro Drug Release Studies

Sl. No.	Time interval of withdrawal hr	Cumulative Percentage drug released formulation 4 ± 1°C	Lyophilised Product 4 ± 1°C
01	1	6.17	1.89
02	2	12.32	4.76
03	6	14.72	6.83
04	8	17.95	11.68
05	10	18.27	19.72
06	12	20.87	20.82
07	24	21.90	28.36
08	36	27.94	43.06
09	48	43.08	48.71
10	72	56.09	56.71

suspended in 25 ml of isotonic saline were used for drug release studies. 2 ml of the dialysate was withdrawn at different time interval. The amount of the drug was estimated colorimetrically using the reaction with aminophenazone and the colour developed was read at 550 nm[7].

IN VIVO STUDIES

Simple and rapid method reported by Lish[8] was followed.

Healthy fasted guinea pigs of either sex weighing 250-350 gm were selected and divided into two groups of three each. The first group received the standard drug while the second group, the lyophilised product.

Guinea pigs were placed in a histamine chamber. Five percent histamine diphosphate mist was fed to the chamber through a 2 cm diameter opening of the chamber. Then they are allowed to recover for 2-4 hours. Then they were treated with the drug and subjected to a second challenge with histamine mist. Freedom from asthma after a 6 minutes exposure to histamine was regarded as protection. Cessation of respiration and the appearance of asphyxial convulsions were taken as end point. Histamine mist was fed to the chamber after 3,6,12,,24 and 48 hours.

RESULTS AND DISCUSSION

Twenty five ml of suspension of the resealed erythrocytes containing 15 mg of Salbutamol sulphate and one gram of the lyophilised product containing 16 mg of Salbutamol sulphate was used for evaluation. At 4° C the rate of drug release was found to be slow and steady. In case of the lyophilised product, the release was found to be more at 24-36 hours when compared to the formulation. The release was found to be 56% in both the formulation and the lyophilised product at the end of 72 hours. But the erythrocytes haemolysed completely after 72 hours in case of formulation. The drug in the formulation was found to be intact as evidenced by

Table - II: In Vivo Studies

Drugs used	Dose	Guinea pig No.	Guinea pig Weight in gms	Visual abdominal respiration rate/min					
				Normal	Histamine Aerosol treatment prior to drug administration	Histamine Aerosol treatment after giving drug			
						3	6	12	24
						(Time in hours)			
1. Standard Salbutamol Sulphate solution	0.093 mg in water for injection	1	350	156	96	154	148	97	-
		2	400	180	108	190	177	-	-
		3	300	144	96	148	123	98	-
2. Lyophilised product	6 mg = 0.093 mg of salbutamol sulphate in isotonic saline	1	350	150	100	150	149	151	144
		2	300	184	102	174	160	172	172
		3	320	162	98	152	121	168	134

IR and UV light absorption scanning spectroscopy and TLC.

From **Table II,** it is evident that the lyophilised resealed erythrocytes protected guinea pigs, from bronchoconstrictor effect of histamine aerosol for nearly 48 hours. This indicates prolonged antihistaminic activity of the product due to controlled release of the drug in guinea pigs. In conclusion,human red blood cells do have the potential for the eventual development of an ideal drug delivery system for controlled drug release.

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