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In vivo evaluation of spray formulations of human insulin for nasal delivery

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Abstract

There are many ongoing investigations to improve the nasal bioavailability of peptide and protein formulations. The presence of bioadhesive polymers in nasal formulations may increase the residence time of the drugs in the nasal cavity. A combination of bioadhesive polymers with permeation enhancers would seem to further improve nasal bioavailability. In this study, insulin spray formulations containing two bioadhesive polymers (1.5% w/v microcrystalline cellulose (MCC) and 70% w/w Plastoid L50) alone or in combination with the enhancers such as sodium taurocholate (ST), ammonium glycyrrhizinate (AG) or glycyrrhetinic acid (GA) at 1% w/v level, were evaluated in diabetic rabbits. A total volume of 100 μl of freshly prepared insulin formulation was sprayed into the nasal cavity of each diabetic rabbit. Glucose levels were monitored using a blood glucose assay and serum insulin levels were analyzed using RIA. 5 U/kg insulin in the MCC suspension alone resulted in an absolute bioavailability of 1.96% while Plastoid L50 alone resulted in 2.25% absolute bioavailability. Insulin in the MCC suspension and ST, AG or GA resulted in 8.36, 7.83 and 2.15% bioavailability, respectively. The same formulations produced a hypoglycemic effect in terms of total glucose reductions of 39.12, 15.96 and 9.36% and the maximal decreases in glucose levels were 58.37, 21.8 and 18.61%, respectively. The Plastoid formulation containing 1% ST provided nasal insulin bioavailability of 5.9% with a total glucose reduction of 17.03% and a maximal glucose decrease of 26.56%. Insulin spray formulations containing 1% ST alone and 1% AG alone resulted in bioavailabilities of 7.25 and 3.57%, respectively. These same sprays provided total glucose reductions of 25.08 and 16.97% with maximal glucose decreases of 44.56 and 19.81%, respectively. The presence of benzalkonium chloride and 2-phenylethanol as preservatives in the MCC suspension resulted in higher insulin absorption than the same formulation without preservatives (6.31% vs 1.96%).

Keywords: Insulin, human; Nasal administration; Spray formulation; Bioadhesive polymer; Microcrystalline cellulose; Plastoid; Ammonium glycyrrhizinate; Glcyrrhetinic acid; Sodium taurocholate

1. Introduction

The nasal administration of peptide formulations has received much attention in the recent past with the advent of new developments in the biotechnology industry (Lee and Longenecker, 1988; Chien et al., 1989; Pontiroli et al., 1989; Harris, 1993; Zia et al., 1993). The nasal route is the preferred route of administration because of the rapid absorption of drug molecules across the

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nasal membrane with less enzymatic degradation and relative ease of administration. At present, most of the peptides and proteins are administered by the parenteral route because of the low bioavailabilities observed following nasal administration. However, the considerable potency and large therapeutic ratios of peptide drugs prompted researchers to find viable alternative routes, or means of delivery. Hence, there are many ongoing investigations aimed at improving the nasal bioavailability of peptide and protein formulations.

The limitations of nasal delivery include the following: local tissue irritation; rapid removal of the therapeutic agent from the site of deposition due to mucociliary clearance; low permeability of the nasal membrane to large molecules; degradation of macromolecules due to the presence of proteolytic enzymes in the nasal cavity; and pathological conditions such as cold or allergies which may significantly alter nasal bioavailability of drugs. It has been reported that by incorporating bioadhesive polymers into a formulation one can increase the residence time of the dosage form in the nasal cavity thereby improving drug absorption (Harris and Robinson, 1990; Leung and Robinson, 1990). To overcome the low permeability of the nasal membrane for large molecules, many permeation enhancers have been investigated (De Ponti and Lardini, 1991; Lee, 1990; Lee et al., 1991; Muranashi, 1991). However, many of these enhancers may alter nasal membrane integrity irreversibly, thereby jeopardizing the patient's health (Schipper et al., 1992; Merkus et al., 1993). Hence, there is a constant search to find a nontoxic enhancer that can promote the nasal absorption of peptides and proteins without compromising the safety of the nasal membrane. Another alternative is the use of a combination of bioadhesive polymers and low concentrations of permeation enhancers to improve the nasal bioavailability of peptides and proteins.

The selection of a proper animal model, suitable dosage form, delivery device and mode of application are critical factors that should be evaluated in optimizing the nasal absorption of drugs. Spray formulations are the preferred delivery systems for nasal delivery and have been successfully used to administer numerous therapeutic agents such as propranolol, progesterone, vasopressin, etc. (Chien et al., 1989). Spray solutions administered, using spray pumps or pressurized metered-dose inhalers, deposit mainly in the anterior portion of the nasal cavity (Aoki and Crawley, 1976; Bond et al., 1984; Hardy et al., 1985; Newman et al., 1987). Since this region is largely nonciliated, clearance is relatively slow when compared to the formulations that are deposited in the ciliated region.

It was reported earlier that by increasing the viscosity of nasal formulation, one can significantly increase the residence time of drug at the site of deposition (Morimoto et al., 1985, 1991; Pennington et al., 1988; Harris et al., 1988a,b, 1989; Lin et al., 1993). Highly viscous bioadhesive formulations having suitable requirements can be formulated but such systems would not be easily administered to the nasal cavity with the existing drug delivery devices.

This project involves evaluation of spray formulations containing two bioadhesive polymer solutions (MCC or Plastoid L50) with or without permeation enhancers (ST, AG, GA) to promote the nasal delivery of insulin in diabetic rabbits. The objectives of this project were: (1) to evaluate various bioadhesive spray formulations of human insulin in diabetic rabbits; (2) to determine the effect of polymer solutions and penetration enhancers on nasal absorption; (3) to compare the permeating capability of three enhancer systems; (4) to elucidate the effect of preservatives on the nasal absorption of insulin; and (5) to compare the pharmacokinetic and pharmacodynamic parameters of various spray formulations following nasal administration in diabetic rabbits with those obtained after i.v. injection of insulin.

2. Materials and methods

2.1. Materials

Human insulin powder (26.8 IU/mg) was obtained as a gift from Novo Nordisk (Denmark). Sodium taurocholate and alloxan were purchased

from Sigma Chemical Co. (St. Louis, MO). Microcrystalline cellulose was kindly provided by FMC Corp. (Philadelphia, PA). Plastoid L50 was donated by Rohm Pharma GmbH (Germany). Ammonium glycyrrhizinate and glycyrrhetinic acid were obtained from Aldrich Co. (Milwaukee, WI). Pentobarbitol (Abbot, Chicago, IL) was used as anesthetic. Materials utilized for preparing buffers (sodium phosphate monobasic and dibasic, sodium chloride, potassium hydroxide) were purchase from Fisher Scientific (Fair Lawn, NJ). All chemicals used were of analytical grade.

Chemstrip bG test strips were purchased from Boehringer Mannheim Diagnostics (Indianapolis, IN). The RIA insulin kits were purchased from ICN Biomedicals Inc. (Costa Mesa, CA). Nasal spray pumps were generously supplied by Pfieffer (Princeton, NJ).

2.2. Animals

New Zealand White male rabbits weighing 2.5 kg were purchased from Charles River Labs (Amherst, MA). The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee, University of Rhode Island, Kingston, RI.

2.3. Methods

2.3.1. Experimental set-up

New Zealand White male rabbits were made diabetic prior to the study by intravenous injection of 80 mg/kg alloxan in isotonic saline solution. Rabbits were considered to be diabetic when the basal glucose levels were > 350 mg/dl. Rabbits were fasted for 16 h prior to the experiment with free access to water. Rabbits were anesthetized with an i.v. dose of 30 mg/kg sodium pentobarbitol. An additional dose of 6 mg/kg sodium pentobarbitol was given after 40 min of initial dose to prolong the anesthesia. A catheter was placed in the rabbit ear artery and 0.5–0.8 ml blood samples were collected at −15, −10, 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240 and 300 min after administration of the formulations. The glucose levels were immediately determined. Serum samples were obtained by centrifuging the blood samples at 3000 rpm for 10 min and were stored at −60° C until assayed. Control experiments were conducted without the insulin formulations.

2.3.2. Study design

Insulin formulations were administered to the rabbits as per the randomized cross-over design. A wash out period of at least 1 week was given between the treatments.

2.3.3. Formulation preparation

Two bioadhesive polymer formulations were prepared as base solutions for nasal administration to evaluate the efficacy of insulin formulations by increasing the residence time of the dosage form. A 1.5% w/v microcrystalline cellulose (MCC) aqueous suspension and a 70% w/w Plastoid L50 aqueous solution were prepared and the pH was adjusted to 6.5 with 0.1 M potassium hydroxide solution. The reason for choosing the polymers at the above-mentioned concentrations is that they are sprayable with a spray pump with consistent reproducibility.

Sodium taurocholate (ST), ammonium glycyrrhizinate (AG), and glycyrrhetinic acid (GA) were used at a 1% w/v level as penetration enhancers. Sodium taurocholate is a bile salt and has been reported to be less toxic to biological membranes as compared with other enhancers such as POE, deoxycholate, etc. (Birket and Silen, 1974; Lee et al., 1991) and hence was chosen as a permeation promoter to improve nasal delivery. Ammonium glycyrrhizinate, and glycyrrhetinic acid were chosen as promoters of insulin because they have been proven to be least harmful to the nasal membrane at the chosen concentration (Reardon et al., 1993b). Benzalkonium chloride and 2-phenylethanol at 0.02% w/v concentration were utilized as preservatives in the nasal formulation.

Insulin formulations were prepared by dispersing the required amounts of insulin (5 U/kg) in polymer solutions alone or with combination of permeation enhancers or preservatives. For intravenous administration, human insulin was dissolved in isotonic phosphate buffer (pH 7.4) and administered through the ear vein at a dose of 0.4 U/kg to the diabetic rabbits.

2.3.4. Administration of nasal formulations to the rabbits

The site of drug deposition in the nose is dependent on the dosage form and dose volume. Since sprays provide reproducible and precise doses of drug, it was decided that a spray pump would be utilized to deliver the required amounts of the insulin dosage form during this investigation (Hughes et al., 1993; Newman et al., 1994).

The volume that can be administered to the nasal cavity is limited. From the literature (Harris et al., 1988a) it was reported that the bioavailability of intranasal desmopressin from 2×50 μl dose was 20% which represented a 2-fold increase over the 11% found with 1×50 μl spray or 9% realized from the 1×100 μl dose. This finding suggests that an optimal dosage may be obtained by spraying the dose twice, once into each nostril. Hence, in this study, it was decided that spray formulation would be delivered at 50 μl to each nostril for a total volume of 100 μl per rabbit.

A nasal spray pump with an ability to deliver 50 μl was characterized prior to the experimentation for reproducible dosing (Dondeti, 1994a). A total volume of 100 μl of freshly prepared insulin formulations were sprayed into both nostrils (50 μl each) of the rabbit.

2.3.5. Analytical methods

2.3.5.1. Blood glucose levels. Blood glucose was measured with Chemstrip bG test strips and an Accu-Chek II blood glucose monitor immediately after sampling. In this method, the glucose concentration in whole blood is determined as a function of the color intensity produced by the glucose oxidase/peroxidase reaction occurring on the Chemstrip bG test strips. The assay was validated as described earlier (Dondeti, 1994a). The assay range was 20–500 mg/dl.

2.3.5.2. Serum insulin levels. Radioimmunoassay kits (ICN Biomedicals) were used to determine serum insulin concentrations in the diabetic rabbits. The assay was validated as described earlier (Dondeti, 1994a). The assay range was 2–310 μU/ml.

2.3.6. Data analysis

Insulin activity was determined as a function of glucose reduction as well as the increase in serum insulin levels over a period of 5 h after administration of insulin formulations. Blood glucose levels were calculated as a percentage of the blood glucose concentrations measured prior to the administration of insulin formulations. The mean differences in serum insulin levels and glucose levels for rabbits receiving the various formulations and those of controls were used to determine the pharmacokinetic and pharmacodynamic parameters. The area under the serum insulin vs time curve (AUC) and the area under the change in blood glucose vs time curve (AUC) was calculated using the trapezoidal rule.

The nasal bioavailability of insulin was calculated relative to the increase in serum insulin levels over a period of 5 h after injecting insulin intravenously. C_{max} values are the peak serum insulin concentrations observed at time T_{max} after administering insulin:

$$\%\ \text{absolute bioavailability} = \frac{\text{AUC}_{in} - \text{AUC}_{control}}{\text{AUC}_{iv} - \text{AUC}_{control}} \times \frac{\text{dose}_{iv}}{\text{dose}_{in}} \times 100$$

The hypoglycemic effect was measured in terms of maximal decrease and total glucose reduction. The maximal decrease was the highest percentage of reduction in glucose levels as compared to the basal values. The time at which this reduction took place was T_{max}. The total glucose reduction ($D\%$) was calculated as stated by Hirai et al. (1981b):

$$D\% = \frac{\text{AUC}_{control} - \text{AUC}_{in}}{\text{AUC}_{control}} \times 100$$

where AUC_{in} and $\text{AUC}_{control}$ denote the areas under the curve for nasal formulations and controls, respectively.

Statistical analysis was performed using a one-way ANOVA with the aid of JMPIN [1] and StatView® software packages. The formulation effects were compared with the controls using

Dunnett's test. Multiple comparisons between the treatment effects were determined using Fisher least significant difference test (LSD). Differences between the treatments were assumed to be significant for values of $p < 0.05$.

3. Results and discussion

The diabetic rabbit was chosen as an animal model as it offers certain advantages such as ease of handling, low cost of maintaining and comparable nasal cavity size with that of humans.

For control experiments without insulin formulations, serum insulin levels were observed in the range of 6–20 μU/ml for diabetic rabbits. The basal glucose levels varied between 350 and 400 mg/dl and remained relatively constant over a period of 5–6 h.

The results of evaluation of various spray formulations for nasal delivery are presented in Table 1 and 2. Intravenous administration of human insulin at 0.4 U/kg resulted in immediate increase in serum insulin levels compared to the control experiments. Peak serum insulin levels of 345.26 ± 26.53 μU/ml were observed at the first sampling time interval (10 min) after the injection. The maximal decrease in blood glucose levels was $58.29 \pm 5.01\%$ from the basal values and the total glucose reduction was 40.19% as compared to the controls.

Drug absorption from the nasal cavity would seem to be dependent upon the time available for drug to be absorbed across the nasal mucosa. While it is generally accepted that residence time can be extended by increasing the viscosity of a formulation, whether this will result in improved absorption of drugs or not remains unclear. In this investigation all the insulin spray formulations were characterized for viscosity and globule size distribution and the same formulations were evaluated in diabetic rabbits.

3.1. Microcrystalline cellulose suspensions

From formulation characterization studies (Dondeti, 1994a), it was found that the viscosity of the MCC formulation was increased significantly when compared to isotonic phosphate buffer (8 to 218 cp). Also, the MCC suspension displayed a non-Newtonian behavior as compared to the Newtonian behavior shown by the phosphate buffer. When characterized using an image analyzer, the 1.5% w/w MCC suspension produced sprays with 80% of globules ranging from 156.87 to 423.14 μm and a mean globule size of 266.63 μm as compared to the smaller globules obtained with phosphate buffer (mean diameter

Table 1
Pharmacokinetic parameters after nasal administration of insulin spray formulations in diabetic rabbits

Formulation	n	$T_{max} \pm$ S.E. (min)	$C_{max} \pm$ S.E. (μU/ml)	Insulin AUC $\pm$ S.E. (min μU ml^{-1})	% bioavailability [a]
Control	5	50 ± 6.32	18.06 ± 3.5	2929.8 ± 406.4	–
0.4 U/kg i.v. injection	5	10 ± 0	345.26 ± 26.53	21174.4 ± 3429.2 [b]	100
HI + MCC suspension	5	22 ± 3.74	61.2 ± 6.81	7402.6 ± 585.7	1.96
HI + MCC suspension + 1% ST	5	16 ± 2.45	317.67 ± 14.74	21985.5 ± 3341.1 [b]	8.36
HI + MCC suspension + 1% AG	5	12 ± 2	254.63 ± 18.42	20790.2 ± 2329.8 [b]	7.83
HI + MCC suspension + 1% GA	5	98 ± 35.4	62.6 ± 14.95	7836.7 ± 2083.6	2.15
HI + Plastoid L50	5	20 ± 0	125.41 ± 48.32	8068.5 ± 1556.9	2.25
HI + Plastoid L50 + ST	4	52.5 ± 14.36	192.85 ± 36.23	16384.1 ± 3666.8 [b]	5.90
HI + 1% ST	5	10 ± 0	309.76 ± 33.4	19453.4 ± 2063 [b]	7.25
HI + 1% AG	5	10 ± 0	257 ± 50.62	11067.1 ± 873.8	3.57
HI + MCC suspension + preservative	4	85 ± 34.28	151.7 ± 40.32	17310.4 ± 4790.8 [b]	6.31

[a] $\%\,\text{bioavailability} = \dfrac{AUC_{in} - AUC_{control}}{AUC_{iv} - AUC_{control}} \times \dfrac{dose_{iv}}{dose_{in}} \times 100.$

[b] Significantly different from controls at 95% confidence level using Dunnett's test.

52.77 μm and 80% of globules were in the range of 29.81–82.09 μm).

When administered to diabetic rabbits at a dose of 5 U/kg, the 1.5% w/v MCC suspension alone resulted in 1.96% of absolute bioavailability (Table 1). The peak serum insulin levels (C_{max}) of 61.2 ± 6.81 μU/ml were seen after 22 ± 3.74 min (T_{max}) following nasal administration (Fig. 1). The same formulation resulted in 19.52% of total blood glucose reduction with a maximal decrease of 30.39 ± 5.61% at 185 ± 27.29 min after administration of the spray (Fig. 2 and Table 2). Insulin was absorbed in negligible amounts when administered alone. However, the MCC spray resulted in absorption of significant amounts of insulin characterized by increases in serum insulin levels and reduction in glucose levels as compared to the controls.

Mucociliary clearance acts as major defense mechanism to protect the nasal cavity against external insults by quickly removing the material that deposits on cilia (Geurkink, 1983). It also acts as major deterrent to the nasal absorption of drugs that are large in molecular size and have low bioavailability such as peptide drugs. In previous investigations, it was hypothesized that by increasing the residence time of a formulation, one may improve the nasal absorption of drugs

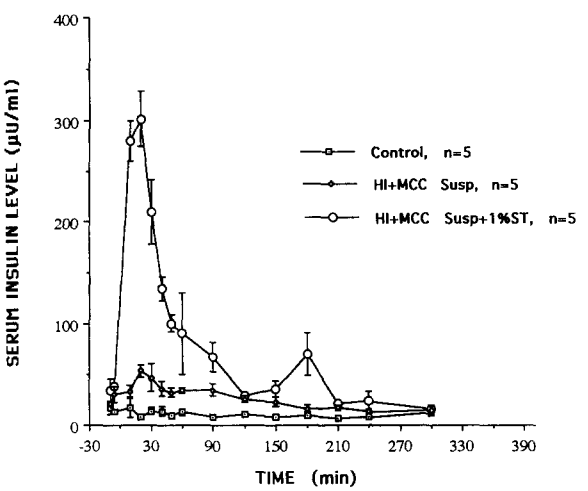

Fig. 1. Serum insulin levels after nasal administration of 5 U/kg insulin spray formulations containing microcrystalline cellulose and sodium taurocholate in diabetic rabbits.

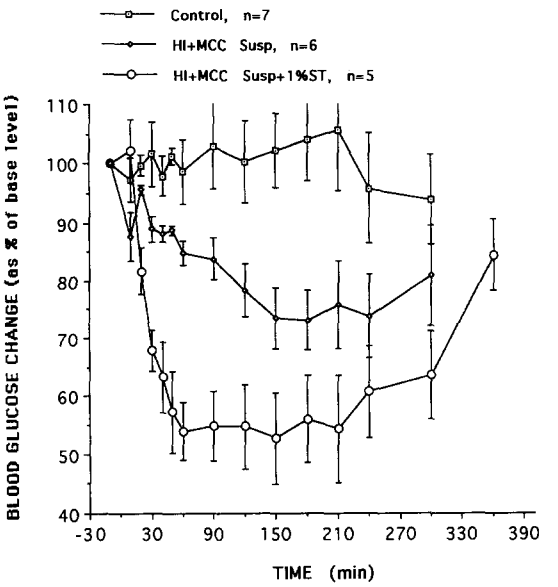

Fig. 2. Hypoglycemic effect after nasal administration of 5 U/kg insulin spray formulations containing microcrystalline cellulose and sodium taurocholate in diabetic rabbits.

(Nagai et al., 1984; Nagai, 1986; Harris and Robinson, 1990; Leung and Robinson, 1990; Dondeti et al., 1994b). Residence time can be increased by manipulating the formulation variables such as incorporating a bioadhesive polymer in the formulation and/or choosing an appropriate delivery device to administer the dosage form to the nasal cavity.

It was reported earlier that the viscosity of a formulation affects the globule size and thus deposition pattern in the nasal cavity (Morimoto et al., 1985, 1991; Harris et al., 1988b, 1989; Pennington et al., 1988; Lin et al., 1993). Increased viscosity of the MCC formulation resulted in a spray that produced large globules (53.96 μm vs 266.63 μm). The large globules tend to give a more localized deposition in the anterior portion of the vestibule than the smaller globules that deposit in the posterior portion of the nose. This results in the lowering of clearance of the dosage form from the site of deposition. Highly viscous preparations have been found to increase the residence time of the drug in the nasal cavity, thereby improving the absorption across the nasal membrane. Pennington and co-workers (1988)

Table 2
Pharmacodynamic parameters after nasal administration of insulin spray formulations in diabetic rabbits

Formulation	n	T_{max} ± S.E. (min)	Maximal decrease ± S.E. (%)	Glucose AUC ± S.E. (min %)	Total glucose reduction (D%) [a]
Control	7	108.6 ± 41.5	11.82 ± 0.95	29753.7 ± 1484.9	–
0.4 U/kg i.v. injection	5	82 ± 20.35	58.29 ± 5.01	17795.3 ± 1868.1 [b]	40.19
HI + MCC suspension	6	185 ± 27.29	30.39 ± 5.61	23945.2 ± 1302.7 [b]	19.52
HI + MCC suspension + 1% ST	5	124 ± 27.13	58.37 ± 4.8	18115.5 ± 1610.3 [b]	39.12
HI + MCC suspension + 1% AG	5	126 ± 6	21.8 ± 2.29	25004.9 ± 1641.6	15.96
HI + MCC suspension + 1% GA	5	150 ± 41.35	18.61 ± 3.19	26969.3 ± 671.9	9.36
HI + Plastoid L50	5	70 ± 10	17.01 ± 6.5	27546.5 ± 1676.9	7.42
HI + Plastoid L50 + 1% ST	4	115 ± 37.53	26.56 ± 1.54	24685.8 ± 2078.6	17.03
HI + 1% ST	9	88 ± 19.85	44.56 ± 6.78	22292.3 ± 1185.6 [b]	25.08
HI + 1% AG	5	146 ± 27.13	19.81 ± 2.65	26363.7 ± 611.6	11.39
HI + MCC suspension + preservative	4	152.5 ± 45.35	32.84 ± 8.52	24818 ± 1616.5	16.59

[a] $D\% = \frac{AUC_{control} - AUC_{in}}{AUC_{control}} \times 100.$

[b] Significantly different from controls at 95% confidence level using Dunnett's test.

compared the clearance of hydroxypropylmethylcellulose solutions (0.6, 0.9 and 1.25% w/v HPMC) having kinematic viscosities of 36, 120 and 430 $mm^2\ s^{-1}$. A gamma-scintigraphic study showed that the areas of deposition were similar for all the solutions. However, the clearance rates decreased with increasing solution viscosity, with retention half-times of 1.0, 1.7 and 2.2 h, respectively.

From the data obtained in this study, it was evident that the presence of bioadhesive polymers alone resulted in improved absorption, though small but significant. Thus, the presence of bioadhesive polymers alone may not provide sufficient absorption of large peptide drugs for therapeutic purposes and hence addition of some kind of penetration enhancer is a necessity. The combination of bioadhesive polymer and penetration enhancer would seem to be advantageous, since by using bioadhesive polymers or viscosity builders, the concentration of penetration enhancer required might be reduced and thus the toxicological concerns associated with compounds may be overcome. Three permeation enhancers, sodium taurocholate (ST), ammonium glycyrrhizinate (AG) and glycyrrhetinic acid (GA), were evaluated in combination with MCC suspension

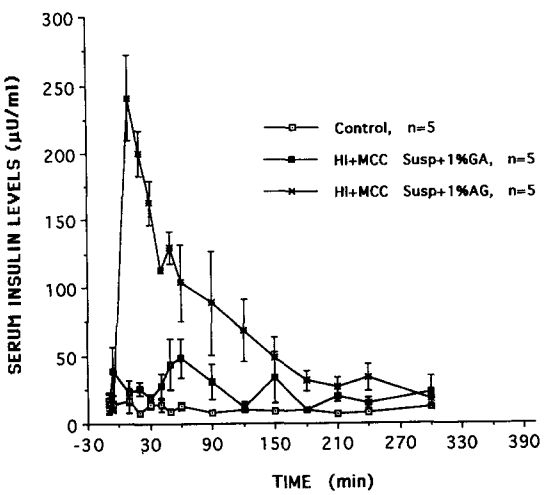

Fig. 3. Serum insulin levels after nasal administration of 5 U/kg insulin spray formulations containing microcrystalline cellulose and ammonium glycyrrhizinate or glycyrrhetinic acid in diabetic rabbits.

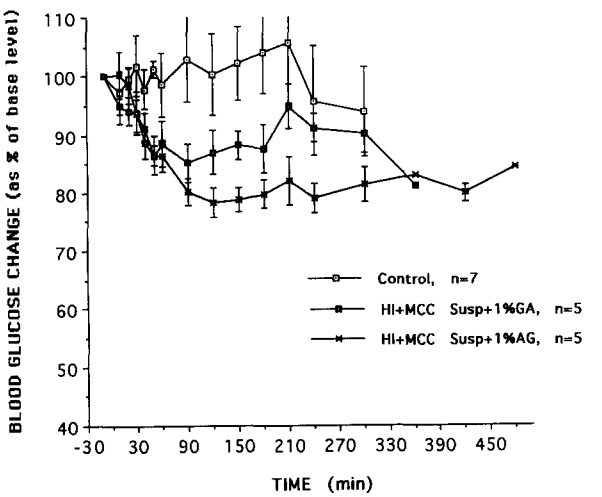

Fig. 4. Hypoglycemic effect after nasal administration of 5 U/kg insulin spray formulations containing microcrystalline cellulose, ammonium glycyrrhizinate or glycyrrhetinic acid in diabetic rabbits.

for their ability to promote the nasal absorption of insulin.

Addition of sodium taurocholate at a 1% w/v level reduced the viscosity of the MCC formulation and also generated a spray with reduced mean globule size (266.63–258.75 μm). This is probably due to the reduced surface activity provided by the surfactant, sodium taurocholate, resulting in smaller droplets with narrower ranges. When administered nasally at a dose of 5 U/kg, the same formulation provided a bioavailability of 8.36% with peak insulin levels of 317.67 ± 14.74 μU/ml observed at 16 ± 2.45 min (Fig. 1 and Table 1). The hypoglycemic effect was characterized by a total glucose reduction of 39.12% and a maximal decrease of 58.37 ± 4.8% observed after 124 ± 27.13 min following nasal dosing (Fig. 2 and Table 2). The reason for improved absorption of insulin may be attributed to the overall permeation effect of sodium taurocholate which surpasses the effect due to viscosity and/or reduction of globule size.

Addition of 1% w/v ammonium glycyrrhizinate (AG) to the MCC solution resulted in an increase in the formulation viscosity from 218 to 734 cp providing an increased mean globule size of 467.79 μm with 80% of the globules in the

range of 157.8–901 μm. These results are consistent with the findings of Harris and co-workers (1988b) that involved nasal administration of 100 μl of solution containing 0, 0.25 and 0.5% w/w methylcellulose using a spray pump. The mean particle size delivered by the spray was measured and found to be 51 μm for 0% w/w solution, 81 μm for 0.25% w/w solution and 200 μm for 0.5% w/w solution. The MCC suspension containing insulin (5 U/kg) and AG (1% w/w) sprayed into the nasal cavities of diabetic rabbits provided a bioavailability of 7.83% (Table 1). As Fig. 3 indicates, the mean peak serum insulin levels of 254.63 ± 18.42 μU/ml were observed at 12 ± 2 min following nasal administration. The same formulation resulted in a total glucose reduction of 15.96% with a maximal glucose decrease of 21.8 ± 2.29% after 126 ± 6 min (Fig. 4 and Table 2). The improved absorption of insulin as compared to MCC alone (7.83% vs 1.96%) can be attributed to the enhancing ability of AG and to the viscosity effect of MCC-AG. It was reported earlier that AG has membrane permeation capability due to its structural similarities to triterpenes (Mishima et al., 1989). Also, the presence of AG significantly increased the overall viscosity of the formulation and thus produced globules of larger size. When this suspension was sprayed into the nasal cavity, the larger globules probably provided more localized distribution in the anterior portion resulting in reduced mucociliary clearance and thereby improving the residence time of the dosage form.

The combination of MCC suspension and 1% w/w GA resulted in an increase of viscosity of the formulation from 218 to 258 cp and produced a spray with a mean globule size of 276.58 μm and 80% of globules were in the range of 88.1–515 μm. When administered to the diabetic rabbits, the spray produced a bioavailability of 2.15% (Table 1). The serum insulin peaks (62.6 ± 14.95 μU/ml) were seen at 98 ± 35.4 min after nasal dosing (Fig. 3). A total glucose reduction of 9.36% with a maximal decrease of 18.61 ± 3.19% of basal glucose levels was witnessed after 150 ± 41.35 min following nasal administration (Fig. 4 and Table 2). AG and GA have similar structures to triterpenes and show surfactant action similar to that of bile salts (Mishima et al., 1989). However, the degree of enhancement of insulin absorption across the nasal membrane varied significantly for these promoters. From the data it was evident that greater absorption of insulin was promoted by inclusion of AG as compared to GA. The reasons for the high degree of enhancement by AG may be due to its ability to increase the viscosity of formulation significantly and its ability to dissolve quickly in the aqueous solution as compared to the sparingly soluble GA.

Thus, the presence of ST, AG, GA in MCC suspension altered the globule size distributions for sprays and as expected improved the nasal delivery of insulin. The addition of ST slightly reduced the viscosity and resulted in a finer spray thereby providing better distribution of insulin in the nasal cavity. In contrast, the presence of glycyrrhetinic acid derivatives significantly increased the viscosity of the formulation and probably increased the residence time of insulin in nasal cavity. The mechanisms by which these agents increase insulin absorption will be discussed in the following sections.

3.2. Plastoid L50 formulations

The rheograms for the 70% w/w Plastoid L50 display non-Newtonian behavior. When sprayed with a nasal spray pump, globules with a mean diameter of 636.66 μm with 80% of globules in the range of 102.5–1364.2 μm were generated. When the same formulation was administered to diabetic rabbits at a dose of 5 U/kg, it provided 2.25% of the absolute bioavailability (Table 1). As Fig. 5 indicates, peak insulin levels of 125.41 ± 48.82 μU/ml were observed 20 min after nasal administration. The total blood glucose reduction was 7.42% with a maximal decrease of 17.01 ± 6.5% at 70 ± 10 min after administration (Fig. 6 and Table 2). As compared to the MCC suspension, the Plastoid L50 solution slightly enhanced insulin absorption. The increase in insulin absorption following Plastoid L50 formulation may be attributed to the larger globules generated by the spray which would slow down the clearance of insulin from the site of deposition thereby providing more time for insulin to be absorbed.

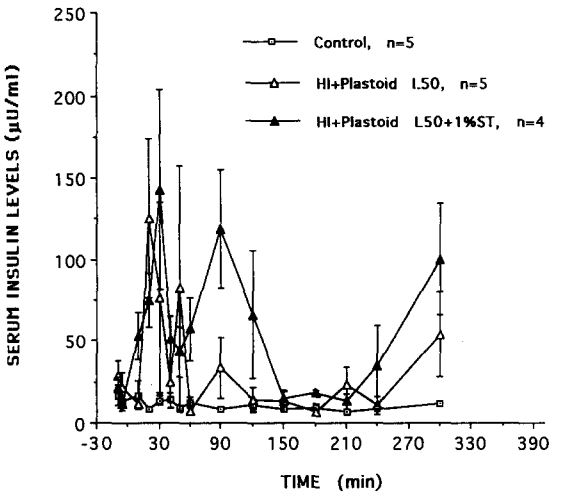

Fig. 5. Serum insulin levels after nasal administration of 5 U/kg insulin spray formulations containing Plastoid L50 and/or sodium taurocholate in diabetic rabbits.

When sodium taurocholate was added to the 70% w/w Plastoid L50 solution at 1% w/v level, the viscosity of the formulation was lowered from 302 to 248 cp. It also reduced the mean globule size of the spray to 584.09 μm with 80% of globules falling in the range of 82.6–1357.5 μm. This same formulation when administered to diabetic rabbits at a dose of 5 U/kg resulted in improved nasal absorption of insulin when compared to the Plastoid formulation alone (5.9 vs 2.25% bioavailability). The peak serum insulin of 192.85 $\pm$ 36.23 μU/ml was noticed at 52.5 $\pm$ 14.36 min after administration (Fig. 5 and Table 1). The total blood glucose reduction was 17.03% when compared to the controls. A maximal decrease of 26.56 $\pm$ 1.54% was observed at 115 $\pm$ 37.53 min after administration (Fig. 6 and Table 2). As expected, the presence of ST significantly improved insulin bioavailability. However, when compared to the MCC-ST formulation, the Plastoid-ST resulted in lower nasal bioavailability (8.36 vs 5.9%). The reason for this may be due to the highly viscous nature of the Plastoid which may retard release of insulin from the globules. Cellulose derivatives are known to reduce the aggregation of protein molecules in solutions (Pearlman and Bewley, 1993). Whether the Plastoid will have a similar or adverse effect on the aggregation of insulin is not known at this time.

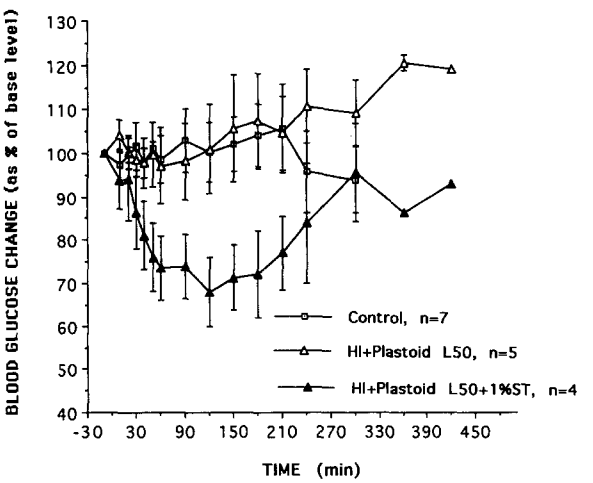

Fig. 6. Hypoglycemic effect after nasal administration of 5 U/kg insulin spray formulations containing Plastoid 50 and/or sodium taurocholate in diabetic rabbits.

3.3. Permeation enhancers alone

Spray solutions having phosphate buffer as base were found to display Newtonian flow behavior. When sprayed from a nasal spray pump, the smallest globules generated with a mean diameter of 52.77 μm and 80% globules were in the range of 29.81–82.09 μm. When 1% sodium taurocholate was added to the buffer, the mean globule diameter was found to be essentially the same (53.65 μm) with 80% of globules varying from 34.62 to 79.83 μm. This insulin formulation, when evaluated in vivo, resulted in 7.25% absolute bioavailability with peak insulin levels of 309.76 $\pm$ 33.4 μU/ml at 10 min following nasal administration (Fig. 7 and Table 1). The same formulation resulted in a corresponding total glucose reduction of 25.08% with a maximal decrease of 44.56 $\pm$ 6.78% at 88 $\pm$ 19.85 min after nasal dosing (Fig. 8 and Table 2).

The exact mechanism of action of sodium taurocholate is not known. There are many mechanisms hypothesized by various investigators (Gibaldi, 1970; Gibaldi and Feldman, 1970; Martin et al., 1978; Hirai et al., 1981a,b; Martin and Marriott, 1981; Pontiroli et al., 1982, 1987; Moses

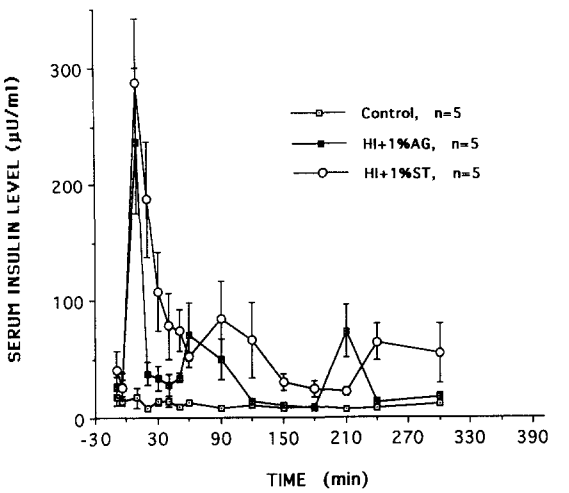

Fig. 7. Serum insulin levels after nasal administration of 5 U/kg insulin spray formulations containing sodium taurocholate or ammonium glycyrrhizinate in diabetic rabbits.

et al., 1983; Gordon et al., 1985; Duchateau et al., 1986; Aungst et al., 1987; Verhoef et al., 1989; Hermens et al., 1990; Lee, 1990; Pontiroli, 1990; Tengamnuay and Mitra, 1990; De Ponti and Lardini, 1991; Lee et al., 1991; Maitani et al., 1991; Muranashi, 1991, Zhou and Po, 1991). It is postulated that bile salts increase absorption of insulin

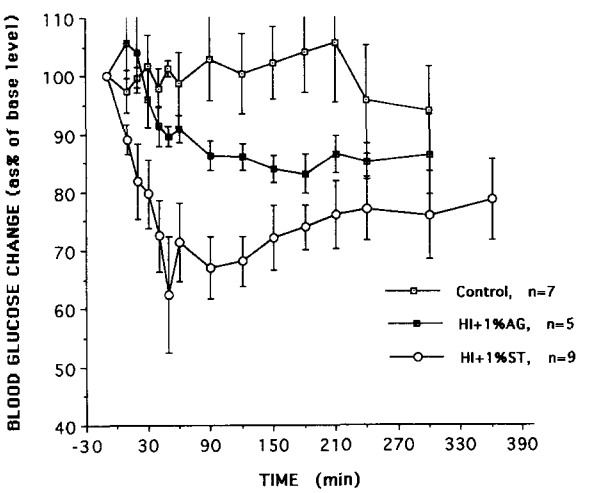

Fig. 8. Hypoglycemic effect after nasal administration of 5 U/kg insulin spray formulations containing sodium taurocholate or ammonium glycyrrhizinate in diabetic rabbits.

by producing high juxtamembrane concentrations of insulin monomers via solubilization in mixed bile salt micelles. These salts form reversed micelles within nasal membrane through which insulin monomers can diffuse from extracellular space. They may also increase the membrane permeability by loosening the intercellular junctions and/or by increasing the number of pores in the cell membrane. Bile salts also seem to reduce the viscosity of mucus layer thereby facilitating peptide diffusion across the membrane. It was reported earlier that bile salts act as stabilizing agents by inhibiting proteolytic enzymes that cause degradation of insulin in the nasal cavity.

Addition of ammonium glycyrrhizinate (1% w/v) to the buffer produced a spray with a mean globule size of 152.43 μm and 80% of globules in the range of 81.5–286.78 μm. The increases in the particle size and size range might be due to the increase in viscosity of the formulation. The same formulation when administered to diabetic rabbits provided a nasal bioavailability of 3.57 and mean peak serum insulin levels of 257 ± 50.62 μU/ml at 10 min from the time of administration of formulation. A corresponding total glucose reduction of 11.39% was observed with a maximal decrease of 19.81 ± 2.65% at 146 ± 27.13 min after nasal administration.

Glycyrrhetinic acid derivatives including ammonium glycyrrhizinate have some properties similar to those of bile salts or saponins (Mishima et al., 1989; Reardon et al., 1993a,b). These compounds enhance nasal transport in a concentration-dependent manner via alteration of tight junctional integrity. From the data available to date, it appears that this change in junctional permeability is limited by size. These agents have been shown to be not as effective as bile salts which was also confirmed in this study. However, for the same reason they are not as destructive to the nasal mucosa as bile salts or other enhancers. It was reported that even at a concentration of 2%, ammonium glycyrrhizinate did not alter the nasal morphology and function and did not produce any irreversible toxic effects (Reardon et al., 1993b). Hence, this enhancer in combination with bioadhesive polymer seems to have a good potential as a nasal delivery system for insulin.

3.4. Effect of preservatives on nasal absorption of insulin

Most nasal drops/sprays are dispensed as multi-dose preparations requiring a preservative to prevent the growth of microorganisms. However, it has been reported in many investigations that almost all of the commonly used preservatives significantly alter ciliary beat frequency (Batts et al., 1989). This effect seem to reduce mucociliary clearance and since the formulations stay in the nasal cavity much longer than usual, improved absorption may be expected.

In this study, the effect of two preservatives, 2-phenylethanol and benzalkonium chloride (0.02% w/v), on the nasal absorption of insulin was investigated.

From in vitro data (Dondeti, 1994a), it was evident that the preservatives slightly reduced the viscosity of the MCC formulation from 218 to 202 cp. Their presence also reduced the globule size of the spray. The measured mean for globules produced by MCC formulation with preservatives was 192.5 μm with 80% of globules varying from 79.76 to 322.52 μm. The decrease in the viscosity and globule size as compared to that provided by the base formulation was would seem to be due to surfactant nature of the preservatives.

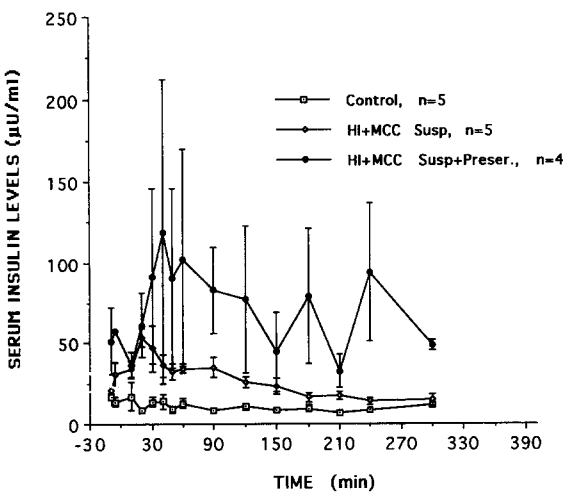

Fig. 9. Serum insulin levels after nasal administration of 5 U/kg insulin spray formulations containing microcrystalline cellulose and/or benzalkonium chloride and 2-phenylethanol.

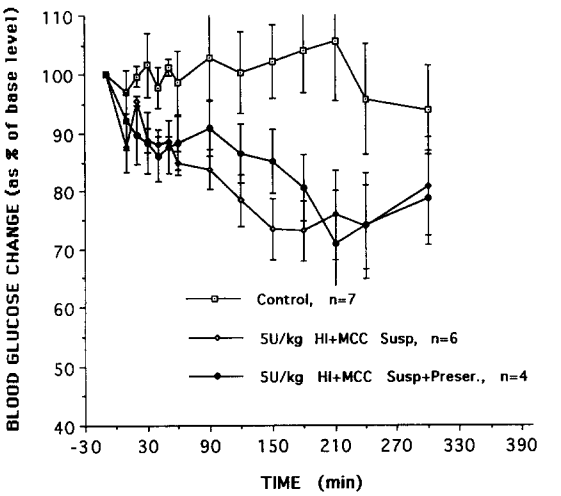

Fig. 10. Hypoglycemic effect after nasal administration of 5 U/kg insulin spray formulations containing microcrystalline cellulose and/or benzalkonium chloride and 2-phenylethanol.

As Fig. 9 indicates, the MCC spray formulation containing the preservatives provided a higher absorption of insulin (6.31%) when compared to the same formulation without the preservatives (1.96%). For the nasal spray containing preservatives, the peak serum insulin levels (151.7 ± 40.32 μU/ml) were noted at 85 ± 34.28 min after administration. A total glucose reduction of 16.59% was observed with a maximal decrease of 32.84 ± 8.52% at 152.5 ± 45.35 min (Fig. 10 and Table 2). The greater absorption of insulin seen with these preservatives may be due to their surfactant nature as well as their ciliotoxicity which allows insulin longer residence in the nasal cavity due to reduced mucociliary clearance. Interestingly, the presence of preservatives resulted in more variable insulin data as compared to the other formulations.

Based on the data obtained here, it is evident that there is no exact correlation between total glucose reduction and percent bioavailability. Interpretation of the hypoglycemic effect is complicated by the counter regulatory response to a falling glucose concentration, involving the secretion of glucagon, cortisol, growth hormone and catecholamines (Brogden and Heel, 1987). Since

this response varies significantly among individual subjects according to the rate of reduction of glucose levels, only an imprecise indication of relative potency of insulin can be obtained. While glucose reduction can be used as a rapid way to determine whether or not a particular formulation is effective, a final decision on the comparative absorption of the different has to be made based on percent bioavailability.

A successful formulation must also overcome toxicity concerns. Based on these considerations, combination formulations with bioadhesive polymers seem to be potentially less toxic as compared to single penetration enhancers such as surfactants, bile salts, fusidic acid derivatives etc. (Dondeti et al., 1994b). Among all the formulations evaluated, the combination of MCC and AG seem to provide improved nasal bioavailability. These formulations would seem to be preferred because of the following factors: (i) less toxic effects at the chosen concentration level as compared to bile salts; (ii) the enhancing effect of AG; and (iii) the increased viscosity effect due to the combination of MCC and AG.

The development of insulin formulation for the nasal cavity can be optimized with a better understanding of the relationship between formulation parameters such as composition and nature of the excipients and their effects on particle/globule size distributions, dosage form design and delivery devices.

4. Conclusions

Different nasal spray formulations of human insulin containing bioadhesive polymers alone, permeation enhancers alone and a combination of bioadhesive polymers and permeation enhancers were evaluated in diabetic rabbits. Insulin absorption was measured in terms of absolute bioavailability and total blood glucose reduction.

A 1.5% w/v MCC suspension alone with a usual dose of 5 U/kg insulin resulted in 1.96% of absolute bioavailability and 19.52% of total blood glucose reduction. The presence of ST, AG and GA in the MCC suspension provided bioavailabilities of 8.36, 7.83 and 2.15%, respectively. The same formulations produced total glucose reductions of 39.12, 15.96 and 9.36%, respectively.

A 70% w/w Plastoid L50 formulation alone with a usual dose of 5 U/kg insulin resulted in 2.25% of absolute bioavailability and 7.42% of total blood glucose reduction. When ST was added at a 1% w/v level to the Plastoid L50 formulation, the nasal bioavailability of insulin was found to increase to 5.9% and the total glucose reduction was 17.03%.

Insulin spray formulations containing 1% ST alone and AG alone provided bioavailabilities of 7.25 and 3.57%, respectively. The same formulations produced hypoglycemic effect in terms of total reduction of 25.08 and 16.97% in glucose levels.

The combination of a bioadhesive polymer and permeation enhancer resulted in greater absorption of insulin in terms of absolute bioavailability and hypoglycemic effect when compared to the polymer solution alone or a spray containing only a permeation enhancer. MCC seems to be more effective when combined with an enhancer as compared to Plastoid L50. The enhancers can be arranged in their permeating capability in increasing order as: ST > AG > GA.

The MCC spray formulation containing 2-phenylethanol and benzalkonium chloride (0.02% w/v) as preservatives provided a much higher bioavailability of insulin (6.31%) when compared to the same formulation without the preservatives (1.96%). The reasons for this may include reduced clearance due to ciliotoxicity, reduced viscosity and also the surface activity of the preservatives.

Thus, the combination of a bioadhesive polymer and permeation enhancer seems to be a viable alternative for these nasal formulations using higher levels of penetration enhancers. Since ammonium glycyrrhizinate is relatively nontoxic when compared to the other enhancers such as bile salts, etc., it is recommended that in future studies, the use of bioadhesive polymers such as cellulose derivatives and ammonium glycyrrhizinate can be of promising potential for developing a nasal spray formulation for peptide drugs. However, the long-term toxic effects of ammo-

nium glycyrrhizinate and bioadhesive polymers on nasal membrane remain to be studied.

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