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Nasal administration of glucagon combined with dimethyl-β-cyclodextrin: comparison of pharmacokinetics and pharmacodynamics of spray and powder formulations

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Abstract

The effect of increasing amounts of dimethyl-β-cyclodextrin (DMβCD) on the nasal absorption and consequent plasma glucose levels of glucagon as spray solution or powder has been examined in rabbits. Nasal spray was prepared by dissolving commercial glucagon in the manufacturer's solvent containing 2 or 5% w/v DMβCD. Powders were obtained by freeze drying of the spray solutions. In general, glucagon was slightly absorbed in the absence of DMβCD. Increasing the enhancer concentration was shown to increase plasma glucagon and glucose levels while decreasing the times for maximum glucagon peaks (t_{max} glucagon) in both spray and powder formulations. Spray solutions produced faster and higher plasma glucagon peaks (C_{max} glucagon) at any enhancer concentration compared to powders, however the $AUC_{0-60\ min}$ glucagon and glucose concentrations were not significantly different. The 5% w/v DMβCD resulted in a more pronounced glucagon absorption with corresponding higher plasma glucose levels. The percentage bioavailability of glucagon from formulations containing 2% w/v enhancer were between 42.78 and 44.61% with corresponding AUC plasma glucose of between 78.89 and 80.47% compared to subcutaneous injection. The values for the 5% w/v DMβCD formulations were about 82% with corresponding AUC plasma glucose between 95 and 97% for spray and powder, respectively. Nasal administration was found to initiate serum glucagon and plasma glucose concentrations earlier than those of subcutaneous injection, but the effect of the latter was more sustained. Spray solution and freeze dried powder were equally effective.

Keywords: Absorption enhancers; Bioavailability; Dimethyl-β-cyclodextrin; Glucagon; Nasal absorption; Pharmacokinetics; Pharmacodynamics

1. Introduction

Severe hypoglycemic attacks are reported to occur in up to 25% of insulin-treated diabetic patients (Goldgewicht et al., 1983). The treatment

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of insulin reactions in diabetic patients can be by orally administered sugar when the patients are conscious or by glucose injection (intravenous) or glucagon intramuscularly or subcutaneously if they are unconscious (Elrick et al., 1958; Hall-Boyer et al., 1984). However, recovery time for oral sugar is about 18 min; intravenous glucose injection requires professional skill, and parenteral administration of glucagon necessitates good training of the relatives and is not always done when the situation arises. Many approaches were initiated in order to simplify glucagon administration and to provide effective glucagon treatment that is less complicated than the parenteral techniques. Nasal administration was reported to be the most desirable alternative route with kinetics resembling those of intravenous administration (Pontiroli et al., 1985). Freychet et al. (1988) showed that intranasal spray of 7.5 mg of glucagon in a solution with 1% deoxicolic acid was able to correct i.v. insulin induced hypoglycemia in type 1 (insulin dependent) diabetic patients. Slama et al. (1990) also demonstrated that 1 mg of intranasal freeze dried mixture of glucagon and 1 mg glycolic acid was as effective as 1 mg of subcutaneous glucagon. The authors concluded that powder formulation was more effective than the spray solutions reported by Freychet et al. (1988). In another study, Pontiroli et al. (1989) showed that enhancers such as 9-lauryl-ether and sodium glycolate are required to obtain significant nasal absorption of glucagon in fasting human volunteers, and both solution and powders did not differ in term of systemic availability in the presence of any of these enhancers. Before intranasal glucagon can be introduced as a realistic alternative in clinical practice, several difficulties need to be overcome, In particular, chemically stable formulations are required to improve the bioavailability and to reduce cost; moreover, the selection of a safer absorption enhancer which may be as effective but less toxic than the surfactants just mentioned (tingling sensations in the nose for 1 or 2 min which may cause sneezing and possible expulsion of the administered dose) has not yet been established.

Cyclodextrins are biocompatible cyclic oligosaccharides. The parent compounds α- β-, and γ-cyclodextrin contain 6, 7, and 8 glucose units, respectively. It has been shown that α-cyclodextrin and to a lesser extent, β- and γ-cyclodextrin were able to improve nasal insulin absorption in rats (Hirai et al., 1983). Addition of 5% of α-cyclodextrin to the nasal preparation resulted in an absolute insulin bioavailability of approx. 30% (Merkus et al., 1991). The derivative dimethyl-β-cyclodextrin (DMβCD) at 5% w/v gave rise to a large increase in insulin absorption, with bioavailability of 100% and concomitant strong hypoglycemic response (Schipper et al., 1992). Although a considerable amount of work has been done to evaluate the effect of DMβCD on the nasal absorption of insulin in solution forms, information about its effect in powder formulations is not available.

In view of the strong effect of DMβCD as a biocompatible enhancer on nasal insulin absorption in rats, it was found appropriate in the present investigation to evaluate its enhancing properties with glucagon. The effect of different concentrations of DMβCD on the pharmacokinetics (immune reactive glucagon serum concentration) and pharmacodynamics (increase in plasma glucose concentration) of glucagon as nasal spray or powder formulations will be examined in relation to subcutaneous administration in rabbits.

2. Materials and methods

2.1. Materials

Porcine lyophilized glucagon (Novo, Copenhagen, Denmark), DMβCD (Janseen Pharmceutica, Goirle, The Netherlands). Other reagents were of analytical grade.

2.2. Preparation of spray solutions and freeze dried formulations

Spray solutions were individually prepared at the time of use by dissolving 1 mg of lyophilized glucagon in 2 ml of the manufacturer's solvent without and with 2 or 5% w/v DMβCD. Powder formulations were prepared as freeze dried mix-

tures from spray solutions containing the different enhancer concentrations.

2.3. Animal model

Groups ($n = 4$) of white male New Zealand rabbits weighing 2.5 kg were maintained on dry rabbit food 3 days before the experiments. Food was withdrawn for 12 h overnight fasting with water allowed. A 1 h time interval was permitted for each group of rabbits to complete the 12 h fasting. This technique was found appropriate as it provided enough time to finish the experiments with one group before the time for the next group was due. The two nostrils for each rabbit were carefully moistened and cleaned with luke-warm normal saline and cotton buds in order to remove any dirt or excessive secretions that may cause additional barriers for the peptide absorption. Spray solutions without and with different concentrations of DMβCD were delivered as a single puff in each rabbits nostril by a manual spray device calibrated to provide 50 μl per puff (total dose equivalent to 20 μg/kg) of glucagon. Similarly, doses of freeze dried glucagon equivalent to 20 μg/kg without and with increasing amounts of the enhancer were given in two divided doses, one in each nostril from a polyethylene tube by blowing air with a 5 ml syringe. For subcutaneous administration, another group were individually injected with equivalent concentrations of glucagon free of the enhancer. The fasting plasma glucagon and glucose levels were determined for each rabbit 10 min before glucagon administration at time 0. Blood samples were then collected for plasma glucose and immunoreactive glucagon (IRG) assay every 2 min for 16 min and every 5–10 min for 60 min. Plasma glucose was assayed with a glucose oxidase method (Beckman Glucose Analyzer 2, Beckman, USA) and plasma IRG by radioimmunoassay reagent kit (Diagnostic Product Corp., Los Angeles, CA, USA) and a gamma counter (Cobra Auto-Gamma, Model B 5010, Packard Instrument Co. Inc., Downers Grove, IL, USA) with intra-assay reproducibility 4.3–4.8%.

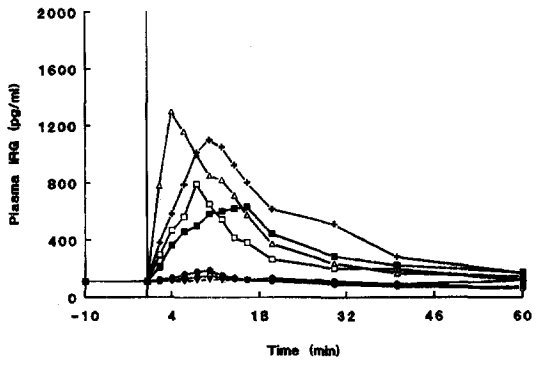

Fig. 1. Plasma IRG levels in response to nasal spray solutions and powder formulations with and without 2 and 5% w/v DMβCD ($\pm$SD from 34 to 299). (▼) Control rabbits; (○) spray with no enhancer; (●) powder with no enhancer; (□) spray with 2% w/v DMβCD; (■) powder with 2% w/v DMβCD; (△) spray with 5% w/v DMβCD; (+) powder with 5% w/v DMβCD.

3. Results and discussion

The plasma IRG and glucose levels of the rabbits receiving spray solutions or powder formulations without and with 2% or 5% w/v DMβCD are shown in Figs. 1 and 2, respectively, while Fig. 3 shows plasma glucagon time curves of the nasal preparations (spray and powder) containing 5% w/v of the additive compared with subcutaneous administration. Corresponding pharmacokinetic data from the results in the three figures are presented in Table 1. The average

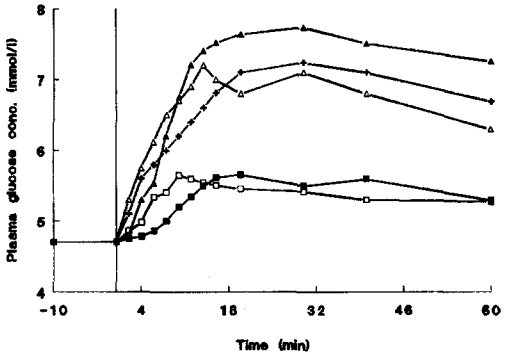

Fig. 2. Plasma glucose levels after nasal and subcutaneous glucagon administrations ($\pm$SD from 0.149 to 0.398). (□) Spray with 2% w/v DMβCD; (■) powder with 2% w/v DMβCD; (△) spray with 5% w/v DMβCD; (+) powder with 5% w/v DMβCD; (▲) subcutaneous administration.

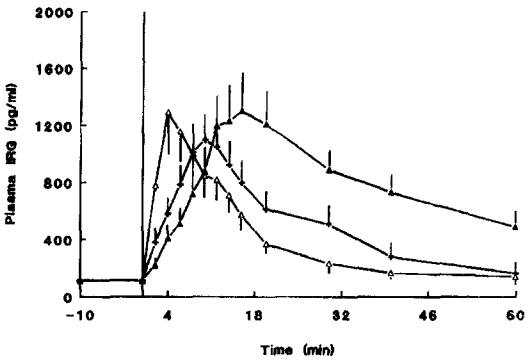

Fig. 3. Mean (±SD) of plasma IRG levels after nasal and subcutaneous administration. (△) Spray with 5% w/v DMβCD; (+) powder with 5% w/v DMβCD; (▲) subcutaneous administration.

basal plasma glucagon and glucose levels measured 10 min before glucagon administration were 112.5 pg/ml and 4.7 mmol/l, respectively. The IRG levels were slightly increased to 151 ± 34 and 187 ± 52.6 pg/ml after 10 min for the spray and powder formulations without DMβCD, then decreased to normal in about 30 min (Fig. 1). Increasing amounts of DMβCD were found to increase plasma IRG and glucose peak concentrations above the basal values, whereas the time to reach the peak glucagon concentrations was decreased (Figs. 1 and 3 and Table 1). Spray solutions were shown to attain the fastest and highest glucagon peak value (791 ± 75.7 pg/ml at 8 min for the 2% w/v DMβCD and 1296 ± 299 pg/ml at 4 min for the 5% w/v DMβCD) compared to powder formulations (630 ± 28.17 pg/ml at 16 min and 1100 ± 176 pg/ml at 10 min). A computed analysis of variance test showed that the differences between means $AUC_{0-60\ min}$ (calculated using the linear trapezoidal rule) of glucagon and glucose time curves for both spray and powder formulations were not significantly different ($p < 0.05$) at any comparable concentration of the incorporated enhancer. The 5% w/v DMβCD resulted in a more pronounced glucagon absorption with corresponding higher plasma glucose levels compared with the 2% w/v of the incorporated enhancer (difference between means were significant at $p < 0.01$). The percentage bioavailability of the nasal preparations (sprays and powders) compared with subcutaneous injection was calculated from the $AUC_{0-60\ min}$ of IRG concentration–time curves illustrated in Figs. 1 and 3 and presented in Table 1 using the equation:

$$(AUC_{i.n.} - AUC_{control})/(AUC_{s.c.} - AUC_{control}) \times dose_{s.c.}/dose_{i.n.} \times 100 \quad (1)$$

where i.n. = intranasal (spray and powder), s.c. = subcutaneous administration and control = rabbits administered no peptide.

The data are presented in the last column of Table 1, and show that the bioavailability values for the spray solution and powder containing no enhancer were 0.77 and 3.52% of the subcutaneous dose. The values for the 2% w/v DMβCD (spray and powder) were respectively between 42.78 and 44.61% of the subcutaneous dose and between 55- and 13-fold higher than those without the enhancer, whereas the values for formulations containing the 5% w/v DMβCD were about 82% for both spray and powder and between 106- and 24-fold higher than those without the enhancer. The AUC plasma glucose time curves in Fig. 2 for the spray solutions and powders were compared with that after subcutaneous administration. The values were 78.89 and 80.47% respectively for spray and powder containing 2% w/v DMβCD, while those for the 5% w/v enhancer were 95 and 97.57%. Although the differences between mean AUC of plasma glucagon for the 5% w/v DMβCD were significant ($p < 0.01$) compared to those after subcutaneous administration (Fig. 3), the differences between mean AUC of plasma glucose concentrations at such an enhancer concentration in Fig. 2 were not significant ($p < 0.05$) compared to the subcutaneous route (see also Table 1). This finding agrees with that reported by Pontiroli et al. (1985), who showed that the metabolic effects of intranasal and intravenous glucagon were equivalent despite lower IRG plasma levels with the intranasal preparation. Also, Freychet et al. (1988) demonstrated an apparent plateau for blood glucose levels with saturation above a value of 7.7 mmol/l after different concentrations of intranasal glucagon. Moreover, Slama et al. (1990) reported a maximum plasma glucose level at different IRG con-

Table 1
Comparison of pharmacokinetics and pharmacodynamics of nasal glucagon with and without 2 and 5% w/v DMβCD with respect to subcutaneous administration (± SD)

Formulation	T_{max} glucagon (min)	C_{max} glucagon (pg/ml) (± SD)	$AUC_{0-60\ min}$ glucagon (pg/ml/min) (± SD)	$AUC_{0-60\ min}$ glucose (mmol/l/min) (± SD)	Bioavailability compared to subcutaneous route (%)
Control		125 ± 22.4	6667.5 ± 830		
Spray solution with no enhancer		151 ± 34[a]	6986 ± 999[a]		0.77[a]
Powder with no enhancer		187 ± 52.6[b]	8127.5 ± 830[b]		3.52[b]
Spray solution with 2% DMβCD	8	791 ± 75.7[c]	24 365 ± 792[c]	313.95 ± 11.54[c]	42.78[c]
Spray solution with 5% DMβCD	4	1296 ± 299[d,h]	40 687 ± 1591[d]	378.16 ± 6.65[d,h]	82.23[d]
Powder with 2% DMβCD	16	630 ± 28.17[e]	25 126 ± 1058 [e]	320.26 ± 4.06 [e]	44.61[e]
Powder with 5% DMβCD	10	1100 ± 176[f]	40 907 ± 1656[f]	388.28 ± 6.61[f,h]	82.76[f]
Subcutaneous glucagon	16	1305 ± 270[g]	48 036 ± 2651[g]	397.94 ± 6.06[g]	100[g]

Significantly different results ($P<0.01$):
a with b, a with c, a with d and a with g;
b with control, b with e, b with f and b with g;
c with control, c with d and c with g;
d with control, and d with g;
e with control e with f and e with g;
f with control and f with g.
Insignificantly different results ($P<0.05$):
a with control, c with e, d with f and h with g.

centrations ranging between 1822 and 5468 pg/ml. The mechanisms of the enhancement of peptide nasal absorption by DMβCD have been examined by many investigators. Irie et al. (1992) demonstrated that cyclodextrins increased the permeability of the nasal mucosa, perhaps through the interaction of the enhancers with lipids and/or divalent cations on the membrane surface; in addition, the enzymatic degradation of peptides such as insulin in rat nasal homogenates was found to be suppressed by cyclodextrins. The combination of increased nasal membrane permeability and reduced proteolysis is therefore responsible for the enhancement of nasal absorption of the administered peptides. The ciliostatic potency of DMβCD on ciliary beating in chicken embryo trachea and human adenoid tissue was examined in vitro (Schipper et al., 1992). It has been demonstrated that DMβCD decreases the ciliary beat frequency (CBF) and the effect was concentration dependent. DMβCD at a concentration of 5% w/v caused ciliostasis in 30 min, whereas a ciliary activity of 40% of the initial value was still present after 60 min incubation with 2% of the enhancer. The CBF seemed to be more influenced by 5 than 2% w/v DMβCD, which may explain the higher increase in glucagon absorption and plasma glucose concentration in the present study with formulations containing 5% DMβCD. The higher and faster glucagon peaks observed with the spray solutions could be attributed to the presence of both the peptide and DMβCD in readily solubilized forms that enable the enhancer to exert its effect on the nasal mucosa and the peptide to quickly penetrate into the blood stream. The relatively delayed absorption of the peptide from the powder formulations is obvious as they have to be solubilized first in the nasal mucosa before being effective. The absorption-enhancement effect of DMβCD was reported to mimic those of bile

salts in regard to the increased membrane permeability being accompanied by the inhibition of proteolysis, although they may be somewhat different from each other in their manner of action on membranes (Billington and Coleman, 1978; Ohtani et al., 1989). The effects of cyclodextrins on nasal mucocilliary movement in rats (Merkus et al., 1991) showed that the ciliotatic potency of DMβCD was less persistent than those found for bile salts and other surfactant-type absorption enhancers; moreover, the effect of DMβCD on ciliary activity was reversible after rinsing with locke-Ringer solution. This could be an advantage of cyclodextrins over other enhancers in promoting peptide nasal absorption especially for patients with sensitive nasal mucosa.

4. Conclusion

DMβCD was found to be an effective absorption promoter for nasal glucagon in rabbits. The enhancement effect was dependent on the enhancer concentration. Both spray solutions and powder formulations were equally effective at any fixed concentration of the enhancer. This should remove some of the concern about the possible harmful effect of the preservatives usually required for spray solutions. The 5% w/v DMβCD was found to be the most effective with bioavailability of about 82% of a comparable subcutaneous dose.

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References

Billington, D. and Coleman, R., Effect of bile salts on human erythrocytes: Plasma membrane vesiculation, phospholipid solubilization and their possible relationships to bile secretion. *Biophys. Acta*, 509 (1978) 33–47.

Elrick, H., Witten, T.A. and Yoshiki, A., Glucagon treatment of insulin reactions. *N. Engl. J. Med.*, 258 (1958) 476–480.

Freychet, L., Rizkalla, S.W., Desplanque, N., Basdevant, A., Zirinis, P., Tchobroutsky, G. and Slama, G., Effect of intranasal glucagon on blood glucose levels in healthy subjects and hypoglycemic patients with insulin-dependent diabetes. *Lancet*, 1 (1988) 1364–1366.

Goldgewicht, C., Slama, G. and Tchobroutsky, G., Hypoglycemic reactions in 172 Type I (insulin-dependent) diabetic patients. *Diabetologia*, 24 (1983) 95–99.

Hall-Boyer, K., Zaloga, G.R. and Chernow, B., Glucagon: hormone or therapeutic agent? *Crit. Care Med.*, 12 (1984) 584–589.

Hirai, S., Okada, H., Yashiki, T. and Uda, Y., European Patent 883302118.1, November 1, 1983.

Irie, T., Wakamatsu, K., Arima, H., Aritomi, H. and Uekama, K., Enhancing effects of cyclodextrins on nasal absorption of insulin in rats. *Int. J. Pharm.*, 84 (1992) 129–139.

Merkus, F.W.H.M., Verhoef, J.C., Romeijn, S.G. and Schipper, N.G.M., Absorption enhancing effect of cyclodextrins on intranasally administered insulin in rats. *Pharm. Res.*, 8 (1991) 588–592.

Ohtani, Y., Irie, T., Uekama, K., Fukunaga, K. and Pitha, J., Differential effects of α-, β- and γ-cyclodextrins on human erythrocytes. *Eur. J. Biochem.*, 186 (1989) 17–22.

Pontiroli, A.E., Alberetto, M., Calderara, A., Pajetta, E. and Pozza, G., Nasal administration of glucagon and human calcitonin to healthy subjects: a comparison of powders and spray solutions and of different enhancing agents. *Eur. J. Clin. Pharmacol.*, (1989) 427–430.

Pontiroli, A.E., Alberetto, M. and Pozza, G., Metabolic effects of intranasaly administered glucagon: comparison with intramuscular and intravenous injections. *Acta Diab. Lat.*, 22 (1985) 103–109.

Schipper, N.G.M., Verhoef, J., Romeijn, S.G. and Merkus, F.W.H.M., Absorption enhancers in nasal insulin delivery and their influence on nasal ciliary functioning. *J. Controlled Release*, 21 (1992) 173–186.

Slama, G., Alamowitch, C., Desplanque, N., Letanoux, M. and Zirinis, P., A new non-invasive method for treating insulin reaction: Intranasal lyophilized glucagon, *Diabetologia*, 33 (1990) 671–674.