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Nasal glucagon delivery using microcrystalline cellulose in healthy volunteers

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Abstract

We developed an intranasal powder form of glucagon to improve metabolic status and fatty liver in patients with pancreatectomy. Microcrystalline cellulose, which is commonly used in commercial preparations for allergic rhinitis was used as an absorption enhancer. We compared the intranasal powder form with some spray solutions of glucagon with regard to glucagon absorption, concentration of blood glucose, stability and nasal irritation. The absorption of glucagon from the spray solution including 1.5% sodium glycocholate or 1% sodium caprate was 1.3- and 2.6-fold higher than that from the powder form mixed with microcrystalline cellulose at a ratio of 1:69, respectively. The C_{max} values of plasma glucose were 2.18, 3.39 and 1.56 mmol l^{-1} in the spray solutions including sodium glycocholate and sodium caprate and in the powder form, respectively. However, glucagon in spray solutions was unstable, but that in the powder form was stable at 5 and 25 °C for at least 84 days. The spray solution caused strong irritation, but the powder form did not. These results suggested usefulness of the powder form of glucagon for treatment of pancreatectomized patients.

Keywords: Glucagon; Microcrystalline cellulose; Intranasal administration; Pancreatectomy; Metabolic status

1. Introduction

The plasma glucose levels in patients after pancreato-duodenectomy are unstable due to postoperative stress and hormone deficiency. Although the levels of glucose in plasma are relatively stable during the convalescent period, other problems such as digestive and absorptive disorders, liver dysfunction, fatty liver, and hyperaminoacidemia arise (Muller et al., 1979; Boden et al., 1980; Hirata et al., 1989). Muller et al. (1983) suggested that glucagon deficiency is the cause of hyperaminoacidemia in duodeno-pancreatec-

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tomized patients. Boden et al. (1980) confirmed that the hyperaminoacidemia was reduced by glucagon infusion. Hirata et al. (1989) reported decreases in the plasma concentrations of gluconeogenic amino acids, triglycerides, and fatty acids after administration of glucagon.

Metabolic disorders of lipids and amino acids and impairment of hepatic function are serious problems. Therefore, the administration of glucagon is necessary for improvement of metabolic status in patients with pancreatectomy (Muller et al., 1979, 1983; Boden et al., 1980; Hirata et al., 1989). However, the duration of glucagon action is so short that it has to be administered several times a day. The injection form of glucagon, which is available as a commercial pharmaceutical preparation, may not be useful for frequent use. Intranasal preparations of glucagon have been developed for the purpose of treating hypoglycemia (Pontiroli et al., 1983, 1989a,b; Freychet et al., 1988; Rosenfalck et al., 1992; Sakr, 1996) mainly as spray solutions. Use of an absorption enhancer is necessary to facilitate the absorption of glucagon via the mucous membrane (Pontiroli et al., 1989c). As enhancers, deoxycholic acid (DOC) (Freychet et al., 1988), dimethyl-β-cyclodextrine (DβC) (Sakr, 1996), sodium glycocholate (SGC) (Pontiroli et al., 1989b) and sodium caprate (SCP) (Hirata et al., 1989) have been used. On the other hand, microcrystalline cellulose (MCC) has been studied as an absorption enhancer of the intranasal powder forms for the other peptides such as insuline (Nagai et al., 1984), leuprolide and salmon calcitonin (Suzuki and Makino, 1999). Therefore, we designed the intranasal powder form of glucagon with MCC from the reasons why the better absorption, higher stability, reduction of irritation and a good dispersion would be obtained. Here, we found that glucagon in the spray solutions was unstable and that the powder form including MCC as an absorption enhancer had high stability and low irritability in the nose. We also confirmed the effects of the powder form on plasma glucagon and glucose concentrations in healthy male subjects.

2. Materials and methods

2.1. Reagents and materials

Each spray solution was delivered by a manual spray device (Fujisawa Pharmaceutical Company, Osaka, Japan) that provided 130 μl per puff. Commercially available glucagon for injection (Ito Ham, Tsukuba, Japan) was used for each spray solution. DOC, DβC, SGC, SCP and other chemicals were at least of analytical grade. Glucagon and MCC (particle size, 40 μm) were purchased from Ito Ham Central Research Institute (Tsukuba, Japan) and Asahikasei Industrial Company (Osaka, Japan), respectively. The powder form of glucagon was prepared by making glucagon adhere to the MCC using the press-on force method, according to the method of Suzuki and Makino (Suzuki and Makino, 1999). The ratios of glucagon to MCC were 1:29, 1:49, 1:59 and 1:69. Each powder form was filled in the capsules which warranted JP standard (glucagon 1mg per capsule) and delivered by a manual spray device (Teijin Limited, Osaka, Japan).

2.2. In vivo study

Five healthy male volunteers (mean age, 31.2 ± 7.9 years; height, 170.8 ± 6.2 cm; weight, 59.4 ± 3.0 kg) participated in this study. They were informed of the methods and purpose of this study both orally and in written form. This study was approved by the regional ethics committee of Kyushu University Hospital, Fukuoka, Japan and informed consent was obtained from all volunteers.

The tests were always started at about 9:00. According to a randomization table and on different days, five subjects sitting on a chair, and after overnight fasting and abstention from smoking, received intranasal glucagon (1 mg) as a powder form with MCC or as a spray solution (glucagon 1 mg ml^{-1}) with 1.5% SGC or 1.0% SCP. The subjects were instructed to take light breaths after puffing to prevent the powder reaching the lungs. Blood samples were collected before and 5, 10, 15, 20, 25, 30, 40, 50, 60 and 90 min after administra-

tion, and immediately centrifuged in chilled tubes containing aprotinin 10 000 kIU ml^{-1} and EDTA. Plasma glucose was assayed by the glucose oxidase method (Hitachi auto-analyzer, Tokyo, Japan) and plasma glucagon was determined by radioimmunoassay using a commercial kit (Daiichi Radioimmunoassay, Tokyo, Japan) and a gamma counter (ARC-950, Aloka, Tokyo, Japan). Intra-assay coefficient of variation (CV) was 6.2–8.9%, and inter-assay CV was 5.1–10.0%.

2.3. Stability test

The stability of the powder form (mixing ratio with MCC 1:69) of glucagon with MCC in the capsule was compared with those of spray solutions including glucagon (1 mg ml^{-1}) in each solution of 1% DOC, 5% DβC, 1% SGC and 1.5% SCP as absorption enhancers. The products were stored at 5 or 25 °C, and each 100 μl and capsule was collected on days 0, 1, 3, 7, 10, 14 and 21 from the spray solutions and on days 0, 7, 14, 21, 28, 42, 63 and 84 from the powder form, respectively. The glucagon contents in the samples were determined by HPLC. The conditions were as follows: column, TSK gel ODS-120T (4.6 mm I.D. × 15 cm, 5 μm, TOSOH, Japan); mobile phase, a mixture of 1-pentane sodium sulfonate solution (10 mM) adjusted to pH 3.05 with acetic acid and acetonitrile (6:4 v/v); flow rate, 1.0 ml min^{-1}; detection, UV at 220 nm; and injection volume, 20 μl. Glucagon in the powder form was extracted with 2 ml of the mobile phase for HPLC by using vortex mixer for 3 min. After centrifugation, the supernatant was injected onto HPLC.

2.4. Statistical analysis

Plasma glucagon and glucose concentrations are expressed as means with S.D. The areas under the serum concentration–time curve from 0 to 90 min ($AUC_{0-90\ min}$) were determined by the linear trapezoidal rule. Statistical analysis between treatments with different enhancers was performed using one-factor ANOVA and Fisher's PLSD.

2.5. Irritation

After intranasal glucagon treatment, the subjects were asked about their condition and requested to record the degree of irritation in the nose on a scale from 0 to 3 (0, no irritation; 1, slight irritation; 2, acceptable; 3, unwilling to accept the treatment again) as reported by Hvidberg et al. (1994).

3. Results

3.1. Effects of the mixing ratio of MCC in the powder form of glucagon on the plasma concentrations of glucagon and glucose

As shown in Fig. 1, plasma concentrations of glucagon and glucose remained unchanged after treatment with the powder form of glucagon in the absence of MCC carrier, but increased in the presence of MCC carrier in a mixing ratio-dependent manner. The mean incremental values of C_{max} of plasma glucose were 0.76, 0.81, 1.18 and 1.56 mmol l^{-1} at mixing ratios of 1:29, 1:49, 1:59 and 1:69 (glucagon:MCC), respectively.

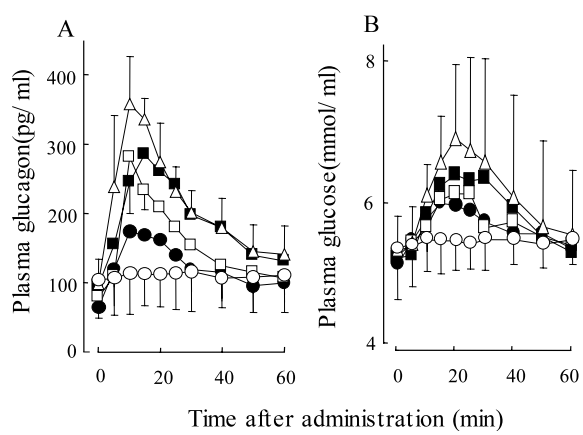

Fig. 1. Changes in the plasma glucagon (A) and glucose (B) concentrations after intranasal intake of powder forms of glucagon mixed with MCC in different ratios in five healthy male adults. (–○–) 1:0; (–●–) 1:29; (–□–) 1:49; (–■–) 1:59; (–△–) 1:69.

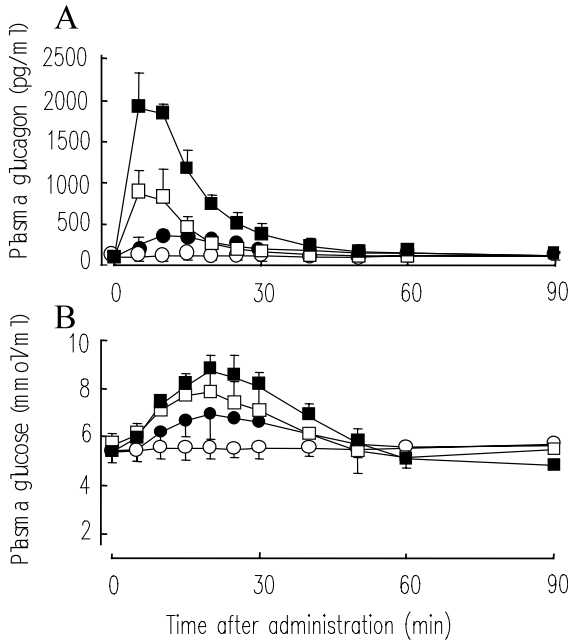

Fig. 2. The effects of the powder form and two kinds of spray solutions of glucagon on plasma concentrations of glucagon (A) and glucose (B) in healthy male adults; (–○–) powder form with no MCC; (–●–) powder form with MCC (1:69); (–□–) 1.5% SGC spray solution; (–■–) 1% SCP spray solution.

3.2. Comparison of plasma profiles of glucagon and glucose after administration of powder form, 1.5% SGC and 1% SCP spray solutions

Fig. 2 shows the effects of two kinds of spray solutions and powder form (mixing ratio with MCC 1:69) of glucagon on plasma concentrations of glucagon and glucose in healthy male subjects. The absorption of glucagon from the spray solutions was higher than that from the powder form. The elevation of plasma glucose accompanied by the absorption of glucagon was also greater for the spray solutions.

The C_{max} values of plasma glucagon after treatment with 1.5% SGC and 1% SCP solutions were 2.4- and 5.3-fold higher, respectively, than that after treatment with the powder form (Table 1). The $AUC_{0-90\ min}$ of glucagon of SGC and SCP solutions were also 1.3- and 2.6-fold higher, respectively. In comparison with the powder without MCC, the $AUC_{0-90\ min}$ of glucagon showed 65, 106 and 325% increase in powder form, 1.5% SGC and 1% SCP spray solutions, respectively. The difference between mean $AUC_{0-90\ min}$ of the powder form and the spray solution including SCP was significant ($P < 0.001$), but that between the powder form and SGC spray solution was not.

The C_{max} values of plasma glucose were 2.18 and 3.39 mmol l^{-1} in the spray solutions including SGC and SCP, respectively, and 1.56 mmol l^{-1} in the powder form. The differences between mean $AUC_{0-90\ min}$ of glucose were not significant for any of the formulations examined.

3.3. Stability of glucagon in the powder form and spray solutions

As shown in Fig. 3, the glucagon content in the powder form (mixing ratio with MCC 1:69) remained unchanged at 5 or 25 °C for 84 days. On the other hand, glucagon in the spray solutions was unstable. As the DOC spray solution gelled at 5 °C, the sample solution could not be collected. Therefore, the glucagon content could not be analyzed at this temperature. The glucagon content in the DOC spray solution at 25 °C was reduced to about 10%, 21 days after preparation. Glucagon in the SCP spray solution was reduced to about 20% at 5 and 25 °C 10 days after preparation. Glucagon in the DβC spray solution was reduced to about 70% and 50% at 5 and 25 °C, respectively, 21days after preparation. Glucagon in the SGC spray solution was rapidly reduced to about 60% at 5 °C 1 day after preparation, but about 80% remained at 25 °C until 21 days after preparation.

3.4. Irritation of the nose by intranasal preparations

All subjects complained of severe nasal irritation after administration of intranasal glucagon in the 1% SCP spray solution, and the mean score of irritation in the nose was 3. For the 1.5% SGC spray solution, all subjects complained of brief nasal irritation, resulting in a mean score of irritation in the nose of 2 (range 1–2). None of the

Table 1
Pharmacokinetic parameters of glucagon and glucose following intranasal administration of powder form with MCC (1:69), spray solution with 1.5% SGC or 1% SCP

Formulation	T_{max} glucagon (min)	C_{max} glucagon (pg ml^{-1}) ($\pm$S.D.)	$AUC_{0-90\ min}$ glucagon (pg ml^{-1} min^{-1}) ($\pm$S.D.)	C_{max} glucose (mmol l^{-1}) ($\pm$S.D.)	$AUC_{0-90\ min}$ glucose (mmol l^{-1} min^{-1}) ($\pm$S.D.)
Powder without MCC	10	111 ± 59.3 ($n=4$)	9945 ± 4630	5.50 ± 0.42	498 ± 34.4
Powder with MCC (1:69)	10	358 ± 68.0 ($n=5$)	$16\,366 \pm 3224$*	6.94 ± 1.06	537 ± 81.6
Solution with 1.5% SGC	5	866 ± 276.0 ($n=5$)	$20\,477 \pm 3479$*	7.82 ± 1.04	548 ± 44.4
Solution with 1% SCP	5	1900 ± 424.3 ($n=4$)	$42\,301 \pm 4870$*†	8.72 ± 0.62	565 ± 33.6

Significantly different results ($P<0.001$), *, compared with powder without MCC; †, compared with powder with MCC.

subjects reported nasal irritation after administration of the intranasal powder form (score 0).

4. Discussion

As the intranasal preparations reported previously (Pontiroli et al., 1983, 1989a,b; Freychet et al., 1988; Rosenfalck et al., 1992; Sakr, 1996) were designed for the purpose of treating hypoglycemia, little attention was paid to the stability of these preparations. This study showed that glucagon was unstable in the spray solutions examined but stable in the powder form. The permanent use of glucagon would be necessary for the improvement of metabolic status in patients with pancreatectomy. Therefore, a high degree of stability would be required for glucagon formulations. Although, the loss of glucagon in the spray solution including SGC was about 20% at 25 °C, it reached 40% at 5 °C. The misty precipitate was found in the spray solution including SGC at 5 °C, but not at 25 °C. Therefore, the remaining content of glucagon might decrease at 5 °C. In terms of stability, the powder form of glucagon was a suitable formulation.

In this study, however, all of the subjects reported that they would be unwilling to accept repeat administration of the SCP spray solution because of strong irritation bordering on pain. Administration of the powder form resulted in scarcely any irritation in the nose. Thus, the intranasal glucagon powder form appeared to be an adequate formulation that does not cause discomfort.

For the treatment of hypoglycemia, a high degree of absorption of glucagon is necessary to cause a rapid increase in the glucose concentration in plasma. It has been reported that an increase of plasma glucose level of 1.9 to 2.2 mmol l^{-1} induced by oral administration of 15 g glucose tablets (Slama et al., 1990) would be necessary for recovery from hypoglycemia. The plasma glucose level was increased to 2.18 and

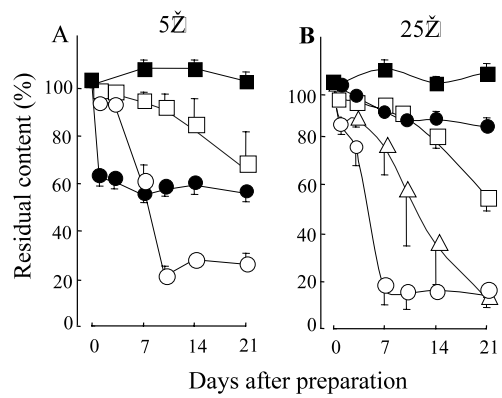

Fig. 3. Stability of glucagon in the powder form and four kinds of spray solutions for intranasal administration at 5 °C (A) and 25 °C (B); (–○–) 1% SCP spray solution; (–●–) 1.5% SGC spray solution; (–□–) DβC spray solution; (–■–) powder form; (–△–) DOC spray solution.

3.39 mmol l^{-1} following administration of spray solutions including SGC and SCP, respectively, but increased only to 1.56 mmol l^{-1} following administration of glucagon powder form. Therefore, the intranasal powder form of glucagon might be unsuitable for the treatment of hypoglycemia. However, for improvement of the metabolic status, glucagon administration is always accompanied by insulin. Therefore, a rapid increase in the plasma glucose concentration caused by higher absorption of glucagon might be inadequate with regard to balance between the actions of insulin and glucagon.

Most important thing is constantly to keep some extent of the levels of glucagon and insulin to maintain the body condition. For these patients, a high level of absorption of glucagon may not be necessary. The patients have to take glucagon frequently and as long as they live. Therefore, the intranasal powder form of glucagon with the high stability and without irritation may be a reasonable formulation for pancreatectomized patients.

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