International Journal of Pharmaceutics, 34 (1986) 157–161
Elsevier

IJP 01154

Trials of rectal insulin suppositories in healthy humans

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(Received 21 November 1985)
(Modified version received 19 March 1986)
(Accepted 23 July 1986)

Key words: Insulin suppository; Adjuvants; Enamine; Salicylate; Healthy human; IRI level; Glucose level

Summary

Administration of an insulin suppository containing adjuvant such as sodium salicylate and L-phenylalanine enamine ethylacetoacetate (enamine) to healthy male human subjects increased serum immunoreactive insulin (IRI) levels significantly. However, to decrease serum glucose concentrations significantly in humans, high serum IRI levels have to be maintained for at least about one hour in humans, rather than being transient. In comparison with enamine, sodium salicylate seems to be feasible as adjuvant in the insulin suppository since its administration to human subjects resulted in high IRI serum levels for a period long enough to lower serum glucose concentrations significantly.

Introduction

Recently we have demonstrated that enamine (Kamada et al., 1981; Yagi et al. 1983) and sodium salicylate (Nishihata et al., 1981, 1983) promote the rectal absorption of insulin in rats, rabbits, dogs and depancreatized dogs (Nishihata et al., 1985; Okamura et al., 1985). In spite of many investigations on the rectal insulin administration in animals (Meshia et al., 1981; Ichikawa et al., 1980; Touitou et al., 1978), there is no report on the rectal insulin absorption in humans except for a rectal insulin suppository containing non-ionic surfactant as adjuvant (Yamasaki et al., 1981). Before clinical trials of the insulin suppository, however, it is necessary to investigate whether insulin from a suppository is absorbed in sufficient amounts in healthy human subjects. In the present study, we examined the effect of either enamine or salicylate on the rectal absorption of insulin in healthy human subjects and its effect on plasma glucose concentrations.

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Materials and Methods

Six healthy male human subjects, 23–36 years old, were fasted for 12 h prior to drug administration, and were divided into two groups: one group for the insulin suppository containing enamine as adjuvant and another group for the insulin suppository containing sodium salicylate (see Fig. 1).

TABLE 1

CODE AND CONSTITUENTS OF SUPPOSITORIES

Code	Adjuvant (mg/supp.)	Base [a] (mg/supp.)	Insulin dose [b] (U/kg)	Volume of 0.5 M citric acid (μl)
A2	0	1000	2.0	60
Enamine				
E0	100	900	0	60
E2	50	950	2.0	60
Sodium salicylate				
S0	300	700	0	60
S1	300	700	1.0	60
S1.5	300	700	1.5	60
S2	300	700	2.0	60

[a] Witepsol H-15 was used as suppository base.

[b] Content of insulin in each insulin suppository was based on the body weight of each human subject. As described in the text, insulin solutions prepared with 0.5 M citric acid solution were added as 60 μl into 1 g of molten mass. For example, the preparation of the insulin suppository for a 60 kg human subject at a dose of 2 U/kg was as follows: 60 μl of the insulin solution containing 2000 U of insulin/ml of 0.5 M citric acid was added to 1 g molten mass composed of base and adjuvant.

After administration of the insulin suppository around 08.00 h, blood samples were taken at designated time intervals and were centrifuged to obtain serum samples.

Constituents of rectal suppositories are listed in Table 1 and suppositories were prepared in the following manner: powdered insulin (porcine monocomponent insulin, 27 U/mg, Nihon Novo Co., Tokyo, Japan) was dissolved at the appropriate concentration in 0.5 M citric acid solution and 60 μl of insulin solution was added to 1 g of molten mass at 40°C, which consisted of Witepsol H-15 and adjuvant (Table 1). After mixing thoroughly, the molten mass was poured into a suppository mold at room temperature. Before use, suppositories were kept at 4°C. As adjuvants, sodium salicylate was obtained from Nakarai Chemicals Co. (Kyoto, Japan) and L-phenylalanine enamine of ethylacetoacetate (enamine) was synthesized according to the method described by Murakami et al. (1981). In a preliminary study, in vitro release of insulin from suppository was determined according to the method described previously (Nishihata et al., 1983). The in vitro release of insulin from each suppository of Code-A2, Code-E2, Code-S1.5 and Code-S2 in Table 1 was 53.4 ± 9.2% ($n = 6$), 52.9 ± 7.1% ($n = 9$), 49.2 ± 6.8% ($n = 9$), and 54.8 ± 7.6% ($n = 9$), respectively, at 1 h after immersion of the suppository in the medium. However, the release of insulin from the suppository of Code-S1 was somewhat lower at 39.1 ± 6.3% ($n = 9$).

Assay of insulin was performed using a radioimmunoassay kit (Daiichi Radioisotope Co., Tokyo, Japan) based on the method described by Nakagawa et al. (1972). Thus, serum insulin concentrations were presented as immunoreactive insulin (IRI) levels. Assay of glucose was carried out using an assay kit based on enzymatic method (Wako Pure Chemicals Co., Osaka, Japan).

Results and Discussion

The administration of the insulin suppository of Code-A2 (Table 1), which did not contain adjuvant, did not change serum IRI levels (Fig. 1A′) and glucose concentrations (Fig. 1A) in human subjects. This result indicates that rectal absorption of insulin did not occur in the absence of an absorption-promoting adjuvant in humans, and was not promoted by citric acid in the suppository. As reported previously (Nishihata et al., 1985; Liversidge et al., 1985), citric acid solution as acidic solution was added to the insulin suppository to improve the dissolution rate of insulin. The administration of the suppository of Code-E0 or Code-S0, which did not contain insulin, also did not change serum IRI levels and glucose concentrations (data are not shown).

The administration of the insulin suppository of Code-E2, which contained enamine as adjuvant, with a dose of 2 U of insulin/kg increased serum IRI levels with transient peak IRI levels at 15–20 min (Fig. 1A′) lowered the serum glucose concentrations only marginally. (Fig. 1A). The additional administration of Code-E0 at 20 min after administration of Code-E2 lowered serum glucose concentration significantly with maintaining serum IRI levels for a longer time (Fig. 1A′).

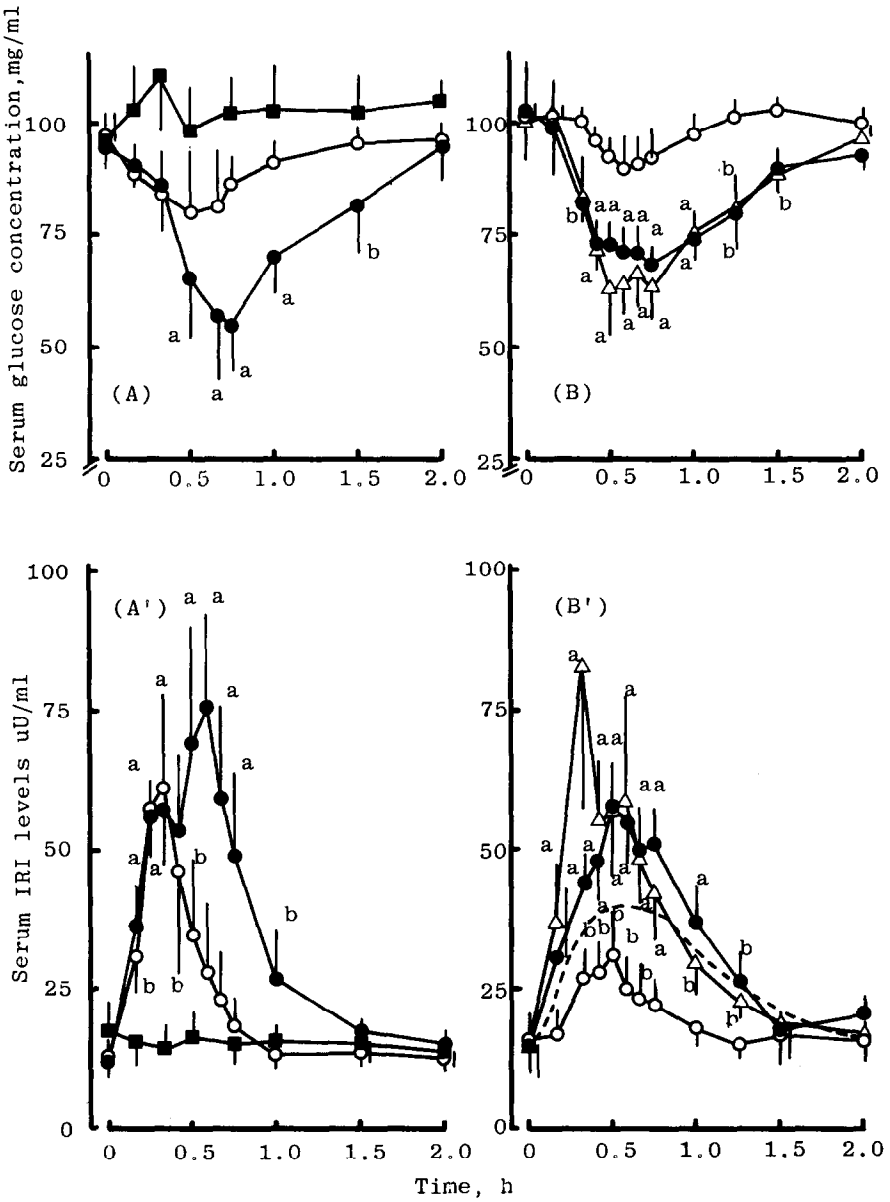

Fig. 1. Serum glucose concentration (A and B) and IRI levels (A′ and B′) in human subjects after administration of the insulin suppository containing either enamine (A and A′) or sodium salicylate (B and B′), as listed in Table 1. A and A′: a cross-over study was carried out with the following three subjects; 24 years old and 60 kg, 33 years old and 65 kg, and 36 years old and 67 kg. The code of insulin suppository was as follows: ■, for Code-A2 (dose of insulin in human; 2 U/kg); ○, for Code-E2 (2 U/kg); and ●, for additional administration of Code-E0 at 20 min after administration of Code-E2 (2 U/kg). B and B′, a cross-over study was carried out with the following three subjects; 23 years old and 54 kg, 26 years old and 58 kg, and 36 years old and 76 kg. The code of the insulin suppository was as follows; ○, for Code-S1 (1 U/kg); ●, for Code-S1.5 (1.5 U/kg); and △, for Code-S2 (2 U/kg). The dotted line in B′ represents the serum IRI levels after an oral glucose tolerance test at a dose of 50 g glucose in healthy human subjects (Kanai and Kanai, 1975). Each value represents the mean ± S.D. ($n = 3$). a = $P < 0.05$ versus the value at zero time (Student's t-test); b = $P < 0.1$ versus the value at zero time.

We have reported that to decrease serum glucose concentrations effectively in depancreatized dogs, serum IRI levels (more than 30 μU/ml) had to remain high for a long period, rather than be transient (Nishihata et al., 1985; Okamura et al., 1985). The present results also indicate that serum IRI levels have to remain high for a long period to lower serum glucose concentrations significantly in human subjects.

A single administration of the insulin suppository of Code-S1.5 or Code-S2 lowered serum glucose concentrations significantly in human subjects (Fig. 1B) while maintaining high serum IRI levels for about 1 h (Fig. 1B′). The long action period of sodium salicylate may be due to the slow clearance of sodium salicylate from the rectum due to its slow absorption in humans as well as in dogs (Liversidge et al., 1985). This may be due to the fact that the adjuvant action of sodium salicylate disappears rapidly after the disappearance of salicylate from the rectum (Sithigorngul et al., 1983).

From the above results, the insulin suppository containing sodium salicylate as adjuvant seems to be a potential dosage form for clinical trials in comparison with an insulin suppository containing enamine as adjuvant, since single administration of the insulin suppository containing sodium salicylate to human subjects resulted in high serum IRI levels for a longer time sufficient enough to lower serum glucose concentrations significantly. It also should be noted that serum insulin profiles as a function of time after administration of the insulin suppository of Code-S1.5 or Code-S2 are close to those after oral glucose tolerance test in healthy adult Japanese (Kanai and Kanai, 1975), as shown in Fig. 1B′. This finding may indicate that the insulin suppository containing sodium salicylate is effective in diabetic patients, who have basic serum IRI levels (10–20 μU/ml) under fasting conditions but have a pancreatic disorder to secrete insulin in response to high serum glucose concentrations after a meal. Many diabetic patients, who are medicated with oral antidiabetic agents such as chlorpropamide, belong to the case of diabetic mellitus described above.

As shown in Fig. 1B and 1B′, the administration of the insulin suppository of Code-S1 to human subjects at a dose of 1 U of insulin/kg lowered serum glucose concentrations only slightly with the increase of serum IRI levels only up to 30 μU/ml. Thus, it may be estimated that a minimum dose of 1.5 U/kg of insulin in the insulin suppository formulation is adequate for a Japanese adult.

Acknowledgement

This work was supported in part by a grant from R.P. Scherer North America, FL, U.S.A.

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