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Effect of gelatin in the formulation on rectal insulin absorption in the presence of enamine in normal rats and depancreatized dogs

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Summary

A significant decrease in plasma glucose levels of normal rats and depancreatized dogs was observed when insulin was rectally administered in conjunction with enamine adjuvants. The enamine employed was derived from ethylacetoacetate and DL-phenylalanine. Higher bioavailability of insulin was observed when it was dissolved and dispersed in a gelatin microenema compared to the crystalline suspension of insulin in a glyceride suppository base. The lower bioavailability from glyceride suppositories has been attributed to the slow dissolution rate of crystalline insulin at the lipid/aqueous interface becoming the rate-limiting step in absorption. However, an aqueous microenema containing a solution of insulin and enamine produced lower bioavailability than gelatin formulations. This reduction in bioavailability has been attributed to greater spreading of the aqueous microenema resulting in a decrease in the amount and concentration of insulin and enamine available at the absorption site.

Introduction

Since its discovery, insulin has been routinely administered by the parenteral route for the treatment of diabetes mellitus. Recently a number of attempts have

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been made to deliver insulin rectally in conjunction with adjuvants. The most popular method, concurrent rectal administration of insulin with surfactant adjuvants, requires large doses of insulin to elicit significant decreases in blood glucose levels, limiting its clinical application (Touitou et al., 1978; Yamasaki et al., 1981; Ichikawa et al., 1980). Recently alternative adjuvants, such as salicylate and enamines, have been used and require much lower doses of insulin to elicit similar reductions in blood glucose levels (Nishihata et al., 1981; Kamada et al., 1981; Kim et al., 1983; Yagi et al., 1983).

Suppositories of either glycerides or polyethylene glycol bases have been extensively used to deliver insulin rectally. The glyceride suppositories generally show less effects on plasma glucose levels both with and without adjuvants present.

In the present paper we report the effects of various rectal formulations, adjuvant concentrations and dosages on the absorption of insulin in the presence of enamine as an adjuvant, using rats and depancreatized dogs.

Materials and Methods

Wistar male rats, 250–275 g, and depancreatized dogs, 9.0–9.5 kg, were fasted for 16 h prior to experiments but water was given freely. Depancreatized dogs were prepared according to the method described by Shichiri et al. (1978). Dogs were conscious, but rats were anesthetized with sodium pentobarbital (60 $mg \cdot kg^{-1}$) and placed on a platform maintained at 38 °C during the experiments. Insulin (amorphous insulin, 24 $U \cdot mg^{-1}$, supplied from NOVO K.K., Japan) was rectally administered in the following forms: aqueous microenema, gelatin microenema, or suppository prepared from a glyceride base, Witepsol H-15, accoring to the method of Nishihata et al. (1983). These preparations contained the designated amount of DL-phenylalanine enamine of ethyl acetoacetate synthesized according to the method of Murakami et al. (1981).

Blood samples were taken at the designated time intervals from the jugular vein of rats and from the femoral vein of dogs after administration of each insulin formulation. Blood samples were centrifuged at 3000 rpm for 10 min. Glucose levels were determined using an *o*-toluidine boric acid method with minor modification (Nishihata et al., 1978). Plasma insulin levels in rats were determined using an enzyme immunoassay kit supplied from Toyo Jozo, (Tokyo, Japan) with some modification and for dogs, a radioimmunoassay was employed (Nakagawa et al., 1972).

Release and dissolution of insulin from 4% gelatin formulation and glyceride suppository in the presence of enamine was examined according to a method described previously (Nishihata et al., 1983).

Results and Discussion

The availability of insulin for absorption from glyceride suppositories is limited by the poor release of crystalline insulin from the molten base and the slow

TABLE 1

THE RELEASE AND DISSOLUTION OF INSULIN FROM 1 ml OF 4% GELATIN FORMULATION AND 1 g OF SUPPOSITORY FORMULATION IN 5 ml OF STIRRED SALINE SOLUTION CONTAINING 1% BOVINE SERUM ALBUMIN AT 37 °C

4% gelatin contained 10 U of insulin·ml^{-1} and 100 mg of enamine·ml^{-1}. Glyceride suppository contained 10 U of crystalline insulin·g^{-1} and 100 mg of enamine·g^{-1}. (n = 3)

Formulation	Concentration of insulin in saline solution after incubation (mU·ml^{-1})	
	30 min	60 min
4% Gelatin	1884 ± 95	1873 ± 84
Glyceride	128 ± 26	407 ± 48

dissolution rate of the released crystalline insulin (Nishihata et al., 1983). To overcome the slow dissolution rate of insulin in the rectal luminal secretory fluid, soluble insulin formulations such as aqueous microenemas and gelatin microenemas were used in this study. As shown in Table 1, release and dissolution of insulin from the 4% gelatin formulation occurred rapidly compared to that from the glyceride suppository. The formulations for the rat study contained 5 U of insulin and 100 mg of enamine per ml of microenema or per g of glyceride base. A dosage of 50 μl of microenema or 50 mg of suppository per animal were administered to rats unless otherwise stated.

Typical insulin and glucose profiles following administration of the 3 rectal formulations are shown in Fig. 1. It can be seen that the gelatin microenema had the

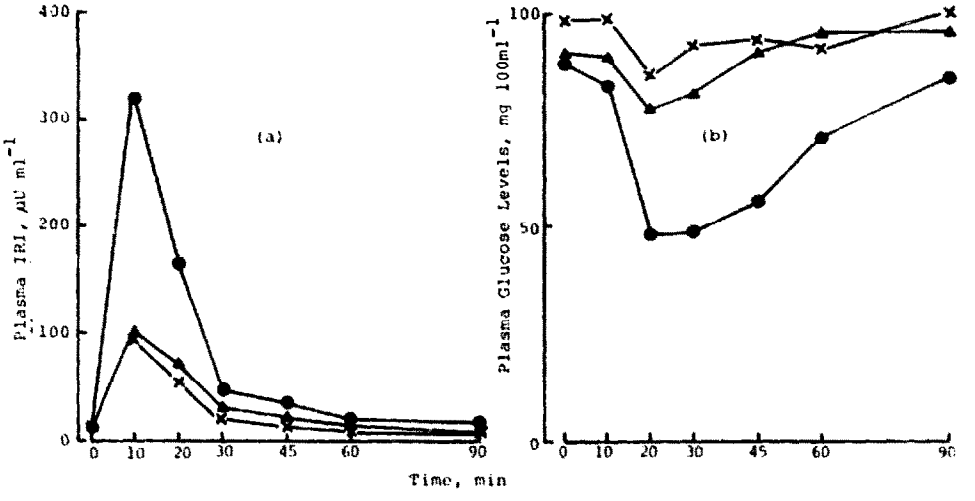

Fig. 1 Typical profiles of plasma insulin levels (a) and plasma glucose levels (b) in rats after rectal administration of insulin at a dose of 0.25 U per animal in aqueous microenema (×) 4% gelatin microenema (●) or glyceride suppository (▲). Aqueous microenema containing 100 mg of enamine·ml^{-1} and 5 U of insulin·ml^{-1}, and 4% gelatin formulation containing 100 mg of enamine·ml^{-1} and 5 U of insulin·ml^{-1} were administered at a dosage volume of 50 μl per animal. Glyceride suppository containing 100 mg of enamine·g^{-1} and 5 U of insulin·g^{-1} was administered at 50 mg per animal.

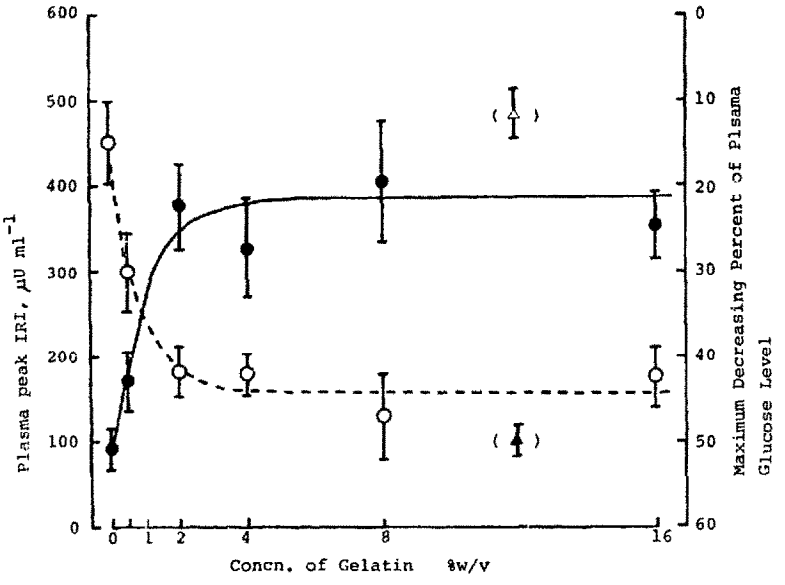

Fig. 2. The effect of gelatin concentration on the rectal absorption of insulin in the presence of enamine expressed as plasma peak insulin level (●) and plasma maximum decreased glucose level (○) in rats after administration of insulin at a dose of 0.25 U per animal and 5 mg of enamine per animal. ▲ and △ represent the plasma insulin peak level and plasma maximum decreased glucose levels, respectively, after administration in suppository. Each value represents the mean ± S.D. $P < 0.001$ versus no gelatin formulation (Student's t-test, $n \geq 5$).

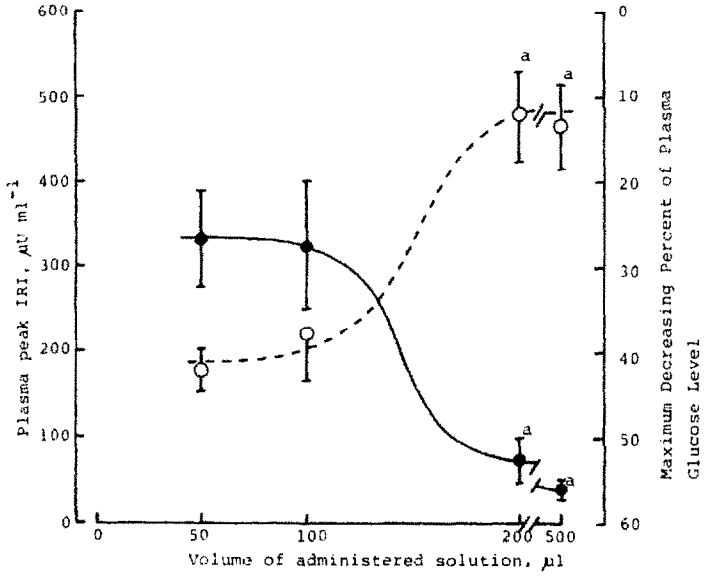

Fig. 3. The effect of volume of 4% gelatin formulation on the plasma insulin peak levels (●) and plasma maximum decreased glucose levels (○) in rats after administration at a dose of 0.25 U of insulin per animal and 5 mg of enamine per animal. Each value represents the mean ± S.D. ($n \geq 5$). [a] $P < 0.001$ versus the dosage of 50 and 100 μl.

greatest effect on plasma glucose levels and gave the highest plasma insulin levels, followed by the glyceride suppository and the aqueous microenema. We would have expected the aqueous microenema to give higher plasma insulin levels than the glyceride suppository, since the rate-limiting dissolution step would not occur in the microenema. This unexpectedly poor effect of the aqueous microenema may be due to the dilution of adjuvant with rectal fluid by rapid spreading of microenema as described in our previous paper (Nishihata et al., 1981).

The superior effects of the gelatin microenema suggested further investigation of this formulation. The effect of gelatin concentration in the microenema on the peak plasma levels of insulin and on the nadir percentage decrease of glucose levels in rats are presented in Fig. 2. The effects are markedly enhanced as the percentage of gelatin in the microenema is increased up to 4% gelatin. We attribute the enhancing effect of gelatin to an increased viscosity of the microenema which probably reduces spreading of the microenema resulting in a decrease in dilution of the insulin and adjuvant in the rectal luminal fluid.

Earlier in this paper we have hypothesized that the consequences of increased spreading are dilution of the drug and adjuvant which result in a reduced absorption of the drug. To test this hypothesis we varied the concentration (not the total amount) of insulin (0.25 U per animal) and adjuvant (5 mg per animal) by varying the volume of the 4% gelatin solution used. Fig. 3 shows the effect of varying the volume of the gelatin microenema vis-a-vis concentration on the insulin levels and percent glucose nadirs. Volumes in excess of 100 μl per animal showed significantly lower plasma levels for insulin. Thus it would appear that the concentration of the

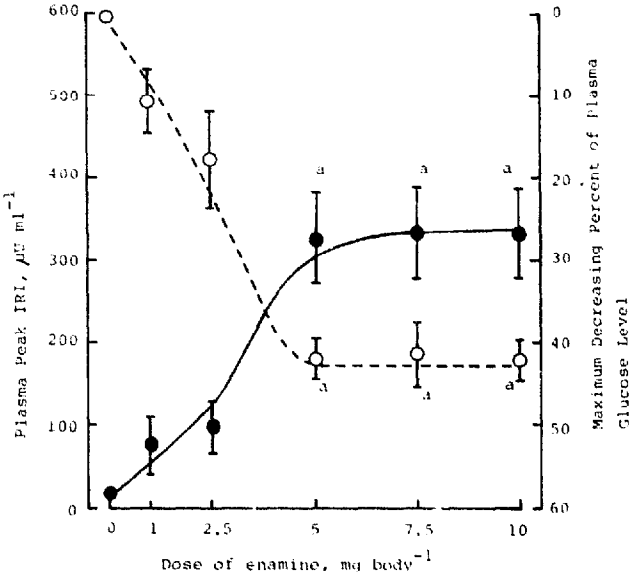

Fig. 4. The effect of enamine dose on the plasma peak insulin levels (●) and the plasma maximum decreased glucose levels (○) in rats after administration of 50 μl of 4% gelatin formulation at a dose of 0.25 U of insulin per animal. Each value represents the mean ± S.D. ($n \geq 5$). $^a P < 0.001$ versus the 1 and 2.5 mg of administered enamine.

drug and adjuvant at the absorptive site are of primary importance in controlling the absorption.

Further studies were undertaken to elucidate the role of enamine concentration on the absorption of a fixed amount of insulin. Dosage forms were formulated such that 5 U of insulin and designated quantities of enamine were dissolved in 1 ml of 4% gelatin solution and 50 μl was administered per rats. Fig. 4 illustrates the effects of increasing the concentration of enamine in the gelatin microenema. Concentrations in excess of 100 mg · ml^{-1} show a marked increase in adjuvant effect. Thus the concentration of adjuvant at the site of absorption has an important role to play in governing the amount of drug absorbed. Consequently, if significant spreading and dilution occur, a marked decrease in insulin absorption will be observed. However, viscous vehicles such as 4% gelatin solution may reduce spreading and dilution and hereby increase absorption of insulin.

Glyceride suppository formulations containing 5 U of insulin and 100 mg of enamine/g suppository when administered at a dose of 50 mg per animal cause an increase in plasma insulin levels and a decrease in plasma glucose levels (Fig. 1). However, these effects are similar to those from aqueous microenemas and are different from results obtained using ampicillin as a drug. When ampicillin was administered to rabbits with sodium salicylate, superior absorption was obtained from glyceride suppositories than from an aqueous microenema (Nishihata et al., 1984a). The ampicillin results were explained by a decrease in spreading of the molten glyceride suppository in comparison to the microenema. Lower insulin absorption from glyceride suppositories than from gelatin microenemas has been attributed to a slow release and dissolution of insulin from the molten glyceride suppository than from the gelatin formulation (see Table 1 and Nishihata et al., 1983). The amount of insulin released and in solution hence available for absorption

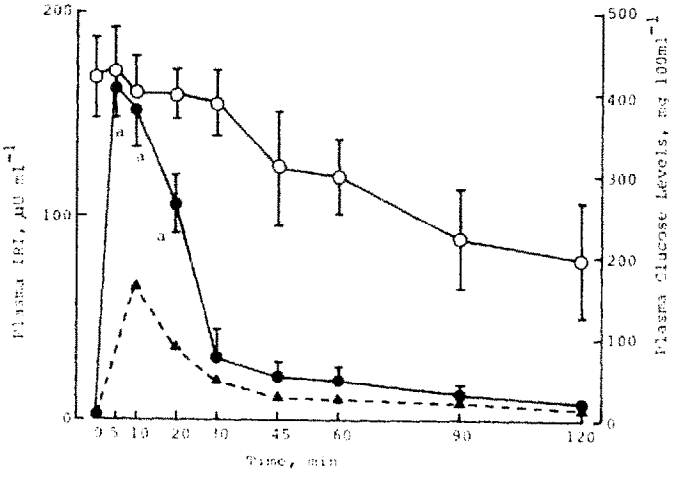

Fig. 5. Plasma insulin profile (● and ▲) and plasma glucose profile (○) as a function of time after administration of insulin to depancreatized dogs in 4% gelatin formulation (● and ○) or in glyceride suppository (▲) at a dose of 1 U of insulin·kg^{-1} and 5 mg of enamine·kg^{-1}. Each value represents the mean ± S.D. (n = 3). [a] $P < 0.001$ versus suppository administration.

from gelatin microenemas will be greater than from glyceride suppositories during the relatively short period when the enamine adjuvant is effective. The enamine is very rapidly absorbed with more than 50% being absorbed within the first 5 min after administration (Nishihata et al., 1984b).

A comparison of the bioavailability of gelatin microenema and glyceride suppositories was undertaken in diabetic dogs to confirm the data obtained in the rat studies. Fig. 5 shows the effects of the two formulations on plasma insulin levels. It can be seen that the gelatin microenema shows significantly higher insulin levels.

The present study has shown the superiority of gelatin formulations over aqueous microenemas and glyceride suppositories on the plasma insulin and glucose levels. However, insulin may prove unstable in gelatin microenemas. Also the inconvenience of administration of a gelatin microenema formulation in the clinical situation may reduce its utility in comparison with glyceride suppositories which are more easily administered to patients. Thus glyceride formulations may have a wide clinical application. We are now attempting to develop glyceride suppository formulations that will address the problems presented in this paper, namely, how to facilitate the release and solubilization of insulin and how to control the release of adjuvant so as to prolong its action.

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