J. Pharm. Pharmacol. 1985, 37: 22–26
Received January 30, 1984

Enhanced bioavailability of insulin after rectal administration with enamine as adjuvant in depancreatized dogs

TOSHIAKI NISHIHATA*, YASUFUMI OKAMURA, AKIRA KAMADA, TAKERU HIGUCHI†, TOSHIHITO YAGI‡, RYUZO KAWAMORI‡, AND MOTOAKI SHICHIRI‡

Faculty of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565, Japan; †Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66044, USA; ‡First Department of Medicine, Osaka University Medical 1-1-50 Fukushima, Fukushimaku, Osaka 553, Japan

Rectal absorption of insulin by depancreatized dogs was significantly enhanced by the coadministration of enamine as a suppository adjuvant and if this was followed by a further suppository containing enamine alone, the insulin absorption was even further enhanced. The additional enamine suppository resulted in high serum insulin concentrations for a longer time and effected a significant decrease in serum glucose concentrations. To decrease serum glucose concentrations effectively in depancreatized dogs, serum insulin levels had to remain high for a long period of time, rather than be transient.

Recently, we reported the results of enhanced rectal absorption of insulin when non-surfactant adjuvants such as enamines (Kamada et al 1981; Yagi et al 1983), acetoacetate esters (Nishihata et al 1983a) and salicylate analogues (Nishihata et al 1981b) were incorporated into microenema preparations and triglyceride suppositories. The bioavailability of insulin after rectal administration of such suppositories in normal dogs was enhanced 25% with salicylate (Nishihata et al 1983b), 27% with enamine (Yagi et al 1983) and 11% with BL-9 (Yagi et al 1983), a non-ionic surfactant.

Rectal absorption of insulin enhanced by non-surfactant adjuvants is much better than that obtained using surfactants. However, more investigations using non-surfactant adjuvants are needed to improve the bioavailability of insulin and to control the serum glucose concentrations in animals.

Since the release of insulin from the suppository is considered to be one of the limiting steps in rectal insulin absorption (Kim et al 1984), it is evident that the release of adjuvants such as the sodium salts of enamines and salicylates from suppositories must be correlated with the release of insulin. Without this correlation, rapidly absorbed adjuvants may be assimilated from the rectal compartment before the release of insulin is complete. In considering the efficacy of insulin suppositories, two important factors must be recognized, namely, (1) facilitation of insulin release from suppositories and (2) control of the adjuvant release from suppositories.

*Correspondence.

We report on the second factor and have made preformulation experiments to develop a new rectal delivery preparation for insulin that uses enamine as the adjuvant. Our aim was to maintain a reasonably high serum insulin concentration for the time required in the treatment of diabetes mellitus.

MATERIALS AND METHODS

Materials

Monocomponent insulin (27·0 U mg^{-1}) was supplied from Nihon NOVO (Tokyo, Japan). The enamine, sodium phenylalanine enamine of ethylacetoacetate, was synthesized using ethanol according to Sollenberger & Martin (1970). Other reagents were of analytical grade.

Preparation of suppositories

These were prepared by melting triglyceride suppository base (Witepsol H-15, Dynamit Novel Chemicals, Troisdorf-Oberlat, West Germany) at 40 °C on a hot plate and adding insulin and/or adjuvants to the melt. The molten mass was poured into disposable plastic moulds (Nichii Packing Co., Ltd., Osaka, Japan). Suppositories were kept at 4 °C before use. An insulin suppository containing insulin and enamine, and an adjuvant suppository containing enamine only were used.

In-vivo study

After pancreatectomy, six dogs, 8·5–10 kg, were injected daily with insulin (10–20U/dog). Insulin was withheld 36 h before the experiments and the serum insulin concentration was undetectable at the begin-

ning of experiments (<3 μU ml^{-1}). The dogs, with fasting serum glucose values of about 500 mg/100 ml, had ketonaemia and insulin-free serum levels which were not detectable by radioimmunoassay (Nakagawa et al 1972). Normal beagle dogs, 9·5–11 kg, with fasting serum glucose values of ca 90 mg/100 ml, showed about 13 μU of insulin ml^{-1} of serum. After the administration of the insulin suppository, blood samples were taken from the femoral vein at designated times and centrifuged at 3000 rev min^{-1} for 10 min to obtain serum samples. The adjuvant suppository was administered at 15, 17·5, 20, 30 or 40 min after the insulin suppository. In a study using a balloon catheter, the catheter was inserted into the rectum to a depth of 4 cm from anus before administration of the suppositories.

RESULTS

In a preliminary study, the insulin suppository (containing insulin, 5 U kg^{-1} and enamine 50 mg kg^{-1}) at a dose of 1 g suppository/10 kg was administered to depancreatized dogs. This resulted in a significant increase in serum insulin concentrations (Fig. 1). The serum glucose values, however, did not decrease significantly in response, and the high serum insulin concentrations lasted only for less than 0·5 h (Fig. 1).

Administration of the adjuvant suppository to the depancreatized dogs 17·5 ± 1 min after administration of the insulin suppository resulted in a high serum insulin concentration over some 60 min and in a gradual but significant fall in serum glucose values during the 2 h experimental period (Fig. 1). The area under the curve (AUC) of serum insulin concentrations for 2 h after rectal administration was calculated to estimate the rectal insulin absorption. The AUC value of 174·4 ± 29·3 μU h ml^{-1} obtained when the adjuvant suppository was administered after the insulin suppository was twice that (84·8 ± 12·8 μU h^{-1} ml^{-1}) when the insulin suppository was administered alone. The adjuvant suppository given 30 min after the insulin suppository sometimes failed to maintain a high serum insulin or to decrease the serum glucose level, giving results similar to those obtained after the single administration of the insulin suppository. As shown in Fig. 1, high serum insulin values (over 30 μU ml^{-1}) were maintained for 60–90 min following the administration of the adjuvant suppository at 17·5 ± 1 min after the insulin suppository, but this time was much less after the insulin suppository alone, or when the adjuvant suppository was given 30 min after the insulin

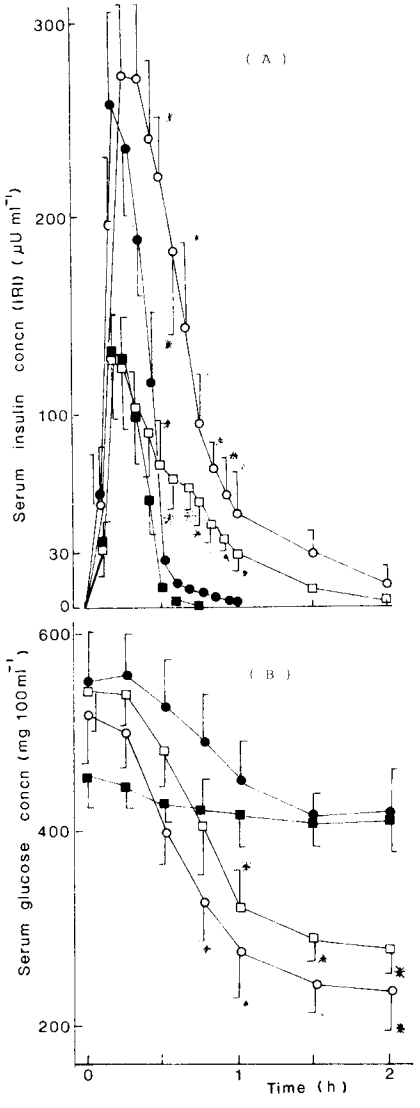

FIG. 1. Serum insulin (A) and glucose (B) concentrations in depancreatized dogs after administration of the insulin suppository (●, ■) and the effect of the adjuvant suppository (○, □), given 17·5 ± 1 min after, on serum insulin and glucose concentrations. The insulin suppository contained 50 mg of enamine and 50 U (○ and ●) or 20 U (□ and ■) of insulin g^{-1} suppository. The adjuvant suppository contained 100 mg of enamine g^{-1}. The dose of each suppository was 0·1 g kg^{-1}. Three depancreatized dogs with initial glucose concentrations of greater than 400 mg/100 ml were used in a cross-over study. Each value represents the mean ± s.d. *$P < 0·001$ versus the results obtained when the insulin suppository alone was administered.

suppository, the serum insulin values remaining at over 30 μU ml^{-1} for only 25 min.

It is well known that intramuscular injection of insulin in the clinical setting effects a high serum insulin concentration for more than 60 min. The

intramuscular injection of 0·4 U kg^{-1} to depancreatized dogs with blood glucose about 500 mg/100 ml showed a peak of about 60 μU ml^{-1} with maintenance of 30 μU ml^{-1} for more than 60 min, which resulted in some 50% decrease of the initial serum glucose concentration (data not shown, AUC value of insulin after this intramuscular injection was 46·2 ± 4·38 h ml^{-1}. When we administered insulin to depancreatized dogs at a dose of 5 U kg^{-1} in a suppository, followed by an enamine suppository 17 min later, a high serum insulin concentration (>30 μU ml^{-1}) was maintained for more than 60 min. The result was a significant decrease in serum glucose (63·3% decrease against the initial level). Further experiments were done in which the adjuvant dose was decreased from 50 to 30 mg g^{-1} suppository to supress the initial high serum insulin concentrations, and the suppository containing 100 mg enamine g^{-1} was administered 17·5 ± 1 min later. As shown in Fig. 2, serum peak insulin values were about 100 μU ml^{-1}, the serum insulin concentration being over 30 μU ml^{-1} for more than 60 min after the treatment. The serum glucose values were significantly decreased (Fig. 2), with an AUC of 96 ± 19·6 μU h ml^{-1} insulin, similar to that of 88·1 ± 16·3 μU h ml^{-1} after the insulin suppository containing 50 mg enamine g^{-1}. However, the drop in the serum glucose from the enamine-sustained high serum insulin values was greater than that effected by the one-suppository treatment, when the insulin was maintained for only short periods.

To confirm the above findings the dose of insulin administered was reduced. When the insulin suppository containing insulin at a dose of 2 U kg^{-1} and 50 mg of enamine g^{-1} suppository was administered with the adjuvant suppository follow-up as shown in Fig. 1, a significant decrease of serum glucose was observed in depancreatized dogs by maintaining the serum insulin at over 30 μU ml^{-1} for about 1 h. This indicates that the mode of administration used to maintain the high serum insulin concentration allows the reduction in the administered dose of insulin for rectal delivery. The estimated bioavailability of insulin after rectal administration was determined by comparison of the AUC of insulin after rectal administration with that after intramuscular administration. Since AUC for insulin after the insulin suppository containing 2 U kg^{-1} (Fig. 1) was 44·8 ± 5·3 μU h^{-1} ml^{-1} while that after intramuscular administration of 0·4 U kg^{-1} was 46·2 ± 4·38 μU h ml^{-1}, the estimated bioavailability from that suppository was 19·4%. But when the enamine suppository was given 17 min after a 2 U kg^{-1} suppository (Fig.

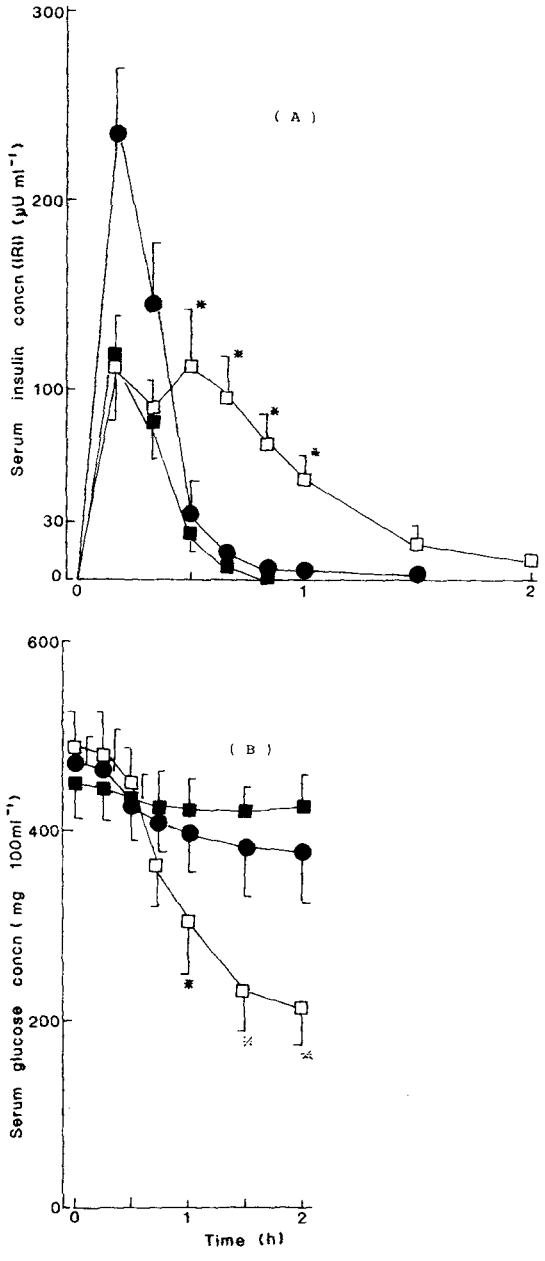

FIG. 2. Serum insulin (A) and glucose (B) concentrations in depancreatized dogs after administration of the insulin suppository (●, ■) and the effect of the adjuvant suppository (□), given 17·5 ± 1 min after, on serum insulin and glucose concentration. The insulin suppository contained 50 U of insulin and 50 mg (●) or 30 mg (□ and ■) of enamine g^{-1} suppository. The adjuvant suppository contained 100 mg of enamine g^{-1}. Dose of suppositories was 0·1 mg kg^{-1}. Three depancreatized dogs, who were different from dogs used in Fig. 1, with initial glucose concentrations of greater than 400 mg/100 ml were used in cross-over study. Each value represents the mean ± s.d. *$P < 0·001$ versus no adjuvant suppository.

1) the estimated bioavailability was 38·2% with AUC of 88·3 ± 8·1 μU h ml^{-1}.

To examine the effect of the enamine suppository further, it was given to normal dogs 15 or 20 min after an insulin suppository (Fig. 3). High serum insulin values were maintained for longer than those after the insulin suppository alone. The adjuvant suppository given 30 min after the insulin suppository failed to maintain the high serum insulin. Unlike the results with depancreatized dogs, the administration of the insulin suppository alone to normal dogs caused a significant decrease in the serum glucose although serum insulin remained high for only a short time (Fig. 3).

To elucidate why administration of the enamine suppository 30 min after the insulin suppository did not maintain high serum insulin, a balloon catheter was inserted at 4 cm depth from the anus before administration of the suppositories to normal dogs. Under this condition, the enamine suppository even 30 and 40 min after the insulin suppository, resulted in sustained high serum insulin values (Fig. 4). It appears that the balloon catheter prevented escape of insulin. Without the catheter, insulin remained at the absorption site for less than 30 min. The adjuvant suppository given 30 or 40 min after the one containing insulin at 5 U kg^{-1} increased the serum insulin significantly only when a balloon catheter was inserted.

DISCUSSION

The lower bioavailability of insulin after adjuvant-insulin administration via the small intestine (Nishihata et al, 1981a) compared with the rectal route (Nishihata et al 1981b) can be explained in terms of insulin being enzymatically degraded in the small intestine, the lining of which presents more of a barrier to permeation of non-surfactant adjuvants than that of the rectum. We have already reported that, in the in-situ loop study, adjuvant activity of enamine at the rectal membrane is much stronger than that at the jejunal membrane using poorly absorbable drugs which are not degradated enzymatically (Nishihata et al 1984). This finding may partially explain why adjuvant-enhanced insulin absorption from the rectum results in insulin bioavailability higher than absorption from the small intestine. The development of formulations for rectal insulin administration therefore appears to have the greater potential clinically.

The rectal absorption of insulin using salicylate as adjuvant is significantly influenced by formulation (Nishihata et al 1983b). In that report, gelatin

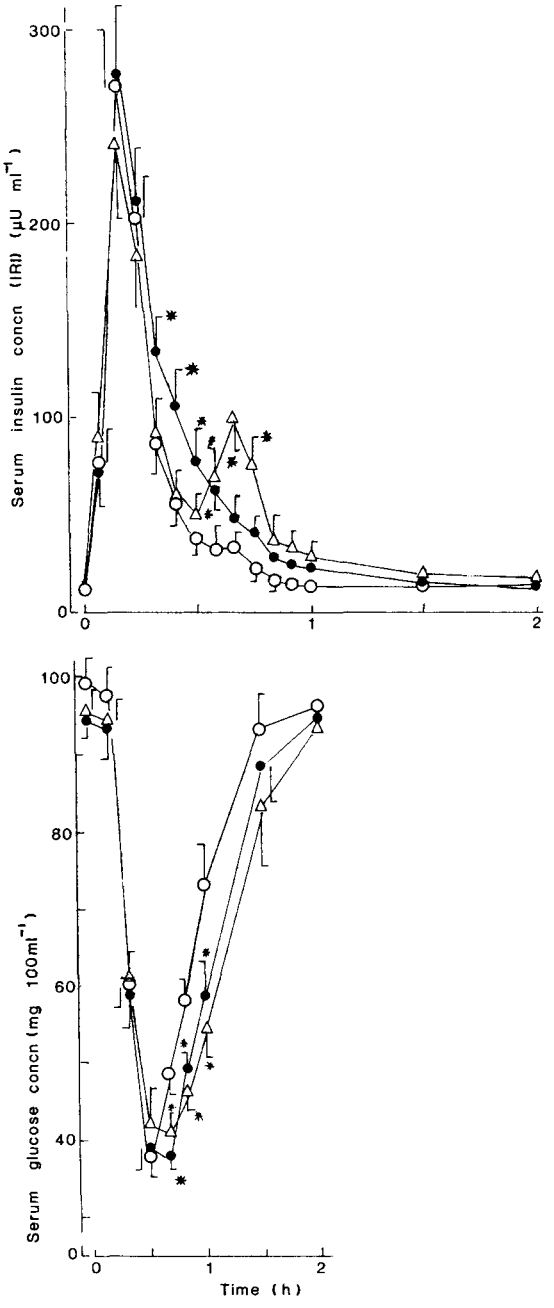

FIG. 3. Effect of the adjuvant suppository administered 15 min (●) or 20 min (△) after administration of the insulin suppository containing 50 mg of enamine g^{-1} suppository weight and an insulin at a dose of 5 U kg^{-1} on (A) serum insulin and (B) serum glucose concentrations in four normal dogs (Cross-over study). the adjuvant suppository contained 100 mg of enamine g^{-1} suppository weight. The dose of suppositories was 0·1 mg kg^{-1}. The open circle (○) shows the effect of the insulin suppository alone. Each value represents the mean ± s.d. *$P < 0·001$ versus no adjuvant suppository.

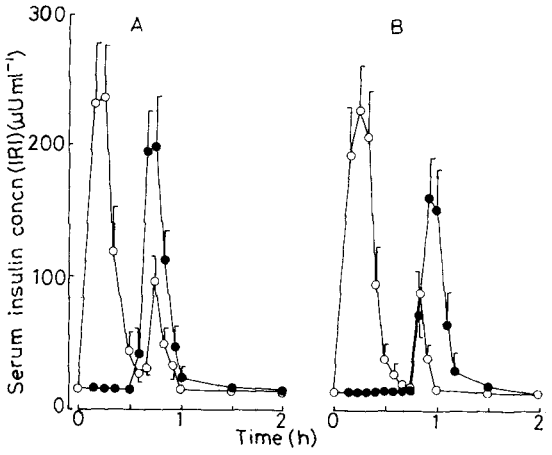

FIG. 4. Effect of the adjuvant suppository at (A) 30 or (B) 40 min after administration of the insulin suppository on serum insulin concentrations in normal dogs with fitted balloon catheter. The insulin suppository (●) contained only 50 U of insulin g^{-1} suppository weight and the insulin suppository (○) contained 50 mg of enamine and 50 U of insulin g^{-1} suppository weight. The adjuvant suppository contained 100 mg of enamine g^{-1} suppository weight. The balloon catheter was inserted to a depth of 4 cm from anus before administration of the insulin suppository to avoid its escape from the administered site. Each value represents the mean ± s.d. (n = 3).

microenemas were found to be superior to triglyceride suppositories since release of insulin from the suppository base was much the slower. Given that adjuvants are rapidly released from the suppository base and absorbed from the site of administration, the rate of insulin dissolution needs to coincide closely with the release of adjuvant in order to effect an increase in the bioavailability of insulin. However, as we have now shown, rapid insulin release from the suppository and subsequent rapid adjuvant-enhanced absorption of insulin does not maintain high serum insulin long enough to effectively decrease serum glucose, especially in depancreatized dogs.

Rather than facilitating the release of insulin from the suppository base, it would seem to be more important to use enamine to prolong the insulin absorption and this was achieved by administration of the enamine suppository after the insulin suppository.

This work was supported in part by a grant from R. P. Scherer North America, Detroit, Michigan USA.

REFERENCES

Kamada, A., Nishihata, T., Kim, S., Yamamoto, M., Yata, N. (1981) Chem. Pharm. Bull. 29: 2012–2019

Kim, S., Nishihata, T., Kawabe, S., Okamura, Y., Kamada, A., Yagi, T., Kawamori, R. Schichiri, M. (1984) Int. J. Pharm. 00: 178–186

Nakagawa, S., Sasaki, T., Horiuchi, T. (1972) Radioisotop. 21: 97–101

Nishihata, T., Rytting, J. H., Kamada, A., Higuchi, T. (1981a) Diabetes. 30: 1065–1067

Nishihata, T., Rytting, J. H., Higuchi, T., Caldwell, L. (1981b) J. Pharm. Pharmacol. 33: 334–335

Nishihata, T., Kim, S., Morishita, S., Kamada, A., Yata, N., Higuchi, T. (1983a) J. Pharm. Sci. 72: 280–285

Nishihata, T., Rytting, J. H., Kamada, A., Higuchi, T, Routh, M., Caldwell, L. (1983b) J. Pharm. Pharmacol. 35: 148–151

Nishihata, T., Kamikawa, K., Takahata, H., Kamada, A. (1984) J. Pharm. Dyn. 7: 143–150

Sollenberger, P. Y., Martin, R. B. (1970) J. Amer. Chem. Soc. 92: 4261–4270

Yagi, T., Hakui, N., Yamasaki, Y., Kawamori, R., Shichiri, M., Abe, H., Kim, S., Miyake, M., Kamikawa, K., Nishihata, T., Kamada, A. (1983) Ibid. 35: 177–178