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An effective formulation for an insulin suppository; examination in normal dogs

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Summary

A solid dispersion of insulin with sodium salicylate or mannitol could result in the rapid release of insulin from suppositories and a significant decrease in plasma glucose concentrations in dogs after the administration of the insulin suppository even at doses as low as 0.5 U/kg or less in dogs. Furthermore, it has been shown that high plasma insulin concentrations can be maintained for more than 1 h. This results in a decrease of plasma glucose concentrations in normal dogs for more than 1.5 h, which was required for effective treatment in decreasing plasma glucose concentrations in depancreatized dogs (Nishihata et al, 1985a; Okamura et al., 1985). The addition of lecithin to the suppository base resulted in a prolonged effect of salicylate as an adjuvant in suppositories (i.e. > 1.5 h.), due to the slow release of sodium salicylate. Thus, a preparation of a solid dispersion of insulin and a triglyceride base containing lecithin seems to be an effective suppository for lowering blood glucose.

Introduction

We have demonstrated that sodium salicylate and enamine derivatives are effective adjuvants in promoting insulin absorption through the rectal mucosa (Nishihata et al., 1981a and b, 1983 and 1985a and b; Kim et al., 1983; Yagi et al., 1983). The insulin suppositories were prepared by the addition of an acidic insulin solution to the triglyceride base to improve the bioavailability of insulin by facilitating the release rate of insulin from the suppository (Nishihata et al., 1985a; Okamura et al., 1985; Liversidge et al., 1985), since the dissolution rate of powdered insulin from such a suppository was very slow (Nishihata et al., 1983). This insulin suppository caused a significant decrease in plasma glucose concentrations in healthy human subjects at a dose of 1.5 U insulin/kg or more (Nishihata et al., 1986), and was effective at a dose of 1.5 U insulin/kg or more in depancreatized dogs (Nishihata et al., 1985a; Okamura et al., 1985). Although it is possible that such an insulin suppository prepared by incorporation of an acidic insulin solution may be effective in human diabetics, a reduction in the insulin dose may be desired for frequent clinical use.

In the present study, a more effective formulation of an insulin suppository was developed to

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reduce the insulin dose. This was done by improving the release of insulin from the suppository as suggested previously (Nishihata et al., 1983 and 1985a; Kim et al., 1984; Okamura et al., 1985; Liversidge et al., 1985). Sodium salicylate was used as an adjuvant. In addition to its effectiveness in enhancing rectal absorption, salicylate has been reported to improve the blood disease which sometimes appears in diabetics (Yue et al., 1984 and 1985).

Materials and Methods

Materials

Human insulin (27.3 U/mg) was supplied by Nihon NOVO (Tokyo, Japan). Sodium salicylate and natural soya lecithin were obtained from Nakarai Chemicals Co., Ltd. (Koyto, Japan) and Wako Pure Chemicals Co., Ltd. (Osaka, Japan), respectively. Pharmasol A-100 (m.p. 41–43 °C) and Pharmasol B-100 (m.p. 33–35 °C) as triglyceride bases for suppositories were supplied by Nippon Oil and Fat Co., Ltd. (Tokyo, Japan). Other reagents used were of analytical grade.

Preparation of the suppositories

Codes and constituents of suppositories used in this study are listed in Table 1. Suppositories of Code 1 to Code 10 were prepared by mixing all constituents thoroughly at 45 °C. The molten mass was poured into a suppository mold at room temperature. Solid dispersions of insulin with sodium salicylate or mannitol were prepared as follows. Insulin powder was dissolved in 0.05 M citric acid solution containing 0.001% polysorbate 80 and then sodium salicylate or mannitol was added to the solution gradually to obtain complete dissolution. The solution was dried to obtain a solid dispersion of insulin. Suppositories of Codes 11 and 12 were prepared as follows. Insulin powder was dissolved in 0.1 M citric acid solution containing 0.001% polysorbate 80 and the insulin solution was added to a molton mass of that suppository base at 45 °C and mixed well. Sodium salicylate was then added to the molten mass and mixed thoroughly after each addition. After the additives were added, the molten mass was poured into suppository molds at room temperature. The suppositories were kept at 4 °C before use. The melting temperature for each suppository (about 10 mg) (Table 2) was measured with a Differential Scanning Calorimeter, Model DSC SSC/560U of Daini Seikosha Co., Ltd. (Tokyo, Japan), with a scanning rate of 0.4 °C/min.

TABLE 1

Codes and constituents of suppositories

Code	Base		Sodium salicylate (mg)	Insulin (U)
	Triglyceride (mg)	Lecithin (mg)		
1	630 *	70	299.93	2
2	630 *	70	299.82	5
3	630 *	70	299.63	10
4	630 *	70	298.76	20
5	630 *	70	298.89	30
6	630 *	70	298.15	50
7	700 *	0	298.89	30
7	700 **	0	298.89	30
8	560 *	140	298.89	30
9	490 *	210	298.89	30
10	540 *	60	300	30
11	630 *	70	300	50
12	700 **	0	300	50

Codes 1–9, use of solid dispersion form of insulin with sodium salicylate (amounts of sodium salicylate and insulin is 300 mg/g suppository).
Code 10, use of solid dispersion form of insulin with mannitol (amounts of mannitol and insulin is 100 mg/g suppository).
Codes 11 and 12, use of acidic insulin solution (volume of the insulin solution added is 50 μl/g suppository)
* Mixture of triglyceride of Pharmasol A-100 and Pharmasol B-100 with a ratio of 40:60. ** Pharmasol B-100.

In vitro dissolution of insulin and sodium salicylate from suppositories

A 1-g suppository wrapped with gauze was immersed in 3 ml sodium phosphate buffer (0.05 M pH 7.4) containing 0.001% polysorbate 80 in a test tube at 37 °C and after 0.5 h and 1 h, 200-μl aliquots were collected through a millipore filter (pore size 0.45 μm) to measure the concentrations of insulin and sodium salicylate dissolved in the buffer. Polysorbate 80 was added to the buffer to inhibit the adsorption of insulin to the glassware. Assays of insulin (Liversidge et al., 1985) and salicylate (Nishihata et al., 1981) were performed

by high performance liquid chromatographic methods.

In vivo study

In the study of rectal insulin absorption, 9 beagle dogs, 11.2–12.9 kg, divided into 3 groups were used, each group involving 3 dogs. After administration of the insulin suppository to the dogs, blood samples were taken from the femoral vein at designated times and centrifuged to obtain plasma. Suppositories of Codes 5 and 7–12 were examined with 3 dogs in group 1. Suppositories of Codes 1–6 were examined with 3 dogs in group 2. In another study using 3 dogs in group 3, the effective periods of salicylate as an adjuvant in suppositories of Codes 5, 7 and 9 were investigated as follows. A ballon catheter was inserted into the rectum to a depth of 4 cm from the anus (Nishihata et al., 1985a) before the administration of each suppository without insulin (referred to as the salicylate suppository). At 0.5, 1.0, 1.5, 2.0 and 2.5 h after the administration of the salicylate suppository, 0.2 ml insulin solution (30 U of insulin dissolved in 0.2 ml saline) was administered and then blood samples were taken at designated times. After centrifugation of blood, plasma glucose concentrations were assayed with an assay kit (Glucose Test Wako, Wako Pure Chemicals Co., Ltd.) based on the enzymatic method.

Statistical analyses

Statistical analyses were performed using Student's *t*-test.

Results

Effect of a solid dispersion of insulin in the suppository

After the administration of suppositories of Codes 11 or 12 containing 50 U insulin to dogs, a significant lowering of glucose concentrations in plasma was observed from 20 min to 90 min, with minimum glucose concentrations of 46.6 ± 6.2 mg/100 ml at 40 min and 59.2 ± 6.9 mg/100 ml at 60 min, respectively (Fig. 1A). The Code 10

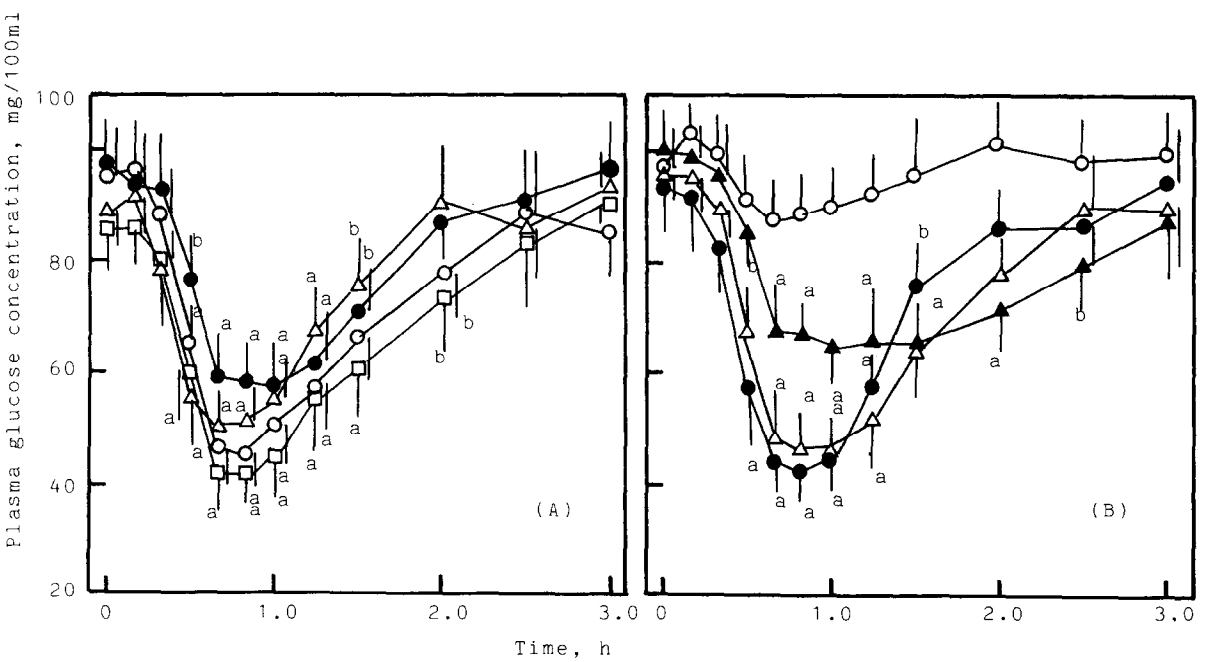

Fig. 1. Plasma glucose concentrations in normal dogs after the administration of each suppository. A: □, Code 5; ○, Code 10; ●, Code 11; △, Code 12. B: ○, Code 7; ●, Code 7′; △, Code 8; ▲, Code 9. Each value represents the mean ±S.D. ($n = 3$, cross-over study). Body weights of the 3 dogs were 11.9, 12.1 and 12.4 kg. a, $P < 0.01$ vs zero time; b, $P < 0.05$ vs zero time.

TABLE 2

Melting ranges of suppositories.

Code	Temperature of melt (°C)	
	Start	End
1	34.5	37.2
2	34.8	37.0
3	35.1	37.2
4	34.1	36.8
5	34.2	36.9
6	34.1	36.7
7	35.9	41.9
7	33.4	35.6
8	33.2	35.8
9	31.1	34.3
10	34.6	36.8
11	34.7	37.0
12	33.1	35.8

Temperature was increased at a rate of 0.4°C/min.

suppository containing 30 U insulin also caused a significant decrease of plasma glucose concentration from 30 min to 120 min, with the minimum glucose concentration of 42.2 ± 4.7 mg/100 ml at 50 min after the administration. This result from the Code 10 suppository, which was similar to Codes 11 and 12 in spite of a lower insulin dose in Code 10, seems to be due to greater release of insulin from the Code 10 suppository containing a solid dispersion of insulin with mannitol (i.e. the amount of insulin released from Code 10 was similar to those from Codes 11 and 12) (Table 3).

TABLE 3

Dissolution of insulin and salicylate from suppositories

Code	Insulin		Salicylate	
	0.5 h	1 h	0.5 h	1 h
1	73.2±5.9	91.4±1.6	62.3±4.8	93.6±2.4
2	71.4±4.9	91.2±5.9	66.3±3.7	97.1±5.2
3	68.6±8.1	90.9±1.6	61.3±3.7	94.2±4.9
4	70.3±6.2	93.2±7.0	61.1±4.6	94.2±4.9
5	67.9±5.1	92.2±7.9	69.1±8.4	90.2±4.6
6	68.2±7.1	90.6±4.4	70.4±2.5	91.1±3.1
7	16.7±4.5	22.1±8.2	21.3±9.6	44.4±7.1
7′	79.4±9.2	96.1±4.7	83.9±5.2	96.6±2.0
8	74.3±6.9	86.7±3.8	64.3±7.2	86.1±7.2
9	49.8±6.1	70.4±3.8	39.2±7.5	72.4±8.1
10	68.4±8.3	89.7±5.9	72.6±5.1	96.9±4.9
11	47.2±7.4	54.6±6.1	86.4±5.2	94.1±3.9
12	46.7±7.3	59.2±7.1	90.3±4.2	95.6±8.1

Values are %.

Another solid dispersion of insulin prepared with sodium salicylate was examined. After the administration of the suppository of Code 5 containing 30 U insulin, significantly lower glucose concentrations in plasma were observed from 30 min to 120 min, with a minimum glucose concentration of 40.9 ± 6.1 mg/100 ml at 40 min (Fig. 1A). No differences between Code 5 and Code 10 supportories were observed in the profiles of plasma glucose concentrations (Fig. 1). Furthermore, no differences in the in vitro dissolution percentages of insulin from each suppository (Table 3) were observed.

Effect of lecithin in the suppository

Using a solid dispersion of insulin with sodium salicylate, the effect of lecithin content in the suppository base was studied at a dose of 30 U insulin. Among the suppositories of Codes 5, 7, 8 and 9 the slowest release of both insulin and salicylate was observed in the Code 7 formulation containing no lecithin. The addition of lecithin at 10% w/w (Code 5) or 20% w/w (Code 8) compositions in the base promoted the release of both insulin and salicylate from the suppository. The effect of lecithin in the base in promoting insulin release may be due to a drop in the melting temperature (Table 2). That is suggested since the suppository of Code 7′ prepared with triglyceride base melts at a low temperature in spite of no lecithin being added caused a rapid release of both insulin and salicylate. However, the addition of lecithin in 30% w/w (Code 9) caused a slow release of both insulin and salicylate in spite of the suppository melting at a low temperature. We did not investigate in detail why the increase of lecithin content (30% w/w) decreased the release percent of both insulin and salicylate. It may be possible that a large lecithin content (30% w/w) in the suppository may increase the viscosity of the base during melting, resulting in the slow release of ingredients from the base. In the present study, it appears that the optimum content of lecithin in the insulin suppository is between 10 and 20% w/w.

In the in vivo study, the administration of a

Code 8 suppository caused a significant decrease in plasma glucose concentrations in a similar fashion with that after the administration of a Code 5 suppository (Fig. 1). The suppository of Code 7′ also caused a significant decrease of plasma glucose concentrations, but the periods of low glucose concentrations observed was shorter than those after the administration of a Code 5 or a Code 8 suppository (Fig. 1B). After the administration of a Code 9 suppository, a significant decrease in plasma glucose concentration was observed for a longer time, i.e. 40–150 min (Fig. 1B). But the minimum glucose concentration was 62.9 ± 8.7 mg/100 ml at 60 min, which was higher than with Codes 5 and 8 (Fig. 1B). The administration of Code 7 suppositories caused only a slight decrease in plasma glucose concentrations. Thus, the release rates of both insulin and salicylate from the suppositories seem to be related to their effectiveness in decreasing plasma glucose concentrations.

Study on the effective period

The effective period of salicylate as an adjuvant was examined with Codes 5, 7′ and 9 suppositories as described in the experimental section. Codes 5 and 9 suppositories without insulin (referred to as the salicylate suppositories) caused a decrease in plasma glucose concentrations following administration of an insulin solution even at 1.5 h and 2.0 h, respectively, after the administration of the salicylate suppository (Fig. 2A and C). The salicylate suppository of Code 7′ also decreases plasma glucose concentrations significantly after administration of an insulin solution up to 1 h after the administration of the salicylate suppository (Fig. 2B). The longer effective time of Code 9 suppositories in decreasing glucose concentrations as shown in Fig. 1B seems to be due to the longer times of effective concentrations of salicylate after Code 9 suppositories which have a higher lecithin content.

Investigation of the effective dose of insulin

Using the suppository base containing 10% w/w lecithin, the effect of the insulin dose was examined. In Table 3, the release rate of neither insulin nor salicylate was influenced by the amount

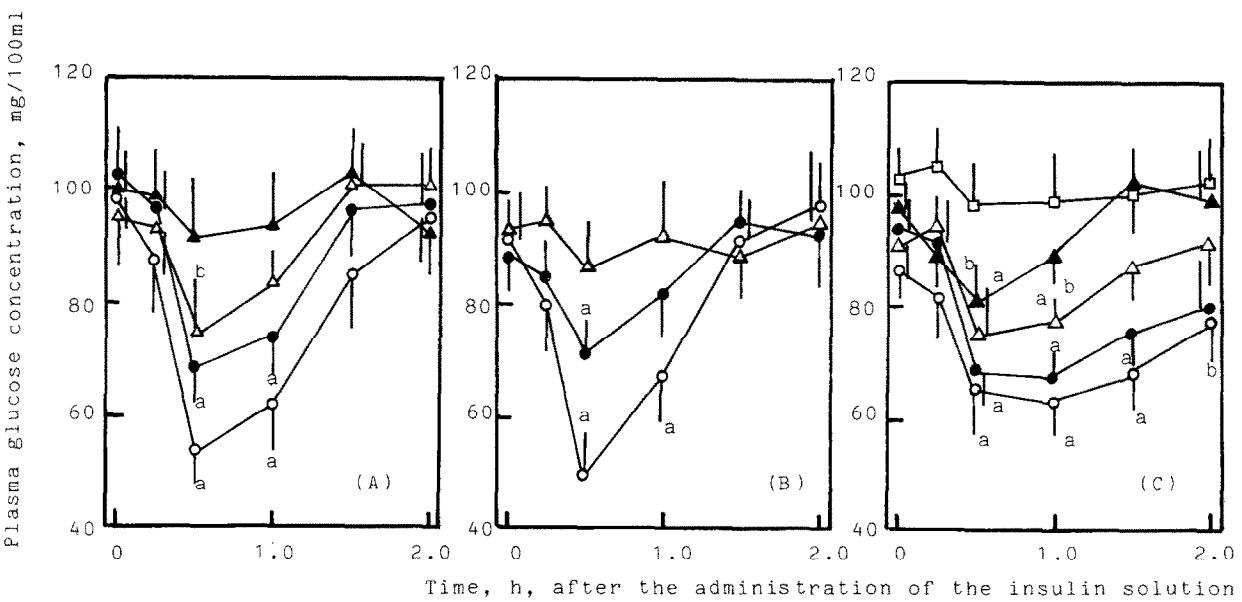

Fig. 2. Plasma glucose concentrations after the administration of 0.2 ml insulin solution at 30 U of insulin at 0.5 (○), 1.0 (●), 1.5 (△), 2.0 (▲) and 2.5 h (□) after the administration of the salicylate suppository of Code 5 (A), Code 7′ (B), or Code 9 (C). Each value represents the mean ± S.D. ($n = 3$, cross-over study). Body weights of the 3 dogs were 11.2, 11.7, and 12.6 kg. a, $P < 0.01$ vs zero time; b, $P < 0.05$ versus zero time.

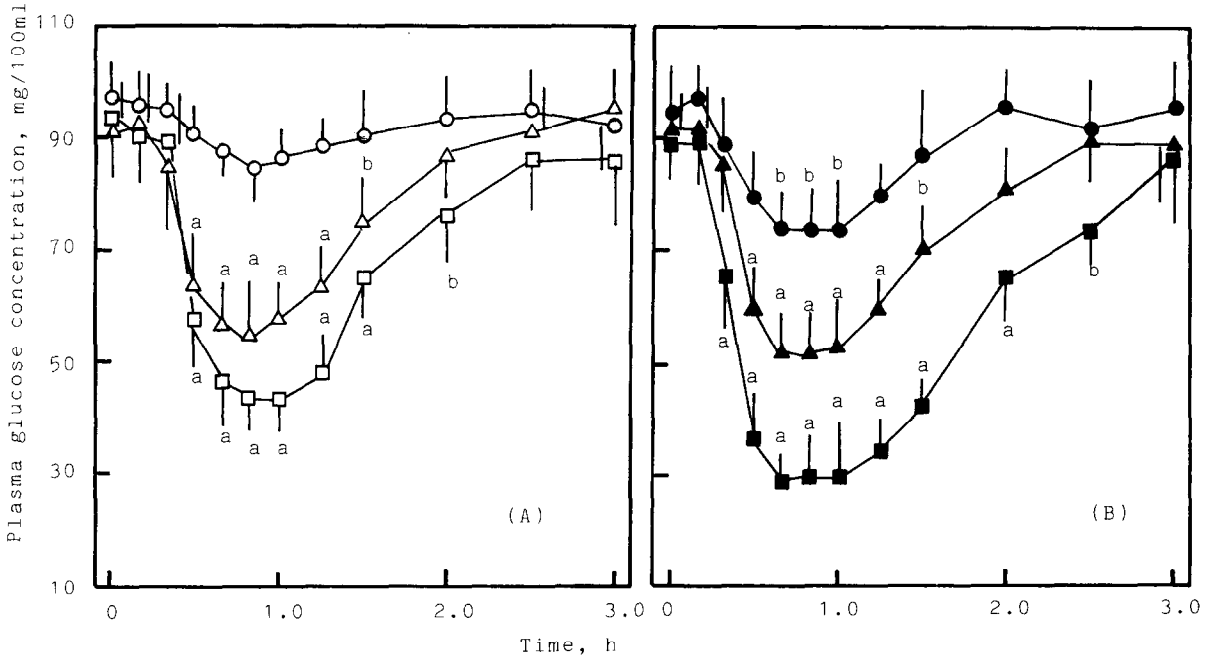

Fig. 3. Plasma glucose concentrations in dogs after the administration of each suppository. A: ○, Code 1; △, Code 3; □, Code 5. B: ●, Code 2; ▲, Code 4; ■, Code 6. Each value represents the mean ± S.D. ($n = 3$, cross over study). Body weights of the 3 dogs were 11.6, 12.2 and 12.9 kg. a, $P < 0.01$ vs zero time; b, $P < 0.05$ vs zero time.

of insulin in the suppositories (Codes 1–6). An increase in insulin dose in the suppositories caused a significant decrease in plasma glucose concentrations (Fig. 3). The minimum effective dose of insulin in this formulation was 5 U insulin in normal dogs (less than 0.5 U/kg b. wt.).

Discussion

An effective formulation of the insulin suppository along with a reduction of insulin dose might be of importance in insulin therapy. We have already reported that an insulin suppository prepared by the addition of an acidic insulin solution in triglyceride base was an effective suppository, with somewhat rapid release of insulin in comparison with the addition of insulin powder, in normal dogs, in depancreatized dogs (Nishihata et al., 1985a; Okamura et al., 1985) and in healthy humans (Nishihata et al., 1986). The administration of the above suppository at a dose of 1.5 U insulin/kg b. wt. or more in dogs and in humans could cause a significant decrease in plasma glucose concentrations. In the present study, the use of a solid dispersion of insulin with mannitol or sodium salicylate in the suppository caused almost complete release of insulin within 1 h in comparison with the earlier suppository formulation which had about 50% dissolution within 1 h (Table 3). With the solid dispersion of insulin with sodium salicylate, even doses of 0.5 U insulin/kg or less were effective as an insulin suppository, because a dose of 5 U insulin in the suppository caused a significant decrease in plasma glucose concentrations in dogs with body weights of 11.2–12.9 kg. Thus, the insulin suppository developed in the present study is an effective form to reduce the necessary insulin dose.

Earlier we reported that a suppository containing lecithin caused a sustained release of sodium diclofenac (Nishihata et al., 1985b). To prepare a suppository which melts between 33 and 37 °C, a mixture of triglycerides melting at low and high temperatures (suppositories of Codes 1–7) were

used. That is because the addition of lecithin resulted in a drop of the melting temperature as shown in Table 2. The addition of 30% w/w lecithin in the base caused a prolonged effect of salicylate, due to the slow release of salicylate from the suppository. Since nearly all salicylate released was absorbed and the effect of salicylate is dependent on its concentration in rectal luminal fluid (Sithingorngul et al., 1983), slow release seems to be required for prolonged action.

It has been reported that portal insulin delivery resulted in less hyperinsulinemia in depancreatized dogs than peripheral delivery (Goriya et al., 1979 and 1980). A substantial part of the insulin rectally absorbed enters the portal vein and consequently the liver (Tiran et al., 1979). Although the liver is the major organ of insulin degradation (Rojmark et al., 1981), it also is the locus of highest insulin utilization. An increase in portal insulin concentration increases the magnitude of net hepatic glucose uptake (Berman and Bucolo, 1974). All these findings substantiate the suggestion of Ritschel and Ritschel (1983) that peripherial s.c. or i.v. insulin administration may not be optimal and have undesirable pharmacological effects (Tiran et al., 1979). Portal insulin delivery is important in normalizing both glycemia and insulinemia postprandially (Yamasaki et al., 1981). Insulin is the first drug where the presence of first-pass effect is essential in eliciting the pharmacological response. Thus, rectal insulin delivery by the administration of the insulin suppository may be a rational dosage form for antidiabetic therapy.

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