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Effect of rectal suppository formulation on the release of insulin and on the glucose plasma levels in dogs

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Summary

The effects of formulation, such as acetic acid content and suppository size, on the plasma levels of glucose and sodium salicylate after rectal administration of a suppository containing insulin and sodium salicylate were studied. An HPLC method is described for measuring insulin content in suppositories. Mechanisms are proposed that account for the differences observed in plasma levels of glucose and sodium salicylate from the various formulations.

Introduction

In recent years, there has been considerable interest in the development of polypeptide drugs. However, most have low oral bioavailability due mainly to degradation by protease enzymes in the gastrointestinal (G.I.) tract. In contrast, rectal administration offers a number of advantages, i.e. reduced polypeptide degradation and much greater availability particularly with the advent of adjuvant enhanced absorption (Nishihata et al., 1983b). As a consequence, we are developing new suppository formulations which will be required for polypeptide drugs. Our

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model drug is insulin, a long established polypeptide of high molecular weight and low dose, which is normally administered by intramuscular or subcutaneous injection for the control of diabetes. A rectal formulation would have considerable advantages over an intramuscular or subcutaneous route.

A number of adjuvants have been used to enhance the absorption of insulin across the rectal membrane with varying degrees of success (Mesiha et al., 1981; Bar-On et al., 1981; Yamasaki et al., 1981; Schichiri et al., 1978). Suppository formulations have been proposed, but these show poor effects on the reduction of plasma glucose levels (Yamasaki et al., 1981; Schichiri et al., 1978) and make no mention of the in vitro release of insulin in the formulations. The present work describes a simple yet sensitive HPLC method to determine insulin release from suppositories and the effect of insulin suppositories on the glucose levels in normal dogs using sodium salicylate as the primary adjuvant.

Materials and Methods

Crystalline porcine insulin (Eli Lilly & Co.), glacial acetic acid analytical grade, Witepsol S55, aprotinin (Sigma), sodium dihydrogen phosphate, phosphoric acid, acetonitrile and hydrochloric acid were used as purchased. Sodium salicylate (99% +) (Aldrich) was subjected to grinding and sieving to obtain particles in the size range of 150–175 μm.

Five normal healthy adult beagle dogs, 10.6–14.5 kg, were used in this study. The animals were fasted with water 24 h, water was allowed ad libitum. Suppositories were inserted so that the lowest portion was 4 cm from the outer rectal sphincter and blood samples were taken from the cephalic vein at the following time intervals: −15, 0, 15, 30, 45, 60, 90, 120, 180 and 240 min. Blood was withdrawn at the same time intervals after intravenous administration of insulin.

Plasma was obtained from blood samples and analyzed for sodium salicylate content by HPLC (Nishihata et al., 1982), and glucose levels were determined by colorimetric analysis using orthotoluidine (Sigma 635 Technical Bulletin).

Suppositories were prepared in the following manner: crystalline insulin (1 mg/23 I.U.) was dissolved in 5% v/v acetic acid solution (pH 2.7) added to molten

TABLE 1

CODE AND CONSTITUENTS OF SUPPOSITORIES LISTED IN TABLES 2 AND 3

Code	Witepsol S55 (mg)	Sodium salicylate (mg)	Insulin (mg)	5% v/v acetic acid solution (μl)
A	317	340	1	11
B	264	330	1	55
C	209	330	1	110
D	1730	340	1	60
E	1498	330	1	312
F	1198	330	1	631

Witepsol S55 at 44°C and mixed well. Sodium salicylate was then added (see Table 1) in aliquots and mixed thoroughly after each addition. When the additions were completed, the molten mass was poured into a suppository mold at room temperature. Suppository samples were tested for uniformity of drug content and were found to be uniform.

HPLC assay of insulin in suppositories

A suppository was melted and mixed thoroughly at 46°C with 20 ml of a mixture of 5% v/v acetic acid and 0.37% v/v hydrochloric acid (pH 2.0) for 30 min. No further insulin or sodium salicylate was released after this time. The mixture was centrifuged and a sample (50 μl) from the aqueous layer was injected into the precolumn loop. The analysis was performed using a column switching method. The first column (precolumn loop) was 30 mm × 4.6 mm reverse-phase column (MPLC RP-GU RP-C18, particle size 10 μm, Brownlee Labs). The initial solvent system consisted of 32% v/v acetonitrile and 68% v/v water with a flow rate at 2 ml/min.

Four minutes after sample injection the mobile phase was changed to 32% acetonitrile, 0.1 M NaH_2PO_4/H_3PO_4 (pH 2.1) and the precolumn loop was connected to the analytical column (analytical column consisted of a 3 cm guard column and a 10 cm analytical column (analytical column consisted of a 3 cm guard column and a 10 cm analytical column, both were reverse-phase C_{18}, particle size 5 μm Brownless Labs). The eluant was monitored using a Waters variable wavelength spectrophotometer detection model 450 at 200 nm. Waters Model M45 pumps were used and column switching was performed using Rheodyne 1725 injector.

The calibration curve for insulin was performed by adding known quantities of insulin and sodium salicylate to a 20 ml mixture of 5% v/v acetic acid/0.37% v/v hydrochloric acid solution. The resulting mixture was centrifuged, and samples (50 μl) from the aqueous layers were analyzed by HPLC. A plot of insulin peak height vs concentration in the range 200–25 μg/ml of the aqueous layer (n = 3 at each of 5 points on the curve) gave a linear correlation coefficient of 0.9971 and passed through the origin. The relative coefficient of variation at 250 μg/ml was 8% and at 25 μg/ml was 15%.

Results and Discussion

In a recent study, Yamasaki et al. (1981) have reported a highly significant correlation between the dose of rectally administered insulin and nadir plasma glucose levels. The glucose levels after the nadir do not bear a relationship to the amount of insulin absorbed. This lack of relationship is attributed to the glycaemic rebound. Consequently, measurements of glucose AUC, unlike glucose nadirs, will not give an indication of the absorption of insulin.

It has been reported (Nishihata et al., 1983a) that suppositories containing insulin crystals and adjuvant show very low insulin bioavailability compared to a microenema. This has been attributed to the slow dissolution of the insulin in the rectal compartment. Thus, with insulin suppositories, the rate-limiting step in the bioavail-

ability appears to be the dissolution of insulin into the aqueous rectal fluid. For this reason, crystalline insulin was dissolved in 5% v/v acetic acid solution then mixed with the molten suppository base. At concentrations lower than 5% v/v of acetic acid in water insulin will not dissolve. If the strong mineral acids, e.g. hydrochloric acid, were used they would cause rectal tissue damage. The suppository base Witepsol S55 was chosen for the following reasons: (1) high proportions of aqueous solutions, e.g. 5% v/v acetic acid, can be incorporated in the base; (2) high proportions of solids, e.g. sodium salicylate, can be readily dispersed in the base; (3) it exhibits a short solidification time which prevents sedimentation of sodium salicylate in the molds; and (4) it is a liquid at body temperature. Using dogs it has been found that the dose of sodium salicylate administered and the amount of insulin absorbed are directly proportional until a limiting quantity of sodium salicylate is reached. Doses in excess of approximately 330 mg of sodium salicylate produce negligible increases in insulin absorption. Consequently, sodium salicylate was incorporated at a dose of approximately 330 mg in each suppository to obtain the optimum adjuvant effect. To facilitate comparison of the present work with that of others, e.g. Yamasaki et al. (1981), a similar quantity of insulin (1 mg/23 I.U.) was used.

A major problem with rectal administration is the inter-patient variability in absorption, which has been attributed to a number of factors the most important one among these being variable spreading of the molten suppository. In an attempt to reduce the variability in spreading one can reduce the suppository size by decreasing the quantity of excipient present. A consequent reduction in the variability of absorption should be shown by a small suppository in comparison to a large suppository. To achieve the comparison a standard 2 g suppository mold was used to manufacture the large suppositories (Table 1, codes D–F). The small suppository mold (550 mg) was arrived at by determining the minimum quantity of Witepsol S55 that could be used to manufacture suppositories that contained 330 mg of sodium salicylate, 110 μl of acetic acid solution and 1 mg of insulin and still retained suppository characteristics, e.g. molten at body temperature, etc. (see Liversidge et al., 1981). A comparison of the bioavailability data using the small and large suppositories showed no significant differences in the variability of absorption. However, the smaller suppositories showed greater bioavailability of insulin (measured as glucose nadirs).

It has been stated above that insulin is more readily absorbed when in solution. We hypothesize the the greater the aqueous content of the suppository the better the absorption of insulin. To test this hypothesis the maximum amount of acetic acid solution that Witepsol S55 could contain was incorporated into two formulations (Table 1, codes C and F) and the minimum amount of acetic acid that would dissolve 1 mg of insulin was incorporated into a further formulation (Table 1, code A). Thus, the two extremes of maximum and minimum acetic acid content were tested. An intermediate suppository was also tested (Table 1, code B and E). A comparison of the bioavailability data from similar-sized suppositories (Table 3) shows that upon increasing the aqueous content an increase in the bioavailability of insulin occurs.

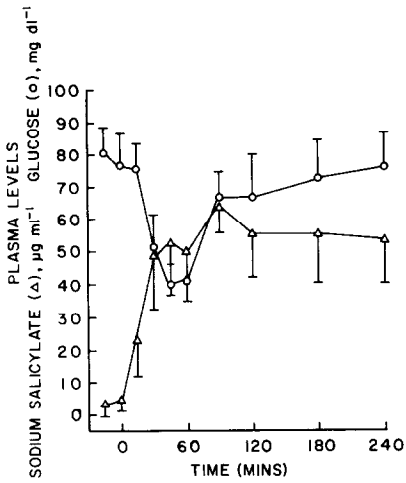

Fig. 1. Plasma levels of glucose and sodium salicylate after rectal administration of Type C suppositories (±S.D. for n = 5 at 95% confidence intervals).

In vivo release

Fig. 1 shows the simultaneous plasma levels of glucose and sodium salicylate after the rectal administration of a suppository whose formulation is given in Table 1. The initial rapid absorption of sodium salicylate is followed by the initial rapid drop in glucose levels. This is again demonstrated by other formulations (see Table 3). It would appear that the passage of insulin across the membrane is dependent upon the rate of flux of the sodium salicylate, glucose nadirs following closely upon the most rapid absorption rates of sodium salicylate. However, it must be noted that the lowering of glucose levels does not bear a simple relationship to the plasma sodium salicylate levels; we have tried unsuccessfully to find a relationship using a large number of suppository formulations.

The decreases in glucose levels using the formulations proposed in this paper are much greater than those shown by suppositories containing crystalline insulin (Nishihata et al., 1983).

TABLE 2

PERCENT OF INSULIN RELEASED FROM SUPPOSITORY FORMULATION (GIVEN IN TABLE 1)

Formulation	Insulin released (%)
A	98
B	109
C	118
D	54
E	74
F	89

TABLE 3

SUMMARY OF IN VIVO DATA FOR SODIUM SALICYLATE AND GLUCOSE AFTER ADMINISTRATION OF A SUPPOSITORY (SEE TABLE 1 FOR FORMULATIONS)

Suppository code	Sodium salicylate					Glucose	
	$AUC_{0-240min}$ ($\mu g \cdot ml^{-1} \cdot min^{-1}$)	C_{max} ($\mu g/ml$)	t_{max} (min)	Max absorption * rate ($\mu g \cdot ml^{-1} \cdot min^{-1}$)	time of max absorption (min)	Glucose nadir % of pr[illegible]ient level	time of nadir (min)
A	5392 ± 1062	30 ± 10	240	0.80	15–30	78 ± 15	60
B	8438 ± 1121	40 ± 15	90	0.80	15–30	73 ± 10	45
C	12415 ± 963	50 ± 8	45	1.67	15–30	58 ± 6	45
D	2282 ± 455	30 ± 12	180	0.67	0–15	78 ± 9	45
E	5815 ± 507	30 ± 14	120	0.67	0–15	78 ± 20	60
F	7012 ± 700	33 ± 7	45	0.93	15–30	70 ± 15	60

* Taken as change in plasma levels.
Values are ± S.D. for n = 5.

Classically, the rate of absorption of a drug is proportional to the surface area available. It would thus be expected upon increasing the size of the suppository, a consequent enhancement of absorption would occur as the available area for absorption is increased due to greater spreading of the larger suppository. However, this relationship proves not to be applicable in this case. Consider Table 3, Formulation C and F, if one looks at the sodium salicylate data, it can be seen that the smaller suppository, with similar proportions of excipient constituents, gives a higher plasma level and more rapid absorption rate. These phenomena may be attributed to a number of factors which can be divided into: (1) formulation factors; and (2) physiological factors. First, consider formulation factors: (i) the mean transit time of a sodium salicylate particle from the interior of a large suppository to the aqueous interface will be greater than for a small suppository; (ii) the rate of dissolution of sodium salicylate particles is related to the rate at which the particles are presented to the lipid/aqueous interface, termed 'drainage time' by Schoonen et al. (1980). As the layers of sodium salicylate particles increase on the lipid side due to sedimentation, the drainage time decreases. Consequently, dissolution is increased. Small suppositories show a greater stacking of particles on the lipid side than do large suppositories due to a higher concentration of particles. Consequently, the interfacial process is more readily overcome giving a higher release of sodium salicylate. (iii) Particles may agglomerate more easily in a small suppository due to their close proximity causing an increased sedimentation rate and a greater ability to penetrate the interfacial surface (Crommelin and De Blaey, 1980; Schoonen et al., 1980). (iv) Differences in melting times of the formulations could account for the differences observed. However, the melting times at 37°C for the formulation ranged from 3 to 6 min and could not account for the large differences observed.

Considering next the physiological factor, a reduction in absorption may occur higher up the rectum. This would cause a reduction in absorption from a large suppository due to a greater spreading of the molten mass up the rectum. We have found in rat studies that increasing the size of the suppository 4-fold causes an increase in spreading up the rectal passage. We would expect similar spreading behavior in dogs when the suppository size is increased 4-fold, thus exposing the molten suppository mass to absorptive sites higher up the rectum.

If one looks at the plasma glucose levels, it can be seen again that the smaller suppository has a greater effect. In explaining this phenomenon, we must consider the rate of presentation of sodium salicylate to the membrane and its effects on insulin absorption. Thus a more complex situation exists as adjuvant effects of both salicylate and acetic acid have to be considered. Obviously, similar arguments to those listed for the formulation and physiological factors above can be applied here. The mechanism of action of sodium salicylate, or in fact any adjuvant, is not yet fully understood and any mechanistic conclusions based upon the formulation would be highly speculative at this time. However, there are some trends that may be interpreted. Consider firstly, the sodium salicylate levels from the small suppository formulations (Table 3, Formulations A, B and C). As the amount of acetic acid in the formulation is increased, the initial rate of absorption and also the plasma levels of sodium salicylate are increased. This may be attributed to either an adjuvant

action of acetic acid upon sodium salicylate or as the aqueous content of the suppository is increased the release of salicylate is increased. It has been shown in a previous paper (Nishihata et al., 1981) that the rate and amount of sodium salicylate absorbed is related to the pH of the solution. As the acetic acid is absorbed, its adjuvant effect on sodium salicylate will be decreased since the pH is increasing. Thus, larger amounts of acetic acid will exert greater adjuvant effects and will tend to increase sodium salicylate absorption. However, when larger amounts of acetic acid are present, the concentration of sodium salicylate in solution will be less. This tends to reduce absorption. Thus, two opposing factors can influence sodium salicylate absorption. Further, acetic acid may itself act as an adjuvant for insulin absorption (Hara et al., 1981). For example, as the amount of acetic acid is increased for the small suppositories, plasma glucose levels decrease with an inverse relationship. However, no such relationship was observed with the large suppositories.

In vitro release

The data on the release of insulin from various suppository formulations are given in Table 2. It can be seen from Table 2 that for the small-sized suppositories, the amount of insulin released is ~ 100%. The trend of increased release with an increase in acetic acid solution content can also be seen from Table 2. This same trend can be noted for the large suppositories. However, the amount of insulin release is lower. This could be attributed to a partitioning of insulin between the triglyceride and aqueous layers. As the triglyceride content is increased, more insulin will be retained in the suppository mass.

It is interesting to note that as the percent of acetic acid is increased, more insulin is released (in vitro data) and a greater decrease of blood glucose levels is achieved (in vivo data). The response of glucose levels to rectally administered insulin in normal dogs is smaller than the response encountered in diabetic dogs (Yamasaki et al., 1981; Shichiri et al., 1978). This phenomenon has also been encountered in rats (Bar-On et al., 1981). Thus, we would expect an even greater decrease of glucose levels when the suppositories were administered to diabetic dogs. The formulations, especially Formulation C, hold great promise in the area of post-prandial hyperglycaemia; and we are presently pursuing the application.

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