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Enhancement of rectal absorption of insulin using salicylates in dogs

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Sodium salicylate and 5-methoxysalicylate both increased the rectal absorption of insulin in dogs when co-administered with insulin in various formulations. Microenema formulations containing 4% gelatin showed the highest insulin bioavailability of the formulations studied whereas microenemas (without gelatin) and suppository formulations were not as effective in enhancing the rectal absorption of insulin.

Since its discovery, insulin has generally been administered parenterally. For many years there has been a search for a practical alternative to this route for the administration of insulin. Shichiri et al (1978), Nishioka & Kawamura (1978) and Patel & Ryman (1976) reported some success using surfactants and insulin encapsulated in liposomes to facilitate rectal absorption. Touitou et al (1978) have reported effective rectal delivery of insulin using a non-ionic, surface-active agent as one of the suppository excipients. In their hands, cetomacrogol 1000 in combination with varying amounts of polyethylene glycol has been effective as a suppository excipient in enhancing the rectal absorption of insulin.

Recently, Nishihata et al (1981b,c) have examined systemic delivery of insulin following rectal or intraduodenal administration in rats using novel water-soluble absorption adjuvants. Insulin absorption from the rat rectum or small intestine was significantly improved in the presence of sodium 5-methoxysalicylate. The objective of the present study was to examine the rectal delivery of insulin in the dog by using sodium salicylate and sodium 5-methoxysalicylate as absorption adjuvants in various insulin formulations.

MATERIALS AND METHODS

Six male beagles, 9·5 to 11·0 kg were used in a cross-over experiment. Dose-response experiments with various levels of insulin or adjuvants were also carried out. Insulin was administered rectally in the form of a 0·5 or a 0·25 ml microenema or in a suppository. Insulin formulations were made up as either 0·9% NaCl (saline) microenemas, saline microenemas with 4% gelatin, or 1 g suppositories with Witepsol H-15 as the suppository base. The microenema, gelatin and suppository formations with and without absorption adjuvants were compared. An intramuscular injection of insulin was used as the control.

Blood samples were taken at intervals from the external jugular vein and treated with EDTA. Blood samples were centrifuged at 4000 *g* for 10 min. Plasma glucose levels were determined at 650 nm using the *o*-toluidine method described by Nishihata et al (1978). Plasma insulin levels were determined at 420 nm using an immunospecific assay (Toyo Jozo Company, Ltd, Japan) (Nakagawa et al 1983). The release rates from suppositories of insulin (Lilly) and sodium salicylate into saline were also measured. A 1 g suppository and a 4% gelatin formulation, each containing 5·0 mg of insulin and 200 mg of sodium salicylate were maintained in 5 ml stirred saline at 37 °C. At regular intervals, 100 µl samples of the solutions were removed, filtered (Millipore, HA, 0·45 µm) and assayed.

RESULTS AND DISCUSSION

A 4% gelatin insulin formulation containing 20 i.u. of insulin with either 150 mg of sodium 5-methoxysalicylate or 300 mg of sodium salicylate was rectally administered to dogs as a 0·5 ml microenema. These dosage forms caused a significant decrease in plasma glucose levels (Fig. 1). Maximum plasma insulin levels occurred within 45 min of rectal administration and correlated closely with plasma levels of the absorption adjuvants which were measured by h.p.l.c. (Nishihata et al 1981a, 1982b). After rectal administration of insulin, the observed

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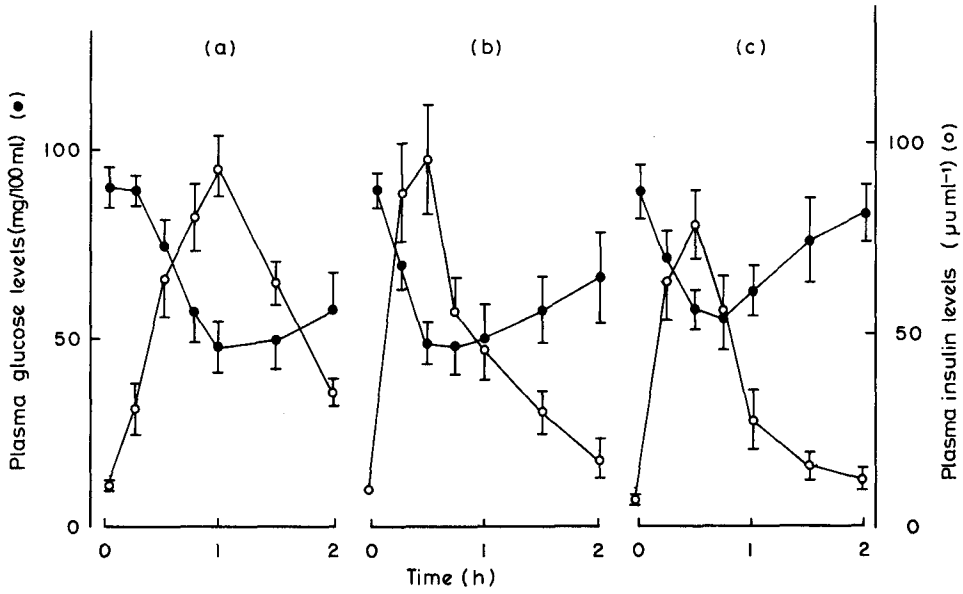

FIG. 1. Mean concentrations of glucose (mg 100 ml^{-1}) and insulin (μ u ml^{-1}) in the plasma of dogs after the administration of (a) an intramuscular injection of 5 u of insulin, (b) a 0·5 ml microenema containing 20 u of insulin with 150 mg of sodium 5-methoxysalicylate in a 0·9% NaCl solution containing 4% gelatin, and (c) a 0·5 ml microenema containing 20 u of insulin with 300 mg of sodium salicylate in a 0·9% NaCl solution containing 4% gelatin. The error bars represent standard errors of the mean with n = 6.

minimum in plasma glucose levels appeared to lag about 15 min behind peak insulin levels. When the adsorption adjuvant was omitted from the rectal dosage form, there was little change in plasma glucose levels.

For comparison purposes, 5 u insulin was administered intramuscularly. This produced a similar delivery profile for insulin except that the changes in plasma glucose and insulin levels occurred about 15 min later than after rectal administration with maximum plasma levels at 1 h post administration (Fig. 1). Peak plasma insulin levels after i.m. injection were roughly the same as for rectal administration; however, the rectal dose was four times the i.m. dosage.

Rectal absorption of insulin from a microenema was significantly promoted after co-administration of 100 mg or more of sodium 5-methoxysalicylate whereas 50 mg of the absorption adjuvant had no significant effect. Sodium salicylate appears to be somewhat less effective as an absorption adjuvant than sodium 5-methoxysalicylate. It was found that 300 mg of sodium salicylate caused a significant increase in rectal insulin absorption whereas 150 mg did not.

With regard to facilitated rectal absorption, there appeared to be a minimum effective insulin dose in the formulation containing sodium 5-methoxysalicylate and 4% gelatin. Insulin in the amount of 5 u produced significant plasma levels concomitant with a decrease in plasma glucose levels. However, 2·5 u of insulin in this same gel formulation was not sufficient to cause a significant decrease in plasma glucose levels after rectal administration in normal dogs.

Rectal administration of an insulin microenema formulation in saline containing 5-methoxysalicylate caused an increase in plasma insulin levels and a decrease in plasma glucose levels (Fig. 2a). However, these changes were not as dramatic as those observed in the corresponding 4% gelatin formulations (Fig. 1b). The efficiency of insulin absorption from the gelatin formulation was further increased when the microenema volume was reduced from 0·5 ml to 0·25 ml which provided an increase in the concentration of the insulin and adjuvant in the microenema (cf Fig. 1b and 2b). These results are consistent with the report by Nishihata et al (1980, 1981b) in which the absorption of drugs was found to increase with an increase in the adjuvant concentration. The rectal administration of the same amount of insulin in a suppository formulation containing 5-methoxysalicylate caused only a small increase in the plasma insulin levels (Fig. 2b) with a simul-

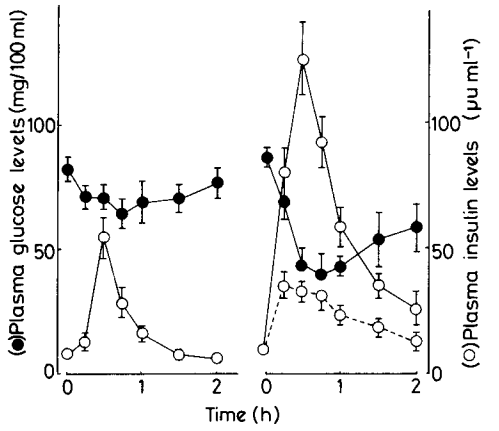

FIG. 2. Mean concentration of glucose (mg 100 ml^{-1}, —●—) and insulin (μ u ml^{-1}, —○—) in the plasma of dogs after the administration of (a) a 0·5 ml microenema containing 20 u of insulin with 150 mg of sodium 5-methoxysalicylate in a 0·9% NaCl solution, (b) a 0·25 ml microenema containing 20 u of insulin and 150 mg of sodium 5-methoxysalicylate in a 0·9% NaCl solution containing 4% gelatin (○——○) or a 1 g suppository (containing 20 u of insulin and 300 mg of sodium 5-methoxysalicylate) with Witepsol H-15 as the base (○- - -○). The error bars represent standard errors of the mean with n = 6.

taneous decrease in the plasma glucose levels. One possible explanation for the apparent lower bioavailability of insulin following the administration of insulin suppositories compared with gelatin formulations may be the relatively slow dissolution rate of crystalline insulin in the suppository formulations compared with the gelatin formulations used in this study (Fig. 3a). Consequently, by the time the insulin has been released from the suppository, the adjuvant may have already been absorbed from the rectal compartment, since it was found that sodium salicylate was rapidly released and absorbed from both these suppository and gelatin formulations.

As previously reported by Nishihata et al (1981a), the drug and adjuvant must be absorbed simultaneously for the adjuvant-enhancing effect to occur. To further examine this hypothesis, a 0·5 g suppository containing 300 mg sodium salicylate was administered 1 h after a suppository containing 20 u of crystalline insulin. As shown in Fig. 3b, a substantial increase in plasma insulin and decrease in plasma glucose concentrations were observed following the administration of the salicylate suppository substantiating the need for adjuvant in the absorption process.

Earlier studies in our laboratory (Nishihata et al 1982a) have indicated that salicylate and related absorption adjuvants do not produce lasting effects on the rectal mucosa when administered as the sodium salt. Using a perfusion technique in the rat, it was shown (Nishihata et al 1981a) that the increased permeability following salicylate treatment was eliminated by washing the rectum with fresh buffer solution. However, enhanced drug absorption persisted after pretreatment with a sodium lauryl sulphate solution followed by washing the rectum with a buffer solution. It was further reported (Nishihata et al 1982a) that only transient changes in the transmembrane potential across the rectal mucosa of both dogs and rats occurred following salicylate type adjuvant administration. The normal transmucosal potential difference of 20–30 mV was unaffected by rectal administration of a pH 7·4 phosphate buffer. When either salicylate or 5-methoxysalicylate was added to the rectal buffer, the potential difference decreased dramatically within 5 min and slowly recovered within 1 h. A rapid (10 min) recovery to the normal potential difference occurred after washing out the salicylate with fresh buffer. On the other hand, sodium lauryl sulphate caused a slow decay of the transmucosal potential difference (30 min) which did not return to initial values even 3 h after wash-out. Histological examination revealed no change in the appearance of the mucosa following acute exposure to 5% sodium salicylate or 5% sodium 5-methoxy salicylate in a 1/15 M phosphate buffer at pH 7·4 for 1 h. Chronic administration for 60 days also showed no change in the mucosa. However, sodium lauryl sulphate at a

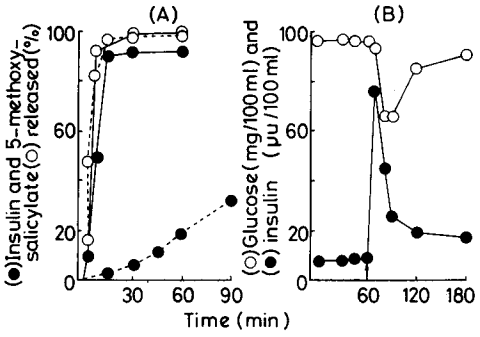

FIG. 3. (a) The release and dissolution of a 5 mg sample of crystalline insulin (●) and 200 mg sodium salicylate (○) from a 1 g suppository (- - -) and a 4% gelatin formulation (——) in a 5 ml stirred solution of normal saline at 37 °C. The insulin was soluble in the gelatin formulation.
(b) Plasma concentrations of insulin (●) and glucose (○) observed after the administration of a 0·5 g suppository containing 20 u of insulin in Witepsol H-15 as the base followed by the administration of a 0·5 g suppository containing 300 mg of sodium salicylate at 60 min (↑).

lesser concentration in the microenema was found to cause mucosal erosion.

In conclusion, the presence of 5-methoxysalicylate appears to markedly enhance the rectal absorption of insulin. In these studies, the greatest absorption occurred from a formulation including 4% gelatin in a microenema. It is tempting to speculate that the enhancing effect might be due to the increased viscosity of the microenema containing gelatin limiting diffusion of the drug and adjuvant from the site of administration. Another possible explanation, is that the enhancement may result from the gelatin inhibiting adsorption of insulin from dilute solutions onto the apparatus used in preparing the microenema. However, assay of insulin in the final dosage forms showed 90% recovery from the dilute solution and 92% recovery from the 4% gelatin formulation. The assay for 5-methoxysalicylate yielded 99% for the solution and 100% for the gelatin formulation.

This work confirms other reports that pharmaceutical modifications of the dosage form can enhance the rectal absorption of insulin. However, considerable progress will be necessary before practical alternatives to parenteral therapy are available.

Acknowledgements

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