Relative Hypoglycemic Effect of Insulin Suppositories in Diabetic Beagle Dogs: Optimization of Various Concentrations of Sodium Salicylate and Polyoxyethylene-9-lauryl Ether

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The effect of insulin suppositories containing different amounts and concentrations of sodium salicylate (50, 100 mg) and polyoxyethylene-9-lauryl ether (POELE 1, 3, 4%), respectively, on the plasma glucose concentration of diabetic beagle dogs was investigated after rectal administration. Comparison of the effects of these formulations was made with that produced after subcutaneous insulin injections. Insulin suppositories containing sodium salicylate (50 mg) produced a maximum reduction of plasma glucose concentration ($C_{max}$) of 55±11%, an area under the curve (AUC) of 252±59% reduction h; and a relative hypoglycemia (RH) of 49±12% relative to subcutaneous injection of insulin (4 U/kg). Increasing sodium salicylate to 100 mg/suppository did not improve the hypoglycemic effect of insulin suppositories further. Investigation of the influence of insulin suppositories containing different concentrations of the nonionic surfactant POELE (1, 3, 4%) showed that; the suppositories containing the lowest concentration (1%) produced the highest hypoglycemic effect with a $C_{max}$ of 68%, AUC of 332±67% reduction h, and RH of 55±11%. Incorporation of sodium salicylate 50 mg in insulin suppositories containing 1% POELE did not improve further the effects found with these suppositories.

In conclusion, a relative hypoglycemic effect of about 50—55% can be achieved using insulin suppositories containing Witepsol W35 as a base, insulin (5 U/kg), and sodium salicylate (50 mg) or POELE (1%) as rectal absorption enhancers.

Key words insulin suppository; sodium salicylate; polyoxyethylene-9-lauryl ether

The efficacy and bioavailability of rectal insulin remained low when compared with intravenous or subcutaneous injection until surfactants and other absorption promoters were introduced. These appear to increase significantly the uptake of high molecular weight polar drugs; such as insulin. The hypoglycemic response was dependent on both the concentration of the surfactant and the dose of the insulin administered. The non surfactant sorption promoters; sodium salicylate, 5-methoxysalicylate, sodium-3-methoxysalicylate and sodium homovanillate were evaluated for insulin delivery. An increase in insulin dose was not accompanied by an increase in response. Ichikawa et al. evaluated the effect of surfactants, bile acids, and phospholipids on the reduction of glucose levels in diabetic rabbits. They found that the most effective sorption promoter was polyoxyethylene-9-lauryl ether (POELE) at a concentration of 1%. Again an increase in insulin dose did not proportionally increase the response. Others found that the greatest reduction in glucose concentration was observed with suppositories containing 3% of surfactant. Yamasaki et al. showed that in dogs with fasting plasma glucose levels below 300 mg/dl; both insulin suppository at a dose of 20 U and subcutaneous insulin at a dose of 0.2 U/kg showed similar effects in reducing fasting glucose levels. In dogs with higher fasting glucose levels, 0.5 U of subcutaneous insulin/kg was less effective in reducing fasting glucose levels than 50 U suppositories.

The effectiveness of rectal administration of insulin was examined in normal and non insulin-dependent non obese diabetic subjects. In normal subjects, when 50 U insulin suppositories containing 3% POELE and 0.02 M HCl were used a reduction of glucose level by about 10% after 45 min, returning to baseline within 90 min was achieved. When insulin 100 U was used, a reduction in plasma glucose of 23% after 45 min was reached. In diabetic patients, 100 U insulin suppositories resulted in a reduction of glucose level by 31% after 120 min. The insulin response after suppository administration demonstrated a significant positive correlation.

In our previous work, the absorption-promoting effect of sodium salicylate, 200 mg/100 U insulin suppository was studied in 4 normal volunteers and 15 insulin-dependent diabetic patients. A hypoglycemic effect and a significant rise in serum insulin concentrations were traced at 15 min; and maintained for 90 min; post administration.

The objective of this study is to investigate the effect of different concentrations of sodium salicylate or POELE in rectal insulin suppositories with the purpose of optimizing the concentration of both enhancers to provide the highest hypoglycemic effect. The reduction in plasma glucose levels of overnight food-deprived hyperglycemic beagle dogs was measured. The hypoglycemia resulting from the suppository formulations was calculated and compared with that produced after subcutaneous injection of regular soluble insulin.

MATERIALS AND METHODS

Materials Insulin crystals HM (ge) was a kind gift from Novo Nordisk A/S (Novo Alle, 2880 Bagsvaerd, Denmark). Sodium salicylate was purchased from BDH Limited (Poole, England), Witepsol W35 from Dynamit Nobel (Northvale, NJ, U.S.A.), POELE from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and glucose GOD-PAP from Randox Laboratories Ltd. (Antrim, U.K.).

Methods Preparation of Insulin Suppositories The suppositories were prepared by the fusion method. The base, Witepsol W35, was melted over a water bath. Sodium salicylate or POELE was added and uniformly distributed in the melted base. After the melted mass was allowed to cool, in-
sulin was added and triturated. The melted mass was then poured into a 2-g mold and cooled. The suppositories were stored at 4 °C until use on the next day.

Induction of Hyperglycemia  Male beagle dogs weighing between 9.5 and 16.5 kg were overnight food-deprived and rendered diabetic with an intravenous injection of a cocktail containing alloxan and streptozotocin (35 mg/kg each). This cocktail was injected on two occasions: In the first day; as 40 mg/kg, and 2 d later; as 30 mg/kg. The diabetic dogs were managed by daily injection of regular insulin 2 U/kg and NPH insulin 1 U/kg subcutaneously.

Subcutaneous Injection of Insulin  Regular human insulin, USP (100 U/ml), was injected subcutaneously at 4 U/kg body weight on different occasions into overnight food-deprived hyperglycemic beagle dogs.

Blood Sampling  Blood samples (1 ml) were drawn into heparinized tubes before and every hour after rectal administration of insulin suppositories for 6 consecutive hours by inserting a disposable intravenous cannula (20G-O.D. 1 × 32 mm luer lock, Distr. Da ARTSANA S.P.A.—Casnate Co., Italy) into the cephalic vein of each dog. Blood samples were immediately centrifuged and aliquots of plasma aspirated and stored at −20 °C for subsequent glucose measurement at the end of the experiment.

Plasma Glucose Measurements  The glucose concentration in plasma was measured according to the principles of the enzymatic colorimetric assay by Trinder, plasma (10 μl) was added to glucose reagent (GOD-PAP) 1 ml. After vortexing for 10 s, the tubes were incubated for 25 min; at room temperature. The absorbency of the standard and plasma glucose samples was measured within 60 min; against reagent blank at 500 nm using a Spectronic 21D Spectrophotometer (Milton Roy, Rochester, NY, USA.). The plasma glucose concentration was calculated as milligrams per deciliter.

Calculations of the Hypoglycemic Effect  The maximum change in plasma glucose concentration (∆C<sub>max</sub>) was obtained from the plasma glucose concentration–time curves (% of initial plasma glucose level at zero time) of each dog which is calculated from the equation:

\[
\% \text{ of initial} = \frac{(F-P_t)}{P} \times 100
\]

where \( F \) is the fasting (initial) plasma glucose level and \( P_t \) is the plasma glucose level at time \( t \) after administration. This was done to minimize the influence of the varying initial plasma glucose levels between dogs. The time to reach this maximum change in plasma glucose levels (\( T_{max} \)) was obtained from the plasma glucose concentration–time curve of the dogs. The plasma glucose concentration was calculated using the equation:

\[
glucose \text{ concentration (mg/dl)} = \left( \frac{A_{\text{sample}}}{A_{\text{standard}}} \right) \times 100
\]

where \( A \) is the absorbency.

The area under % glucose reduction–time profile (∆AUC<sub>0→6h</sub>) was determined using the linear trapezoidal rule. The relative hypoglycemia (RH) of insulin suppository formulations was calculated by comparing their ∆AUCs relative to that after subcutaneous injections. All data are expressed as mean ± S.D.

Statistical Analysis  Plasma glucose levels (0—6 h) after rectal or subcutaneous administration of insulin suppositories or injection, respectively, were compared in each group with the respective initial values using repeated-measure analysis of variance (ANOVA) followed by the Bonferroni multiple comparison test. Differences between groups in \( C_{max}, T_{max}, \) and ∆AUC analysed using Student’s t-test to compare two values, or by one-way ANOVA followed by the Tukey–Kramer multiple comparison test in the case of more than two values. These statistical calculations as well as; correlation and regression analyses were performed using the Graph Pad Instat computer program (1990—1993; Graph Pad Software, V2.04, San Diego, CA, U.S.A.).

RESULTS AND DISCUSSION

Effect of Sodium Salicylate  Sodium salicylate is probably the most extensively studied enhancer for improving rectal drug absorption. Sodium salicylate has been shown to stimulate the rectal absorption of insulin in rats, dogs, and humans.

Figure 1 and Table 1 show the effect of insulin suppositories containing sodium salicylate 50 and 100 mg on the plasma glucose levels, \( C_{max}, T_{max}, \) AUC, and RH of hyperglycemic beagle dogs. The results show that there is no significant difference (\( p>0.05 \)) in AUC, RH, \( C_{max} \), and \( T_{max} \) between these two formulations. Insulin suppositories containing sodium salicylate 50 mg were efficient in reducing plasma glucose levels by a value of 55±11% at \( T_{max} \) of 2 h producing RH of 49±12% relative to that of insulin injection of 4 U/kg subcutaneous. It is to be noted that increasing the amount of sodium salicylate to 100 mg in insulin suppositories did not improve the effect found with suppositories containing sodium salicylate 50 mg any further. This could be explained by the fact that increasing the amount of sodium salicylate increased the hydrophilic tendency of the suppository mass, leading to decreased drug release. Also, it should be mentioned that sodium salicylate at dose of 25 mg/suppository did not result in any improvement in insulin absorption manifested as hypoglycemia.

Sodium salicylate stimulates the absorption of insulin by acting on both the apical cell membrane (transcellular pathway) and the tight junction between cells (paracellular pathway). Salicylate also acts on the protein components of plasma membrane, red blood cell membranes, and retinal brush border membrane vesicles.

Non protein thiols such as glutathione are yet another membrane component on which salicylates might act. Nishihata et al. and Suzuka et al. showed that salicylate decreased the levels of non protein thiols in intestinal tissues and isolated enterocytes. Sodium salicylate has been shown to increase insulin solubility 7875 times, permitting the preparation of an aqueous solution of 630 mg/ml of insulin. Thus the interference between sodium salicylate and insulin self-association behavior; by increasing drug solubility may substantially contribute to the improved drug bioavailability.

Effect of Polyoxyethylene-9-lauryl Ether  Surfactants are one of the most important classes of adjuvant in pharmaceutical preparations. They may affect the rate and/or extent of absorption of drugs. Several studies have shown that drug release from suppository bases is influenced by inclusion of surfactants in the formulations and may result in an increase or decrease in the rate of release, depending on the nature and concentration of the surfactant. Surfactants could increase the amount of drug absorbed by solubilizing...
the drug or by affecting the permeability of the epithelial membrane\(^2\)) but could damage the rectal membrane when added in high concentration.\(^2\)

Table 2 shows the effect of insulin suppositories containing different concentrations (1, 3, 4%) of POELE; on plasma glucose levels, \(\Delta C_{\text{max}}\), \(T_{\text{max}}\), \(AUC\), and \(RH\) of diabetic dogs. The results show that POELE at 1% concentration was effective in promoting insulin absorption, resulting in reduction of plasma glucose levels by 68±18% in 2 h. This hypoglycemic effect was maintained for the experimental time, and at 6 h; the reduction in plasma glucose level was still 60%. This formulation resulted in an \(AUC\) of 332±67% reduction h and \(RH\) of 55±11% compared to injection of insulin 4 U/kg subcutaneously.

As the concentration of POELE in insulin suppositories increased to 3% the \(AUC, RH\), and \(\Delta C_{\text{max}}\) were reduced significantly (\(p<0.05\)) to 19±7% reduction h, 3±1.2%, and 6±2.7%, respectively. With 4% POELE these values remained low at 96±30% reduction h, 16±5.0%, and 24±5.9%, respectively. This could be due to micellar solubilization of insulin when the critical micelle concentration of surfactant is reached, leading to decreased free concentration of insulin. Consequently, a decrease in the release rate and retardation effect of insulin is expected as it is clear from prolonging the \(T_{\text{max}}\) from 2 h at 1% concentration to 3 and 4 h at 3 and 4% concentrations, respectively.

In a trial to improve the hypoglycemic effect of these insulin suppositories containing 1% POELE, sodium salicylate 50 mg was incorporated. This addition did not improve further the hypoglycemic effect of the insulin suppositories con-

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**Table 1.** Plasma Glucose Levels, \(C_{\text{max}}, T_{\text{max}}, AUC\), and \(RH\) in Hyperglycemic Beagle Dogs after Rectal Administration of Witepsol W35 Suppositories Containing Human Insulin (5 U/kg) in the Presence of Sodium Salicylate (NaSal, 50, 100 mg) and after Injection of Insulin 4 U/kg Subcutaneously

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>NaSal; 50 mg</th>
<th>NaSal; 100 mg</th>
<th>Insulin s.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
</tr>
<tr>
<td>0</td>
<td>219±6.6</td>
<td>230±17.7</td>
<td>216±13.0</td>
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<tr>
<td>1</td>
<td>119±17.0*</td>
<td>123±14.4*</td>
<td>73±30.0*</td>
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<td>2</td>
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<td>119±44.9*</td>
<td>60±12.0*</td>
</tr>
<tr>
<td>3</td>
<td>100±25.5*</td>
<td>123±35.9*</td>
<td>47±13.9*</td>
</tr>
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<td>4</td>
<td>115±22.9*</td>
<td>139±27.1*</td>
<td>47±11.9*</td>
</tr>
<tr>
<td>5</td>
<td>130±20.7*</td>
<td>143±19.2*</td>
<td>41±16.4*</td>
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<tr>
<td>6</td>
<td>175±12.9*</td>
<td>147±19.1*</td>
<td>68±17.6*</td>
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</table>

\(\Delta C_{\text{max}}\): maximum reduction (% of initial) in plasma glucose concentration, \(T_{\text{max}}\): time to reach \(C_{\text{max}}\), \(AUC\): area under % glucose reduction–time curve, \(RH\): relative hypoglycemia. \(*p<0.001\) compared with respective initial plasma glucose level (0 h). \(**p<0.05\) compared with subcutaneous insulin injection.

**Table 2.** Plasma Glucose Levels, \(C_{\text{max}}, T_{\text{max}}, AUC\), and \(RH\) in Hyperglycemic Beagle Dogs after Rectal Administration of Witepsol W35 Suppositories Containing Human Insulin (5 U/kg) in the Presence of Different Concentrations (1, 3, 4%) of Polyoxyethylene-9-lauryl Ether (POELE) and after Injection of Insulin 4 U/kg Subcutaneous

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>POELE 1%</th>
<th>POELE 3%</th>
<th>POELE 4%</th>
<th>Insulin s.c.</th>
</tr>
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<td>(n=6)</td>
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<td>211±34.1</td>
<td>206±26.1**</td>
<td>33±1.9*</td>
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<tr>
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<td>186±26.4*</td>
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<tr>
<td>4</td>
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<tr>
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<td>6</td>
<td>107±30.2</td>
<td>225±51.0</td>
<td>182±10.1*</td>
<td>16±1.5*</td>
</tr>
</tbody>
</table>

\(\Delta C_{\text{max}}\): maximum reduction (% of initial) in plasma glucose concentration, \(T_{\text{max}}\): time to reach \(C_{\text{max}}\), \(AUC\): area under % glucose reduction–time curve, \(RH\): relative hypoglycemia. \(*p<0.001\), \(**p<0.05\) compared with respective initial plasma glucose level (0 h). \(**p<0.001\) compared with 1% POELE.

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![Fig. 1. Effect of Insulin Suppositories (5 U/kg) Containing Sodium Salicylate (NaSal, 50, 100 mg) on the Mean (±S.D.) Plasma Glucose Levels (% of Initials) of Hyperglycemic Beagle Dogs after Rectal Administration Compared with Insulin Subcutaneous Injection (Ins SC, 4 U/kg)](image1)

![Fig. 2. Effect of Insulin Suppositories (5 U/kg) Containing Polyoxyethylene-9-lauryl Ether (POELE 1, 3, 4%) on the Mean (±S.D.) Plasma Glucose Levels (% of Initials) of Hyperglycemic Beagle Dogs after Rectal Administration Compared with Insulin Subcutaneous Injection (Ins SC, 4 U/kg)](image2)
taining 1% POELE, giving a ΔC_{max} of 72±47%, AUC of 334±10% reduction h, and RH of 56±1.7%.

These results show that POELE is an efficient absorption promoter of insulin from rectal suppositories when used at 1% concentration. The potency of polyoxyethylene alkyl ethers in enhancing nasal absorption of insulin in rats was maximal with POELE and diminished gradually with smaller or larger numbers of ethylene oxide units.29

The ultimate development of a clinically useful rectal insulin suppository for the treatment of diabetes is likely to depend in part on the possible local or systemic toxicity of the transport-enhancing system for long-term treatment. Nishihata et al.,27 using a perfusion technique in the rat, showed that the increased permeability following salicylate treatment was eliminated by washing the rectum with fresh buffer solution. It was further reported by Sithigorngul et al.,28 using a ligation method and light and electron microscopic methods that 2% sodium salicylate does very little damage to rectal epithelial cells at pH 7.0, an effect that is reversed upon washing out the sodium salicylate. The major cellular change induced by salicylate was a reduction in the length or distribution of glycocalyx filaments on microvilli of epithelial cells.28,29

Yamasaki et al.,5 also demonstrated that no pathologic changes occurred in the rectal specimens of alloxan-diabetic dogs controlled with insulin suppositories containing POELE for 6—9 d using light and electron microscopy. Hirai et al.,29 found that; after a 1-month application of insulin preparation containing surfactant to the nasal cavity, slight morphological changes in the nasal cavity, slight morphological changes in the nasal mucosa were observed in the case of POELE.

In conclusion, sodium salicylate 50 mg/suppository and POELE at 1% concentration are efficient absorption promoters in enhancing the rectal delivery of insulin from suppositories formulated with Witepsol W35.

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REFERENCES