Drug Development and Industrial Pharmacy, 27(8), 837–845 (2001)

RESEARCH PAPER

Effect of Different Bile Salts on the Relative Hypoglycemia of Witepsol W35 Suppositories Containing Insulin in Diabetic Beagle Dogs

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ABSTRACT

Insulin suppositories were formulated using Witepsol W35 as a base to investigate the effect of various bile salts/acids on the plasma glucose concentration of diabetic beagle dogs. Comparison of the effect of these formulations was made with that produced by insulin subcutaneous injections. Of the bile salts/acids studied, incorporation of 100 mg of deoxycholic acid (DCA), sodium cholate (NaC), or sodium deoxycholate (NaDC) with insulin (10 U/Kg) showed that suppositories containing NaDC produced the highest area under the curve (AUC) and relative hypoglycemia (RH) of 290 ± 83 mg%h and 28% ± 8.1%, respectively. To study the optimum amount of NaDC in insulin suppositories to produce the highest RH, 50–200 mg/suppository were used, and we found that 150 mg NaDC produced 35% ± 13% RH. We also studied the influence of different doses of insulin (5–20 U/kg) in the presence of NaDC (100 mg). It was found that increase of the insulin dose was accompanied by an increase in AUC and maximum reduction in plasma glucose level C_{max}. A combination of NaDC (100 mg) and NaC (50 mg) produced an AUC of 252 ± 13 mg%h and an RH of

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49% ± 2.6%, which were higher than produced by either of its individual components (NaC 50 mg or NaDC 100 mg) when used alone or when compared with an equivalent amount of NaDC (150 mg). When the effect of sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC) was studied, it was found that an insulin suppository containing 100 mg of either NaTC or NaTDC produced an RH equivalent to that produced previously with a mixture of NaDC (100 mg) and NaC (50 mg). On the other hand, NaC (50 mg) did not improve the hypoglycemic effect of NaTC any further. In conclusion, a relative hypoglycemia of about 50% can be reached using insulin suppositories containing Witepsol W35 as a base and NaDC plus NaC (100 mg plus 50 mg, respectively), NaTDC (100 mg), or NaTC (100 mg) as rectal absorption enhancers of insulin. A desirable hypoglycemia, expressed as C_{max}, and/or AUC can be reached by adjusting the insulin dose in the formulation according to the degree of hyperglycemia.

Key Words: *Beagle dogs; Bile salts; Insulin suppositories*

INTRODUCTION

In spite of newer, more potent and highly purified insulin; development of human insulin; introduction of portable infusion pumps; and pancreatic transplantation, diabetes is still a high-risk disease, far from being controlled or cured. The present mode of insulin administration is parenteral by subcutaneous (s.c.), intramuscular (i.m.), or (less frequently) intravenous and intraperitoneal injection with syringes or pumps. This insulin is introduced into the body in a nonphysiological manner. It is peripherally administered to the systemic circulation instead of entering the portal circulation.

Although in the great majority of cases these parenteral routes are satisfactory in terms of efficacy, these routes result in peripheral hyperinsulinemia. Stout (1) concluded that this hyperinsulinemia stimulates smooth muscle cell proliferation and the incorporation of glucose into lipid in arterial walls and thus might be a causative factor in diabetic microangiopathy. In addition, the burden of daily injections, physiological stress, costs, risks, infections, inability to handle, and inconvenience all make it highly desirable to find alternative routes for administration that would allow convenient administration and efficient absorption to achieve the hypoglycemic activity of insulin.

During the past few years, considerable interest has arisen in the rectal route for insulin administration. This route is regarded as a more physiologic route for applying insulin. It is known that about 50% of insulin delivered is degraded in the liver, which is also the locus of the highest insulin utilization (2). About 30% of insulin rectally absorbed enters into the portal vein (3). An open-loop portal insulin delivery study applied to depancreatized dogs fed regular meals demonstrated less hyperinsulinemia (4) than that observed in a peripheral infusion study (5). On the other hand, the increment of portal insulin concentration is shown to intensify the magnitude of the net hepatic glucose uptake induced by portal glucose infusion (6).

These facts indicate that portal insulin delivery is important in normalizing both glycemia and insulinemia postprandially. Yamasaki et al. (7) concluded from their study that insulin suppositories attenuated the postprandial glycemic rise in diabetic subjects, and that the peripheral insulinemia was similar to that present in normal subjects after meals. The studies presented a possibility that insulin suppositories could control postprandial glycemia in a more physiological manner than conventional insulin therapy because substantial amounts of insulin absorbed from the rectum enter directly into the portal vein.

The efficacy and bioavailability of rectal insulin were very low compared to intravenous or subcutaneous injection. This situation was modified when surfactants and other absorption promoters were introduced as they appear to increase significantly the uptake of high molecular weight polar drugs such as insulin (8).

Rectal gels consisting of emulsion systems prepared from pH 8 buffer solution containing insulin, an oleaginous phase, a surface-active agent (bile

salts, Myrj, or Brij), and a viscosity-increasing agent were tested in a parallel and a crossover design in nondiabetic and diabetic rabbits (9). The selected rectal gel in nondiabetic and diabetic rabbits resulted in a pharmacologic availability of about 25%. By addition of Azone, the pharmacologic availability was further increased, although not significantly. In a nondiabetic human, the pharmacologic availability was about 32%, whereas the bioavailability (measured from plasma insulin) was only about 11%.

The effect of the bile salt derivative sodium tauro-24,25-dihydrofusidate (STDHF) on rectal insulin absorption was investigated in rats (10). At concentrations of 1% and 4% (w/v), it enhanced insulin bioavailability from $0.2\% \pm 0.2\%$ (control) to $4.2\% \pm 3.2\%$ and $6.7\% \pm 2.1\%$, respectively, as assessed by radioimmunoassay. Insulin preparations with STDHF reduced blood glucose concentrations considerably in a concentration-dependent manner. Coadministration of STDHF with Na_2EDTA (0.25% w/v) tended to increase further insulin bioavailability and hypoglycemic response. Varying the site of rectal administration did not influence these parameters.

Insulin suppositories using polyethylene glycol (molecular weight [MW] 4000) as a base and containing 50 U insulin incorporated with 50 mg of deoxycholic acid (DCA), sodium taurocholate (NaTC), or both were placed in the rectum of alloxan-induced hyperglycemic rabbits (11). A significant decrease in plasma glucose concentrations was observed, and the relative hypoglycemias were calculated to be 38.0%, 34.9%, and 44.4%, respectively, compared with insulin subcutaneous injection (40 U).

The objective of this study was to formulate insulin suppositories for rectal administration to obtain a formulation that could be a practical alternative to the parenteral route of insulin administration. Therefore, the influence of rectal insulin suppositories containing various bile acids/salts (DCA, sodium cholate [NaC], sodium deoxycholate [NaDC], taurodeoxycholic acid–sodium salt, taurocholic acid–sodium salt, or their combinations) on the reduction of plasma glucose levels were studied using hyperglycemic beagle dogs deprived overnight of food. The hypoglycemia that resulted from the suppository formulations was calculated and compared with that produced after subcutaneous injection of soluble insulin.

EXPERIMENTAL

Materials

Deoxycholic acid was from Fluka Chemie (AG, CH-9470 Buchs, Switzerland). Sodium deoxycholate came from BDH Limited (Poole, England). Cholic acid–sodium salt was from Serva Feinbiochemica GmbH and Company (Carl-Benz-Straoße 7, Germany). Witepsol W35 was from Dynamit Nobel (Northvale, NJ). Taurocholic acid–sodium salt and taurßodeoxycholic acid–sodium salt were from Sigma Chemical Company (St. Louis, MO). Glucose GOD-PAP came from Randox Laboratories Limited (Antrim, UK).

Induction of Hyperglycemia

Male beagle dogs weighing between 9.5 and 16.5 kg were deprived of food overnight 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; on the first day, it was injected as 40 mg/kg, and 2 days later, it was given as 30 mg/kg. This modification of injection from the method of Black et al. (12) was carried out as the percentage of death for our dogs using Black's method for induction of diabetes was 100%. By using this method of dividing the dose of the cocktail, no death of dogs was reported.

The diabetic dogs were managed by daily subcutaneous injections of 2 U/kg of regular insulin and 1 U/kg of NPH insulin (Eli Lilly and Company, Indianapolis, IN).

Subcutaneous Injection of Insulin

Regular human insulin injection, USP (100 U/ml), was injected subcutaneously to hyperglycemic beagle dogs deprived overnight of food. Insulin was injected as 4 U/kg body weight on different occasions.

Preparation of Insulin Suppositories

The suppositories were prepared by the fusion method. The base, Witepsol W35, was melted over a water bath. The bile salts/acids were added and uniformly distributed in the melted base. After the mixture was allowed to cool, insulin was added and

triturated. The melted mass was then poured into a 2-g mold and cooled. The suppositories were kept at 4°C until used on the next day. The displacement values of all the additives used in the formulations were determined in Witepsol W35 and considered during preparation of suppositories.

Blood Sampling

Blood samples (1 ml) were taken into heparinized tubes before and every hour after rectal administration for 6 consecutive hours by inserting disposable intravenous cannula (20G O.D. 1×32 mm luer lock). (distributed by Da Artsana SPA, Casnate Co., Italy) into the cephalic vein of each dog. The blood samples (1 ml) were immediately centrifuged, and aliquots of plasma were aspirated and stored at -20°C for subsequent glucose measurement at the end of the experiment.

Plasma Glucose Measurements

Plasma (10 µl) was added to 1 ml glucose reagent (GOD-PAP). After vortexing for 10 s, the tubes were incubated for 25 min at room temperature. The absorbance of the standard and plasma glucose samples was measured within 60 min against a reagent blank at 500 nm using a Spectronic 21D spectrophotometer (Milton Roy, Rochester, NY). The plasma glucose concentration was calculated as milligrams per deciliter (mg/dl).

Calculations of the Hypoglycemic Effect

The maximum reduction in plasma glucose concentration C_{max} and the time to reach this reduction T_{max} were obtained from the plasma glucose concentration-time curves (percentage of initial) for each dog. The area under the percentage glucose reduction-time profile $AUC_{0-6\,h}$ 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 and taking dose differences into consideration. All the data are expressed as mean ± standard deviation (SD).

Statistical Analysis

Statistical analyses for differences between groups in C_{max}, T_{max}, and AUC were carried out using the Student t test to compare two values or by one-way analysis of variance (ANOVA), followed by a Tukey-Kramer multiple comparison test in case of more than two values. These statistical calculations were performed using the Graph Pad Instat computer program (1990–1993; Graph Pad Software, V2.04, San Diego, CA).

RESULTS AND DISCUSSION

Effect of Bile Acids and Salts

Figure 1 and Table 1 show the hypoglycemic effect of insulin suppositories containing 10 U of insulin and 100 mg of DCA, NaC, or NaDC in comparison to that produced after subcutaneous injection of 4 U insulin into hyperglycemic beagle dogs deprived of food overnight. The use of bile salts was to inhibit the mucosal proteolytic activity (13,14) and to promote insulin absorption (15,16).

Figure 1 shows that the plasma glucose concentration decreased to about 66% of its initial value

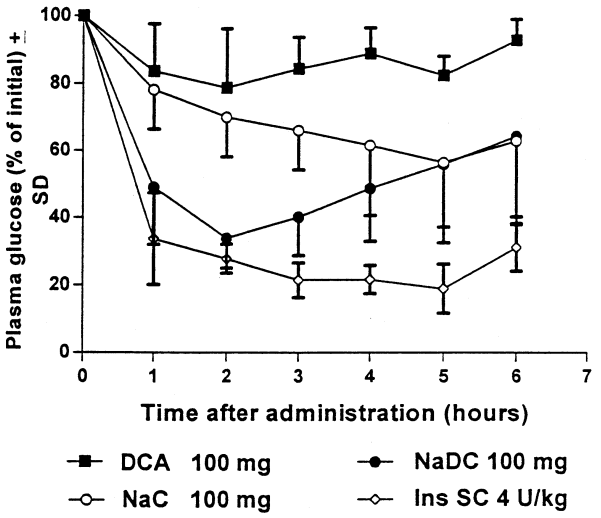

Figure 1. Effect of 100 mg of deoxycholic acid (DCA), sodium cholate (NaC), or sodium deoxycholate (NaDC) on the mean plasma glucose levels (% of initial value) of hyperglycemic beagle dogs after rectal administration of Witepsol W35 suppositories containing human insulin (10 U/kg) compared with insulin subcutaneous injection (Ins SC, 4 U/kg). Mean initial fasting plasma glucose levels, mg% ± SD (No), were as follows: Ins SC, 216 ± 13 (6); DCA, 204 ± 17.4 (5); NaC, 205 ± 20.5 (5); NaDC, 188 ± 19.4 (6).

Table 1

Hypoglycemic Effect of Witepsol W35 Suppositories Containing Human Insulin and Various Absorption Enhancers Expressed as $C_{max,}$ $T_{max,}$ AUC, and RH in Hyperglycemic Beagle Dogs Compared with Insulin Subcutaneous Injection

Enhancer (mg)	No. (Dogs)	Insulin (U/kg)	C_{max} (% Reduction)	T_{max} (h)	AUC (mg%h)	RH (%)
Insulin sc injectable	6	4	81 ± 7.3	5	411 ± 33	100
1. DCA (100)	5	10	20 ± 19	2	85 ± 54	8 ± 5.2
2. NaC (100)	5	10	43 ± 24	5	186 ± 92	18 ± 8.9
3. NaDC (100)	6	10	$66 \pm 8.9^{a,b}$	2	$290 \pm 83^{a,b}$	28 ± 8.1^{a}
4. NaDC (50)	6	5	6 ± 2.6	2	23 ± 7.3	5 ± 1.4
5. NaDC (100)	6	5	36 ± 21^{c}	6	126 ± 79^{c}	25 ± 15^{c}
6. NaDC (150)	6	5	37 ± 13^{c}	3	179 ± 65^{c}	35 ± 13^{c}
7. NaDC (200)	4	5	30 ± 0.4^{c}	2	132 ± 4.6^{c}	26 ± 0.9^{c}
8. NaDC (100)	6	20	78 ± 7.8^{b}	2	369 ± 94^{b}	18 ± 7.9
9. NaC (50)	6	5	48 ± 3.6	1	207 ± 7.1^{b}	40 ± 1.9^{b}
10. NaDC (100) + NaC (50)	6	5	54 ± 5.8	2	252 ± 13^{b}	49 ± 2.6^{b}
Insulin sc injectable	6	4	83 ± 6.4	5	479 ± 45	100
11. NaTDC (100)	6	5	55 ± 14	4	277 ± 61	46 ± 10
12. NaTC (100)	6	5	60 ± 5	4	301 ± 28	50 ± 4.7
13. NaTC (100) + NaC (50)	6	5	57 ± 21	4	295 ± 95	49 ± 16

sc, insulin subcutaneous injection; DCA, deoxycholic acid; NaC, sodium cholate; NaDC, sodium deoxycholate; NaTDC, sodium taurodeoxycholate; NaTC, sodium taurocholate; C_{max}, maximum reduction (% of initial) in plasma glucose concentration; T_{max}, time to reach C_{max}; AUC, area under percentage glucose reduction-time curve; RH, relative hypoglycemia.

[a] $P < 0.01$ compared with group 1.

[b] $P < 0.05$ compared with group 5.

[c] $P < 0.05$ compared with group 4.

after 1 h of subcutaneous injection of 4 U/kg of regular insulin. This reduction continued slowly to reach a C_{max} of $81\% \pm 7.3\%$ at T_{max} of 5 h. This type of rapid reduction in plasma glucose is not recommended as this rapid hypoglycemia may lead to death. The results in Fig. 1 also show that bile salts such as NaC and NaDC are more effective than DCA in promoting insulin absorption after rectal administration of suppositories. The bile salts produced an AUC of 186 ± 92 mg%h ($P > .05$) and 290 ± 83 mg%h ($P < .05$), respectively, compared to 85 ± 54 mg%h after the rectal administration of insulin suppositories containing DCA (Table 1). The promotion of insulin absorption could be due to the enhancement of paracellular absorption that results not only from an expansion in the dimensions of the tight junction and the intercellular space, but also from an increase in water influx through that space (17). The increase in water flux is sodium dependent, as indicated by reduction in its effect by ouabain (18). The increase in water flux may affect drug absorption by an increase in concentration gradient for penetration, an increase in solvent drag, or an increase in blood flow at the absorption site (18).

Table 1 also shows that NaDC is more effective in promoting insulin absorption than NaC; the RHs were $28\% \pm 8.1\%$ and $18\% \pm 8.9\%$ ($P > .05$), respectively, relative to that of subcutaneous injection of 4 U/kg insulin. This is in agreement with the finding that the potency of bile salts in enhancing the nasal absorption of insulin in human volunteers was correlated with increasing hydrophobicity of the steroid nucleus of the bile salts (19). Specifically, the rank order of effectiveness was as follows:

Deoxycholate > Chenodeoxycholate > Cholate > Ursodeoxycholate.

Figure 2 and Table 1 show the effect of the addition of different amounts of NaDC in insulin suppositories containing 5 U/kg body weight. The results show that, as the amount of the bile salt increased from 50 to 100, 150, and 200 mg per suppository, the AUC increased significantly from

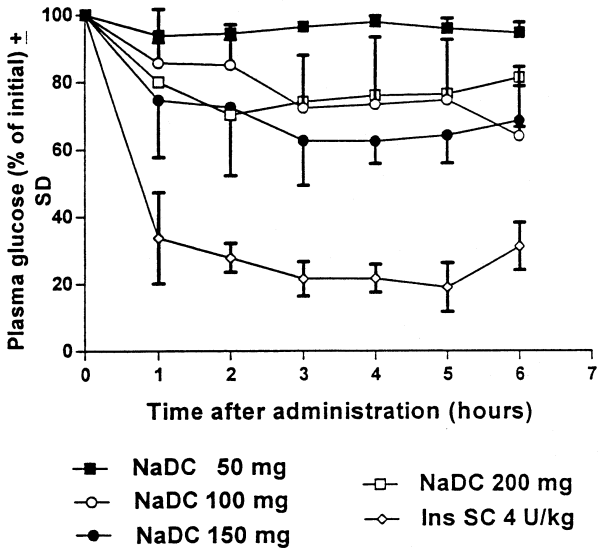

Figure 2. Effect of sodium deoxycholate (NaDC; 50, 100, 150, 200 mg) on the mean plasma glucose levels (% of initial values) of hyperglycemic beagle dogs after rectal administration of Witepsol W35 suppositories containing human insulin (5 U/kg) compared with insulin subcutaneous injection (Ins SC, 4 U/kg). Mean initial fasting plasma glucose level, mg% ± SD (No), were as follows: Ins SC, 216 ± 13 (6); NaDC 50 mg, 216 ± 9.0 (6); NaDC 100 mg, 187 ± 6.2 (6); NaDC 150 mg, 241 ± 11.9 (6); NaDC 200 mg, 225 ± 0.5 (4).

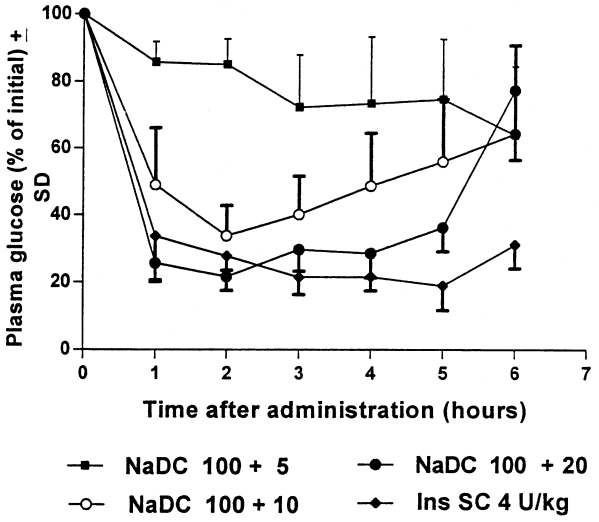

Figure 3. Effect of sodium deoxycholate (NaDC, 100 mg) on the mean plasma glucose levels (% of initial values) of hyperglycemic beagle dogs after rectal administration of Witepsol W35 suppositories containing human insulin (5, 10, 20 U/kg) compared with insulin subcutaneous injection (Ins SC, 4 U/kg). Mean initial fasting plasma glucose level, mg% ± SD (No), were as follows: Ins SC, 216 ± 13 (6); NaDC with insulin 5 U/kg, 187 ± 6.2 (6); NaDC with insulin 10 U/kg, 188 ± 19.4 (6); NaDC with insulin 20 U/kg, 206 ± 31.6 (6).

23 ± 7.3 mg%h to 126 ± 79 mg%h ($P < .05$), 179 ± 65 mg%h ($P < .001$) and 132 ± 4.6 mg%h ($P < .05$), respectively. The RH increased from 5% ± 1.4% to 25% ± 15% ($P < .05$), 35% ± 13% ($P < .001$), and 26% ± 0.9% ($P < .05$), respectively, relative to that of subcutaneous injection of 4 U/kg insulin. By increasing the amount of NaDC from 100 mg to 200 mg per suppository, the AUC and the RH, however, were not significantly improved. This could be explained by the fact that bile acids and salts are biological detergents that are synthesized by the liver and are secreted with the bile into the duodenum. At physiologic pH values, bile acids and salts are present as anions that exhibit detergent properties. These bile acids and salts at pH values above their pK_a reversibly form aggregates at concentrations above 2–5 mM. These aggregates are called *micelles*, and the bile salt molecules in the micelles are in equilibrium with the free bile salt in solution (20). Therefore, the decrease in efficiency of NaDC in promoting insulin absorption from the rectum when its amount was increased to 200 mg per suppository may be due to micelle formation.

Figure 3 shows the effect of increasing insulin dose in the suppository formulations that contain a fixed amount of NaDC (100 mg). The RH nonsignificantly changed from 25% ± 15% to 28% ± 8.1% and 18% ± 7.9% as the insulin dose increased from 5 to 10 and 20 U/kg, respectively. It is to be noted that the increase in C_{max} and AUC, however, was significant as the insulin dose increased (Table 1), but these changes were not correlated linearly with the changes in RH.

Also, it is clear from Table 1 that the C_{max} and AUC from the suppositories containing 20 U insulin and 100 mg NaDC are not significantly different ($P < .05$) from those obtained after subcutaneous injection, although the RH of the suppositories was only 18% of that of the subcutaneous injection. So, it is seen that the hypoglycemic profile obtained after subcutaneous injection can be mimicked by rectal suppository administration using higher doses of insulin.

Effect of Combinations of Bile Salts

Since in some instances enhancers are more effective when used in combination than alone, combinations may be a more effective method than increasing one enhancer concentration to increase the effectiveness of penetration enhancers. Indeed, increasing the enhancer concentration may not necessarily lead to enhanced drug penetration if the enhancer can form micelles, thereby entrapping drug and reducing its activity. As shown in Table 1, increasing the NaDC amount from 150 mg to 200 mg per insulin suppository resulted in a decrease of the AUC and the RH from 179 ± 65 mg%h and $35\% \pm 13\%$ to 132 ± 4.6 mg%h and $26\% \pm 0.9\%$, respectively. Since 150 mg NaDC seemed to be the maximum amount of the enhancer in the suppository formulation, we tried to formulate suppositories containing 5 U/kg insulin and 100 mg of NaDC or 50 mg of NaC, alone or in combination.

As shown in Fig. 4 and Table 1, insulin (5 U/kg) suppositories containing 100 mg NaDC produced an AUC of 126 ± 79 mg%h and an RH of $25\% \pm 15\%$.

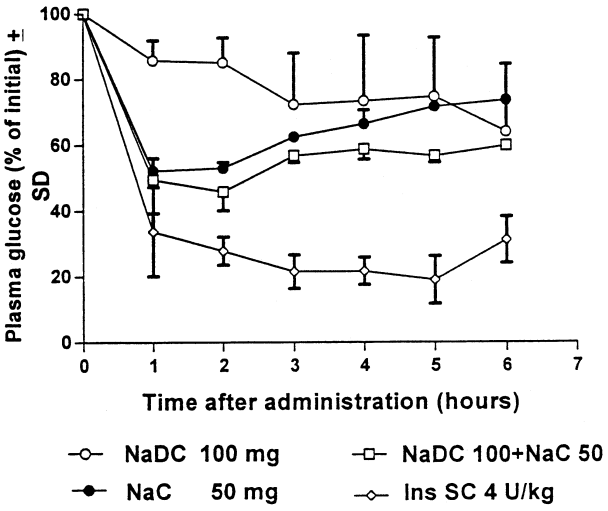

Figure 4. Effect of sodium deoxycholate (NaDC; 100 mg), sodium cholate (NaC; 50 mg) alone or in combination on the mean plasma glucose levels (% of initial values) of hyperglycemic beagle dogs after rectal administration of Witepsol W35 suppositories containing human insulin (5 U/kg) compared with insulin subcutaneous injection (Ins SC, 4 U/kg). Mean initial fasting plasma glucose level, mg% ± SD (No), were as follows: Ins SC, 216 ± 13 (6); NaDC, 187 ± 6.2 (6); NaC, 250 ± 3.5 (6); NaDC + NaC, 245 ± 6.8 (6).

On the other hand, insulin (5 U/kg) suppositories containing 50 mg NaC resulted in a rapid reduction in plasma glucose concentration of about $48\% \pm 3.6\%$ of its initial value in the first hour. Furthermore, an AUC of 207 ± 7.1 mg%h and an RH of $40\% \pm 1.9\%$ were found. Those values were significantly higher than those produced by NaDC. Insulin suppositories that contained a combination of NaC (50 mg) and NaDC (100 mg) produced an AUC of 252 ± 13 mg%h and an RH of $49\% \pm 2.6\%$, which were only significantly higher than those produced by NaDC.

This indeed shows that using a combination of bile salts is more effective than using a higher concentration of only one enhancer to increase the effectiveness of penetration. This is clear when comparing the effect of the combination with the formula containing 150 mg NaDC (Table 1), for which the RH reached was $35\% \pm 13\%$. This was the case for MGK and Na_2EDTA (ethylenediamine tetraacetic acid disodium salt) in the rectal absorption of des-enkephalin-γ-endorphin. Whereas the rectal bioavailability of this peptide was 0%–1% in the presence of EDTA and 8%–20% in the presence of MGK, it increased to 10%–44% in the presence of the combination (21).

Effect of Conjugated Bile Salts Alone or in Combination with Sodium Cholate

The hypoglycemic effect of insulin suppositories containing 100 mg of either sodium taurocholate (NaTC) or sodium taurodeoxycholate (NaTDC) is shown in Fig. 5. From the data in Table 1, NaTC and NaTDC seem to be very efficient in promoting insulin absorption from rectal suppositories. The suppositories containing NaTC produced an AUC of 301 ± 28 mg%h and resulted in an RH of $50\% \pm 4.7\%$. These suppositories also produced a C_{max} of $60\% \pm 5\%$ with a T_{max} of 4 h, and this effect lasted until the end of the sampling time. The bile salt conjugate NaTDC also produced an AUC of 277 ± 61 mg%h and an RH of $46\% \pm 10\%$ when compared to that of subcutaneous injection of 4 U/kg of insulin. The C_{max} was $55\% \pm 14\%$ after 4 h and lasted through the duration of the experiment. NaTDC has been found to reduce the viscosity and elasticity of bronchial mucus and presumably other types of mucus as well (22), while NaTC is shown to increase mucus secretion (23). The presence of mucous layers that coat all

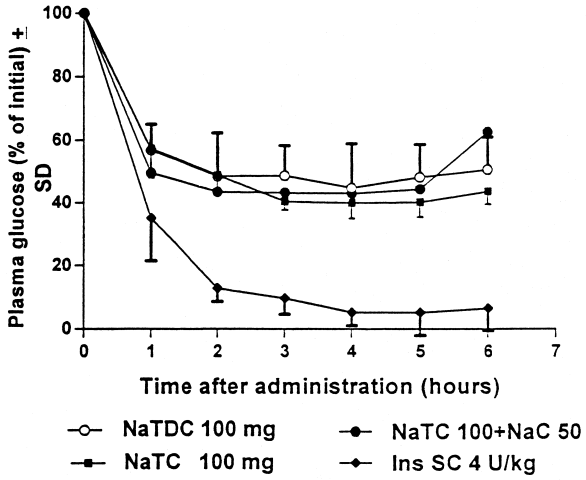

Figure 5. Effect of sodium taurodeoxycholate (NaTDC; 100 mg) or sodium taurocholate (NaTC; 100 mg) alone or in combination with sodium cholate (NaC; 50 mg), on the mean plasma glucose levels (% of initial values) of hyperglycemic beagle dogs after rectal administration of Witepsol W35 suppositories containing human insulin (5U/kg) compared with insulin subcutaneous injection (Ins SC, 4 U/ kg). Mean initial fasting plasma glucose level, mg% ± SD (No), were as follows: Ins SC, 253 ± 37.9 (6); NaTDC, 207 ± 18.3 (6); NaTC, 207 ± 10.3 (6); NaTC + NaC, 209 ± 12.4 (6).

epithelial surfaces has been overlooked in elucidation of penetration enhancement mechanisms. This is partly because the role of mucus in the absorption of peptide and protein drugs has not yet been established. Therefore, the exact mechanism of absorption promotion of insulin when NaTC or NaTDC was used in rectal insulin suppositories is not known, but it is reported that bile salts both increase membrane permeability and inhibit some proteolytic enzymes at the absorption site (24).

Figure 5 and Table 1 also show that using a combination of 100 mg of NaTC and 50 mg of NaC in insulin suppositories did not produce any significant change in AUC, RH, C_{max}, and T_{max} compared to that produced by insulin suppositories containing 100 mg NaTC.

In conclusion, a relative hypoglycemia of about 50% can be reached using insulin suppositories containing Witepsol W35 as a base and NaDC plus NaC (100 mg plus 50 mg, respectively), NaTDC (100 mg), or NaTC (100 mg) as rectal absorption enhancers of insulin. A desirable hypoglycemia, expressed as C_{max} and/or AUC, can be reached by adjusting the insulin dose in the formulation according to the degree of hyperglycemia.

ACKNOWLEDGMENT

We would like to acknowledge the financial support and the valuable suggestions of King Abdulaziz City for Science and Technology (KACST) to carry out this project through their grant AT/17/11. We are also grateful to Mr. Abdullah A. Abdulrazig for his expert technical assistance throughout this work.

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