

Evaluation of efficiency of insulin suppository formulations containing sodium salicylate or sodium cholate in insulin dependent diabetic patients

Ehab A. Hosny^{a*} • Zohair M.H. Al-Marzouki^a • Mohammed E.-S. Metwally^a • Mamdouh Y.S. Souaida^a • Abdel Rhman A.M. Alshaik^b

Pervenuto in Redazione il 26 giugno 2003

Accettato il 9 ottobre 2003

a) College of Pharmacy, King Abdulaziz University, Jeddah, SAUDI ARABIA

b) College of Medicine, King Abdulaziz University, Jeddah, SAUDI ARABIA

*Correspondence

Key Words: insulin-suppository-formulation; sodium-salicylate; sodium cholate

Abstract

Two formulations of insulin suppositories were prepared to contain different amounts of sodium salicylate and sodium cholate as absorption promoters and also of insulin with the purpose of obtaining the most effective formulation in reducing plasma glucose levels after rectal administration to diabetic patients.

The results show that insulin suppositories containing 100 mg sodium salicylate and 100 or 200 U of crystalline insulin showed no significant difference in AUC, C_{max} and T_{max} and both formulations showed significant reduction in plasma glucose level compared to initial values within 1.5-2 h. The results from experiments carried out in health volunteers showed that 100 mg sodium salicylate is the optimum amount to be included in insulin suppositories producing significantly higher C_{max} and AUC compared to those produced after rectal administration of insulin suppositories containing 50 or 200 mg sodium salicylate.

The results also show that using sodium cholate in 50 mg amount did not produce any significant reduction in plasma glucose levels of insulin dependent diabetic patients given suppositories containing 100 U of insulin, but this amount in suppositories containing 200 U of insulin was able to produce significant ($p < 0.05$) reduction in plasma glucose level within 1 h which lasted till end of experiment producing C_{max} of $29.7 \pm 6.61\%$ at T_{max} of 1.5 ± 0.61 h. On increasing the amount of sodium cholate to 100 mg in the suppositories, a marked ($p < 0.01$) reduction in plasma glucose level took place and the C_{max} increased to $47.7 \pm 12.24\%$ at T_{max} of 1.5 ± 0.63 h. This resulted in AUC of 86.7 ± 22.4 mg%h which was non significantly higher from that produced after administration of suppositories containing 50 mg sodium cholate and 200 U insulin (62.5 ± 17.6 mg%h).

The results also show that insulin suppositories containing

100 mg sodium cholate and 200 U insulin resulted in a non significant differences in C_{max} and AUC from those produced by S.C. injection of insulin (20 U) but significantly ($p < 0.001$) shorter T_{max} . This formulation also shows non significant differences in T_{max} and AUC and significantly ($p < 0.05$) higher C_{max} than from those produced after rectal administration of suppositories containing 100 mg of sodium salicylate and same amount of insulin. Further more this formulation produced severe hypoglycemia in control healthy volunteers within 1 h of administration producing C_{max} of $57.0 \pm 18.8\%$ at T_{max} of 0.75 ± 0.35 h.

The results of this study showed that the formulation containing 100 mg of sodium cholate and 200 U of insulin tested in fasted insulin dependent diabetic patients produced a maximum % reduction in plasma glucose levels (C_{max}) of $47.7 \pm 12.24\%$ at t_{max} of 1.5 ± 0.63 h compared to C_{max} of $50.56 \pm 6.8\%$ at t_{max} of 2.93 ± 0.19 h resulted after subcutaneous injection of 20 U insulin. These suppositories produced an area under the curve (AUC) of 87 ± 22.4 mg%h compared to an AUC of 81 ± 13.4 mg%h obtained after subcutaneous injection. This formulation of suppositories studied in 7 insulin dependent diabetic patients was found to abolish the 2-h post-prandial significant rise in plasma glucose levels after meal. These results show that these insulin suppositories containing 100 mg of sodium cholate and 200 U of insulin can serve as effective buffer against meal related hyperglycemia. The suppositories were safe, effective, accepted and well tolerated by the tested individuals.

Introduction

Absorption of insulin from subcutaneous tissue is variable both within & between various insulin preparations. The same dose of insulin may even have quite different effects on different days in the same patient. This makes it necessary not only to individualize therapy, but also to follow trial and error, supported to some extent by clinical judgement based on experience.

Ideally exogenous insulin should be administered in a way which mimics the physiological pattern, i.e., maintaining a continuous low basal level of serum insulin throughout the 24 hours, delivering an appropriate bolus as blood glucose levels rise after a meal, and returning to basal levels when the blood glucose falls in the post-absorptive phase.

Figure 1. Percent reduction of initial plasma glucose level in insulin-dependent diabetic patients after rectal administration of Witepsol W35 suppositories containing sodium salicylate compared with S.C. insulin injection

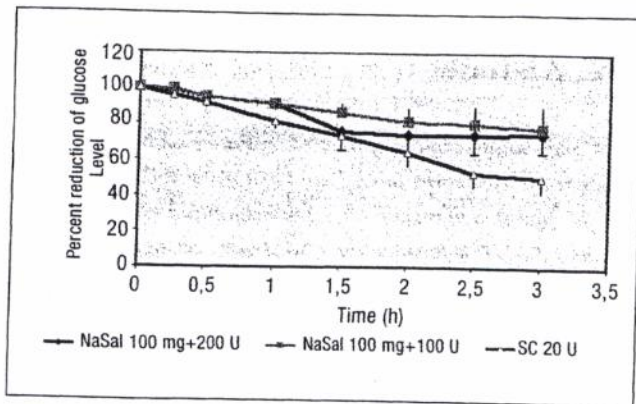


Figure 2. Percent reduction of initial plasma glucose level in healthy volunteers after rectal administration of Witepsol W35 suppositories containing sodium salicylate

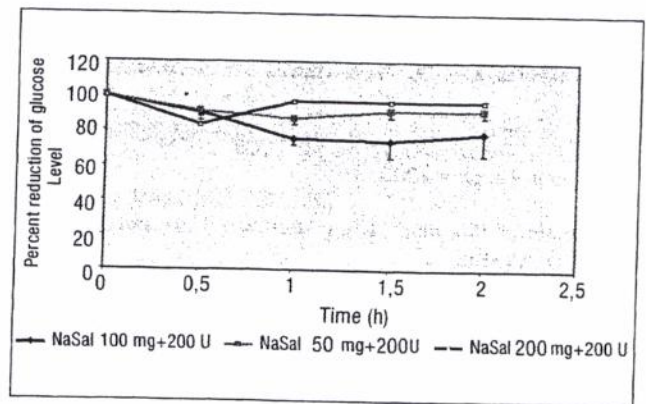


Figure 3. Percent reduction of initial plasma glucose level in insulin-dependent diabetic patients after rectal administration of Witepsol W35 suppositories containing sodium cholate

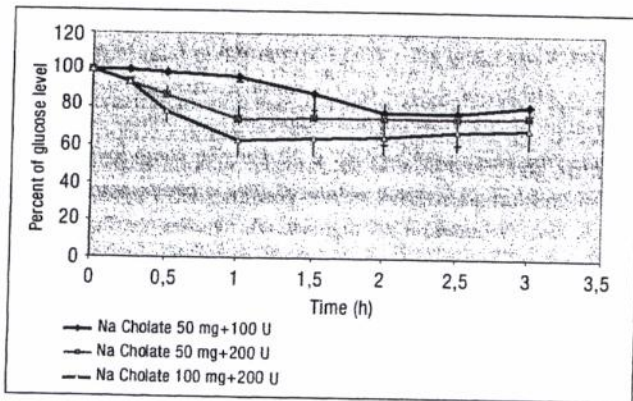


Figure 4. Percent reduction of initial plasma glucose level in healthy volunteers after rectal administration of Witepsol W35 suppositories containing sodium cholate

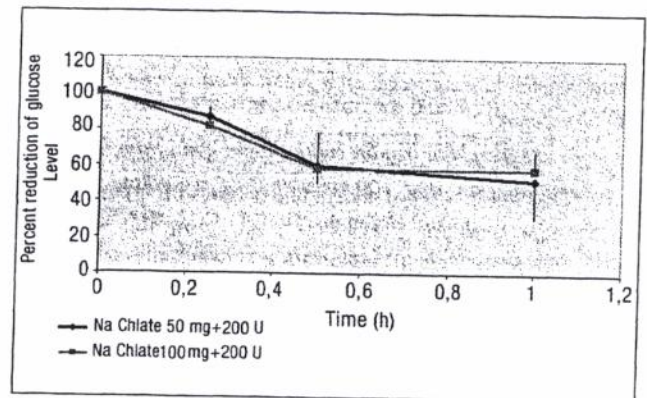
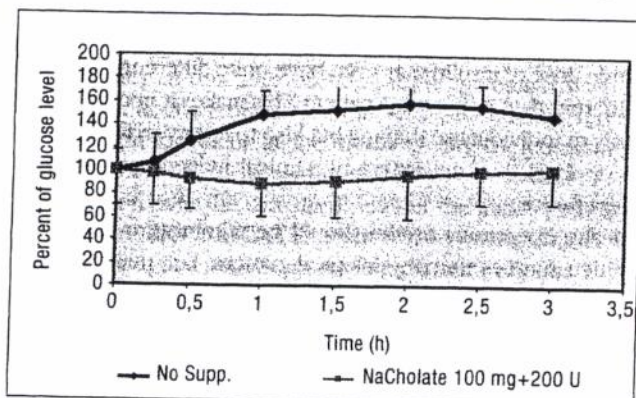


Figure 5. Comparison between percent plasma glucose level in insulin-dependent diabetic patients post-prandial after rectal administration of Witepsol W35 suppositories containing sodium cholate and control diabetic patients after eating



In conventional insulin therapy, twice-daily mixtures of fast and intermediate insulins are usually used in an effort to achieve this pattern, keeping a relatively fixed time relationship between injections and meals. This fixed relationship often results in a temporal mismatch between levels of insulin and blood glucose values. Insulin levels are inappropriately high or low at various times of the day. Furthermore, the insulin is delivered to the peripheral circulation in contrast to the normal intraportal secretion, thus resulting in peripheral hyperinsulinaemia. This hyperinsulinaemia stimulates smooth muscle cell proliferation and the incorporation of glucose into lipid in arterial walls, thus might be a causative factor in diabetic microangiopathy (1).

The belief that maintenance of euglycaemia has a beneficial effect on the long-term complication rate, and the realization that systemic hyperinsulinaemia may be harmful, led to the development of new strategies in the management of the insulin-dependent diabetic patients. Intensive control, using

better methods of insulin delivery have been thought of, studied and produced.

During the past 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 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 the depancreatized dogs fed regular meals demonstrated a less hyperinsulinaemia (4) than that observed in the 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 glycaemia and insulinaemia postprandially. Yamasaki et al, (1881) (7), concluded from their study that insulin suppositories attenuated the postprandial glycaemic rise in diabetic subjects and that the peripheral insulinaemia was similar to that present in normal subjects after meals. The studies presented a possibility that insulin suppositories could control the postprandial glycaemia 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 absorption promoting effect of sodium salicylate for insulin from suppositories was studied in normal volunteers and insulin dependent diabetic patients (8) and also in hyperglycaemic rabbits and dogs (9,10) and the results proved that sodium salicylate is effective in enhancing the rectal absorption of insulin and were effective in reducing plasma glucose levels.

The effect of insulin suppositories containing various bile salts/acids on the plasma glucose concentration of diabetic beagle dogs (11) and alloxan-induced hyperglycemic rabbits (12) was studied. The results show that insulin could be well absorbed due to the absorption enhancing effect of bile salts.

The objective of this research is to test the promoting effect of sodium salicylate and sodium cholate on the rectal absorption of human crystalline insulin from suppositories and determining the optimum formula that produces the largest reduction of plasma glucose level after clinical studies on fasting adult insulin-dependent diabetic patients. Also, perform clinical studies on these patients to test the efficacy of the best formulation chosen with respect to its ability to abolish the 2-h post-prandial significant rise in plasma glucose levels after meal.

■ Experimental

Materials

Insulin crystals HM (ge) from Novo Nordisk A/S (Novo Alle, 2880 Bagsvaerd, Denmark). Sodium salicylate from

BDH limited (Poole, England), Cholic acid-sodium salt from Serva Feinbiochemica GmbH & Co. (Carl-Benz-SträBe 7, Germany), Witepsol W35 from Dynamit Nobel (Northvale, NJ, USA), 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 at moderate temperature on a water bath. The absorption promoters sodium salicylate or sodium cholate then will be added and triturated until uniformly distributed in the melted base. After the melted mass was allowed to cool, insulin was added and triturated. Finally, the melted homogeneous mass was then poured into a 2-g mold and cooled. The suppositories were kept at 4°C until used on the next day.

Subcutaneous injection of insulin

Regular human insulin, Actrapid HM (100 IU/ml) from Novo Nordisk, Bagsvaerd, Denmark was injected subcutaneously as 20 U/ fasted insulin dependent diabetic patients.

Human studies

Twenty one insulin dependent diabetic patients (7 males and 14 females) and 3 health subjects, as controls, aged between 37 to 70 years with mean age of 52.77 ± 10.37 years were participated in this part of study. Their weight ranged from 50 to 108 kg with mean body weight of 75.57 ± 17.17 kg and their diabetes history ranged from 3 to 22 years with mean history of 14.40 ± 6.98 years. They were subjected to full clinical examination and found to be free from renal, hepatic, cardiac and neurologic diseases as well as bowel habits disturbances. The volunteers were asked not to take any insulin the day prior to study and no food after dinner. They were given in the morning the insulin formulation and blood samples were withdrawn at designed times. With regard post-prandial study the fasted patients were given suppositories just before given breakfast. The data obtained after rectal administration of the suppositories were compared to that after sc injection.

Blood sampling

Blood samples were withdrawn from the cannulated cubital vein immediately before dose administration and at different time intervals afterwards (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 hours). The blood samples for plasma glucose determination were centrifuged at 4000 rpm for 5 min. The plasma was collected and plasma glucose concentration was estimated within few hours of collection. Also continuous monitoring of plasma glucose levels was done during experiments using Accu-Chek Active. Also, fasting and post-prandial plasma glucose level was measured.

Table 1. Pharmacokinetic parameters of insulin-dependent diabetic patients and healthy volunteers after rectal administration of Witepsol W35 suppositories containing human insulin in presence of sodium salicylate (Nasal) or sodium cholate (NaC)

Enhancer (mg)	Insulin (U)	# of patients	Volunteers	C _{max} (%)	T _{max} (h)	AUC (mg% h)
-	20, SC	6	diabetic	51 ± 6.8	2.9 ± 0.19	81 ± 13.4
Nasal, 100	100	5	diabetic	24 ± 11.7***a	2.3 ± 0.67***a	35 ± 13.8***a
Nasal, 100	200	5	diabetic	26 ± 10.6***a	1.9 ± 0.22**a	51 ± 19.2**a
Nasal, 50	200	3	healthy	14 ± 2.9	1.2 ± 0.29	17 ± 6.2
Nasal, 100	200	3	healthy	28 ± 4.7**b,c	1.3 ± 0.29	35 ± 9.9**b,**c
Nasal, 200	200	3	healthy	16 ± 4.9	1.0 ± 0.0	9 ± 3.4
NaC, 50	100	5	diabetic	23 ± 16.7	2.2 ± 0.76	34 ± 27.2
NaC, 50	200	5	diabetic	30 ± 6.6	1.5 ± 0.61	63 ± 17.6
NaC, 100	200	6	diabetic	48 ± 12.2**d	1.5 ± 0.63	87 ± 22.4**d
NaC, 50	200	3	healthy	67 ± 11.7	1.3 ± 0.35	21 ± 7.8
NaC, 100	200	3	healthy	57 ± 18.8	0.75 ± 0.35	31 ± 8.4

*p < 0.05, **p < 0.01, ***p < 0.001

a compared with S.C. injection

**b compared to Nasal 50 mg and **c compared to Nasal 200 mg.

*d, **d compared to NaC 50 and 100 U insulin.

Plasma glucose estimation

Glucose reagent prepared by reconstituting the content of GOD-PAP reagent (Randox, Randox Laboratories, Antrim, U.K.) with phosphate buffer will be added to the plasma samples, mixed well by vortexing for 1 min and then incubated for 30 min at room temperature (25 °C). The absorbance (Ab) of the samples and standard against reagent blank was measured at 500 nm using UV/VIS. spectrophotometer. The blood glucose was calculated in mg/dl using the equation:

$$C = \frac{\text{"Ab" sample}}{\text{"Ab" standard}} \times 100 = \text{mg}/100 \text{ ml}$$

Pharmacokinetic calculations

1. The percent change in plasma glucose was calculated in order to minimize the effect of the varying initial plasma glucose levels of the patients. The change in plasma glucose was evaluated relative to the initial value of each individual.

$$\% \text{ change at time } t = \frac{P_0 - P_t}{P_0} \times 100$$

Where

P₀ is the initial plasma glucose concentration (fasting).

P_t is the plasma glucose concentration at time t after dose administration.

2. The maximum reduction in plasma glucose concentration and the corresponding time were taken as C_{max} and t_{max}, respectively.

3. The area under plasma glucose concentration-time curve (AUC) up to the last sampling time was calculated using the linear trapezoidal method. The AUC for % glucose reduction-time curve was calculated from % change in plasma glucose level where the fasting (initial) was considered as 100%. So after administration of insulin dosage forms the reduction in plasma glucose create a curve and the area between that curve and the 100% line was calculated using the linear trapezoidal rule. So, as the reduction in plasma glucose level increases, the area between the curve and the 100% line increases. All the data are expressed as mean + SD.

Statistical Analysis

Plasma glucose levels (0-3 h) after rectal administration of insulin suppositories and after subcutaneous injection were compared in each group with the respective initial values using repeated measures of variance (ANOVA) followed by Bonferroni multiple comparison test. Difference between groups in C_{max}, T_{max} and AUC were evaluated using Student's t test to compare two values or by one way ANOVA followed by Tukey-Kramer multiple comparison test in case of more than 2 values. These statistical calculations were performed using microcomputer statistical package (SAS, Statistical Analysis System). The difference will be considered significant at p values 0.05 or less. All data will be expressed as mean ± standard deviation (X±S.D).

Results and Discussion

Figure 1 and table 1 show the effect of sodium salicylate 100 mg/suppository on the hypoglycemic response of insulin suppositories containing 100 or 200 U of insulin. These insulin suppositories showed a significant (p < 0.05) reduction in plasma glucose level compared to the initial values (0 h) within 1.5-2 h and lasted till end of experiment (3 h) producing a C_{max} of 23.9 ± 11.7 and 26.4 ± 10.6 % at T_{max} of 2.3 ± 0.67 and 1.9 ± 0.22 h and AUC of 35 ± 13.8 and 51 ± 19.2 mg%h., respectively. The maximum reduction in plasma glucose levels produced after S.C. injection of soluble commercial insulin was significantly (p < 0.001 and p < 0.01) higher than that produced after rectal administration of these insulin suppositories. The S.C. injection of 20 U of insulin produced a significant (p < 0.05) reduction in plasma glucose levels by 1st hour and increased from 1.5 h (p < 0.01) and lasted till end of experiment. There is no significant difference in AUC, C_{max} and T_{max} between these suppository formulations containing 100 mg of sodium salicylate and 100 or 200 U of insulin.

Sodium salicylate is probably the most extensively studied enhancer for increasing rectal drug absorption (13-15). Sodium salicylate has been shown to stimulate the

rectal absorption of insulin in rats (16) dogs (14) and humans (8, 17).

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) (18, 19). Salicylate also acts on the protein components of plasma membranes (20). Similar results were obtained with red blood cell membranes and rectal brush border membrane vesicles (21, 22).

Non-protein thiols such as glutathione are yet another membrane components where salicylates might act upon (18, 23). Nishihata et al. (18) and Suzuka et al. (23) 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 (24) 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.

Fig. 2 shows the plasma glucose levels expressed as % of initial produced after rectal administration of insulin suppositories containing 200 U of insulin and different amounts of insulin 50, 100 and 200 mg of sodium salicylate to healthy non diabetic volunteers. The results in table 1, show that the suppositories containing 100 mg salicylate produced a significant ($p < 0.01$) reduction in plasma glucose level within 1 h and lasted to end of experiment (2 h) producing C_{max} of $27.7 \pm 4.7\%$ at T_{max} of 1.33 ± 0.29 h which was significantly higher than that produced by suppositories containing 50 and 200 mg of sodium salicylate (13.7 ± 2.9 and $16.0 \pm 4.9\%$ at T_{max} of 1.2 ± 0.29 and 1.0 ± 0.0 h, respectively). Also, the AUC (35 ± 9.9 mg%h) resulting after rectal administration of insulin suppositories containing 100 mg sodium salicylate was significantly higher than that produced after rectal administration of suppositories containing 50 ($p < 0.05$) or 200 mg ($p < 0.01$) sodium salicylate. These results indicate that addition of 100 mg salicylate is the ideal amount to be included in insulin suppositories to produce maximum reduction in plasma glucose levels.

Figure 3 shows that using sodium cholate in 50 mg amount did not produce any significant reduction in plasma glucose levels of insulin dependent diabetic patients given suppositories containing 100 U of insulin, but this amount in suppositories containing 200 U of insulin was able to produce significant reduction ($p < 0.05$) in plasma glucose level within 1 h which lasted till end of experiment (3 h) producing C_{max} of $29.7 \pm 6.61\%$ at T_{max} of 1.5 ± 0.61 h.

On increasing the amount of sodium cholate to 100 mg in the suppositories, a marked ($p < 0.01$) reduction in plasma glucose level took place and the C_{max} increased to $47.7 \pm 12.24\%$ at T_{max} of 1.5 ± 0.63 h. This resulted in AUC of 86.7 ± 22.4 mg%h which was non significantly higher from that produced after administration of suppositories containing 50 mg sodium cholate and 200 U insulin (62.5 ± 17.6 mg%h) as shown in table 1.

The results show that insulin suppositories containing 100

mg sodium cholate and 200 U insulin resulted in a non significant differences in C_{max} and AUC from those produced by S.C. injection of insulin but significantly ($p < 0.001$) shorter, T_{max} . This formulation also showed non significant differences in T_{max} and AUC and significantly ($p < 0.05$) higher C_{max} than from those produced by rectal administration of suppositories containing 100 mg of sodium salicylate and same amount of insulin. Further more this formulation produced severe hypoglycemia in control healthy volunteers within 1 h of administration producing C_{max} of $57.0 \pm 18.8\%$ at T_{max} of 0.75 ± 0.35 h as show in table 1 and Fig. 4.

The reduction in plasma glucose level is due to promotion of insulin absorption by the enhancement in paracellular absorption due to presence of bile salt 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 (25). The increase in water flux is sodium dependent, as indicated by reduction in its effect by ouabain (26). 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 absorption site (26). Bile salts are known to inhibit the mucosal proteolytic activity (27, 28) and to promote insulin absorption (29).

Sodium taurodeoxy cholate (NaTDC) has been found to reduce the viscosity and elasticity of bronchial mucus and presumably other types of mucus as well (30). While, sodium tauro cholate (NaTC) is shown to increase mucus secretion (31). The presence of mucus layers that coats all epithelial surfaces have 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 (32).

Ikemoto et al. (33) showed that addition of 0.5% cholate to high-safflower oil diet completely prevented high fat-induced hyperglycemia and obesity in C57BL/6J mice with a slight decrease of energy intake but with no inhibition of fat absorption. Furthermore, the addition of cholate decreased blood insulin levels and prevented high-fat diet-induced decrease of glucose uptake in epitrochlearis. However, there was no change in the unsaturation index of fatty acids in skeletal muscles and in GLUT-4 levels by cholate. In liver, cholate addition resulted in cholesterol accumulation and completely prevented high-fat diet-induced triglyceride accumulation.

Fig. 5 shows the changes in plasma glucose levels after eating for control diabetic patients and post-prandial after rectal administration of the chosen suppository formulation that contains 100 mg of sodium cholate and 200 U of insulin where it is given to diabetic patients just before eating breakfast. The data show that the plasma glucose levels of the control group increased significantly by 150 to 160 % of the initial fasting levels after eating. While for the dia-

betic group who administered the suppositories the 2 h post-prandial plasma

The study presented a possibility that insulin suppositories could control the postprandial hyperglycemia 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 suppositories were very well tolerated and accepted by all the diabetic patients involved in this study. These suppositories could be a viable alternative to existing therapies and are believed to be suitable for meal time administration with the goal of tight control of post-prandial hyperglycaemia in diabetic population. These suppositories will also be recommended in critical conditions where repeated doses of short acting

insulin are needed as in diabetic ketoacidosis. These suppositories will be suitable for children as well as adults. This route of administration of insulin will overcome most of the drawbacks associated with parenteral delivery of insulin e.g. peripheral hyperinsulinaemia which may lead to diabetic microangiopathy, burden of daily injections, reactions at site of injection, lack of patient compliance, cost, risks, infections, inconvenience and inability of some patients to self administer their insulin injections.

Acknowledgement

The investigators would like to acknowledge the financial support of Research Center, King Abdulaziz University.

References

- 1) Stout, R.W. *Diabetologia*, 1979, 16, 141.
- 2) Rojmark, S., Bloom, G., Chou, M.C.Y. and Field, J.B. *Endocrinology*, 1978, 102, 806.
- 3) Tiran, J., Avruch, L.I. and Albisser, A.M. *Am. J. Physiol.* 1979, 237, 231.
- 4) Goriya, Y., Bahoric, A., Marliss, E.B., Zinman, B. and Albisser, A.M. *Diabetologia*, 1980, 19, 452.
- 5) Goriya, Y., Bahoric, A., Marliss, E.B., Zinman, B. and Albisser, A.M. *Diabetes* 1979, 28, 558.
- 6) Berman, R.N. and Bucolo, R.J. *Am. J. Physiol.* 1974, 227, 1314.
- 7) Yamasaki, Y., Shichiri, M., Kawamori, R., Morishima, T., Hakui, N., Yagi, T. and Abe, H. *Can. J. Physiol. Pharmacol.* 1981, 59, 1.
- 8) Hosny, E., El-Ahmady, O., El-Shattamy, H., Nabih, A., El-Damacy, H. and El-Kabbany, N. *Drug Res.* 1994, 44, 611.
- 9) Hosny, E.A., Khan Ghilzai, N.M. and Elmazar, M.M. *Saudi Pharm. J.* 1998, 6, 235.
- 10) Hosny, E.A., Al-Shora, H.I. and Elmazar, M.M. *Biol. Pharm. Bull.* 2001, 24, 1294.
- 11) Hosny, E.A., Al-Shora, H.I. and Elmazar, M.M. *Drug Dev. Ind. Pharm.* 2001, 27, 837.
- 12) Hosny, E.A. *Drug Dev. Ind. Pharm.* 1999, 25, 745.
- 13) Nishihata, T., Rytting, J., and Higuchi, T. *J. Pharm. Sci.* 1982, 17, 869.
- 14) Nishihata, T., Rytting, J.H., Kamada, A., Higuchi, T., Routh, M. and Caldwell, L.J. *Pharm. Pharmacol.* 1983, 35, 148.
- 15) Aungst, B.J. and Rogers, N.J. *Pharm. Res.* 1980, 5, 305.
- 16) Nishihata, T., Rytting, J.H., Higuchi, T. and Caldwell, L.J. *Pharm. Pharmacol.* 1981, 33, 334.
- 17) Nishihata, T., Okamura, Y., Inagaki, H., Sudho, H. and Shichiri, M. *Int. J. Pharm.* 1986, 34, 157.
- 18) Nishihata, T., Nghiem, B.T., Yoshitomi, H., Lee, C.S., Dillsaver, M., Higuchi, T., Choh, T., Suzuka, T., Furuay, A. and Kamada, A. *Pharm. Res.* 1986, 3, 345.
- 19) Kajii, H., Horie, T., Hayashi, M. and Awazu, S. *Life Sci.* 1985, 37, 208.
- 20) Kajii, H., Horie, T., Hayashi, M. and Awazu, S. *J. Pharm. Sci.* 1986, 75, 475.
- 21) Nishihata, T. and Higuchi, T. *Biochim. Biophys. Acta*, 1984, 775, 269.
- 22) Nishihata, T., Higuchi, T. and Kamada, A. Possible mechanism. *Life Sci.* 1984, 34, 437.
- 23) Suzuka, T., Yata, N., Sakai, K. and Nishihata, T. *J. Pharm. Pharmacol.* 1988, 40, 469.
- 24) Touitou, E., Alhaque, F., Fisher, P., Memoli, A., Riccieri, F.M. and Santucci, E. *J. Pharm. Sci.* 1987, 76, 791.
- 25) Freel, R.W., Hatch, M., Earnest, D.L., and Goldner, A.M. *Am. J. Physiol.* 1983, 245, G816.
- 26) Shiga, M., Hayashi, M., Hori, T., and Awazu, S. *Chem. Pharm. Bull.* 1986, 34, 2254.
- 27) Zhou, X.H. and Wan Po, A. Li. *Int. J. Pharm.* 1991, 69, 29.
- 28) Hayakawa, E., Chien, D.S., Inagaki, Y., Yamamoto, A. and Lee, V. *Pharm. Res.* 1992, 9, 769.
- 29) Kidron, M., Bar-On, H., Berry, E.M. and Zin. *E. Life Sci.*, 1982, 31, 2837.
- 30) Martin, G.P., Marriott, C. and Kellaway, I. *W. Gut.* 1978, 19, 103.
- 31) Poelma, E.G.J., Tukker, J.J. and Grommelin, D.J.A. *J. Pharm. Sci.* 1989, 78, 285.
- 32) Hirai, S., Yashiki, T. and Mima, H. *Int. J. Pharm.* 1981, 9, 173.
- 33) Ikemoto, S., Takahashi, M., Tsunoda, N., Maruyama, K., Itakura, H., Kawanaka, K., Tabata, I., Higuchi, M., Tange, T., Yamamoto, T.T. and Ezaki, O. *Am. J. Physiol.* 1997, 273. (1 Pt 1) E37.