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Development of a thermo-reversible insulin liquid suppository with bioavailability enhancement

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Abstract

The purpose of this work is to develop a thermo-reversible insulin liquid suppository, which undergoes a phase transition to bioadhesive gels at body temperature and enhances the bioavailability of insulin. The effects of insulin and sodium salicylate on the physicochemical properties of a liquid suppository composed of poloxamer P 407, P 188 and polycarbophil were investigated. The pharmacodynamic study and quantitative histological assessment of the rectal mucosa of rats were carried out after the dose of insulin-loaded liquid suppositories with different amounts of sodium salicylate into streptozotocin-treated rats. Only thermo-reversible insulin liquid suppository [insulin/P407/P188/polycarbophil/sodium salicylate (100 (IU/g)/15/20/0.2/10%)] showed the optimal physicochemical properties and good safety in rats. It gave significantly lower plasma glucose levels, $AUC_{0\rightarrow 4h}$ (the area below basal glucose level) and C_{nadir} (the plasma glucose levels at the nadir) than did the solid and liquid suppositories without sodium salicylate in rats, indicating that the insulin from liquid suppository with sodium salicylate could be well absorbed in rats due to the absorption enhancing effect of sodium salicylate. It is concluded that thermo-reversible insulin liquid suppository [insulin/P 407/P 188/polycarbophil/sodium salicylate (100 (IU/g)/15/20/0.2/10%)], which was easy to administer without any pain during insertion and remained at the administered sites, could have a potential to be developed as a more convenient, safe and effective rectal delivery system of insulin.

Keywords: Liquid suppository; Thermo-reversible; Insulin; Poloxamer; Polycarbophil; Sodium salicylate; Pharmacodynamics

1. Introduction

Insulin, 51-amino acid polypeptide, is difficult to orally administer because it is inactivated by proteolytic digestion. It was attempted to absorb insulin through the mucosa of the duodenum by in vitro experiments using isolated intestinal loops of rats, rabbits and dogs. In vivo absorption studies in rabbits and rats were also carried out with surfactants, proteolysis inhibitors or emulsions (Hosny et al., 1994). Although some promising experimental results have been reported, the

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gastrointestinal route of insulin delivery has not yet been proven successful in the clinical setting due to its low bioavailability and high variability (Scott-Moncrieff et al., 1994). Therefore, insulin is still administered in the form of an injection, a conventional dosage form. The diabetic patients appeal to various problems such as physical and psychic pain, hypertrophy or atrophy of the subcutaneous fat at an injection site. In an attempt to overcome these problems, many alternative dosage forms including infusion (Goriya et al., 1980), implant (Burczak et al., 1996), transdermal (Hoffman and Ziv, 1997) and mucosal delivery (Asada et al., 1995) have been developed. The insulin rectal suppository, which was a safer and more convenient dosage form, has also been developed (Hosny et al., 1994).

The ideal suppository would be easy to administer with good patient compliance and remain at the administered sites avoiding the first pass effect in the liver and the gastrointestinal tracts. The conventional suppository is in a solid dosage form which melts or softens in the rectum. Such a solid suppository can give a feeling of alien, discomfort and refusal to the patients, possibly lowering patient compliance. Furthermore, a solid suppository, which might reach the end of the colon, has a loss of drug at colonic level and may also allow the carried drugs to undergo the first-pass effect. Recently, an attempt was made to develop in-situ gelling and mucoadhesive, namely 'thermo-reversible liquid suppository' which exists as a liquid in vitro but a gel in vivo, by modulating the gelation temperature of the liquid suppository base. The thermo-reversible liquid suppository was easy to administer to the anus, since it was in a liquid form at room temperature and turned into a gel instantly at physiological temperature and was also mucoadhesive to the rectal tissues without leakage after the dose. Furthermore, it showed the enhanced bioavailability of drug with good safety in rats and human subjects (Miyazaki et al., 1987; Choi et al., 1998a,b,c; Kim et al., 1998; Miyazaki et al., 1998).

In the formulation of insulin suppository, enhancers such as enamine derivatives (Kim et al., 1984), salicylate derivatives and bile salts (Scott-Moncrieff et al., 1994) were used to promote the rectal absorption of insulin. Sodium salicylate has been found to be the most effective enhancer for rectal systemic delivery of insulin and has been studied in rats (Richardson et al., 1992), dogs (Liversidge et al., 1985) and humans (Nishihata et al., 1986), showing a significant improvement in insulin absorption.

Thus, in this study, thermo-reversible insulin liquid suppositories were developed with poloxamers, polycarbophil and sodium salicylate. Furthermore, we carried out the quantitative histological assessment of the rectal mucosa of rats and evaluated the pharmacodynamic profiles of insulin after the administration of insulin-loaded liquid suppositories with different amounts of sodium salicylate into streptozotocin-treated rats.

2. Materials and methods

2.1. Materials

Porcine insulin (26.1 U/mg) and streptozotocin were purchased from Green Cross (Seoul, South Korea) and Sigma (St Louis, MO), respectively. Sodium salicylate was of USP grade. Poloxamers (P 407, P 188) and polycarbophil were supplied from BF Goodrich (Breesville, OH) and BASF (Ludwigshafen, Germany), respectively. Glucose-E kit was obtained from International Reagent Co. (Kobe, Japan). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of liquid suppository

Aqueous solutions of polycarbophil, sodium salicylate and insulin were prepared by dispersing or dissolving in distilled water at room temperature and the solutions were cooled down to 4°C. Poloxamer 407 and 188 were then slowly added to the solution with continuous agitation. The liquid suppository was left at 4°C until a clear solution was obtained.

Four different liquid suppositories were obtained with the following compositions; Liquid suppository A [insulin/P 407/P 188/polycarbophil

(0.38/15/15/0.6%)], B [insulin/sodium salicylate/P 407/P 188/polycarbophil (0.38/10/15/20/0.2%)], C [insulin/sodium salicylate/P 407/P 188/polycarbophil (0.38/20/15/20/0.2%)] and D [insulin/sodium salicylate/P 407/P 188/polycarbophil (0.38/30/15/20/0.2%)] containing 100 IU/g insulin, respectively.

2.3. Determination of insulin contents in liquid suppository

Each liquid suppository (0.5 g) was dissolved in 250 ml distilled water and then filtered. The solutions were analyzed using a high-performance chromatograph (Waters, Model TM 717) equipped with a Lichrosorb RP-18 column (0.5 μm, 25×0.46 cm i.d.) and an ultraviolet spectrophotometric detector (Model SPD-6A) at 214 nm. The mobile phase consisted of 0.2 M anhydrous sodium sulfate adjusted to pH 2.3 with phosphoric acid and acetonitrile (74:26, volume ratio). The flow rate of eluent was 1.2 ml/min (Khaksa et al., 1998).

2.4. Measurement of gelation temperature

A 20-ml transparent vial containing a magnetic bar and 10 g of liquid suppository was placed in a low-temperature thermostat water bath (Heto, Scandinavia). A digital thermosensor (Ika Labortechnik, RET digi-visc) connected to a thermistor was immersed in the liquid suppository which was heated at a constant rate with constant stirring. When the magnetic bar stopped moving due to gelation, the temperature displayed on the thermistor was determined as the gelation temperature.

2.5. Measurement of gel strength

Liquid suppository (50 g) was put in a 100-ml graduated cylinder and gelled in a thermostat at 36.5°C. The apparatus for measuring gel strength (weight: 35 g) was then placed onto the liquid suppository. The gel strength, which means the viscosity of liquid suppository at physiological temperature, was determined by the time (s) the apparatus took to sink 5 cm down through the liquid suppository. In cases, it took more than 10 min to drop the apparatus into the suppository, various weights were placed on top of the apparatus and gel strength was determined by the minimal weights that pushed the apparatus 5 cm down though the suppository (Choi et al., 1998b).

2.6. Determination of bioadhesive force

The bioadhesive force of liquid suppository was determined by using the measuring device in Fig. 1. In brief, a section of tissue was cut from the fundus of rabbit rectum and secured with the mucosal side out onto each glass vial (C) using a rubber band and an aluminum cap. The vials with the rectal tissues were stored at 36.5°C for 10 min. Next, one vial with a section of tissue (E) was connected to the balance (A) and the other vial was placed on a height-adjustable pan (F). The liquid suppository (D) was added onto the rectal tissue on the other vial. Then, the height of the vial was adjusted so that the liquid suppository could be placed between the mucosal tissues of both vials. The weights (B) were kept raised until two vials were attached. Bioadhesive force, the detachment stress (dyne/cm^2), was determined from the minimal weights that detached two vials. The rectal tissue pieces were changed for each measurement.

2.7. In vivo experiments

2.7.1. Streptozotocin-treated rats

Male Sprague–Dawley rats weighing 280 ± 20 g were supplied from Experimental Animal Breeding Center of Seoul National University (Seoul, South Korea). Diabetes was induced by i.p. injection of a freshly prepared solution of streptozotocin (100 mg/kg) in saline. After 2–3 days, the induction of diabetes was assessed by the weight and blood glucose level of the rats (Sato et al., 1991; Kim et al., 1999).

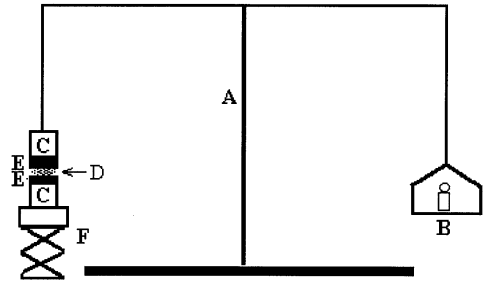

Fig. 1. Bioadhesive force-measuring device: (A) modified balance; (B) weights; (C) glass vial; (D) liquid suppository; (E) rectal tissue; (F) height-adjustable pan.

2.7.2. Treatment groups

Twenty-five streptozotocin-treated rats were divided into five groups. The rats in each group were administered with conventional solid suppository [insulin/polyethylene glycol 4000 (9.62/0.38%)], and liquid suppository A, B, C and D containing 100 IU/g insulin, respectively.

2.7.3. Administration and blood collecting

Before administration of suppository, the streptozotocin-treated rats were fasted overnight but allowed free access to water. Each rat, anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position with a thread, followed by eliminating the feces from the anus of rats with a stomach sonde needle. A polyethylene tube was inserted into the right femoral artery of the rat. Liquid suppositories (1 g/kg equivalent to insulin 100 IU/kg) were administered into the rectum 4 cm above the anus through a stomach sonde needle fitted on a glass syringe. The solid suppository was administered with a dose of 1 g/kg (equivalent to insulin 100 IU/kg) into the rectum 4 cm above the anus.

2.7.4. Analysis of glucose in plasma

Blood samples were collected from the right femoral artery at designated time intervals during 4 h after the dose, centrifuged to obtain plasma. Plasma samples were stored at -20°C until analysis of glucose by the following procedure. The level of glucose in plasma was determined using glucose-E kit (Hosny et al., 1994). The mixture of glucose oxidase (24 U/ml) and 4-aminoantipyrine (0.1 mg/ml) was reconstituted with *p*-hydroxybenzoic acid solution (1.66 mg/ml). Three milliliters of the reconstituted solution were then added to 20 μl of plasma sample and standard solution, respectively. The resulting mixture was incubated at 37°C for 10 min and the optical density was read at 540 nm. Plasma glucose levels were expressed as a percentage of the basal concentration, which was calculated as the mean value of five samples taken prior to the rectal administration of the insulin suppository (Richardson et al., 1992).

2.7.5. Morphology test of rectal tissues

At 4 h after the dose, the rectum was isolated, rinsed with a saline solution, fixed in 10% neutral carbonate-buffered formaldehyde, embedded in paraffin using an embedding center and cut into slices. The slices were stained with hematoxylin–eosin and observed under a light microscope (Leitz; Laborlux 12 Pols, Germany). The observation rate of the three types of gland changes in the rectal epithelium was evaluated and compared with those in fresh rectal epithelium without drug treatment as a control (Reid et al., 1987).

3. Results and discussion

3.1. Effect of insulin and sodium salicylate on the physicochemical properties of liquid suppository

Since insulin is an active material and sodium salicylate is an absorption enhancer, their effects on the physicochemical properties of the liquid suppository need to be studied.

Various concentrations of insulin (0.10–0.38%, 25–100 IU/g) and sodium salicylate (10–30%) were added to a liquid suppository base [P 407/P 188/polycarbophil (15/20/0.2%)], and the physicochemical properties such as gelation temperature, gel strength and bioadhesive force of liquid suppositories were evaluated (Tables 1 and 2).

Liquid suppositories loaded with more than 10% sodium salicylate and more than 0.4% of polycarbophil underwent phase separation. Therefore, 0.2% polycarbophil was used to prepare the liquid suppositories. The results indicated that insulin did not affect the physicochemical properties of liquid suppository possibly due to a negligible amount of insulin (Table 1). However, sodium

Table 1
Effect of insulin on the physicochemical properties of liquid suppositories[a]

	Gelation temperature (°C)	Gel strength (s, g)	Bioadhesive force ($\times 10^2$ dyne/cm^2)
P 407/P 188 (15/20%)	27.0 ± 0.5	187 ± 23 (450 g)	124.6 ± 11.3
Insulin (IU/g)			
25	27.0 ± 0.3	184 ± 28 (450 g)	124.6 ± 38.7
50	27.0 ± 0.3	181 ± 16 (450 g)	121.5 ± 46.8
100	27.2 ± 0.4	180 ± 33 (450 g)	121.0 ± 42.0

[a] Each value represents the mean $\pm$ S.D. ($n = 6$).

salicylate increased the gelation temperature and decreased the gel strength and bioadhesive force (Table 2). As a possible mechanism by which sodium salicylate affected the physicochemical properties of gel, it is conceivable that the binding force (hydrogen bonding) of cross-linked reticular poloxamer gel (liquid suppository) became weaker by placing sodium salicylate in the gel matrix.

Insulin liquid suppository A without sodium salicylate and B with 10% sodium salicylate in Table 2 had the gelation temperature, gel strength and bioadhesive force suitable for liquid suppository. Previously, it was reported that the optimal liquid suppository should have the suitable range of gelation temperature (30–36°C), gel strength (10–50 s) and bioadhesive force to administer easily and to remain at the administered site without leakage after the dose (Choi et al., 1998b).

3.2. Stability of insulin in liquid suppository

Before the pharmacodynamic studies, we investigated the stability of insulin during the preparation and gelation of liquid suppository at body temperature. After the preparation and the subsequent storage of liquid suppository B over 12 h at 37 ± 0.5°C, it contained 99.5 ± 2.1 and 98.1 ± 1.8 IU/g insulin, respectively. Furthermore, the contents of insulin in the liquid suppository B were monitored over 3 months at 4°C. No significant changes in the insulin contents were observed during the storage period. Our results suggested that insulin was stable in the liquid suppository base during preparation, gelation in the anus and storage over 3 months at 4°C.

3.3. Pharmacodynamic studies

The induction of diabetes was assessed by body weight and plasma glucose level of rats. After treatment with streptozotocin, rats showed a slightly decreased body weight (280 ± 22 vs 253 ± 13 g) and more than threefold increase of plasma glucose level (124 ± 30 vs 355 ± 44 mg/dl). The higher glucose level of streptozotocin-treated rats indicated that these rats could be used as model animals of diabetes.

Plasma glucose level-time profiles and pharmacodynamic parameters are shown in Fig. 2 and Table 3. Liquid suppository A [insulin/P 407/P 188/polycarbophil (0.38/15/15/0.6%)] prepared

Table 2
Physicochemical properties of insulin liquid suppositories with and without sodium salicylate[a]

Liquid suppository (sodium salicylate)	Gelation temperature (°C)	Gel strength (s, g)	Bioadhesive force ($\times 10^2$ dyne/cm^2)
A (0%)	32.7 ± 0.5	39.83 ± 7.62	152.2 ± 34.2
B (10%)	33.5 ± 0.7	30.34 ± 7.53	120.3 ± 39.2
C (20%)	40.1 ± 0.3	7.48 ± 3.14	107.2 ± 11.4
D (30%)	44.6 ± 0.4	4.33 ± 2.00	86.8 ± 9.3

[a] Each value represents the mean $\pm$ S.D. ($n = 6$).

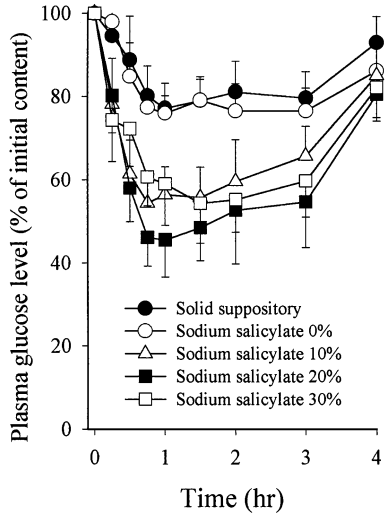

Fig. 2. Change in basal plasma glucose level (%) after the rectal administration of insulin-loaded solid suppository, liquid suppositories (100 IU/kg) to streptozotocin-treated rats. Conventional solid suppository and liquid suppository A were composed of [insulin/PEG 4000 (0.38/99.62%)] and [insulin/P 407/P 188/polycarbophil (0.38/15/15/ 0.6%)], respectively. Liquid suppository B, C and D were composed of [insulin/sodium salicylate/P 407/P 188/polycarbophil (0.38/10 (20 and 30)/15/20/0.2%)], respectively. Each value represents the mean ± S.D. ($n = 6$). *$P < 0.05$ compared to solid suppository and liquid suppository A.

Table 3
Pharmacodynamic parameters of insulin delivered by the solid suppository and liquid suppositories[a]

Parameters	C_{nadir} (%)	T_{nadir} (h)	$AUC_{0\rightarrow 4h}$ (%·h)[b]
Solid suppository	79.02 ± 5.70	0.95 ± 0.10	333.68 ± 32.69
Liquid suppository			
A (0%)[c]	75.94 ± 4.26	0.95 ± 0.10	322.53 ± 23.41
B (10%)	54.37 ± 3.18*	0.75 ± 0.16	262.91 ± 5.77*
C (20%)	46.25 ± 7.12*	0.75 ± 0.17	234.46 ± 24.83*
D (30%)	54.39 ± 4.53*	1.25 ± 0.21	255.78 ± 12.67*

[a] Each value represents the mean ± S.D. ($n = 6$).
[b] Area was calculated by the trapezoidal method over 4 h.
[c] The percentages in parentheses indicate the contents of sodium salicylate.
* $P < 0.05$ compared to solid suppository and liquid suppository A.

without sodium salicylate was inserted into the anus of rats instead of [insulin/P 407/P 188/polycarbophil (0.38/15/20/0.2%)], since the latter was impossible for insertion into the anus of rats due to very strong gel strength (Table 2).

Solid suppository and liquid suppository A did not show a significant difference in the plasma glucose level-time profiles over 4 h after the dose (Fig. 2). The $AUC_{0\rightarrow 4h}$ (the area below basal glucose level), C_{nadir} (the plasma glucose levels at the nadir) and T_{nadir} (the time to reach the nadir) of liquid suppository A did not significantly differ from those of solid suppository (Table 3). These results indicated that liquid suppository A, which was easy to administer to the anus without any pain during insertion and remained at the administered site, was biologically equivalent to solid suppository.

Liquid suppository B–D with 10–30% sodium salicylate showed lower plasma glucose levels in rats compared with liquid suppository A without sodium salicylate (Fig. 2). The $AUC_{0\rightarrow 4h}$ and C_{nadir} of liquid suppository B–D were lower than those of liquid suppository A without sodium salicylate ($P < 0.05$). The mean T_{nadir} of liquid suppository B–C was faster than that of liquid suppository A, although there was no significant difference between T_{nadir} (Table 3). These results indicated that the insulin from liquid suppository with more than 10% sodium salicylate could be well absorbed compared with suppositories without sodium salicylate in rats due to the absorption enhancing effect of sodium salicylate.

Liquid suppository C with 20% of sodium salicylate gave a lower mean plasma glucose level, $AUC_{0\rightarrow 4h}$ and C_{nadir} compared with liquid suppository B as shown in Table 3, even if there was no significant difference ($P < 0.05$). On the other hand, liquid suppository D with 30% of sodium salicylate tended to increase the mean plasma level of glucose, $AUC_{0\rightarrow 4h}$ and C_{nadir} compared with liquid suppository C (Fig. 2). It appeared that more than 20% of sodium salicylate tended to reduce the enhancing effect on the absorption of insulin in rats.

Our pharmacodynamic results with rats proved that the insulin liquid suppository with more than 10% sodium salicylate enhanced the bioavailabil-

Table 4
Observation rate of the three types of changes observed in the rectal epithelium at 4 h after the rectal administration of insulin liquid suppositories[a]

Liquid suppository (sodium salicylate)	Classification (%)			
	Normal	Type I	Type II	Type III
Control[b]	72.38 ± 21.54	20.26 ± 8.51	4.19 ± 2.34	3.17 ± 1.22
A (0%)	74.44 ± 34.23	9.73 ± 4.67	10.32 ± 6.71	5.51 ± 4.26
B (10%)	67.35 ± 23.77	11.27 ± 8.18	15.38 ± 7.53	6.00 ± 3.38
C (20%)	31.27 ± 9.45	19.51 ± 17.80	25.56 ± 9.34	23.66 ± 10.17
D (30%)	12.31 ± 4.56	25.44 ± 11.53	15.68 ± 8.53	46.57 ± 21.53

[a] Each value represents the mean ± S.D. ($n = 6$).
[b] Control means fresh rectal epithelium without drug administration.

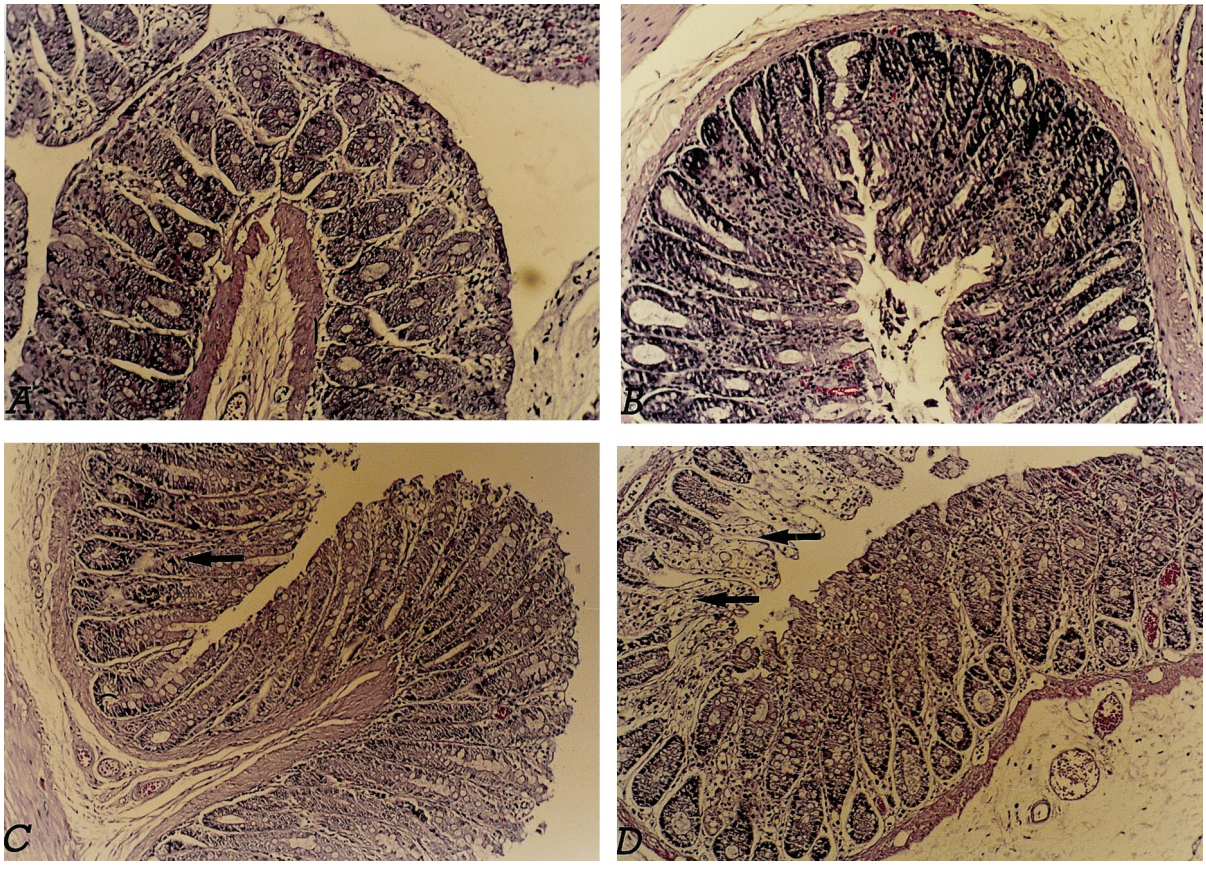

Fig. 3. Morphology of rectal mucosa of streptozotocin-treated rats after the rectal administration of insulin liquid suppositories with various concentrations of sodium salicylate (× 250): (A) 0%, (B) 10%, (C) 20%, (D) 30%.

ity of insulin. Therefore, the bioavailability of insulin from liquid suppository in human subjects is very feasible.

3.4. Histological assessment of rectal tissues

Previously, liquid suppository composed of acetaminophen, poloxamers and polycarbophil was reported to give no damage to mucous membranes (Choi et al., 1998a,b), whereas sodium salicylate, an absorption enhancer, was reported to be an irritatant to mucous membranes (Richardson et al., 1992). Histological assessment was performed by observing any irritation of insulin liquid suppository on the rectal tissues followed by evaluating the observation rate of the three types of gland changes in the rectal epithelium (Reid et al., 1987).

Liquid suppository A and B with 0 and 10% sodium salicylate showed similar observation rates of normal gland to control (72.38 ± 21.54 vs 74.44 ± 34.23 and $67.35 \pm 23.77\%$, respectively). However, liquid suppository C and D with 20 and 30% sodium salicylate exhibited significantly lower observation rates of normal gland compared with control (72.38 ± 21.54 vs 31.27 ± 9.45 and $12.31 \pm 4.56\%$, respectively) (Table 4). It indicated that liquid suppository A and B did not damage the rectal tissues but liquid suppository C and D damaged the rectal tissues, in spite of relatively weak gel strength and bioadhesive force (Fig. 3). Presumably, 10% sodium salicylate was the tissue-damaging threshold level in this rectal suppository system. Liquid suppository D with 30% sodium salicylate gave the higher mean plasma glucose level, $AUC_{0\rightarrow 4h}$ and C_{nadir} than liquid suppositories C, probably due to the reduction of the enhancing effect by 30% sodium salicylate on the absorption of insulin in rats caused by serious damage of the rectal tissues.

4. Conclusion

Based on the results of pharmacodynamic profiles of insulin and histological assessment of the rectal tissues of rats after the dose, it is concluded that a thermo-reversible insulin liquid suppository with 10% sodium salicylate, which was easy to administer without any pain during insertion and remained at the administered site, could have potential to be developed as a more convenient, safe and effective rectal delivery system of insulin.

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