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# Effect of formulation on the systemic absorption of insulin from enhancer-free ocular devices

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**Abstract**

Several Gelfoam® (absorbable gelatin sponge, USP) based surfactant free devices containing either sodium or zinc insulin were prepared with diluted acetic or hydrochloric acid. They were evaluated by the lowering of the blood glucose concentration in rabbits. The systemic absorption of insulin from the device can be enhanced by using a 5% or higher concentration of acetic acid solution as well as 1% HCl solution. The results indicate that the proposed device prepared with up to 30% of acetic acid solution produced no eye irritation. A single device containing 0.2 mg of insulin is sufficient to control the blood glucose levels in a uniform manner (60% of initial) for over 8 h. 

*Keywords:* Insulin; Ocular device; Gelfoam

## 1. Introduction

In recent years, eye drop solutions for the systemic delivery of insulin have been extensively studied in rats (Pillion et al., 1991, 1995), rabbits (Chiou et al., 1989a,b, 1990; Yamamoto et al., 1989; Chiou, 1991; Chiou and Li, 1993; Sasaki et al., 1994, 1995), cats (Hopper et al., 1991; Morgan 1995) and dogs (Morgan and Huntzicker, 1996) as well as in humans (Bartlett et al., 1994a,b). All of the reported data indicate that an absorption enhancer (usually a surfactant or chelating agent) is required for the systemic absorption of insulin. Even with an enhancer, the physiological responses to insulin given by conventional eye drops are characterized by rapid onset, short duration (Pillion et al., 1995), and low bioavailability (Bartlett et al., 1994a,b; Morgan and Huntzicker, 1996). Also, the use of a surfactant or chelating agent as an enhancer in the eye drop may be associated with side effects. For these reasons, it has been suggested by Hoffman and Ziv (1997) that the ocular delivery of insulin by solution has not reached the acceptable level of practical utility.

It has been proposed that the use of an ocular insert could improve the therapeutic efficacy of insulin delivered by the ocular route (Yamamoto

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et al., 1989). The systemic delivery of insulin from an ocular device is similar to that from an eyedrop. Both utilize the dynamics of the lachrymal system to transport the drug to the absorption site, which is believed to be the nasal cavity (Yamamoto et al., 1989). Unlike eyedrops, the device can remain in the conjunctival sac and significantly increase the contact time of insulin with the absorption site.

Gelfoam®, absorbable gelatin sponge, USP, has been introduced by our laboratory as a carrier for the systemic delivery of insulin via the ocular route (Simamora et al., 1996; Lee et al., 1997a). It was shown that the Gelfoam device along with 20 μg of Brij-78 (polyoxyethylene-20-stearyl ether) is effective in producing prolonged systemic delivery of sodium bovine insulin in rabbits. The device produces a five-fold longer duration and a ten-fold larger area above the blood glucose suppression curve than eyedrops.

More recently, absorption enhancer-free insulin eye inserts were introduced (Lee et al., 1997b). The manufacturing procedure for the enhancer-free insulin devices is relatively simple. These devices were prepared by dissolving 0.2 mg of either sodium or zinc insulin into 30 μl of 10% acetic acid–water, sorbing the solution into a Gelfoam sponge and evaporating the solvent. The acetic acid was initially used only as a solvent to facilitate the dissolution of zinc insulin, which is not soluble in either water or 30% ethanol. Interestingly, the acidified insulin-Gelfoam devices produced a uniform blood glucose reduction (60% of initial) for well over 8 h (Lee et al., 1997b). The objective of this report is to investigate the role of the concentration and type of acid in enhancing the absorption of insulin from eye devices.

## 2. Experimental section

### 2.1. Materials

Sodium bovine insulin and zinc bovine insulin were purchased from Calbiochem Corporation (La Jolla, CA) and Sigma Chemical Company (St. Louis, MO), respectively. Gelfoam®, absorbable gelatin sponge, USP, size 100, was obtained from Pharmacia & Upjohn Company (Kalamazoo, MI). The ONE TOUCH® BASIC™ blood glucose meter was generously provided by the Lifescan Company (Mountain View, CA) for the measurement of blood glucose concentration. Humulin® R (recombinant DNA origin) was obtained from Eli Lilly & Company (Indianapolis, IN). The concentration of Humulin® R is 100 IU/ml prepared from zinc–insulin crystals. All other solvents and chemicals were of reagent or HPLC grade and were used as received from commercial suppliers.

### 2.2. Fabrication of the insulin delivery systems

The 14 different devices studied are described in Table 1. All devices were 6.0 mm in diameter. Devices were punched from a 2.0 mm thick Gelfoam sponge with the aid of a common hole punch.

#### 2.2.1. Insulin loaded devices

A total of 0.2 mg of either sodium or zinc insulin was dissolved in 30 μl of either 0, 1, 5, 10, or 30% (v/v) aqueous acetic acid or 30 μl of 1% (v/v) aqueous HCl. Each of the insulin containing solutions was placed on a Gelfoam disc with the aid of a Pipetteman pipettor. The wet matrices were then dried under vacuum for at least 72 h to evaporate the solvent. Note that 60 μl of Humulin® R (equivalent to 0.2 mg zinc insulin) was used to make zinc insulin devices containing 0% acetic acid solution.

#### 2.2.2. Placebo devices

A total of 30 μl of either 10% acetic acid or 1% HCl solutions was sorbed into Gelfoam for the blank device preparation. The rest of the manufacturing procedure is the same as for the acid treated insulin delivery systems as described above.

### 2.3. In vivo glucose lowering evaluation

The in vivo evaluation of insulin delivery was described in a previous study (Lee et al., 1997a). Briefly, the insulin device was inserted into the lower conjunctival sac of rabbits. Blood samples

were collected from the marginal ear veins and the glucose concentrations were determined by the ONE TOUCH® BASIC™ blood glucose meter. The reduction of blood glucose levels as a percentage of the initial value was used to assess the absorption of insulin. The study was terminated if the blood glucose concentration of the subject fell below 40 mg/dl. If this occurred, the eye device was removed and instant glucose was administered orally.

## 3. Results

All of the devices listed in Table 1 were well tolerated by rabbits. No physical signs of irritation (e.g. redness, lachrimation, or ulceration) were observed during or after the experimental period. The AAC, Area Above the glucose Concentration-time curve (0–6 h), calculated by trapezoidal rule, is assumed to be a measure of the effectiveness of the various devices. Two rabbits developed hypoglycemia response during the study (one after 6 h post-dosing with 30% HAc treated Zn–insulin device and one after 8 h post-dosing with 10% HAc treated Nz–insulin device). These animals were treated with glucose orally and removed from the study. Only the data up to 6 h were used to calculate AAC.

The blood glucose lowering versus time profiles of 10% acetic acid solution, treated placebo devices, and 0–30% acetic acid solution treated sodium insulin devices are plotted in Fig. 1. Neither the placebos nor the devices prepared with 0 or 1% acetic acid produced any significant alternation in blood glucose concentration, while those containing 5% or more acetic acid produced similar blood glucose lowering profiles. Note that the data in Fig. 1 is only shown for up to 8 h and that the blood glucose levels of most of the rabbits returned to normal within 10–12 h.

Fig. 2 shows that all of the zinc insulin devices produce blood glucose suppression similar to their sodium insulin counterparts. The glucose levels from devices prepared with more than 5% acetic acid were also maintained at approximately 60% of initial for over 8 h following device instillation and returned to normal values within 10–12 h. Note that the 1% acetic acid zinc insulin devices were made from a zinc insulin suspension rather

Table 1
Summary of studies performed on 0.2 mg insulin devices and placebos

| No. | Insulin | Solvants | Figure | Symbol | AAC ± S.D.[a] | *N* |
|---|---|---|---|---|---|---|
| 1[b] | Placebo | 10% HAc | 1 | + | 55 ± 45 | 3 |
| 2 | Na | Water | 1 | × | 29 ± 57 | 3 |
| 3 | Na | 1% HAc | 1 | △ | 110 ± 22 | 3 |
| 4 | Na | 5% HAc | 1 | □ | 224 ± 57 | 3 |
| 5 | Na | 10% HAc | 1 | ○ | 202 ± 40 | 3 |
| 6 | Na | 30% HAc | 1 | ◇ | 193 ± 35 | 3 |
| 7 | Zn | Water[c] | 2 | × | 43 ± 23 | 3 |
| 8[d] | Zn | 1% HAc | 2 | △ | 94 ± 63 | 3 |
| 9 | Zn | 5% HAc | 2 | □ | 207 ± 15 | 3 |
| 10 | Zn | 10% HAc | 2 | ○ | 201 ± 40 | 3 |
| 11 | Zn | 30% HAc | 2 | ◇ | 204[e] | 2 |
| 12 | Placebo | 1% HCl | 3 | × | −32 ± 5 | 3 |
| 13 | Na | 1% HCl | 3 | △ | 167 ± 64 | 3 |
| 14 | Zn | 1% HCl | 3 | □ | 179 ± 34 | 3 |

[a] Area from 0 to 6 h (calculated by the trapezoidal rule) above the glucose concentration curve.
[b] Two placebo devices in the same eye.
[c] Humulin®.
[d] Devices were made from zinc insulin suspension due to the low solubility of zinc insulin in either 1% acetic acid solution or water.
[e] AAC was calculated from two rabbits and only the mean presented.

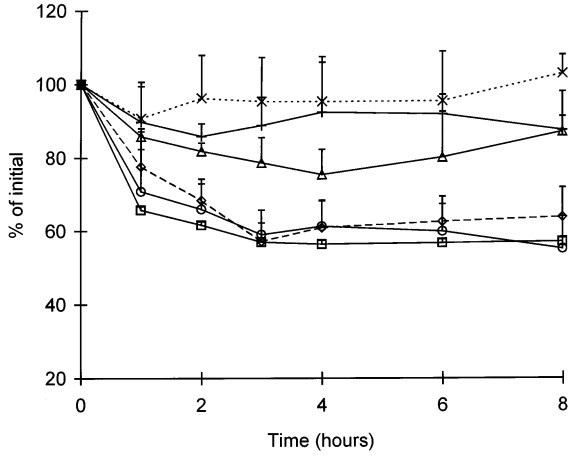


Fig. 1. Mean percentage of initial blood glucose level after ocular instillation of placebo and 0.2 mg sodium insulin devices prepared with: 0% ( × ), 1% (△), 5% (□), 10% (○), and 30% (◇) acetic acid solution. Each value represents the average of three rabbits ± S.D.

than from a clear solution. Humulin® R was used for the 0% acetic acid (i.e. water only) treated device because zinc insulin could not be dissolved in pure water at the desired concentration.

Fig. 3 shows that the blood glucose lowering produced by either sodium or zinc insulin devices

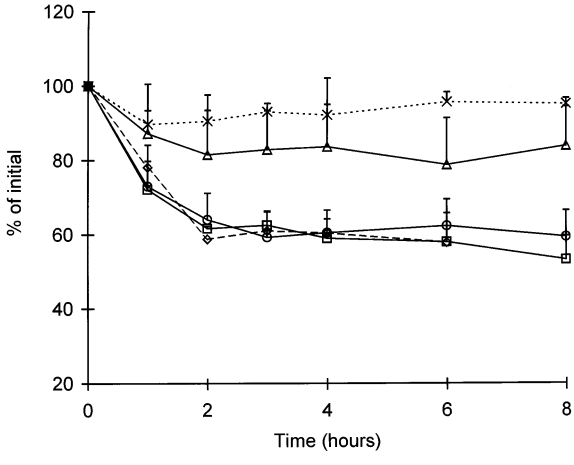


Fig. 2. Mean percentage of initial blood glucose level after ocular instillation of 0.2 mg Zn–insulin devices prepared with: 0% ( × ), 1% (△), 5% (□), 10% (○), and 30% (◇) acetic acid solution. Each value represents the average of three rabbits ± S.D., except the 30% acetic acid formulation, which was carried out by two rabbits and only mean be presented.

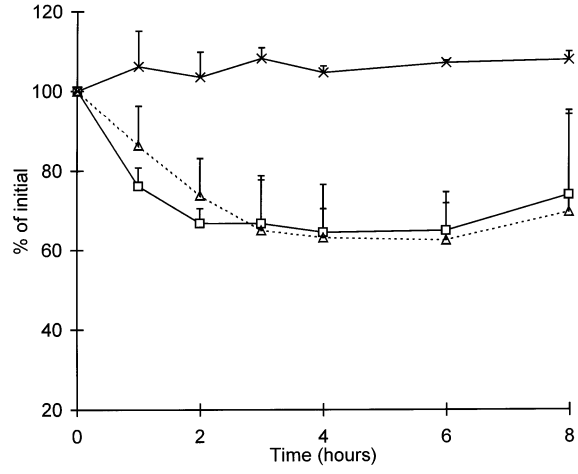


Fig. 3. Mean percentage of initial blood glucose level after ocular administration of 0.2 mg of insulin: 1% HCl acidified placebo device ( × ), 1% HCl acidified sodium insulin–Gelfoam device (△), 1% HCl acidified zinc insulin–Gelfoam device (□). Each value represents the average of three rabbits ± S.D.

prepared with 1% HCl are comparable to each other and to those produced by acetic acid concentrations greater than 5%. In both cases the blood glucose levels were well maintained at about 65% of the normal level between 2 and 8 h after device instillation and gradually returned to normal after 8 h. The placebo HCl treated device produced no blood glucose lowering.

## 4. Discussion

Although the proposed device was treated with different concentrations of acetic acid as well as a 1% HCl solution, no visible eye irritation such as redness, lachrimation and ulceration has been observed during or after the experiments. This is consistent with only a trace amount of volatile acid remaining in the device after the evaporation and the fact that after the instillation of the device, the pH of the tears changed from pH 7 to 5 and returned to neutral pH within 5 min. Note that non-volatile acids were not studied because they would be irritating to the eye. These results along with other reports on the local delivery of pilocarpine (Simamora et al., 1998) phenylephrine

and tropicamide (Negvesky et al., 1999) and systemic delivery of peptides such as melanotan II (Pinsuwan et al., 1997) and insulin (Simamora et al., 1996; Lee et al., 1997a) clearly indicate that the Gelfoam sponge is a useful vehicle for both local and systemic drug delivery via the ocular route.

Fig. 4 shows the dependency of AAC, listed in Table 1, on the concentration of acetic acid for both sodium and zinc insulin formulations. This figure clearly indicates that acidified insulin–Gelfoam devices with at least 5% acetic acid are sufficient to substantially enhance the efficacy of the insulin devices and that higher concentrations offer little or no improvement, and thus are not needed. As can be seen in Fig. 3, the blood glucose lowering profiles produced by 1% HCl treated Na– and Zn–insulin are virtually identical to each other and are comparable to the profiles produced by acetic acid treated devices shown in both Figs. 1 and 2. This suggests that the systemic absorption of insulin from the eye device can be enhanced by acid in general rather than acetic acid only. While the use of dilute acid to prepare the insulin device can enhance the systemic absorption of insulin, the mechanism of this enhancement is not fully understood. We suspect that the interaction between acid and Gelfoam might produce an absorption enhancer which promotes the systemic absorption of insulin from the proposed device. This is consistent with the fact that Imai et al. (1989) proposed that hydrolyzed gelatin is an absorption enhancer.

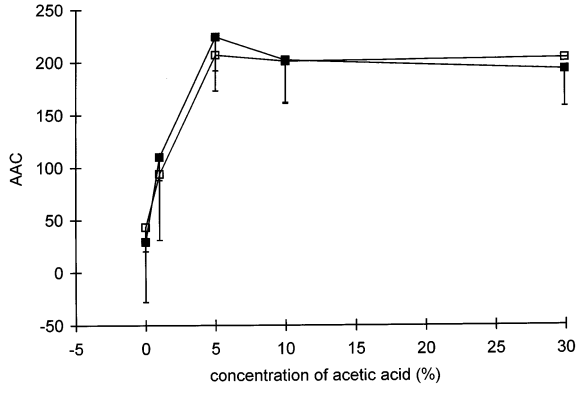


Fig. 4. The AAC values produced by sodium (■) and zinc (□) insulin versus the concentration of acetic acid. Each value represents the average of three rabbits ± S.D., except the 30% acetic acid formulation, which was carried out by two rabbits and only mean be presented.

Previously, the dependency of the systemic absorption of insulin from the proposed device upon the dose of insulin and amount of surfactant has been reported (Lee et al., 1997a). In that report, devices containing 1.0 mg of insulin with 20 μg of Brij-78 produced 12 h of uniform blood glucose lowering profiles in rabbit. This current report demonstrates that the efficacy and safety of Gelfoam as a carrier for the systemic delivery of insulin can be improved greatly by the addition followed by the evaporation of dilute acetic or hydrochloric acid and eliminates the use of a surfactant. In fact, a 0.2 mg acid treated insulin device produced a blood glucose lowering profile that is fairly similar to that of a 1.0 mg insulin device with 20 μg of Brij-78 as an absorption enhancer.

## 5. Conclusions

Several insulin ocular formulations were prepared to evaluate the dependency of the concentration and type of acids on the systemic absorption of insulin. The glucose lowering responses produced by 0.2 mg of insulin devices prepared with 5, 10, or 30% acetic acid as well as 1% HCl are similar to each other and are well controlled for over 8 h. These findings confirm that insulin can be delivered into systemic circulation without the aid of either a surfactant or chelating agent to improve adsorption.

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