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# Systemic absorption of insulin from a Gelfoam® ocular device

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**Abstract**

In previous reports (Lee et al., 1997b; Lee and Yalkowsky, 1999), it has been shown that insulin, delivered by an acidified Gelfoam (absorbable gelatin sponge, USP) based ocular device, can be efficiently absorbed into the systemic circulation without the aid of an absorption enhancer. The role of acid in the enhancer-free absorption of insulin is investigated in this report. Gelfoam ocular devices containing 0.2 mg of sodium insulin prepared with either water or 10% acetic acid were evaluated in rabbits. The results suggest that a change in the Gelfoam upon treatment with acid is responsible for the efficient systemic absorption of insulin from these enhancer-free devices. 

*Keywords:* Insulin; Ocular device; Gelfoam

## 1. Introduction

It is generally believed that an enhancer is required for the systemic absorption of insulin delivered by the ocular route (Chiou and Chuang, 1989; Chiou et al., 1989, 1990; Yamamoto et al., 1989; Chiou, 1991; Hopper et al., 1991; Pillion et al., 1991, 1995; Chiou and Li, 1993; Bartlett et al., 1994a,b; Sasaki et al., 1994, 1995; Morgan 1995; Morgan and Huntzicker 1996; Simamora et al., 1996; Lee et al., 1997a). Recently, Simamora et al. (1996) introduced a Gelfoam based ocular device for the systemic delivery of insulin. Lee et al. (1997a) showed that the device could give a uniform blood glucose reduction for well over 8 h with the aid of Brij-78 as an absorption enhancer. Lee et al. (1997b) also showed that similar results can be obtained from enhancer free devices to which acetic acid has been added and then removed by evaporation. This study will investigate how acetic acid treatment promotes the therapeutic activity of insulin even though it is removed through vacuum drying for 72 h prior to administration of the device.

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## 2. Experimental section

### 2.1. Materials

Sodium bovine insulin was purchased from Calbiochem (La Jolla, CA). Gelfoam®, absorbable gelatin sponge, USP, size 100, was generously supplied by Pharmacia & Upjohn (Kalamazoo, MI). The ONE TOUCH® BASIC™ blood glucose meter was generously provided by Lifescan (Mountain View, CA) for the measurement of blood glucose concentration. All 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

#### 2.2.1. Formulation 1: acidified Gelfoam placebo devices

A total of 30 μl of 10% acetic acid solution was sorbed into each 6.0-mm diameter 2.0-mm thick Gelfoam disc. The wet matrices were vacuum dried for at least 72 h to remove the solvents.

#### 2.2.2. Formulation 2: water treated insulin-Gelfoam devices

A total of 0.2 mg of sodium insulin was dissolved in 30 μl of distilled water. The mixture was placed on and sorbed into Gelfoam discs. The wet devices were then dried by evaporation under vacuum for at least 72 h to remove the solvents.

#### 2.2.3. Formulation 3: acid pretreated insulin devices

A total of 0.2 mg of sodium insulin was dissolved in 30 μl of 10% (v/v) aqueous acetic acid solution. This insulin-acetic acid solution was dried under vacuum. The dry acid pretreated insulin powder was reconstituted with 30 μl of distilled water. This was placed on and sorbed into Gelfoam discs. The wet devices were dried under vacuum for at least 72 h to remove the solvents.

#### 2.2.4. Formulation 4: acidified insulin-Gelfoam devices

A total of 0.2 mg of sodium insulin was dissolved in 30 μl of 10% (v/v) aqueous acetic acid. This solution was placed on and sorbed into Gelfoam discs. The wet matrices were then dried under vacuum for at least 72 h to remove the solvents.

### 2.3. In vivo glucose lowering evaluation

Each of the above devices was placed into the lower conjunctival sac of one eye of the rabbit. As in previous studies by Simamora et al. (1996), blood glucose levels were measured with the aid of ONE TOUCH® BASIC™ blood glucose meter, and the extent and duration of blood glucose lowering in rabbits was taken as a measure of the therapeutic efficacy of the devices. In order to distinguish between the effect of acetic acid on insulin and the effect of acetic acid on Gelfoam, it was necessary to simultaneously administer two devices into the same eye in one set of experiments. All experiments were performed in triplicate.

## 3. Results

The pharmacological responses to the instillation of the devices into the eyes of rabbits are shown in Fig. 1. As shown in Fig. 1, the rabbits exhibited no significant blood glucose lowering after receiving either formulation 1 (+), 2 (×) or 3 (□). Interestingly, the blood glucose levels of the rabbits were substantially suppressed after the instillation of one acidified placebo and one insulin-loaded device treated with water into the same eye (▲). This combination of the two devices in the same eye gave well-controlled glucose depression profiles between 2 and 8 h postdosing. These profiles were similar to those produced by the single acidified insulin-Gelfoam devices (△) reported by Lee and Yalkowsky (1999). The area above the glucose concentration curve (AAC), calculated by trapezoidal rule, is assumed to be a measure of the effectiveness of the various devices. In order to facilitate comparisons, these values are given in Table 1.

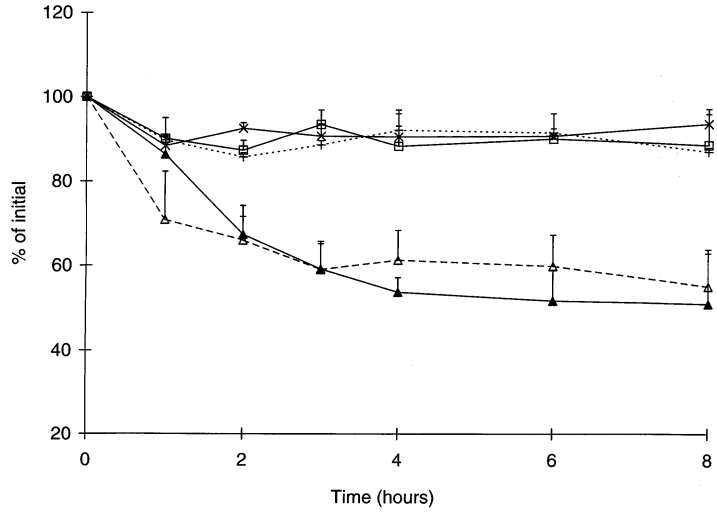


Fig. 1. Mean percentage of initial blood glucose level after ocular instillation of acidified placebo device (+), water treated insulin-Gelfoam devices (×), acid pretreated insulin device (□), acidified insulin-Gelfoam device (△), one acidified placebo device and one water treated insulin-Gelfoam device (▲). Each value represents the average of three rabbits ± S.D.

## 4. Discussion

It has been shown by Lee and colleagues (Lee et al., 1997b; Lee and Yalkowsky, 1999) that acetic acid enhances the systemic absorption of insulin from gelatin devices by the ocular route. There are a number of possible explanations for the effectiveness of insulin devices in lowering blood glucose. Some of these are listed below:

A. Acetic acid is a glucose lowering agent or an absorption enhancer.
B. Gelfoam is a glucose lowering agent or an absorption enhancer.
C. The interaction of insulin and Gelfoam produces a more potent glucose lowering agent or an absorption enhancer.
D. The interaction of acetic acid and insulin produces a more potent glucose lowering agent or an absorption enhancer.
E. The interaction of acetic acid and Gelfoam produces a more potent glucose lowering agent or an absorption enhancer.
F. The three-way interaction of insulin, Gelfoam and acetic acid produces a more potent glucose lowering agent or an absorption enhancer.

Table 1
Summary of studies performed on insulin devices and placebos

| Regimen | Na-insulin, mg | Insulin treatment | Gelfoam treatment | Symbol | AAC[a] ± S.D. | *n* |
|---|---|---|---|---|---|---|
| Formulation 1[b] | Placebo | – | 10% HAc | + | 75 ± 31 | 3 |
| Formulation 2 | 0.2 | Water | Water | × | 65 ± 10 | 3 |
| Formulation 3 | 0.2 | 10% HAc | water | □ | 76 ± 36 | 3 |
| Formulation 4[b] | 0.2 | 10% HAc | 10% HAc | △ | 278 ± 53 | 3 |
| Combination of | Placebo | – | 10% HAc | | | |
| Formulations 1 and 2[c] | 0.2 | Water | Water | ▲ | 324 ± 6 | 3 |

[a] Area (calculated by the trapezoidal rule) above the glucose concentration curve.
[b] Data obtained from Lee et al. (1998).
[c] Two devices in the same eye.

It is generally believed that an enhancer such as a surfactant or chelating agent is required for the systemic absorption of insulin via the ocular route. However Hirai et al. (1972, 1981) have shown that insulin can be absorbed into the systemic circulation effectively from nasal solutions buffered at pH 3.1–3.7 with acetic acid but not from pH 5–7 solutions. They also showed that the delivery of a pH 5.5 insulin nasal solution without absorption enhancer has virtually no blood glucose lowering effect in the rat. They suggested that the absorption efficacy of insulin nasal solution is dependent upon the solubility and aggregation of insulin, and that these properties are dependent on the pH of medium. Since they did not use a pH 3.1–3.7 placebo, it is not possible to state whether or not acetic acid is an absorption enhancer.

Previously, Lee et al. (1997b) reported that dilute acetic acid treated insulin devices without surfactant significantly lowered the blood glucose levels in rabbits for over 8 h. The acetic acid in these devices was intended to be a solvent for insulin rather than to act as an absorption enhancer. The pH of the tears changed from pH 7 to pH 5 immediately after the instillation of the acid treated device and returned to pH 7 within 5 min while absorption continued for over 8 h. Clearly, tear pH is not responsible for enhancing the absorption of insulin from its major absorption site, the nasal cavity (Yamamoto et al., 1989), as proposed by Hirai et al. (1978). Finally, the administration of formulation 1, the acidified placebo device, produces no significant blood glucose lowering. This outcome proves that acetic acid is not a glucose lowering agent. Accordingly, possibility A is excluded for any further consideration.

Lee et al. (1997a) demonstrated that neither a 30% ethanol water solution treated Gelfoam placebo nor the same device containing up to 2.0 mg of sodium insulin, could lower the blood glucose levels in rabbit. On the other hand, if 20 μg of Brij-78 (a polyoxyethylene-20-stearyl ether surfactant) is incorporated into the insulin devices, the blood glucose concentration of rabbits is drastically depressed to below 40% of initial concentration after 2 h of dosing. These data suggest that the low efficacy of the 2.0-mg Na-insulin device without an absorption enhancer is not due to denaturation of insulin in the presence of 30% of ethanol, but due to the lack of an absorption enhancer. In the current study, formulation 2, i.e. water treated 0.2 mg sodium insulin, was used. As expected, this dose, which is ten times lower than the dose used in the previous study, produced no significant blood glucose lowering. According to these observations, Gelfoam alone is neither an absorption enhancer nor a glucose lowering agent, and the interaction between Gelfoam and insulin does not produce enhanced absorption or blood glucose lowering. Thus, possibilities B and C can be eliminated. The Gelfoam in these devices appears to function only as a comfortable and biocompatible carrier.

The instillation of the acetic acid pretreated insulin device, formulation 3, did not significantly alter the blood glucose levels in rabbits. These data imply that the interaction of acetic acid with insulin dose not produce a more potent glucose lowering agent or an absorption enhancer, and thus possibility D can be eliminated.

As seen in Fig. 1, the blood glucose levels of rabbits are significantly suppressed after they receive an acidified insulin-Gelfoam device (formulation 4). Interestingly, blood glucose levels are also lowered significantly in rabbits that receive one acidified placebo device (formulation 1) plus one water treated insulin device in the same eye (formulation 2). These results suggest that possibilities E and/or F may be responsible for the enhancer-free absorption of insulin from the device. However, in the two-device instillation study, the trace of acid in the placebo devices did not come into direct contact with the insulin. Therefore, it could neither alter the structure or conformation of the insulin nor participate in a three-way interaction with the carrier. Since the enhancement of absorption could not be attributed to the interaction of insulin either with acid or with a combination of acid and gelatin, possibility F can be eliminated. The only remaining explanation for the enhancement of insulin absorption from acid treated Gelfoam ocular devices is possibility E, i.e. that the acid interacts with the gelatin to produce an absorption en-

hancer or a glucose lowering agent. The second possibility in E can be eliminated on the basis of the lack of activity of formulation 1 and also of a previous study in which Lee and Yalkowsky (1999) showed that the administration of a 1% HCl treated placebo device did not alter the blood glucose levels in rabbits. Overall, these data suggest that the enhancement of the absorption of insulin is due to a change in the Gelfoam upon acid treatment. This is confirmed by the fact that devices which are not treated by acid (formulation 2) are inactive whereas those that are acid treated (formulation 4) are active.

Hydrolyzed gelatin fragments (mean molecular weight 6000) have been reported to promote the absorption of some poorly water soluble drugs after oral or rectal administration (Imai et al., 1989; Kimura et al., 1990, 1991). While neither the chemical composition nor the mechanism by which it acts are known, it is clear that the interaction of gelatin with dilute acetic acid produces a potent enhancer that promotes the systemic absorption of insulin delivered via the ocular route.

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